

AVERY'S DISEASES *of the* NEWBORN

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TENTH EDITION

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Avery's Diseases of the Newborn

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Tenth Edition

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*To the babies—our patients—who humble and inspire us.
To their families, who encourage us to keep moving our field forward.
To neonatal caregivers everywhere, with gratitude for all you do.*

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Preface

“The neonatal period ... represents the last frontier of medicine, territory which has just begun to be cleared of its forests and underbrush in preparation for its eagerly anticipated crops of saved lives.” Introduction to the first edition of Schaffer’s Diseases of the Newborn

History

The first edition of *Diseases of the Newborn* was published in 1960 by Dr. Alexander J. Schaffer, a well-known Baltimore pediatrician who coined the term *neonatology* to describe this emerging pediatric subspecialty that concentrated on “the art and science of diagnosis and treatment of disorders of the newborn infant.” Schaffer’s first edition was used mainly for diagnosis, but also included descriptions of new neonatal care practices (i.e., the use of antibiotics, temperature regulation, and attention to feeding techniques)—practices that had led to a remarkable decrease in the infant mortality rate in the United States, from 47 deaths per 1000 live births in 1940 to 26 per 1000 in 1960. But a pivotal year for the fledgling subspecialty of neonatology came 3 years later in 1963, with the birth of President John F. Kennedy’s son, Patrick Bouvier Kennedy, at 35 weeks’ gestation (i.e., late preterm). His death at 3 days of age, from complications of hyaline membrane disease, accelerated the development of infant ventilators that, coupled with micro-blood gas analysis and expertise in the use of umbilical artery catheterization, led to the development of intensive care for newborns in the 1960s on both sides of the Atlantic. Advances in neonatal surgery and cardiology, along with further technological innovations, stimulated the development of neonatal intensive care units and regionalization of care for sick newborn infants over the next several decades. These developments were accompanied by an explosion of neonatal research activity that led to improved understanding of the pathophysiology and genetic basis of diseases of the newborn, which in turn has led to spectacular advances in neonatal diagnosis and therapeutics—particularly in the care of preterm infants. Combined, these efforts led to continued improvements in the infant mortality rate in the United States, from 26 deaths per 1000 live births in 1960 to 5.8 deaths per 1000 live births in 2014. Current research efforts are focused on decreasing the striking regional, ethnic, and global disparities in infant mortality rates, improving neonatal outcomes, advancing neonatal therapeutics, preventing newborn diseases, and finally—teaming with our obstetrical colleagues—preventing prematurity. We neonatologists would like to begin downsizing, instead of continually expanding, our neonatal intensive care units!

Dr. Mary Ellen Avery joined Dr. Schaffer for the third edition of *Diseases of the Newborn* in 1971. For the fourth edition in 1977, Drs. Avery and Schaffer recognized that their book now needed multiple contributors with subspecialty expertise and they became co-editors, rather than sole co-authors, of the book. In the preface

to that fourth edition, Dr. Schaffer wrote, “We have also seen the application of some fundamental advances in molecular biology to the management of our fetal and newborn patients”—referring to the new knowledge of hemoglobinopathies. Dr. Schaffer died in 1981 and Dr. H. William Taeusch joined Dr. Avery as co-editor for the fifth edition in 1984. Dr. Roberta Ballard joined Drs. Taeusch and Avery for the sixth edition in 1991, with the addition of Dr. Christine Gleason for the eighth edition in 2004. Drs. Avery, Taeusch, and Ballard retired from editing the book in 2009, and became “editors emeriti.” Dr. Sherin Devaskar joined Dr. Gleason as co-editor for the ninth edition, bringing a wonderfully fresh perspective, as well as new contributors to the book. For this, the tenth edition, Dr. Sandra “Sunny” Juul teamed with Dr. Gleason as co-editor—the first time that co-editors have been faculty at the same institution since the fifth edition was published in 1984.

What’s New and Improved About This Edition?

We are thrilled that the book is now in full color—no need to flip back and forth from the chapter text to the color plates at the front of the book! Also new to this edition are several Key Points that contributors have added to the beginning of each chapter, providing readers with a quick summary of the most important content. The Expert Consult eBook version includes new features, such as ultrasound videos, and has been enhanced to make content more easily searchable, shareable (via a new Social Media feature), portable, and perpetual.

The book continues to be thoroughly (and sometimes painfully) revised and updated by some of the best clinicians and investigators in their field—several of whom are new contributors to this edition. Some chapters required more extensive revision than others, particularly those that deal with areas in which we have benefitted from new knowledge and/or its application to new diagnostic and therapeutic practices. This is particularly true in areas such as neurology, hematology, global health and neonatal screening, and genomics. Several new chapters have been added that reflect the continued growth and development of our subspecialty. These include chapters on brain injury (both preterm and term), palliative care, gastroesophageal reflux, platelet disorders, transfusion therapy, neonatal hypertension, and the ear/hearing disorders.

With the incredible breadth and depth of information immediately available to neonatal caregivers and educators on multiple online sites, what’s the value of a printed textbook? We, the co-editors of this tenth edition, believe that textbooks such as *Diseases of the Newborn* and all forms of integrative scholarship, will always be needed—by clinicians striving to provide state-of-the-art neonatal care, by educators striving to train the next generation of caregivers, and by investigators striving to advance neonatal research and

scholarship. A textbook's content is only as good as its contributors and this textbook, like the previous editions, has awesome contributors. They were chosen for their expertise and ability to integrate their knowledge into a comprehensive, readable, and useful chapter. They did this despite the demands of their day jobs in the hopes that their syntheses could, as Ethel Dunham wrote in the foreword to the first edition, "spread more widely what is already known ... and make it possible to apply these facts."

Although the online versions of this and other textbooks enjoy increasingly popular use, in 2017—a full 57 years after the publication of the first edition of this book—we still find copies of this and other textbooks important to our subspecialty lying dog-eared, coffee-stained, annotated, and broken-spined in places where neonatal caregivers congregate. These places, these congregations of neonatal caregivers, are now present in nearly every country around the world. The tentacles of neonatal practice and education are spreading—ever deeper, ever wider—to improve the outcome of pregnancy worldwide. Textbooks connect us to the past, bring us up to date with the present, and prepare and excite us for the future. We will always need them, in one form or another, at our sites of practice. To that end, we have challenged ourselves to meet, and hopefully exceed, that need—for our field, for our colleagues, and for the babies.

Acknowledgments and Gratitude

We wish to thank key staff at Elsevier—Dee Simpson, senior developmental editor, Kate Dimock, our original publishing director, Sarah Barth, our new senior content strategist, and Sharon Corell, senior project manager. Each demonstrated patience, guidance, and persistence; without them, we would still be hard at work, trying to make this book a reality! We also wish to thank our staff and colleagues at our academic institution, the University of Washington, especially our Department Chair, F. Bruder Stapleton, whose leadership and unwavering support have meant a great deal to us both. We are indebted to our contributors, who actually *wrote* the book and did so willingly, enthusiastically, and (for the most part) in a timely fashion—despite myriad other responsibilities in their lives. Finally, we are deeply grateful for the support of our families throughout the long, and often challenging, editorial process.

Christine Gleason and Sandra Juul

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1

Neonatal and Perinatal Epidemiology

NIGEL PANETH AND TRACY THOMPSON

KEY POINTS

- Population-level study of pregnancy and infancy has been an important component of the success of newborn care.
- Disease, mortality, and later outcomes patterns are complex. Some factors (i.e., preterm birth and birthweight) are stable, while others (i.e., cesarean section and twinning rates) can undergo rapid change.
- The success of newborn intensive care is well established and has substantially lowered mortality rates in a short period of time primarily because of the evidence-based nature of neonatal practice.
- Survivors of neonatal intensive care face educational and rehabilitative needs. Recent interventions have reduced the burden of brain damage.
- Sudden infant death syndrome (SIDS), through careful epidemiologic study and active discouragement of prone sleeping, has been reduced by 70% in the United States.
- Observational research and randomized trials have led to increased folate intake and a substantial reduction in neural tube birth defects.

The period surrounding the time of birth, the *perinatal* period, is a critical episode in human development, rivaling only the period surrounding conception in its significance. This time period is when the infant makes the critical transition from its dependence upon maternal and placental support (oxidative, nutritional, and endocrinologic) and establishes independent life. That this transition is not always successful is signaled by a mortality risk in the neonatal period that is not exceeded until age 75–84 and risks for damage to organ systems, most notably the brain, that can be lifelong (Murphy et al., 2013). The developing human organism often does not manifest the immediate effects of even profound insults. Years must pass before the damage to higher cortical functions of insults and injuries occurring during the perinatal period can be reliably detected. Epidemiologic approaches to the perinatal period must therefore be bidirectional: looking backwards from birth to examine the underlying causes of adverse health conditions that arise or complicate the perinatal period and looking forward to later life to see how these conditions shape disorders of health in childhood and adulthood.

Health Disorders of Pregnancy and the Perinatal Period

Key Population Mortality Rates

Maternal and child health in the population has traditionally been assessed by monitoring the two key rates of maternal mortality and infant mortality (IM). Maternal mortality is defined by the World Health Organization (WHO) as the death of a woman during pregnancy or within 42 days of pregnancy, denominated either to live births or to all births (this must be specified) in the population being studied (WHO, 2010). Because pregnancy can contribute to deaths beyond 42 days, some have argued for examining all deaths within a year of a pregnancy but later deaths are not included in standard tabulations of maternal mortality (Hoyert, 2007). When the cause of death is attributed to a pregnancy-related condition, it is described as *direct*. When pregnancy has aggravated an underlying health disorder present before pregnancy, the death is termed an *indirect* maternal death. The WHO recommends that both direct and total (direct plus indirect) maternal mortality rates be provided.

Deaths unrelated to pregnancy, but taking place in women within 42 days of pregnancy, are termed *incidental* maternal deaths and are not included in maternal mortality (Khlal, 2006). But even incidental deaths may bear a relation to pregnancy; homicide and suicide, for example, are more common during pregnancy and shortly thereafter and might not be entirely incidental to it (Shadigian and Bauer, 2005; Samandari et al., 2010).

In most geographic entities, IM is defined as all deaths occurring from birth to 365 days of age in a calendar year divided by all live births in the same year. This approach makes for imprecision, as some deaths in the examined year occurred in the previous year's birth cohort, and some births in the examined year will die as infants in the following year. In recent years, birth–death linkage has permitted vital registration areas in the United States to provide IM rates that avoid this imprecision. The standard IM rate reported by the National Center for Health Statistics (NCHS) links *deaths* for the index year to all births, including those taking place the *previous* year. This form of IM is termed *period* IM. An alternative procedure is to take births for the index year and link them to

infant deaths, including those taking place the *following* year. This is referred to as *birth cohort* IM and is not used for regular annual comparisons because it cannot be completed in as timely a fashion as period IM (Mathews, 2015).

Infant deaths are often divided into deaths in the first 28 days of life (*neonatal* deaths) and deaths later in the first year (*postneonatal* deaths). Neonatal deaths, which are largely related to preterm birth and birth defects, tend to reflect the circumstances of pregnancy whereas postneonatal deaths, when high, are nearly all from infection, often in the setting of poor nutrition. Thus in underdeveloped countries, postneonatal deaths dominate; in industrialized countries, the reverse is true. In the United States, neonatal deaths have been more frequent than postneonatal deaths since 1921. In recent years, the ratio of neonatal to postneonatal deaths in the United States has consistently been about 2:1.

Perinatal mortality is a term used for a rate that combines stillbirths and neonatal deaths in some fashion (Box 1.1) Stillbirth reporting prior to 28 weeks, even in the United States, where such stillbirths are required to be reported in every state, is probably incomplete. Nonetheless, stillbirths continue to be reported at a level not much lower than that of neonatal deaths, and our understanding of the causes of stillbirth remains very uncertain (Paneth, 2012; Lawn et al., 2016).

Sources of Information on Mortality–Vital Data

All US mortality data depend upon the collection of information about all births and deaths. Routinely collected vital data are the

• BOX 1.1 Glossary of Terms

Preterm birth – less than 37 weeks' gestation

- **Very preterm** – less than 32 weeks' gestation
- **Extremely preterm** – less than 28 weeks' gestation

Low birth weight – infant weighs less than 2500 g (5 lb 8 oz) at birth regardless of gestational age

- **Moderately low birth weight** – an infant weighing at least 1500 g but less than 2500 g at birth regardless of gestational age
- **Very low birth weight** – an infant weighing less than 1000 g (2 lb 3 oz) at birth regardless of gestational age

Maternal mortality ratio – death of a woman during pregnancy or within 42 days of pregnancy compared with either live births or with all births in the population

- **Direct maternal mortality** – a maternal death attributed to a pregnancy-related cause
- **Incidental maternal mortality** – a maternal death occurring during the defined time period for maternal mortality but unrelated to pregnancy
- **Indirect maternal mortality** – a maternal death caused by the pregnancy aggravating an underlying health disorder present before pregnancy

Infant mortality rate – all deaths occurring from birth to 365 days of age in a calendar year divided by all live births in the same year

Birth cohort infant mortality – births for the index year are linked to infant deaths including those taking place the following year

Neonatal mortality – infant deaths within the first 28 days of life

Perinatal mortality rate – number of stillbirths that occur after 22 weeks' gestation and deaths in the first week of life per 1000 total births

Period infant mortality – all infant deaths in a calendar year linked to births, including births that took place in the previous year

Postneonatal mortality – infant deaths after the first 28 days of life but before the 366th day of life

nation's key resource for monitoring progress in caring for mothers and children. Annual counts of births and deaths collected by the 52 vital registration areas of the United States (50 states, District of Columbia, and New York City) are assembled into national data sets by the NCHS. Unlike data collected in hospitals or clinics, or even from nationally representative surveys, birth and death certificates are required by law to be completed for each birth and death. Birth and death registration have been virtually 100% complete for all parts of the United States since the 1950s. The universality of this process renders many findings from vital data analyses stable and generalizable, although formatting changes in 2003, affecting both the death and birth certificates, have created some difficulties in interpretation.

For example, since 2003 the US Standard certificate of death, which is recommended for adoption by US vital registration areas, has included a special requirement for identifying whether the decedent, if female, was pregnant or had been pregnant in the previous 42 days. This simple check box on the death certificate has been shown to increase the number of deaths recognized as maternal in states that have followed the 2003 model and incorporated questions about pregnancy in their death certificates (Mac et al., 2011).

Fig. 1.1 illustrates the most recent (2003) nationally recommended standard for birth certificate data collection, which had been adopted for use by 33 states by 2010 (Curtin et al., 2013). The remaining states use birth certificates formatted according to the 1989 standard. While, as we discuss below, some items are collected differently on the two certificate templates, unlike maternal mortality, these changes do not affect the number of reported deaths.

The limitations of vital data are well known. Causes of death are subject to certifier variability and perhaps more importantly to professional trends in diagnostic categorization. The accuracy of recording of conditions and measures on birth certificates is often uncertain and variable from state to state and from hospital to hospital. Yet the frequencies of births and deaths in sub-groups defined objectively and recorded consistently, such as birthweight and mode of delivery, are likely to be valid.

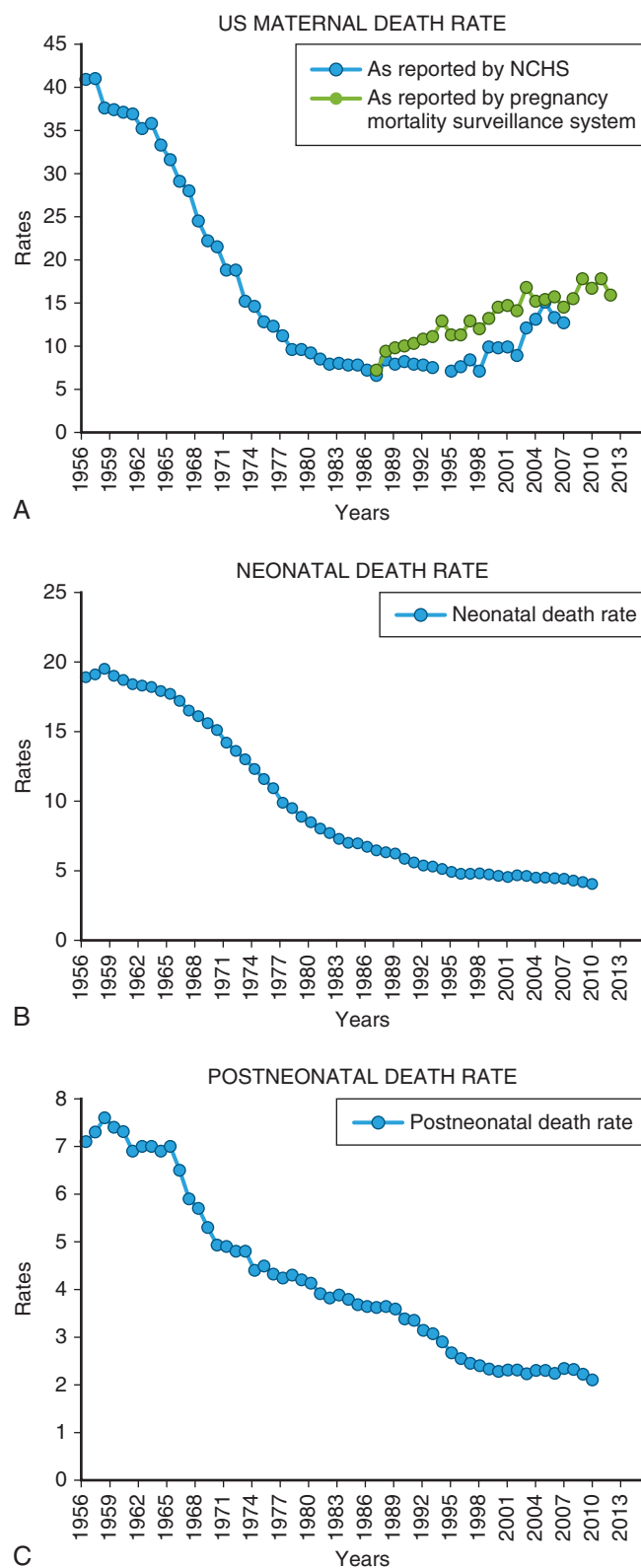
Time Trends in Mortality Rates of the Perinatal Period in the United States

Maternal mortality and IM declined steadily through the 20th century. By 2000, neonatal mortality was 10% of its 1915 value, postneonatal mortality less than 7%, and maternal mortality less than 2%. The contribution to these changes of a variety of complex social factors, including improvements in income, housing, birth spacing, and nutrition, has been widely documented, as has the role of ecologic-level public health interventions that have produced cleaner food and water (Division of Reproductive Health, 1999). Public health action at the individual level, including targeted maternal and infant nutrition programs and immunization programs, has made a lesser but still notable contribution. Medical care per se was, until recently, less critically involved, with the exception of the decline in maternal mortality, which was very sensitive to the developments in blood banking and antibiotics that began in the 1930s. To this day hemorrhage and infection account for a large fraction of the world's maternal deaths (Khan et al., 2006).

A notable feature of the past half-century or so is the sharp decline in all three mortality rates beginning in the 1960s following a period of stagnation in the 1950s (Fig. 1.2) The decline began with maternal mortality, followed by postneonatal, and then

| | | | | | | |
|---|--|--|---|---|--|--|
| MOTHER | 29a. DATE OF FIRST PRENATAL CARE VISIT MM / DD / YYYY No Prenatal Care | | 29b. DATE OF LAST PRENATAL CARE VISIT MM / DD / YYYY | | 30. TOTAL NUMBER OF PRENATAL VISITS FOR THIS PREGNANCY _____ (If none, enter A0".) | |
| | 31. MOTHER'S HEIGHT _____ (feet/inches) | | 32. MOTHER'S PREPREGNANCY WEIGHT _____ (pounds) | | 33. MOTHER'S WEIGHT AT DELIVERY _____ (pounds) | |
| | 35. NUMBER OF PREVIOUS LIVE BIRTHS (Do not include this child) | | 36. NUMBER OF OTHER PREGNANCY OUTCOMES (spontaneous or induced losses or ectopic pregnancies) | | 37. CIGARETTE SMOKING BEFORE AND DURING PREGNANCY For each time period, enter either the number of cigarettes or the number of packs of cigarettes smoked. IF NONE, ENTER A0". | |
| | 35a. Now Living Number _____ None | | 35b. Now Dead Number _____ None | | 36a. Other Outcomes Number _____ None | |
| MEDICAL AND HEALTH INFORMATION | 35c. DATE OF LAST LIVE BIRTH MM / YYYY | | 36b. DATE OF LAST OTHER PREGNANCY OUTCOME MM / YYYY | | 39. DATE LAST NORMAL MENSES BEGAN MM / DD / YYYY | |
| | 38. PRINCIPAL SOURCE OF PAYMENT FOR THIS DELIVERY Private Insurance Medicaid Self-pay Other (Specify) _____ | | 40. MOTHER'S MEDICAL RECORD NUMBER | | | |
| | 41. RISK FACTORS IN THIS PREGNANCY (Check all that apply) Diabetes Prepregnancy (Diagnosis prior to this pregnancy) Gestational (Diagnosis in this pregnancy) Hypertension Prepregnancy (Chronic) Gestational (PIH, preeclampsia) Eclampsia Previous preterm birth Other previous poor pregnancy outcome (Includes perinatal death, small-for-gestational age/intrauterine growth restricted birth) Pregnancy resulted from infertility treatment-If yes, check all that apply: Fertility-enhancing drugs, Artificial insemination or Intrauterine insemination Assisted reproductive technology (e.g., in vitro fertilization (IVF), gamete intrafallopian transfer (GIFT)) Mother had a previous cesarean delivery If yes, how many _____ None of the above | | 43. OBSTETRIC PROCEDURES (Check all that apply) Cervical cerclage Tocolysis External cephalic version: Successful Failed None of the above | | 46. METHOD OF DELIVERY A. Was delivery with forceps attempted but unsuccessful? Yes No B. Was delivery with vacuum extraction attempted but unsuccessful? Yes No C. Fetal presentation at birth Cephalic Breech Other D. Final route and method of delivery (Check one) Vaginal/Spontaneous Vaginal/Forceps Vaginal/Vacuum Cesarean If cesarean, was a trial of labor attempted? Yes No | |
| | 42. INFECTIONS PRESENT AND/OR TREATED DURING THIS PREGNANCY (Check all that apply) Gonorrhea Syphilis Chlamydia Hepatitis B Hepatitis C None of the above | | 44. ONSET OF LABOR (Check all that apply) Premature Rupture of the Membranes (prolonged, ≥12 hrs.) Precipitous Labor (<3 hrs.) Prolonged Labor (≥20 hrs.) None of the above | | 45. CHARACTERISTICS OF LABOR AND DELIVERY (Check all that apply) Induction of labor Augmentation of labor Non-vertex presentation Steroids (glucocorticoids) for fetal lung maturation received by the mother prior to delivery Antibiotics received by the mother during labor Clinical chorioamnionitis diagnosed during labor or maternal temperature ≥38°C (100.4°F) Moderate/heavy meconium staining of the amniotic fluid Fetal intolerance of labor such that one or more of the following actions was taken: in-utero resuscitative measures, further fetal assessment, or operative delivery Epidural or spinal anesthesia during labor None of the above | |
| NEWBORN INFORMATION | | | | | | |
| NEWBORN | 48. NEWBORN MEDICAL RECORD NUMBER | | 54. ABNORMAL CONDITIONS OF THE NEWBORN (Check all that apply) | | 55. CONGENITAL ANOMALIES OF THE NEWBORN (Check all that apply) | |
| | 49. BIRTHWEIGHT (grams preferred, specify unit) _____ 9 grams 9 lb/oz | | Assisted ventilation required immediately following delivery | | Anencephaly Meningocele/Spina bifida Cyanotic congenital heart disease Congenital diaphragmatic hernia Omphalocele Gastroschisis Limb reduction defect (excluding congenital amputation and dwarfing syndromes) Cleft Lip with or without Cleft Palate Cleft Palate alone Down Syndrome Karyotype confirmed Karyotype pending Suspected chromosomal disorder Karyotype confirmed Karyotype pending Hypoplasia None of the anomalies listed above | |
| | 50. OBSTETRIC ESTIMATE OF GESTATION: _____ (completed weeks) | | Assisted ventilation required for more than six hours | | | |
| | 51. APGAR SCORE: Score at 5 minutes: _____ If 5 minute score is less than 6, Score at 10 minutes: _____ | | NICU admission | | | |
| Mother's Name Mother's Medical Record No. | 52. PLURALITY - Single, Twin, Triplet, etc. (Specify) _____ | | Newborn given surfactant replacement therapy | | | |
| | 53. IF NOT SINGLE BIRTH - Born First, Second, Third, etc. (Specify) _____ | | Antibiotics received by the newborn for suspected neonatal sepsis | | | |
| | | | Seizure or serious neurologic dysfunction | | | |
| | | | Significant birth injury (skeletal fracture(s), peripheral nerve injury, and/or soft tissue/solid organ hemorrhage which requires intervention) | | | |
| 56. WAS INFANT TRANSFERRED WITHIN 24 HOURS OF DELIVERY? 9 Yes 9 No IF YES, NAME OF FACILITY INFANT TRANSFERRED TO: _____ | | 57. IS INFANT LIVING AT TIME OF REPORT? Yes No Infant transferred, status unknown | | 58. IS THE INFANT BEING BREASTFED AT DISCHARGE? Yes No | | |

• Fig. 1.1 United States National Standard Birth Certificate 2003 Revision.



• **Fig. 1.2** Maternal, Neonatal, and Postneonatal Mortality Rates 1956–2013. (A) United States maternal death rate. (B) Neonatal death rate. (C) Postneonatal death rate. NCHS, National Center for Health Statistics; US, United States. (From the pregnancy surveillance system <https://www.cdc.gov/reproductivehealth/maternalinfanthealth/pmss.html> and Martin JA, Hamilton BE, Ventura SJ, et al. Births: Final data for 2010. National vital statistics reports; vol 61 no 1. Hyattsville, MD: National Center for Health Statistics. 2012.)

neonatal. The contribution of medical care of the neonate was most clearly seen in national statistics in the 1970s, a decade that witnessed a larger decline in neonatal mortality than in any previous decade of the century. All of the change in neonatal mortality between 1950 and 1975 was in mortality for a given birthweight; no improvement was seen in the birthweight distribution (Lee et al., 1980). This finding suggested that the effectiveness of newborn intensive care has had a striking impact on mortality in very small babies. Prior to the development of newborn intensive care, survival at birthweights less than 1000 g was very rare. In 2013, the US survival rate to 1 year for infants with a birthweight between 501 and 999 g was 75%, and the number of survivors at age 1 was over 16,000.

In retrospect, three factors seem to have played critical roles in the rapid development of the newborn intensive care programs that largely accounted for the rapid decline in birthweight-specific neonatal mortality that characterized national trends in the last third of the 20th century. The first was the willingness of medicine to provide more than nursing care to marginal populations such as the premature infant. While the death of the mildly premature son of President Kennedy in 1963 provided a stimulus to the development of newborn intensive care, it should be noted that the decline in IM that began in the 1970s was paralleled by a similar decline in mortality for the extremely old (Rosenwaik et al., 1980). This was, perhaps, an indicator that the availability of federal funding through Medicare and Medicaid enabled previously underserved populations at the extremes of age to receive greater medical attention than they had before. The Medicaid program, adopted in 1965, may have made it feasible for the first time to pay for the intensive care of premature newborns, among whom the medically indigent are over-represented. While financial support for newborn intensive care may have been a necessary ingredient in its development, finances would have not been sufficient to improve neonatal mortality had not new medical technologies, especially those supporting ventilation of the immature newborn lung, been developed at about the same time (Gregory et al., 1975).

Advances in newborn care have ameliorated the impact of premature birth and birth defects on mortality. Unfortunately, the underlying disorders that drive perinatal mortality and the long-term developmental disorders that are sometimes their sequelae have shown no tendency to abate. With the very important exception of neural tube defects, whose prevalence has declined with folate fortification of flour in the United States and programs to encourage intake of folate in women of child-bearing age (Mathews et al., 2002), the major causes of death (preterm birth and birth defects) have not declined, nor has cerebral palsy, the major neurodevelopmental disorder that can be of perinatal origin (Paneth et al., 2006). Progress has come from improved medical care of the high-risk pregnancy and the sick infant, rather than through understanding and prevention of the disorders themselves.

The pace of decline in infant, neonatal, and postneonatal mortality in the United States began to slow in 1995 and changed little in the following decade. A modest decline was seen, however, between 2005 and 2010 (Table 1.1). Data from the Vermont Oxford Neonatal Network encompassing more than a quarter of a million newborns from hundreds of largely North American neonatal units showed a decline in mortality of 12.2% for infants of 501–1500 g for 1990–1999 (Horbar et al., 2002) and a further 13.3% decline for 2000–2009 (Horbar et al., 2012). These declines are more modest than in the early days of newborn intensive care. From 1960–1985, a greater than 50% decline in mortality for

TABLE 1.1 United States Perinatal Mortality, Morbidity, Interventions, and Pregnancy Health Conditions and Behaviors, 1990–2005

| | 1995 | 2000 | 2005 | 2010 | Net Change 1995–2010 (%) |
|---|-------------------|------|-------------------|------|-----------------------------|
| Deaths^a | | | | | |
| Maternal mortality ratio | 12.9 | 13.2 | 15.2 | 17.8 | + 38.0 |
| Infant mortality rate | 7.6 | 6.9 | 6.9 | 6.1 | – 19.7 |
| Neonatal mortality rate | 4.9 | 4.6 | 4.5 | 4.0 | – 18.4 |
| Postneonatal mortality rate | 2.6 | 2.3 | 2.3 | 2.1 | – 19.2 |
| Fetal mortality ratio ^b | 6.9 | 6.6 | 6.2 | 6.0 | – 13.0 |
| Morbidity | | | | | |
| Preterm birth (<37 weeks, %) | 11.0 | 11.6 | 12.7 | 12.0 | + 9.0 |
| Very preterm birth (<32 weeks, %) | 1.9 | 1.9 | 2.0 | 2.0 | + 5.2 |
| Extremely preterm birth (<28 weeks, %) | 0.70 | 0.72 | 0.76 | 0.74 | + 5.7 |
| Moderately low birth weight (%) | 6.6 | 7.1 | 7.3 | 6.7 | + 1.5 |
| Low birth weight (%) | 7.3 | 7.6 | 8.2 | 8.2 | + 12.3 |
| Very low birth weight (%) | 1.4 | 1.4 | 1.5 | 1.5 | + 1.5 |
| Pregnancy-associated hypertension (%) | 3.3 | 3.9 | 4.0 | — | |
| Diabetes in pregnancy (%) | 2.4 | 2.9 | 3.8 | 5.1 | + 212 |
| Interventions | | | | | |
| Cesarean section (%) | 20.8 | 22.9 | 30.3 | 32.8 | + 57.7 |
| Induction of labor (%) | 15.8 | 19.9 | 22.3 | 23.4 | + 48.1 |
| Health Behaviors | | | | | |
| Smoking (%) | ^c 13.7 | 12.2 | ^d 10.7 | — | |
| Alcohol intake (%) | ^e 1.5 | 0.9 | ^f 0.7 | — | |
| Inadequate weight gain (<16 lb) at 40 weeks (%) | 9.3 | 11.6 | 11.4 | — | |
| Weight gain of >40 lb (%) | 17.5 | 19.2 | 20.6 | 20.8 | + 18.9 |
| Late or no prenatal care (%) | 4.2 | 3.9 | 3.5 | — | |
| Unmarried (%) | 32.1 | 33.2 | 36.9 | 40.8 | + 27.1 |
| Multiple births (%) | 26.1 | — | 33.8 | 34.5 | + 32.2 |
| Fertility rate (women 15–44, %) | 64.6 | 65.3 | 66.7 | 64.1 | – 0.77 |

^aMortality rates denominatored to 1000 live births, except for maternal mortality, denominatored to 100,000 live births.
^bFetal deaths with stated or presumed period of gestation of 20 weeks or more.
^c1995 smoking data include 46 states, New York City, and District of Columbia.
^d2005 smoking data based on 36 states using only yes/no (pre-2003 revision).
^e1995 alcohol intake has no data from California or South Dakota.
^f2005 alcohol use data that includes 36 states, New York City, and District of Columbia.

infants of 501–1500 g was recorded in national data (Buehler et al., 1987; Prager, 1994), even though much of the first decade of that interval preceded the use of newborn intensive care technology in all but a few pioneering centers. The pace of advances in newborn medicine and the expansion of newborn intensive care to populations previously underserved, factors that have exerted a constant downward pressure on IM since the 1960s, have lessened in the past two decades or so.

Reported maternal mortality has actually climbed substantially in recent years, but this is almost certainly the effect of the improved reporting described above. The Centers for Disease Control (CDC) has a special unit dedicated to the problem of maternal mortality, the Pregnancy Mortality Surveillance System (CDC, 2017). Established in 1987, its counts of maternal deaths, based on more in-depth exploration than is possible from a vital registration system alone, have provided consistently higher estimates of maternal

mortality than data reported by the NCHS, as shown in Fig. 1.2. Recognizing this, and the variation in reporting resulting from the variable use by states of the 2003 recommendation for identifying recent pregnancies on death certificates, the NCHS stopped reporting maternal mortality in 2008 (Minino et al., 2011). All recent maternal mortality data in the US are produced by the Pregnancy Mortality Surveillance System.

The risk of preterm birth (<37 weeks' gestation) increased steadily in the first years of the present century, peaked in 2007, and has declined by 8% since (Hamilton et al., 2015). The increase was largely in moderately preterm babies and likely reflected an increased willingness on the part of obstetricians to deliver fetuses earlier in gestation who were not doing well in utero, as well as the increased prevalence of twins and triplets, who are generally born preterm, resulting from in vitro fertilization. The newer data suggest a reversal of these earlier practices.

The recording of diabetes in pregnancy more than doubled from 1995 to 2010, but the NCHS has suggested that some of this might reflect more complete reporting on the 2003 birth certificate revision (Martin et al., 2010b). The differences in the two forms of birth certificate in circulation, and the uneven implementation of the newer version in vital registration areas, led NCHS, in 2008 (Martin et al., 2010a), to omit regular reporting of smoking, alcohol intake, weight gain, late prenatal care, and pregnancy-associated hypertension, among other variables, from its regular tabulations in annual natality reports, and these are not provided for 2010 in Table 1.1.

The cesarean section rate continues its long-term increase, from 5% in 1970 to 23% in 1990, peaking at nearly 33% in 2010, with a faint decline since (Hamilton et al., 2015). The reasons for this increase are multifactorial and include pressures from patients, physicians, and the medical malpractice system. The steady reduction in smoking in pregnancy is likely to be real, whereas trends in the self-reporting of alcohol use in pregnancy may be influenced by societal norms and expectations. Fewer women seem to have late or no prenatal care in recent years, but perhaps surprisingly, more women are found to have inadequate pregnancy weight gain at term. A very slight uptick in the fertility rate follows a long-term (since about 1960) decline in fertility in the United States. More than 4 in 10 mothers in the United States are now unmarried when they give birth.

International Comparisons

The US lag in IM, in comparison to other developed nations, is well known; the United States ranked 26th in IM among the Organization for Economic Co-operation and Development countries in 2010 (MacDorman et al., 2014). This surprising phenomenon, in light of more favorable socioeconomic and medical care circumstances in the United States than in many nations with lower IM, cannot be attributed to inferior neonatal care. Mortality rates for low birth weight infants are generally lower in the United States than in European and Asian nations, though mortality at term may be slightly higher. The key difference, however, is that the United States suffers from a striking excess of premature births. While the US African-American (AA) population is especially vulnerable to prematurity, and especially severe prematurity, prematurity rates are also considerably higher in white Americans than in most European populations. It is likely that the recording of marginally viable small infants as live births rather than stillbirths is more pronounced in the United States than in Europe (Kramer et al., 2002). While this practice does make a

contribution to our higher prematurity and IM rates, it cannot fully explain them.

Premature birth, fetal growth retardation, and IM are tightly linked, in every setting in which they have been studied, to most measures of social class, especially to maternal education. However, uncovering precisely what it is about lower social class that drives these important biologic differences has been elusive. Factors such as smoking have at times been implicated but can only explain a small fraction of the social class effect. It is unlikely that this situation will change until a better understanding of the complex social, environmental, and biologic roots of preterm birth are achieved.

Health Disparities in the Perinatal Period

In 2010, 54.1% of all US births were to non-Hispanic white mothers; 23.6% were to Hispanic mothers; 14.7% were to AA mothers; and the remainder were to mothers of other ethnic groups (Table 1.2). Health disparities are especially prominent in the perinatal period, with AA infant IM stubbornly remaining about double that of white IM in the United States, even as rates decline in both populations. Preterm birth is the central contributor to this racial disparity in IM, and the more severe the degree of prematurity, the higher the excess risk for AA infants. The risk of birth before 37 weeks of gestation was 1.5 times higher in AA mothers than among non-Hispanic whites in 2010, but the risk of birth before 32 weeks was twice as high. Reduction in IM disparities in the United States thus requires better understanding of the etiology and mechanisms of preterm birth. Birth defect mortality shows a less pronounced gradient by ethnic group and does not contribute in a major way to overall IM disparities (Yang et al., 2006).

The *Hispanic paradox* is a term often used to describe the observation that IM is the same or lower in US citizens classified as Hispanic than in non-Hispanic whites, in spite of the generally lower income and education levels of US Hispanics (Hessol and Fuentes-Afflick, 2005). The IM experience of Hispanics in the United States reflects the principle that premature birth and low birth weight are key determinants of IM, as these parameters are also favorable in Hispanics. Smoking is much less common among Hispanics in the United States, but this factor alone does not fully explain the paradox.

Major Causes of Death

Cause-of-death analysis, a staple of epidemiologic investigation, has limitations when applied to the perinatal period. Birth defect mortality is probably reasonably accurate, but causes of deaths among prematures are divided among categories such as respiratory distress syndrome, immaturity, and a variety of complications of prematurity. Choice of which particular epiphenomenon of preterm birth should be chosen to be listed as the primary cause of death is to some extent arbitrary. Some maternal complications, such as preeclampsia, are also occasionally listed as causes of newborn death, and birth defects and preterm birth often overlap. However categorized, prematurity per se accounts for at least a third of infant deaths (Callaghan et al., 2006).

In the period before prenatal ultrasound permitted reasonably accurate gestational age estimation, a high fraction of neonatal deaths were attributed to low birth weight, but most of these deaths occurred in premature infants, because premature birth is much more important as a cause of death than is fetal growth restriction. Extreme prematurity makes a contribution to IM well

TABLE 1.2 Ethnic Disparities in Key Perinatal Outcomes and Exposures in 2010

| | Non-Hispanic White | African-American | RR Compared With Whites | Hispanic | RR Compared With Whites |
|--|--------------------|------------------|-------------------------|----------|-------------------------|
| Births (percent of all births, %) | 54.1 | 14.7 | — | 23.6 | — |
| Deaths^a | | | | | |
| Maternal mortality ratio | 11.8 | 41.1 | 2.5 | — | — |
| Infant mortality rate | 5.2 | 11.5 | 2.2 | 5.3 | 1.0 |
| Neonatal mortality rate | 3.4 | 7.5 | 2.21 | 3.6 | 1.1 |
| Postneonatal mortality rate | 1.8 | 4.0 | 2.2 | 1.7 | 0.94 |
| Fetal mortality ratio | 4.8 | 10.8 | 2.3 | 5.2 | 1.1 |
| Morbidity | | | | | |
| Preterm birth (%) | 11.1 | 16.9 | 1.5 | 11.8 | 1.1 |
| Early preterm birth (%) | 3.0 | 6.0 | 2.0 | 3.3 | 1.1 |
| Late preterm birth (%) | 8.0 | 10.9 | 1.36 | 8.5 | 1.1 |
| Low birth weight (%) | 7.1 | 13.2 | 1.9 | 7.0 | 0.98 |
| Very low birth weight (%) | 1.2 | 2.9 | 2.4 | 1.4 | 1.2 |
| Diabetes in pregnancy (%) | 4.8 | 4.5 | 0.94 | 5.2 | 1.1 |
| Interventions | | | | | |
| Cesarean section (%) | 32.3 | 35.3 | 1.1 | 31.8 | 0.98 |
| Induction of labor (%) | 24.2 | 21.7 | 0.90 | 17.8 | 0.74 |
| Health Behaviors | | | | | |
| Inadequate weight gain (<16 lb) at 40 weeks (%) ^b | 7.6 | 13.3 | 1.75 | 9.5 | 1.25 |
| More than 40 lb weight gain at 40 weeks (%) | 21.3 | 20.5 | 0.96 | 16.5 | 0.77 |
| Unmarried (%) | 35.9 | 72.1 | 2.0 | 53.4 | 1.5 |
| Fertility rate (women 15–44, %) | 64.4 | 66.3 | 1.03 | 80.2 | 1.25 |
| Multiple births (per 100,000) | 181.7 | 133.7 | 0.74 | 98.7 | 0.54 |

^aMortality rates denominators to 1000 live births, except for maternal mortality, denominators to 100,000 live births.

^bExcludes California, which does not record weight gain.

Sources: NCHS Natality Reports 2010 and Pregnancy Mortality Surveillance System <https://www.cdc.gov/reproductivehealth/maternalinfanthealth/pmss.html>. RR, Relative risk.

beyond its frequency in the population. The 1.9% of births born prior to 32 weeks in 2010 contributed 53% of all infant deaths.

After premature birth, the next most important group of causes of death is congenital anomalies. With the signal exception of the folate–neural tube association, we have no clearly effective primary prevention program for any birth defect. Pregnancy screening and termination of very severe defects are, however, an option for many mothers and there is evidence that this practice does contribute to a reduced prevalence of chromosomal anomalies at birth (Loane et al., 2013).

The major postneonatal cause of death since about the 1970s in the United States is the sudden infant death syndrome (SIDS). This cause of death has declined substantially in the United States in parallel with successful public health efforts to discourage prone sleeping in infancy (Mitchell, 2009).

Major Morbidities Related to the Perinatal Period

The principal complications of preterm birth involve five organs – lung, heart, gut, eye, and brain. Management of respiratory distress syndrome and its short-term and longer-term complications is the centerpiece of neonatal medicine. Surgical or medical management of symptomatic patent ductus arteriosus is the major cardiac challenge for the premature, and we have yet to understand the quite striking variations, by time and place, of necrotizing enterocolitis, a disorder that in its most extreme forms can cause death or substantial loss of bowel function. Retinopathy of prematurity (ROP) is closely related to arterial oxygen levels, and the epidemic levels of this disorder encountered in the 1950s, when oxygen was freely administered without monitoring, was a major setback for

neonatal medicine (Silverman, 1980). However, even with much more careful management of oxygen, ROP continues to occur.

The largest unsolved problem in neonatal medicine remains the high frequency of brain damage in premature survivors. The extraordinary decline in mortality rates has not been paralleled by similar declines in rates of neurodevelopmental disabilities in survivors. Indeed, the key epidemiologic feature of cerebral palsy rates in population registries toward the end of the 20th century was a modest overall increase in the prevalence of that disorder attributable entirely to the increasing number of survivors of very low birth weight. There are suggestions that this rise has leveled off since the 1990s (Smithers-Sheedy et al., 2016).

Factors Affecting Perinatal Health

Health States in Pregnancy

The major causes of neonatal morbidity (prematurity and birth defects) generally occur in pregnancies free of antecedent complications. Having a previous birth with an anomaly or a previous preterm birth both raise the maternal risk for recurrence of the condition. Indeed, for preterm birth, no other known risk factor carries as much risk as having previously delivered preterm.

More than a quarter of preterm birth is iatrogenic, the result of induced labor in pregnancies in which the fetus is severely compromised (Morken et al., 2008). Generally the reason is preeclampsia with attendant impairments in uterine blood flow and poor fetal growth, but poor uterine blood flow and impaired fetal growth can also occur independently of diagnosed preeclampsia. The other major complication of pregnancy is diabetes, most often gestational but at times preexisting. Insulin resistance in the mother promotes the movement of nutrients toward the fetus, and typically the infant of the diabetic mother is large for gestational age. Severe diabetes, however, can be accompanied by fetal growth retardation.

Health Behaviors

The most carefully studied and well-established health behavior affecting newborns is maternal cigarette smoking, which more than doubles the prevalence of babies with intrauterine growth retardation and increases the risk of premature birth by 20%–50% (Dietz et al., 2010). Infants growth retarded from smoking paradoxically survive slightly better than do infants of the same weight whose mothers did not smoke, but the net effect of smoking, which also shortens gestation slightly, is to increase perinatal mortality. The risk of SIDS is also increased in the babies of smoking mothers (Mitchell and Milerad, 2006). Although the subject is much debated, it has not been conclusively shown that prenatal smoking has independent long-term effects on child cognitive capacity (Breslau et al., 2005).

Mothers who drink alcohol heavily in pregnancy are at risk of having infants with the cluster of defects known as the fetal alcohol syndrome (Jones et al., 2013). Cocaine use in pregnancy is a severe growth retardant (Janisse et al., 2014) and may affect neonatal behavior, but the long-term effects of this exposure on infant cognition and behavior are not as great as initially feared (Bandstra et al., 2010).

Perinatal Medical Care

In light of the potent effects of medical care on the neonate, it has been important to develop systems of care that ensure, or at

least facilitate, provision of care to neonates in need. This concept was first promoted by the March of Dimes Foundation, which in its committee report of 1976 recommended that all hospitals caring for babies be classified as either Level 1 (care for healthy and mildly ill newborns), Level 2 (care for most sick born-in newborns but not accepting transfers), and Level 3 (regional centers caring for complex surgical disease and receiving transfers) (Health, 1976). This concept of a regional approach to neonatal care, with different hospitals playing distinct roles in providing care, was endorsed by organizations such as the American College of Obstetricians and Gynecologists, the American Academy of Pediatrics, and by many state health departments. While it is important to transfer sick babies to Level 3 centers when needed, it is preferable, if at all possible, to transfer mothers at risk of delivering prematurely or of having a sick neonate, because transport of the fetus in utero is far superior to any form of postnatal transport. Birth at a Level 3 center has consistently been shown to produce lower mortality rates in low birth weight infants than birth in other levels of care (Lasswell et al., 2010).

Epidemiologic Study Designs in the Perinatal Period

Epidemiologic studies have contributed substantially to better understanding of patterns of risk and prognosis in the perinatal period, to tracking patterns of mortality and morbidity, to assessment of regional medical care, and to assisting physicians and other providers to evaluate the efficacy of treatments. The use of vital data to provide a picture of the overall health of mothers and infants and to monitor important time trends has already been mentioned, but cohort studies and randomized controlled trials have been essential to advances in neonatology.

Cohort Studies in Pregnancy/Birth

Studies that follow populations of infants over time, beginning at birth or even before birth, continuing to hospital discharge, to early childhood, or even into adult life, are the leading sources of information about perinatal risk factors for disease and adverse outcomes. As with all observational studies, cohort studies produce associations of exposures and outcomes whose strength and consistency must be carefully judged in the light of other biologic evidence and with attention to confounding and bias. Collaborations across centers in assembling such data are very valuable. One such notable collaboration is the Vermont–Oxford Network, which provides continuous information on the frequency of conditions observed and diagnoses made in hundreds of US overseas hospitals, with a particular emphasis on using these data for improving care (Horbar et al., 2010). The National Institute of Child Health and Human Development (NICHD)-supported neonatal network has not only been a rich source of randomized trials but also has produced observations about prognosis based on very large samples of low birth weight babies (Stoll et al., 2015). The above collaborations focus mainly on the period until hospital discharge.

Multicenter cohort studies focusing on diagnosis and follow-up of brain injury in prematures, such as the Developmental Epidemiology Network Study (DEN) (Kuban et al., 1999), Neonatal Brain Hemorrhage Study (NBH) (Pinto-Martin et al., 1992), and Extremely Low Gestational Age Newborn Study (ELGAN) (O'Shea et al., 2009), have contributed much to our understanding of the

prognostic value of brain injury imaged by ultrasound in the neonatal period because they include follow-up to age 2 or later. Of particular value have been regional or population-wide studies of low birth weight infants with follow-up to at least school age, among which are included the NBH study from the United States and also important studies from Germany (Breeman et al., 2015; Bruin et al., 2015), Great Britain (Petrou et al., 2013), and Canada (Van Lieshout et al., 2015).

Newborn intensive care has been in place long enough that the first reports of adult outcomes in very small infants are now emerging (Saigal and Doyle, 2008). These studies paint a picture that is perhaps less dire than many had anticipated.

From 1959 to 1966, the National Collaborative Perinatal Project assembled data on approximately 50,000 pregnancies in 12 major medical centers and followed them to age 7 (Niswander, 1972). This highly productive exercise, one of whose major contributions was to show that birth asphyxia is a rare cause of cerebral palsy, has now been followed by the development of even larger pregnancy cohort studies. These studies, all of which archive biologic material such as blood and/or urine in pregnancy, should, in principle, permit us to learn a great deal about the unrecognized pregnancy factors that lead to adverse perinatal and child health outcomes. For reasons not entirely clear, a sample size of 100,000 has been universally adopted in studies in Norway (Magnus et al., 2006), Denmark (Olsen et al., 2001), and most recently Japan (Nitta, 2016). Efforts to mount a similar study in the United States were, unfortunately, not successful (Duncan et al., 2015).

Randomized Controlled Trials

Few areas of medicine have adopted the randomized trial as wholeheartedly as newborn medicine. The number of trials mounted has been large and their influence on practice strong. A notable influence on this field has been the National Perinatal Epidemiology Unit (NPEU) at Oxford University, established in 1978, which prioritized randomized trials among their several investigations of perinatal care practices and other circumstances affecting maternal and newborn outcomes. The NICHD neonatal research network was established in 1986, principally to support trials in *newborns*. Hundreds of trials have been mounted by just these two

organizations alone, but many other centers have contributed to the trial literature.

Trials in *pregnancy or in labor* have also been supported by the NPEU and by a network of obstetric centers supported by NICHD, the maternal-fetal network. These trials have often had important implications for newborns as well as for mothers; most notably, the one trial that has thus far successfully reduced the risk of preterm birth is the administration of 17 alpha-hydroxyprogesterone caproate in mid-gestation to high-risk women (Meis et al., 2003). Vaginal progesterone may also be effective (O'Brien and Lewis, 2016).

Most newborn trials have focused on outcomes evident in the newborn period, such as mortality, chronic lung disease, brain damage visualized on ultrasound, duration of mechanical ventilation, and/or hospital stay. Recently, however, trials extending into infancy or even to early childhood that incorporate measures of cognition or neurologic function have been a welcome addition to the trial arena. In the past few years we have learned from such trials that moderate hypothermia can reduce mortality and disability in asphyxiated term infants (Shankaran et al., 2005), caffeine treatment for apnea may reduce cerebral palsy (Schmidt et al., 2007), and magnesium sulfate administered in labor may reduce the risk of cerebral palsy (Rouse et al., 2008).

Trials in which both mortality and later outcome are combined raise complex methodologic issues. Imbalance in the frequency of the two outcomes being combined can result in random variation in the commoner outcome overwhelming a significant finding in the other. Providing the same weight in a trial to a disability and to a death raises ethical questions. Precisely how best to conduct such dual or multi-outcome trials is the subject of discussion and debate in the neonatal and epidemiologic communities.

As trials multiply, not all of them sufficiently powered, the methodology for summarizing them and drawing effective conclusions has become increasingly important to neonatologists. The terms *systematic review* and *metaanalysis* have firmly entered the research lexicon, especially the randomized trial literature. The Cochrane collaboration is an international organization that uses an army of volunteers to systematically review trial results in all fields of medicine. The collaboration, established in 1993, began in the field of perinatal medical trials. Systematic reviews of neonatal trials reviewed by the Cochrane Collaboration are hosted on the website of NICHD <https://www.nichd.nih.gov/cochrane>.

Summary and Conclusions

The patterns of disease, mortality, and later outcome in the perinatal period are complex. Some factors, such as the long-term trends in preterm birth and birthweight, are reasonably stable, while others, such as the rates of cesarean section and twinning, can undergo rapid change. The success of newborn intensive care is well established. No other organized medical care program, targeted at a broad patient population, has had such remarkable success in lowering mortality rates in such a short period of time. Much of that success is owed to the evidence-based nature of neonatal practice.

Nonetheless, this success has opened the door to new problems as survivors of intensive care face the challenges of the information age. Resource allocations similar to those that permitted the development of newborn intensive care are now needed to address the educational and rehabilitative needs of survivors. A hopeful sign is the success of some recently studied interventions in reducing the burden of brain damage.

On the nontechnologic front, targeted epidemiologic efforts to address perinatal disorders have yielded progress as well. Careful study of the circumstances surrounding infant sleep patterns led to active discouragement of prone sleeping, which has produced a halving of mortality from SIDS. Observational research, followed by two important randomized trials in Europe, led to interventions that increased folate intake in women of child-bearing age and a substantial reduction in the birth prevalence of neural tube defects.

The population-level study of health events occurring in pregnancy and infancy, their antecedents, and long-term consequences have been an important component of the success of newborn care. Careful self-evaluation through monitoring of vital data and of collaborative clinical data, rigorous assessment of new treatments through randomized trials, and alertness to opportunities to implement prevention activities following discovery of important risk factors should continue to guide care of the newborn.

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2

Biomedical Informatics in Neonatology

JONATHAN P. PALMA AND PETER TARCZY-HORNOCH

KEY POINTS

- Although health care remains a quintessentially human endeavor, computers are playing a growing role in information management, particularly in neonatology.
- *Biomedical informatics* can be defined as “the interdisciplinary field that studies and pursues the effective uses of biomedical data, information, and knowledge for scientific inquiry, problem solving, and decision making, driven by efforts to improve human health” (Shortliffe and Blois, 2014).
- The trend in electronic health record (EHR) adoption is moving away from niche systems specific to neonatology and toward enterprise-wide systems, highlighting the importance of neonatologist involvement in the selection, implementation, and optimization of EHRs, which are not configured out of the box for the care of critically ill neonates.
- In evaluating therapeutic recommendations, at least 50% of studied treatments are of uncertain benefit. This gap is an opportunity for both evidence-based medicine (clinical trials) and exploration of “practice-based evidence” generated from EHRs.

Background

At a fundamental level, the practice of neonatology can be considered an information management problem. The care provider is combining patient-specific information (history, physical examination findings, and results of physiologic monitoring, laboratory tests, radiologic evaluation) with generalized information (medical knowledge, practice guidelines, clinical trials, personal experience) to make medical decisions (diagnostic, therapeutic, and management). The Internet has made possible a revolution in the sharing and disseminating of knowledge in all fields, including medicine, with continued growth and maturation of online clinical information resources and tools.

Although medicine remains a quintessentially human endeavor, computers play a vital role in information management, particularly in neonatology. Patient-specific and generalized information (medical knowledge) are increasingly available in electronic form. In the United States, a majority of hospitals have implemented electronic health record (EHR; also known as an *electronic medical record*) systems to manage patient-specific information, with levels of EHR adoption ranging from nursing documentation on electronic flow sheets (93.7%) to entirely paperless hospitals (at least 4.2%) (Healthcare Information and Management Systems Society, 2016) (Table 2.1). The American Recovery and Reinvestment Act of

2009 (ARRA; i.e., the federal economic stimulus plan) included the Health Information Technology for Economic and Clinical Health (HITECH) act, a provision for the investment of \$19 billion in health information technology to motivate physicians to adopt EHRs and \$1.1 billion to research the effectiveness of certain healthcare treatments. These ARRA provisions were predicated on the belief that quality, safety, and efficiency of clinical care can be improved through electronic medical records and evidence-based practice, a notion emphasized in the Institute of Medicine (IOM) reports *To Err is Human* (2000), which suggested that up to 98,000 patients die annually as a result of preventable medical errors, and *Crossing the Quality Chasm* (IOM, 2001), which identified health information technology as one approach to mitigating these errors.

To encourage substantive EHR implementation, the Centers for Medicare and Medicaid Services (CMS) established [Meaningful Use](#) objectives necessary to qualify for federal incentive payments. In 2011, Stage 1 of Meaningful Use encouraged the implementation of basic EHR systems; in 2014, Stage 2 was designed to promote more advanced EHR functionality; in 2018, Stage 3 objectives, which focus on improving quality, safety, and efficiency to achieve improved health outcomes, will be required in order to avoid penalties (<https://www.healthit.gov/providers-professionals/how-attain-meaningful-use>). Due to challenges in meeting objectives without undue burden on providers, the requirements for each stage continue to evolve. Recently, CMS leadership suggested major changes to Stage 3, adding flexibility and shifting the focus from use of technology to achievement of health outcomes.

Parallel with and related to the adoption of information technology is the growth of societal pressures to improve the quality of medical care while controlling costs. These pressures are beginning to affect the way in which medicine and neonatology are practiced. In turn, it is becoming important for neonatologists to understand basic principles related to biomedical and health informatics, databases, electronic medical record systems, and evaluation of therapeutic recommendations.

This expansion of information technology in clinical practice and the concurrent growth of medical knowledge hold great promise but have potential pitfalls. One pitfall that must not be underestimated, and which is as great a danger today as when Blois (1984) first cautioned against it, is the unquestioning adoption of information technology:

And, since the thing that computers do is frequently done by them more rapidly than it is by brains, there has been an irresistible urge

TABLE 2.1 Healthcare Information and Management Systems Society EMR Adoption Model

| UNITED STATES EMR ADOPTION MODEL SM | | |
|---|---|--------------|
| Stage | Cumulative Capabilities | 2015 Final % |
| Stage 7 | Complete EMR; CCD transactions to share data; data warehousing; data continuity with ED, ambulatory, OP | 4.2 |
| Stage 6 | Physician documentation (structured templates), full CDSS (variance & compliance), full R-PACS | 27.1 |
| Stage 5 | Closed loop medication administration | 35.9 |
| Stage 4 | CPOE, clinical decision support (clinical protocols) | 10.1 |
| Stage 3 | Nursing/clinical documentation (flow sheets), CDSS (error checking), PACS available outside radiology | 16.4 |
| Stage 2 | CDR, controlled medical vocabulary, CDS, may have document imaging; HIE capable | 2.6 |
| Stage 1 | Ancillaries – lab, rad, pharmacy – all installed | 1.7 |
| Stage 0 | All three ancillaries not installed | 2.1 |
| n = 5460 | | |
| <small>CCD, continuity of care document; CDR, clinical data repository; CDS, clinical decision support; CDSS, clinical decision support systems; CPOE, computerized provider order entry; ED, emergency department; EMR, electronic medical record; HIE, health information exchange; OP, outpatient; PACS, picture archive and communication systems; R-PACS, Radiology picture archive and communication systems. Data from HIMSS Analytics™ Database © 2016.</small> | | |

to apply computers to medicine, but considerably less of an urge to attempt to understand where and how they can best be used.

Adoption and meaningful use of EHRs is not simply a technology challenge: it is a people and process challenge as well. The recently established Clinical Informatics medical subspecialty aims to train physicians in the skills required to address each of these challenges.

Another present and real challenge is information overload, both in terms of the exponentially increasing corpus of medical knowledge and the large amounts of data available within EHRs. As early as 1995, [Bero and Rennie](#) observed:

Although well over 1 million clinical trials have been conducted, hundreds of thousands remain unpublished or are hard to find and may be in various languages. In the unlikely event that the physician finds all the relevant trials of a treatment, these are rarely

accompanied by any comprehensive systematic review attempting to assess and make sense of the evidence.

The potential of just-in-time information at the point of care is thus particularly appealing, especially considering that the growth in published literature continues at an accelerating rate, with a flood of new knowledge coming from the latest research in genomics, proteomics, metabolomics, and systems biology. In addition, the adoption of EHRs has resulted in the collection of large amounts of clinical data as a byproduct of routine care. A vision to address this was articulated by one of the editors of the *British Medical Journal*: “New information tools are needed: they are likely to be electronic, portable, fast, easy to use, connected to both a large valid database of medical knowledge, and the patient record” ([Smith, 1996](#)). More recently, the IOM published *Best Care at Lower Cost: The Path to Continuously Learning Health Care in America* (2012), which calls for transformation of the healthcare system into one that learns from each patient treated and makes the information produced from prior patient encounters (along with evidence from the literature) available at the point of care.

Although progress in healthcare information technology tools is being made, there is significant opportunity to develop, implement, and optimize electronic tools for neonatal care.

Biomedical and Health Informatics

In the 1970s, clinicians with expertise in computers became intrigued by the potential of these tools to improve the practice of medicine, and thus the field of medical informatics was born ([McDonald, 1976](#)). The importance of this field in addressing the issues of information management in health care is growing rapidly, as evidenced by the activities of the American Medical Informatics Association ([AMIA](#); www.amia.org), including the establishment of Clinical Informatics as a subspecialty by the American Board of Medical Specialties (ABMS) in 2011. *Biomedical informatics* can be defined as “the interdisciplinary field that studies and pursues the effective uses of biomedical data, information, and knowledge for scientific inquiry, problem solving, and decision making, driven by efforts to improve human health” ([Shortliffe and Blois, 2014](#)). A more extensive definition can be found at the AMIA website under About AMIA, including professional and training opportunities. The University of Washington (Seattle, Washington, USA) website (<http://bime.uw.edu/>) contains a review of the discipline (found under History, About Us, Vision). The field includes both applied and basic research, with the focus of this chapter being on the applied aspects. Examples of basic research are artificial intelligence in medicine, genome data analysis, and data mining (analyzing data to identify patterns and establish relationships). As our knowledge of the genetic mechanisms of disease expands and more data about patients and outcomes are available electronically, the role of informatics in medicine will expand, particularly in the field of neonatology.

The applied focus of the field in the 1960s and 1970s was data oriented, focusing on signal processing and statistical data analysis. In neonatology, the earliest applications of computers were for physiologic data monitoring in the neonatal intensive care unit (NICU). As the field matured in the 1980s, applied work focused on systems to manage patient information and medical knowledge on a limited basis. Examples include laboratory systems, radiology systems, centralized transcription systems, and, probably the best-known medical knowledge management system, the database of published medical articles maintained by the [National Library of Medicine](#) known first as MEDLARS, then as MEDLINE, and

currently as PubMed (www.ncbi.nlm.nih.gov/pubmed). In addition, neonatologists began to develop tools to aid in the management of patients in the NICU, such as computer-assisted algorithms to help manage ventilators, although the algorithms have not been successfully deployed on a large scale in the clinical setting.

As computers and networking became mainstream in the workplace and home in the 1990s, informatics researchers began to develop integrated and networked systems (Fuller, 1992, 1997). With the explosion of information from the Human Genome Project, the intersection between bioinformatics and medical informatics began to blur, leading to the adoption of the term *biomedical informatics*. The 1990s saw the development of a number of important systems. In terms of patient-specific information retrieval, these systems included integrated electronic medical record systems that in their full implementation can encompass—in a single software application—interfaces to physiologic monitors; electronic flow sheets; access to laboratory and radiology data; tools for electronic documentation (charting), electronic order entry, and integrated billing; and modules to help reduce medical errors. The Internet has permitted ready access and sharing of this information within healthcare organizations and limited secured remote access to this information from home. In terms of patient-population information retrieval, a number of tools were developed to help clinicians and researchers examine aggregate data in these electronic medical records to document outcomes and help to improve quality of care.

The Internet, particularly the World Wide Web, transformed access to medical knowledge (Fuller et al., 1999). Health sciences libraries are increasingly digital, rather than paper repositories. Journals are available online; some are offered exclusively online. Knowledge is available at the point of care in ways that were not previously possible (Tarczy-Hornoch et al., 1997). In 2004, in recognition of the unfulfilled potential of healthcare information technology, the Office of the National Coordinator for Health Information Technology (ONC) was established (<https://www.healthit.gov/>) to achieve the following vision:

Health information technology (HIT) allows comprehensive management of medical information and its secure exchange between health care consumers and providers. Broad use of HIT has the potential to improve health care quality, prevent medical errors, increase the efficiency of care provision, and reduce unnecessary health care costs, increase administrative efficiencies, decrease paperwork, expand access to affordable care, and improve population health.

Throughout the first decade of the 21st century, the focus shifted from demonstrating the promise of electronic medical record and information systems toward implementing them more broadly to realize their benefit (e.g., the ARRA legislation). The pursuit of evidence-based practice can benefit from informatics as an approach to support evaluation of therapeutic recommendations and their implementation (see later discussion) and was part of the ARRA and other approaches to healthcare reform being proposed in 2009. The enormous amount of data being generated through routine use of electronic medical records has the potential to serve as “practice-based evidence,” adding to the medical knowledge base when high quality evidence either doesn’t exist or isn’t applicable to the patient population of interest.

The establishment of Clinical Informatics as a subspecialty in 2011 is further evidence of the evolution of informatics as a medical discipline (Frequently Asked Questions website ([https://](https://www.amia.org/clinical-informatics-board-review-course/faq)

www.amia.org/clinical-informatics-board-review-course/faq). The core content of the subspecialty was defined by AMIA in 2008, and in 2009 the American Board of Preventive Medicine agreed to sponsor the application to the ABMS for Clinical Informatics as a new subspecialty. The ABMS ultimately approved the proposal in 2011, and the first board examination was offered in 2013. In order to sit for the subspecialty boards, candidates must be certified in another ABMS clinical specialty, hold a valid medical license, and (through 2017) qualify either via a practice pathway (at least 25% of a full-time equivalent for 3 years) or the fellowship training pathway. In 2014, the Accreditation Council for Graduate Medical Education (ACGME) released the Program Requirements for Graduate Medical Education in Clinical Informatics, and beginning in 2018, completion of an ACGME-accredited fellowship training program will be required for subspecialty board eligibility (<https://www.amia.org/programs/academic-forum/clinical-informatics-fellowships>).

The practice of neonatal intensive care can clearly benefit from the support of information technology tools; as such, neonatologists have long been involved in informatics. In 1988, as one of the earlier specialties to develop a national database of clinical care, neonatologists established and expanded the Vermont Oxford Network (VON) (www.vtoxford.org) to improve the quality and safety of medical care for newborn infants and their families. As part of their activities, the VON established and maintained a nationwide database about the care and outcome of high-risk newborn infants. Duncan (2015) maintains a continually updated bibliographic database on the history of computer applications in neonatology.

Databases

In broad terms, a database is an organized, structured collection of data designed for a particular purpose. Thus a stack of 3×5 cards with patient information is a database, as is the increasingly rare paper prenatal record. Most frequently, the term *database* is used to refer to a structured electronic collection of information, such as a database of clinical trial data for a group of patients in a study. Databases come in a variety of fundamental types, such as single-table, relational, and object-oriented.

A simple database can be built using a single table by means of a spreadsheet program such as Microsoft Excel or a database program such as Microsoft Access (Microsoft, Redmond, Washington, USA). The advantage of such a database is that it is easy to build and maintain. For an outcomes database in a neonatology unit, each row can represent a patient and each column represents information about the patients (e.g., name, medical record number, gestational age, birth date, length of stay, patent ductus arteriosus [yes/no], necrotizing enterocolitis [NEC; yes/no]). The major limitation of such a database is that a column must be added to store the information each time the researcher wants to track another outcome (e.g., maple syrup urine disease [MSUD]). This limitation can result in tables with dozens to hundreds of columns, which can become difficult to maintain. The challenges can be illustrated with a few examples. One type of challenge results from adding a new column (e.g., MSUD); one must either review all records (rows) already in the spreadsheet for the presence or absence of MSUD or flag all existing records (rows) in the spreadsheet as *unknown* for MSUD status. Another set of challenges results from the logistics of managing an extremely wide spreadsheet – imagine not adding the 10th column but the 1000th column.

The majority of databases and electronic medical records in neonatology are built using relational database software. To build a simple outcomes relational database that permits easy adding of

Tables in the database:

| | | |
|--|--|--|
| DEMOGRAPHIC DATA Name Hospital_Number Gestational_Age Birthdate Admit_Date Discharge_Date | DIAGNOSES Hospital_Number Diagnosis_Code Value | DIAGNOSIS DICTIONARY Diagnosis_Code Description |
|--|--|--|

Example entries in the tables:

| DEMOGRAPHIC DATA | | | | | |
|------------------|-----------------|-----------------|-----------|------------|----------------|
| Name | Hospital_Number | Gestational_Age | Birthdate | Admit_Date | Discharge_Date |
| John Smith | 00-00-01 | 27 | 1/1/2003 | 1/1/2003 | 3/21/2003 |
| Jane Doe | 00-00-02 | 24 | 1/2/2003 | 1/3/2003 | 4/15/2003 |

| DIAGNOSES | | |
|-----------------|----------------|-------|
| Hospital_Number | Diagnosis_Code | Value |
| 00-00-01 | 1 | 1 |
| 00-00-01 | 2 | 1 |
| 00-00-02 | 1 | 2 |
| 00-00-02 | 10234 | |

| DIAGNOSIS DICTIONARY | |
|----------------------|---------------------------------|
| Diagnosis_Code | Description |
| 1 | PDA (1 = small, 2 = large) |
| 2 | NEC (1 = medical, 2 = surgical) |
| | |
| 10234 | Maple Syrup Urine Disease |

• **Fig. 2.1** Example of a Relational Database. *PDA*, patent ductus arteriosus; *NEC*, necrotizing enterocolitis.

new outcome measures, one could use a three-table database design (Fig. 2.1). The DEMOGRAPHIC DATA table contains demographic information for each patient (e.g., Name, Hospital Number [medical record number], Gestational Age). The DIAGNOSIS DICTIONARY table assigns a code number to each diagnosis or outcome being tracked (e.g., patent ductus arteriosus [PDA] = 1; NEC = 2; MSUD = 10234) and defines additional diagnosis descriptors. The DIAGNOSES table links patients (using Hospital Number [i.e., medical record number]) to their diagnoses and assigns a Value to the Diagnosis Code as a descriptor. Adding a new diagnosis to track simply requires adding an entry to the DIAGNOSIS DICTIONARY table. To add a diagnosis to a patient, one would add an entry to the DIAGNOSES table. For example, Girl Smith (medical record number 00-00-01) has a diagnosis of NEC. To add the diagnosis, add to the DIAGNOSES table an entry of “00-00-01” in the Hospital Number column, “2” (the code for NEC) in the Diagnosis Code column, and “2” in the Value column (corresponding to a Description for surgical in the DIAGNOSIS DICTIONARY). Although relational databases are harder to build, they provide greater flexibility for expansion and maintenance and thus are the preferred implementation for clinical databases. They address the challenges in a simple spreadsheet by tracking dates that new diagnostic codes were added and with user interfaces that allow one to easily view only diagnoses present for a given patient rather than all potential diagnoses.

The distinction between a NICU quality assessment–quality improvement (i.e., outcomes) database and an electronic medical record is largely a matter of degree. Some characteristics typical of a neonatal outcomes database are data collection and data entry after the fact, limited amount of data collected (a small subset of

the information needed for daily care), lack of narrative text, lack of interfaces to laboratory and other information systems, and the episodic (e.g., quarterly) use of the system for report generation. Some characteristics typical of an electronic medical record are real-time (many times daily) data entry, a large amount of data collected (approximating all the information needed for daily care in a fully electronic care environment), narrative text (e.g., progress notes, radiology reports, pathology reports), interfaces to laboratory and other information systems, and, most important, the use of the system for daily patient care, including features such as results review, messaging or alerting for critical results, decision support systems (drug dosage calculators, drug–drug interaction alerts, among others), and computerized electronic order entry.

In the past, the majority of neonatal databases and first-generation NICU electronic medical record systems were developed locally by and for neonatologists. A review describing these efforts is available online at www.neonatology.org/technology/computers.html (Duncan, 2015). Unfortunately the majority of these systems were never published or publicly documented, and thus a number of important and useful innovations have been lost or must be repeatedly rediscovered. Anyone considering building their own neonatology database would be well advised to review the existing literature and existing commercial products before embarking on this path. That said, there is room for improvement of the existing products, and neonatologists continue to develop their own databases today. With the trend toward enterprise-wide, interoperable EHRs, it is likely that the market will continue to transition from external niche applications toward a smaller number of major EHR vendors whose applications include integrated neonatology specific tools.

The largest neonatal outcomes database is the centralized database maintained by the VON with the mission of improving the quality and safety of medical care for infants and their families. One of the key activities of the network is their outcomes database, which involves more than 900 participating intensive care nurseries both in the United States and internationally collecting data on over 60,000 very low birth weight infants each year. Other activities of the network are clinical trials, follow-up of extremely low birth weight infants, and NICU quality and safety studies. Although the initial focus of VON was very low birth weight infants (401–1500 g), a newer “Expanded Database” also includes data on infants in neonatal intensive care units weighing more than 1500 g. Currently the network collects data on the majority of very low birth weight infants born in the United States. The databases focus on tracking outcomes. With the passage of the Health Insurance Portability and Accountability Act of 1996 (HIPAA) and federal regulations governing the confidentiality of electronic patient data, some of the anonymous demographic data that were collected by the network in the past have decreased, due to concern that in combination with the identity of the referring center, these data could be used to uniquely identify patients.

Participants in the network submit data and in return receive outcome data for their own institution and comparative data from other nurseries nationwide, including custom reports, comparison groups, and quality management reports. Members also have the ability to participate in collaborative research projects and collaborative multicenter quality-improvement activities. All data except their own are anonymous for all participants. The network does have access to both the individual and aggregate data. The network database is maintained centrally, and data quality monitoring and data entry are centralized. Initially the process involved paper submission of data by participating nurseries. Now, most commercial and custom NICU databases and electronic medical record systems can be used to export much of the information required by the VON, but identification of some clinical details still relies on chart review. Members have the option to submit data electronically in two ways: either directly using the custom eNICQ software developed by VON or by transmitting spreadsheet files to VON for entry into the database.

Certainly, other neonatology outcomes databases exist for purposes ranging from quality improvement to clinical research. Examples include the [Children's Hospitals Neonatal Database](http://thechnic.org/CHND-resources.php) (<http://thechnic.org/CHND-resources.php>) for improving quaternary neonatology care and the [National Institute of Child Health and Human Development \(NICHD\) Neonatal Research Network Generic Database](https://neonatal.rti.org/about/gdb_background.cfm) (https://neonatal.rti.org/about/gdb_background.cfm), which in addition to the clinical trials conducted by the Neonatal Network has been enrolling infants at Network sites in an observational study since 1987.

Electronic Health Record

The EHR, also known as an *electronic medical record*, is much more complex than an outcomes database, because the system is intended to be used continually in real time to replace electronically some, if not all, of the record keeping, laboratory result review, and order writing that occur in a NICU (or more generically in any inpatient or outpatient clinical setting). The complexity of this task becomes evident if one imagines that, for a paperless medical record environment, every paper form in a nursery would need to be replaced with an electronic equivalent. Even more challenging is the

conversion of each paper-based workflow element and process into an electronic one.

Most US healthcare organizations have adopted EHR functionality because of a combination of forces, such as the desire to reduce error and to control the spiraling costs of health care. These reasons were addressed at great length in two reports from the IOM ([Institute of Medicine Committee on Improving the Patient Record, 1997](#); [Kohn et al., 2000](#)). Benefits are typically achieved when information is available electronically (e.g., results of laboratory tests, radiology procedures, transcription) and input into the system (e.g., problem lists, allergies) and when both sets of information are combined and checked against electronic orders. The adoption of computerized provider order entry (CPOE) has been shown to reduce errors and affect care provider behavior ([Radley et al., 2013](#)). Combining just electronic laboratory results (e.g., creatinine level) and electronic order entry (e.g., a drug order), for example, enables one to verify that drug dosages have been correctly adjusted for renal failure. This approach is more straightforward in adults, but in neonates, whose renal function is more difficult to assess and for whom drug dosage norms depend on gestational age and post-delivery age, the system must be able to capture additional information (e.g., urine output, gestational age) and requires more sophisticated logic. Nearly two decades following the aforementioned Institute of Medicine reports, and supported by stimulus funding through the ARRA HITECH act, nearly 75% of US hospitals have implemented CPOE ([Healthcare Information and Management Systems Society, 2016](#); [Adler-Milstein, 2015](#)).

Results of review systems include basic demographic data, such as name, age, and address from the hospital registration system. These systems require a moderate amount of work to tie them to the various laboratory, radiology, and other systems and to train users. The benefits are hard to quantify, but users typically prefer them to the paper alternative because of the more efficient access to information. The challenge in moving beyond the results review level to the integrated system level is that some degree of electronic documentation and CPOE are essentially prerequisites but are challenging to implement in terms of both human and financial costs. Implementation of integrated systems requires significant work, including infrastructure demands like the presence of computers at each bedside and network availability, as well as significant effort to educate and train users. The benefits accrue mainly to the organization, in the form of reduced costs of filing, printing, and maintaining paper records and, if providers enter notes electronically instead of dictating them, significant savings in transcription costs. Unfortunately, much of the burden of integrated systems is passed on to end users, who often find that it takes much longer to do their daily work with electronic documentation. Without implementation of CPOE and associated clinical decision support (CDS) systems, the users and patients do not realize major day-to-day benefits.

The benefits of EHR adoption start to accrue more clearly at the next level of electronic order entry. The complexity of implementing and deploying an electronic order entry system cannot be overstated. Interfaces need to be built with all the systems that are part of results review in addition to other systems. Furthermore, a huge database of possible orders must be created. Finally, and most important, training end users presents a significant challenge, because writing orders electronically is more complex and time consuming than writing them by hand. The change in management issues becomes apparent when one considers that typically these systems take the unit assistant out of the loop; therefore much of the oversight that can occur at the unit assistant level does not,

or the burden of oversight is borne by the person entering the orders. Further complicating matters, despite the mitigation of issues like illegibility and order of magnitude math errors, CPOE systems introduce new types of errors, such as juxtaposition errors (e.g., clicking on the wrong patient or selecting the wrong order in a list) (Ash, 2007; Longhurst et al., 2013).

After overcoming the barriers to electronic order entry, organizations can start to benefit from integrated systems. For this reason, the trend today is not a stepwise move from results review to documentation of integrated systems. Instead, organizations are moving from results review directly to integrated systems. Interestingly, the technical complexities and the training and usage complexities of integrated systems are not much higher than those for order entry. Integrated systems add tools to make life easier for care providers using all the data in the system. As an analogy, an integrated EHR system is like an office software suite that encompasses a word processor, a spreadsheet, a slide presentation tool, a graphic drawing tool, and a database, all of which can communicate with one another, making it easy to put a picture from the drawing tool or a graph from a spreadsheet into a slide show.

Integrated systems include (1) checking orders for errors, (2) alerts and reminders triggered by orders or by problems on the problem list or other data in the system, (3) care plans tied to patient-specific information, (4) charting modules customized to the problem list, (5) charting and progress notes that automatically import information (e.g., from laboratory tests, flow sheets) and that help generate orders for the day as the documentation occurs, (6) modules to facilitate hyperalimentation ordering, and (7) modules to assist in management (Palma et al., 2011b, 2011c; Palma and Arain, 2016). For example, a system could be configured such that reminders for screening studies (e.g., for retinopathy of prematurity, intraventricular hemorrhage, and brainstem auditory evoked response) are triggered by gestational age, a problem list, and previous results of screening studies. Similarly, admitting a neonate at a particular gestational age with a particular set of problems could trigger pathways, orders, and reminders specific to that clinical scenario. An important caveat is that all such systems are only as good as the data and rules put into them. The issues raised in the section on evaluation of therapeutic recommendations are important to consider in the context of electronic order entry and integrated systems. Additionally, enterprise-wide (non-niche) EHRs are not sufficiently configured out of the box for optimal care of critically ill and premature neonates.

The EHR market is continually evolving; this is true of products designed specifically for the NICU and more generic products designed to be used throughout a hospital or healthcare system. The ONC was established, in part, to affect the EHR marketplace by creating standards and certification bodies. In particular, the ONC created the Certification Commission for Healthcare Information Technology (CCHIT) and the Health Information Technology Standards Panel to begin to bring more standardization to the marketplace. The CCHIT examined criteria for certifying systems, including functionality, security, and interoperability. Facilitated by ARRA HITECH incentives, integrated systems are becoming more broadly implemented. However, the needs of different healthcare systems and the clinical services within a single health system vary significantly; in general, existing products are not configured to meet all these needs out of the box. Furthermore, the trend among healthcare organizations, EHR developers, and vendors is to move away from niche systems tailored to particular subsets of care providers, such as neonatology, and toward a focus

on systems that are generically useful. There are two important drivers behind this trend.

The first and most important reason for adopting a single integrated system is that the benefits of an EHR system begin to accrue only when an entire organization uses the same one. Consider the following scenario: a woman receives prenatal care in the clinic of an institution and is then admitted to the emergency department in preterm labor. Her infant is delivered in the labor and delivery department, hospitalized in the NICU, and discharged to an affiliated pediatric follow-up clinic. In the era of paper medical records, paper is used to convey information from one site to the other. In an integrated EHR system, all the information for both mother and infant is in one place for all providers to see. Interoperability is important as well if the care described crosses organizational boundaries, such as an outpatient-focused health maintenance organization contracting inpatient obstetric care to one hospital system and neonatal or pediatric care to a children's hospital in a different healthcare system. If a single unified institution were to adopt niche software tailored to the needs of each site, a provider caring for the infant might need to access an emergency department system, an obstetric system, a NICU system, and an outpatient pediatric system to gather all the pertinent information. Each system would require the user to learn a separate piece of software. Learning a site-specific piece of software is a considerably greater burden on care providers than learning to use a site-specific paper form. If care crosses organizations with electronic systems that are not interoperable, then care transitions most often remain on paper.

The second factor driving adoption of integrated systems is economies of scale. The ideal EHR system contains electronic interfaces that automatically import the system data from laboratory, pharmacy, radiology, transcription, integrated electronic orders, error checking, and electronic documentation by care providers. Given that development of these interfaces, training, and maintenance cost more than the purchase of the software itself, it is far more cost-effective to install one system with one set of interfaces and one set of training and maintenance issues than to replicate the process multiple times.

The neonatal intensive care environment poses some unique challenges for EHRs. As a result, it is important to ensure that when healthcare systems are making decisions about the purchase of an EHR, neonatologists and other neonatal healthcare providers are involved in the process. An excellent source of historical information about NICU medical record systems and databases is an article by Stavis (1999). More recently, Palma et al. reviewed several aspects of EHRs related to neonatology, including computerized provider order entry (Palma et al., 2011a), handoffs (Palma et al., 2011c), and clinical data entry and display (Palma et al., 2012b). Neonatal care providers who are helping to select an EHR system should acquire the necessary background knowledge through reading some basic introductory texts on medical informatics, focusing on EHRs. It is then critical that they survey other organizations similar to their own to understand the benefits and drawbacks of various EHR systems for neonatal care. For example, the needs of a level III academic nursery that performs extracorporeal membrane oxygenation are different from those of a community level II hospital that does not perform mechanical ventilation. Systems that work well in teaching hospitals with layers of trainees may not work well in private practice settings and vice versa. Importantly, when using a medical informatics framework, neonatal care providers should develop a list of prioritized criteria specific to their institution and evaluate available products in the marketplace using this list, while also considering whether the system meets CCHIT standards as described earlier.

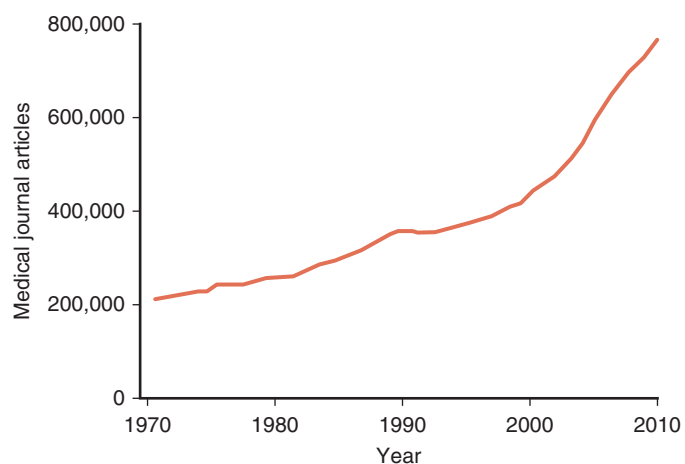
All end users' needs must also be considered. If residents, nurse practitioners, nutritionists, pharmacists, and respiratory therapists are expected to use the system, their input must be solicited. Ensuring broad-based input is especially relevant as the EHR system is likely to require significant data entry by healthcare providers (e.g., electronic nursing and respiratory therapist [RT] documentation, note writing, medication administration records, order entry). Involvement of all stakeholders is an important component of change management; in addition, following implementation, their input is critical for optimizing the system for care in the NICU. Unfortunately there is little literature on this issue, because institutions rarely publicize and publish their failures in this arena, although the situation is beginning to change. A review of some of these challenges and a theoretical framework for looking at them is provided by Pratt et al. (2004), and an evidence-based approach to mitigating unintended consequences of EHR implementation is discussed by Longhurst et al. (2013).

The final step in evaluating and testing a potential system is to develop a series of scenarios and to have potential users test the scenarios. Evaluating usage scenarios prior to vendor selection typically involves visits to sites that have installed the EHR system under consideration. An example scenario might be for a nurse, a respiratory therapist, a resident, and an attending physician to try to electronically replicate, on a given system under consideration, the bedside charting, progress note charting, and order writing for a critically ill patient who undergoes extracorporeal membrane oxygenation from hospital admission through decannulation. Developing and testing such scenarios are the best way to ensure that aspects of charting, note writing, and documentation unique to the NICU are supported by the system.

Evaluating Therapeutic Recommendations

Once all the data about a patient, whether in electronic or paper form, are in hand, the clinician is faced with the challenge of medical decision making and applying all that he or she knows to the problem. It is vital that clinicians understand what is known and what is still uncertain in terms of the validity of therapeutic recommendations. The evaluation of new recommendations arising from a variety of sources, including journal articles (Fig. 2.2), metaanalyses, and systematic reviews, is a critical skill that all neonatologists must master. Broadly speaking, this approach has been termed *evidence-based medicine*. Although a full discussion is beyond the scope of this chapter, there are two outstanding sources of information on the subject, one by Guyatt and Rennie (2015) and the other by Straus et al. (2010). A useful discussion of clinical practice guidelines as they relate to neonatology is provided by Polin and Lorenz (2015). An important caveat is that evidence-based medicine is not a panacea. It is not helpful when the primary literature does not address a particular clinical situation, such as one that is rare or complex. This approach also does not necessarily address broader concerns, such as clinical importance or cost-effectiveness, although it sometimes does. There are potential challenges when combining evidence-based medicine with the emerging humanistic approach to health care, which can heavily weight patient preferences potentially over the evidence base.

In the early days of medicine, the standard practice was observation of individual patients and subjective description of aggregate experiences from similar patients. As the science of medicine evolved, formal scientific methods were applied to help to assess possible therapeutic and management interventions. Important tools in this effort are epidemiology, statistics, and clinical trial design. Currently medicine in general and neonatology in particular are

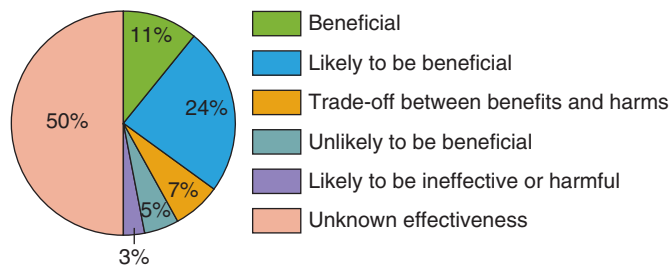


• **Fig. 2.2** Yearly Citation Totals From 2015 MEDLINE/PubMed From 1970 to 2016. (Redrawn from https://www.nlm.nih.gov/bsd/licensee/2017_stats/2017_Totals.html.)

faced with an interesting paradox. For some areas, there is a wealth of information in the form of randomized controlled clinical trials, whereas for others, there is scant information to guide clinical practice. A wealth of well-designed clinical trials on the use of surfactant have been published, for example, but there are essentially no trials addressing the management of chylothorax. The lack of studies of (and therefore evidence for) treatments in neonatology has led to newborns being described as “therapeutic orphans” (Stiers and Ward, 2014).

One might assume that the practice of medicine reflects the available evidence, but this is not the case. McDonald (1996) summarized the problem as follows: “Although we assume that medical decisions are driven by established scientific fact, even a cursory review of practice patterns shows that they are not.” A study of 3000 treatments (as reported in randomized controlled trials) in the *British Medical Journal's* Clinical Evidence database (<http://clinicalevidence.bmj.com>) shows that, as of 2016, 50% of treatments are of unknown effectiveness, 11% are clearly beneficial, 24% are likely to be beneficial, 7% are a tradeoff between beneficial and harmful, 5% are unlikely to be beneficial, and 3% are likely to be ineffective or harmful (Fig. 2.3). As a result, neonatologists have a responsibility to identify what knowledge is available in the literature and elsewhere and to critically evaluate this information before applying it to practice. Furthermore, because this information is constantly evolving, practitioners must continually revisit the underlying literature as it expands (e.g., the recommendations regarding the use of steroids for chronic lung disease).

For the 50% of treatments with known (whether beneficial or harmful) effectiveness, the evidence-based practice of medicine is an approach that addresses these issues. It is helpful to consider the process as involving two steps: first, a critical review of the primary literature and second, the synthesis of information in the primary literature to determine the implications on one's practice. Most neonatologists have significant experience with critical review of the literature through journal clubs and other similar forums. The approach involves systematically reviewing each section of an article (i.e., background, methods, results, discussion) and asking critical questions for each section (e.g., for the Methods section: Is the statistical methodology valid? Were power calculations made? Was a hypothesis clearly stated? Do the methods address the hypothesis? Do the methods address alternative hypotheses? Do the methods address confounding variables?). The formal evaluation of each section must then be synthesized into conclusions. A helpful



Effectiveness of 3000 treatments as reported in randomized controlled trials selected by Clinical Evidence. This does not indicate how often treatments are used in healthcare settings or their effectiveness in individual patients.

• **Fig. 2.3** Effectiveness of 3000 Treatments Reported in Randomized Controlled Trials. (Redrawn from <http://clinicalevidence.bmj.com/x/set/static/cms/efficacy-categorisations.html>.)

question to ask is, “Does this paper change my clinical practice, and if so, then how?” Additional resources for systematic review of the primary literature are listed in the Suggested Readings. It is important to note that guidelines for systematic review of a single article differ according to whether it describes a preventive or therapeutic trial (e.g., use of nitric oxide for chronic lung disease), evaluation of a diagnostic study (e.g., use of C-reactive protein level for prediction of infection), or prognosis (e.g., prediction of outcome from a Score for Neonatal Acute Physiology score).

The second, and arguably more important, step is to determine not the effect of one article on one’s practice but the overall effect of the body of relevant literature on one’s practice. For example, if the preponderance of the literature favors one therapeutic recommendation, then a single article opposing the recommendation must be weighed against the other articles that favor it. This task is complex, and the most complete and formal statistical approach to combining the results of multiple studies (i.e., metaanalysis) requires significant investment of time and effort. Part of the evidence-based practice of medicine approach therefore involves the collaborative development of evidence-based systematic reviews and metaanalyses by communities of care providers. Within the field of neonatology, Sinclair et al. (1992) laid the seminal groundwork for this approach; their textbook *Effective Care of the Newborn Infant* remains an important milestone, but it illustrates the problem of information currency. Because the book was published in 1992, none of the clinical trials in neonatology in the last decade and a half are included. The Internet has permitted creation and continual maintenance of up-to-date information by a distributed group of collaborators, lending itself well to the maintenance of a database of evidence-based medicine reviews of the literature. This international effort is the Cochrane Collaboration, which began in 1993 with the *Cochrane Neonatal Review*. A limitation of the Cochrane approach is illustrated by the relatively restricted scope of topics covered at the NICHD website (<https://www.nichd.nih.gov/cochrane/Pages/default.aspx>). The existence of a review requires adequate literature on a topic and a dedicated and committed clinician to create and update the review.

It is important to distinguish between these formal approaches to reviewing the literature (i.e., systematic literature reviews and metaanalyses) that have specific methodologies and more ad hoc reviews of the literature. Evidence-based medicine aggregate resources such as the Cochrane Collaboration take a more systematic approach, but review articles published in the literature vary in their approach. Metaanalyses are easy to distinguish, but systematic reviews versus

ad hoc reviews are harder to distinguish. Systematic reviews focus on quality primary literature (e.g., controlled studies rather than case series or case reports) and must include a Methods section for the review article that (1) explicitly specifies how articles were identified for possible inclusion and (2) the criteria used to assess the validity of each study and to determine whether to include or exclude primary literature articles in the systematic review. Systematic reviews also tend to present the literature in aggregate tabular form, even when metaanalyses of statistics of all the articles cannot be done. One commonly used source of overview information in neonatology—the *Clinics in Perinatology* series—is a mix of opinion (written in the style of a book chapter), ad hoc literature review, systematic literature review, and metaanalysis. Clinical practice guidelines (e.g., screening recommendations for group B streptococcal infection), although based on a primary literature review, are typically neither metaanalyses nor systematic reviews of the literature. Whereas formal methods are used to derive conclusions with metaanalyses and systematic reviews, frequently guidelines are developed instead by consensus among committee members; this is true of both national and local practice guidelines. General textbooks of neonatology are typically based on ad hoc literature reviews that include both primary literature and systematic literature reviews. When reading overviews of the aggregate state of current knowledge on a given topic in neonatology, it is important to keep these distinctions in mind.

Anyone interested in developing evidence-based reviews on a particular topic should review the textbooks on evidence-based practice referenced at the end of this chapter. Initially, it is a good idea to collaborate with someone with experience in systematic review and metaanalysis. The process consists of the following steps: (1) identifying the relevant clinical question (e.g., management of bronchopulmonary dysplasia); (2) narrowing the question to a focus that enables one to determine whether a given article in the primary literature answers it (e.g., does prophylactic high-frequency ventilation have positive or negative effects on acute and chronic morbidity – pulmonary and otherwise?); (3) extensively searching the primary literature (frequently in collaboration with a librarian with expertise searching the biomedical literature) and retrieving the articles; (4) critically, formally, and systematically reviewing each article for inclusion, validity, utility, and applicability; and (5) formally summarizing the results of the preceding process, including conclusions valid throughout the body of included primary literature.

Online Resources

A number of online resources are valuable for neonatologists. For accessing the primary literature, the most valuable resource is the National Library of Medicine’s database of the published medical literature accessible (PubMed; www.ncbi.nlm.nih.gov/pubmed) along with many other databases accessible from the Health Information Website (www.nlm.nih.gov/hinfo.html). The PubMed system is continually being enhanced; therefore it is useful to review the help documentation online and regularly check the New/Noteworthy section to see what has changed. One of the most powerful yet underused tools is the Similar Articles tool that is available as a link below each article listed on PubMed and is visible as a list adjacent to an article or abstract as it is being viewed (Liu and Altman, 1998). The PubMed system applies a powerful statistical algorithm to each word in the title, each author, each major and minor keyword (Medical Subject Heading terms), and each word in the abstract in order to find similar articles in the database. In general,

this system outperforms novice-to-advanced healthcare providers performing a complex search and approaches the accuracy of an experienced medical librarian. Another powerful search tool within PubMed is the Clinical Query (www.ncbi.nlm.nih.gov/pubmed/clinical). This tool facilitates searches for papers by clinical study category (e.g., etiology, diagnosis, therapy, prognosis), focuses on systematic reviews, and performs Medical Genetics searches – the last of which is particularly useful in the context of neonatology. All the major pediatric journals are available online either through a package at local hospital libraries or by subscription, instead of or in addition to a print subscription. The best free online source of information on evidence-based practice is the *Cochrane Neonatal Review*. Subscriptions to the full Cochrane database can be purchased online as well.

Given the growing role of genetics in health care, and the particular importance of genetic diseases in infants, two notable genetics databases are available free of charge online (in addition to the Medical Genetics search within PubMed Clinical Queries). The first is *Online Mendelian Inheritance in Man* (www.ncbi.nlm.nih.gov/omim). This database, which is a catalog of human genes and genetic disorders, is an online version of the textbook by the same name. It is a diachronic collection of information on genetic disorders, meaning that each disease entry chronologically cites and summarizes key papers in the field. The second is *GeneTests* (www.genetests.org), a directory of genetic testing (what testing is available on a clinical and research basis, where, and how one sends a specimen) and a user's manual (how to apply genetic testing). The user's manual section consists of entries for a growing number of diseases or clinical phenotypes of particular importance. Entries are written by experts, peer reviewed both internally and externally, subjected to a formal process similar to a systematic review, and updated regularly online. As of the spring of 2016, the GeneTests database includes GeneReviews (user's manual entries) for 658 diseases, and the directory includes 1067 genetics clinics, 680 laboratories, and information on 79,004 tests for 4548 diseases covering 5385 genes.

An excellent site that maintains links to the majority of locally developed, neonatology-specific content around the country is maintained by Duncan (2015). In addition to a database of links to clinical resources around the country, the site also has a job listing and a database of the literature on computer applications in medicine.

The clinician must realize that, unlike journals, textbooks, and guidelines, material on the World Wide Web (whether accessed from Duncan's site or using a search engine) is not necessarily subject to any editorial or other oversight; therefore as stated by Silberg et al., as early as 1997, "caveat lector" (reader beware). A number of articles and online resources address criteria for assessing the validity and reliability of material on a website (*Health on the Net Foundation*, www.hon.ch; *American Public Health Association*, 2001). With caution in mind, a search of the World Wide Web using a sophisticated search engine (e.g., Google, Google Scholar) can yield valuable information, though search results typically include a lower proportion of quality resources compared with curated resources.

Future Directions

Computers and biomedical informatics continue to play an increasing role in neonatology – for information management and because EHRs are fundamental to the provision of clinical care (Palma, 2012a). While significant levels of EHR adoption have been achieved in the United States, the benefits of digitizing the medial record

are not yet fully realized, and both providers and patients are keenly aware of the challenges and shortcomings of many current computer-based workflows (Toll, 2012; Wachter, 2015).

One significant and largely unrealized opportunity of EHR adoption is the generation of new knowledge from the vast amounts of clinical data collected electronically (McGregor, 2013). As stated previously, the *British Medical Journal's* Clinical Evidence database suggests that 50% of treatments (likely an even greater proportion in neonatology) are of unknown effectiveness. These "gaps in the evidence—where there are no good (randomized controlled trials [RCTs]) or no RCTs that look at groups of people or at important patient outcomes" (BMJ Clinical Evidence)—represent areas of opportunity. It is not possible for all of these gaps to be filled by RCTs, a study design that may not be feasible for the treatment or population of interest. On the other hand, with the increasing adoption of EHRs, a wealth of clinical data is being collected as a byproduct of routine care (so-called *practice-based evidence*).

The 2012 IOM report *Best Care at Lower Cost: The Path to Continuously Learning Health Care in America* (2012) calls for and outlines an approach to transformation of the healthcare system into one that learns from practice-based evidence, generating knowledge from earlier patient encounters and making it available (along with evidence from the literature) at the point-of-care. Steps toward the concept of a continuously learning healthcare system are being made, with some examples of practice-based evidence being used for improvement in neonatal care (Spitzer et al., 2010; Gale and Morris, 2016; Palma and Arain, 2016). Capitalizing upon clinical data generated in EHRs for the purposes of discovery and improved care will increasingly become a focus of biomedical informatics in neonatology.

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3

Ethics, Data, and Policy in Newborn Intensive Care

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Philosophy

Ethics in the neonatal intensive care unit (NICU), as in all clinical contexts, starts with the traditional triangular framework of principles. We consider autonomy (do what the patient, or in this case the parent, thinks is right), paternalism (do what the doctor thinks is right), and the complementary concepts of beneficence and nonmaleficence (do the right thing). These principles, independent of context or data, are timeless. Many applications of these traditional ethical principles occur daily in the NICU (e.g., avoid futility, do not torture, and intervene when the data provide compelling evidence to do so).

The problem with timeless principles is that we all have to act in real time and, sometimes, in real time, the timeless principles conflict with one another and we have to decide quickly which compromises or combinations are best. What exactly is the right thing? What facts should be brought to bear in the decision? What weight should be given to each fact? And whose opinion should count in the end? Unfortunately, much of NICU care falls between the relatively straightforward decisions to not resuscitate babies born at 21 weeks, providing obligatory support to babies born at 28 weeks, or not performing cardiopulmonary resuscitation on infants with lethal anomalies. Faced with a difficult case, it is rare that simply applying principles will help to devise a solution. Difficult cases are usually ones in which the principles themselves are in conflict, or their application to the case is ambiguous.

The traditional ethical solution to medical dilemmas is to ground concerns in context, take data into account, and be sympathetic to patient preferences when the balance of benefits and burdens is not clear.

In the NICU, health professionals are constantly and anxiously aware that the burdens of treatment are real, immediate, long term, and significant, whereas the benefits of NICU interventions are distant, statistical, and unpredictable. Babies must undergo months of painful procedures such as intubation, ventilation, and intravenous catheterization. Often, they are left with lifelong sequelae. How should we decide whether we did the right thing in order to help decide whether, in similar circumstances, we should again do the same thing?

NICU success is often viewed as “all or none.” In most of the NICU follow-up literature a Bayley Mental Developmental Index (MDI) or Psychomotor Developmental Index (PDI) greater than 70 is classified as *normal*, whereas an MDI or PDI less than 70 is

classified as an *adverse outcome*. We strongly disagree with this dichotomization. There is no single measure of success or failure. Each baby is different, each family is different, and each life course is different.

In order to make good decisions, we need good data.

Getting Good Data

What kind of information would parents, physicians, or judges want to know about the babies in the NICU in order to decide when treatment ought to be obligatory and when it ought to be optional?

The essential truth at the intersection of NICU epidemiology and ethics is that survival depends sharply on gestational age (GA), within relatively precise boundaries. In the United States, as in virtually all the industrialized world, infants born after 25 weeks' gestation have survival rates that are high enough that treatment is generally considered obligatory. For these infants, the ethical principle of best interests requires their resuscitation, in the same way that sick children born at term deserve resuscitation.

Conversely, for infants born before 22 weeks' gestation, survival is essentially zero. Consequently, these infants and their parents deserve our compassion but not our interventions, on the ethical grounds of strict futility.

In between, spanning roughly one gestational month, from 22–25 weeks, we will require not only data but also interpretation. Tyson et al. (2008), using the vast database of the National Institute of Child Health and Human Development network, attempted to go “beyond gestational age” and quantify additional risk factors for both mortality and neurologic morbidity in infants born on the cusp of viability. Their analysis revealed that singleton status, appropriate in utero growth, antenatal steroids, and female gender all improve the likelihood of survival and intact neurologic outcome, independent of GA. By considering these other factors, all of which are available at the time of birth, doctors are able to more accurately estimate the chances that a baby will survive or that survivors will have neurodevelopmental impairment.

However, two problems remain. First, for many infants the predictive value of the Tyson algorithm is still not very good – that is, many of the lower-risk patients will still die, and many of the higher-risk patients will survive. Second, the Tyson algorithm, like GA, ignores a potentially important feature of NICU care – time. The algorithm uses only data that are available at the time of birth.

It does not account for prognostic features that might become available as the infant's course unfolds in the NICU. This is an important limitation that has ethical implications.

There are distinct advantages to making decisions over the first few days of the NICU stay, rather than in the delivery room at the time of birth. The first is emotional. Parents often appreciate the opportunity to get to know their baby as an individual, as opposed to making decisions based only on the anonymous population-based prognostications that are available at the time of birth. Second, there is valuable information to learn while the baby is in the NICU. Two time-sensitive prognostic features have been evaluated in the context of infants born at the border of viability – serial illness severity algorithms (Score for Neonatal Acute Physiology [SNAP]) and intuitions that the patient would “die before NICU discharge” (Meadow et al., 2008). Unfortunately, although SNAPS on the first day of life have good prognostic power for death or survival, their power diminishes over time. Intriguingly, serial intuitions that an individual baby will die before discharge – offered by medical caretakers for patients who require mechanical ventilation and for whom there is an ethical alternative to continued ventilation, namely extubation and palliative care – are remarkably accurate in predicting a combined outcome of either death or survival with neurologic impairment (MDI or PDI <70). Babies with abnormal results from a cranial ultrasound examination whose doctors agree with one another that the babies are likely to die have a less than 5% chance of surviving with both MDI and PDI greater than 70 at 2 years, independent of their GA. The predictive power of these data, acquired over time during an individual infant's NICU course, though not perfect, is greater than any algorithm available at the time of birth.

What do prospective parents or medical caretakers consider when they are asked to decide whether or not to resuscitate their micro-preemie? They may not want the precise prognostic estimates that we try to offer. For many parents, the death of their baby in the NICU is not necessarily the worst outcome. Instead, it may be worse to not even try to save the baby. That decision may leave parents with a life of self-doubt about whether, had they only tried, their baby might have survived. For such parents, trying and failing might be preferable to not trying at all.

If trying and failing is seen as a positive process, then the preferable choice might best be made not by looking at the percentage of intact survivors among all births (the current practice in many studies, including the model, noted above, developed by Tyson et al.) but instead as a function of only those infants who survive to discharge.

Numerous studies analyzing various populations in several countries have converged on the same surprising observation: the incidence of neurologic morbidity in NICU survivors is not very different when comparing infants at 23, 24, 25, and 26–27 weeks' gestation. The essential epidemiologic difference for infants born in this gestational range appears to be whether the baby will survive at all. Once the baby leaves the NICU, the risk of severe morbidity is largely the same; this is true in single-center and multicenter studies, in the United Kingdom, Canada, Europe, and the United States (Tyson et al., 2008; Johnson et al., 2009). Paradoxically, if avoiding survival with permanent crippling neurologic injury is the driving force behind resuscitation decisions, it appears that we should not be worrying about 23- and 24-weekers; rather we should not be resuscitating 26- or 27-weekers. Many more of these more mature babies will survive. Thus even if the rate of disability among survivors is lower, the absolute number of survivors with disability will likely be higher.

A fascinating insight has been offered by Janvier et al. (2008), who have done extensive surveys comparing responses to requests for resuscitation of sick micro-preemies with resuscitation of comparably sick patients at other ages (from term infants to 80 year olds). Consistently, it appears that micro-preemies are devalued – that is, for comparable likelihood of survival and comparable likelihood of neurologic morbidity in survivors, more people would let a micro-preemie die first or at least offer to resuscitate them last. There is no theory to account for these findings.

Finally, there is epidemiologic difficulty in assigning value to morbidity in surviving infants in the NICU. Verrips et al. (2008) have attempted to assess the effects of permanent residual disability for NICU survivors and their immediate families; they have demonstrated consistently that children with disabilities and their parents place a much higher value on their lives, and the quality of those lives, than do either physicians or NICU nurses. The vast majority of infants who survive the NICU, even those with significant permanent neurologic compromise, have “lives worth living,” as judged by the people most affected by those lives.

GA-specific mortality seems to preclude resuscitation for infants born before 22 weeks' gestation and require resuscitation for infants born after 27 weeks' gestation. In between, the outcomes are murky, prognostic indices are imperfect, and sociologic analyses of human behaviors (of parents and physicians) appear inadequate to develop any uniform approach that is satisfactory.

Public Policy: the Baby Doe Case

In the 1980s, the federal government attempted to change the rules for neonatal decisions about babies with congenital anomalies.

In 1982, a baby with Down syndrome and esophageal atresia was born in Bloomington, Indiana (USA). Baby Doe's parents refused to consent to surgery and chose palliative care instead. The court sided with the parents. The doctor and hospital appealed. The Indiana Supreme Court refused to hear the appeal, and the baby died after 8 days (Lantos, 1987).

This led to a national controversy that eventually resulted in amendments to the federal Child Abuse and Treatment Act (Annas, 1986; Kopelman, 1988). While this law has limited authority in regulating clinical decisions, it symbolically endorses the idea that life-sustaining treatment should not be withheld only on the basis of anticipated disability.

There is still controversy when treatments enable survival but have a high likelihood, or certainty, that survival will be accompanied by severe neurologic impairment. As a result, two questions must be asked: How severe will the neurologic impairment be? What is the likelihood that the child will have the most severe possible impairment?

The shift in moral standards regarding babies with Down syndrome was not related to technology but rather sociology. The capacity to repair Arnold–Chiari malformation and duodenal atresia existed long before it was applied to children with myelomeningocele and Down syndrome. What has changed the mood of the country is a growing recognition that disability is as much a social construct as a medical construct, although it is always both and not one or the other.

Malpractice Cases Against Neonatologists

There are also malpractice cases against neonatologists that have shaped the decision-making climate in the NICU. In one, Miller

versus Hospital Corporation of America Inc. (2003) the doctors were sued for resuscitating a tiny preemie over the objections of the parents. The case focused on events that had occurred in 1990, when Mrs. Miller came to a Hospital Corporation of America hospital in Texas in labor at 23 weeks' gestation. The fetus was estimated to weigh 500–600 g. No baby born that size had ever survived at that hospital. Mrs. Miller, her husband, and the attending physicians agreed that the baby was previable and that no intervention was indicated. The baby was born, but a different physician performed resuscitation, and the infant survived with brain damage. As a result, the Millers sued the hospital for a breach of informed consent and were awarded \$50 million by a trial jury. The case wound its way to the Texas Supreme Court, which dismissed the verdict and articulated an “emergency exception” for physicians – that is, if a Texas physician finds himself or herself in the emergency position of needing to resuscitate a patient to prevent immediate death, the physician can try to perform resuscitation without being obligated to obtain consent from anyone. Whether it would be acceptable for a physician not to perform resuscitation in an emergency was left unarticulated by the Texas court.

In Wisconsin, the case of *Montalvo versus Borkovec* (2002) took the legal obligations of neonatologists and parents to a different place. The case derived from the resuscitation of a male infant born between 23 and 24 weeks' gestation, weighing 679 g. The parents claimed a violation of informed consent, arguing that the decision to use “extraordinary measures” should have been relegated to the parents. The Wisconsin Appellate Court disagreed, holding that “in the absence of a persistent vegetative state, the right of a parent to withhold life-sustaining treatment from a child does not exist.” Because virtually no infant is born in a persistent vegetative state, this decision would apparently eliminate the ethical possibility in Wisconsin of a “gray zone” of parental discretion. No other jurisdiction in the United States has adopted this position. The Wisconsin Appellate Court, like the Texas Supreme Court, was silent on whether physicians have discretion not to resuscitate. However, in Texas and Wisconsin, physicians are apparently not liable if they choose to do so.

A number of other state courts have addressed issues of treatment or nontreatment. In general, the courts are permissive of physicians who resuscitate infants. If courts are asked to sanction decisions to allow infants to die, most will do so only if there is consensus among physicians and parents and occasionally ethics committees. Courts are not eager to punish physicians who treat infants over parental objections or to empower physicians to stop treatment when parents want it to continue.

Future Directions

A number of recent developments may change the way we think about ethical issues in the newborn period. These include the rise of fetal medicine and expanded genomic screening of newborns.

Fetal Medicine Centers

Many children's hospitals are now developing fetal medicine centers. The goal of these centers is to identify fetuses at risk – particularly those with congenital anomalies – and to care for those fetuses and their mothers in centers where there is expertise in fetal diagnosis, therapy, and neonatal care. The hope is that such centers will allow more timely, and therefore more effective, intervention for babies with congenital heart disease, congenital diaphragmatic hernia, or other anomalies.

The medical effectiveness of these fetal medicine centers will depend on two distinct developments. First, on a population basis, these centers will only be as effective as fetal screening and diagnosis. The existence of these centers will almost certainly create an expectation and a demand for better fetal screening. Such screening is likely to include both better imaging and better screening tests that can be performed on maternal blood; both will lead to earlier diagnosis of fetal anomalies. These diagnoses will create more complex dilemmas for perinatologists and parents who will need to decide, in any particular case, whether to terminate the pregnancy, offer fetal therapy, or offer either palliative care or interventions after birth. Ironically, better fetal diagnosis may increase the likelihood of pregnancy termination, even when postnatal treatment is possible, such as in hypoplastic left heart syndrome.

Second, the effectiveness of fetal centers will depend on the effectiveness of fetal interventions. To date, fetal interventions have only been effective in a relatively few conditions. Vascular ablation for twin-to-twin transfusion syndromes clearly improves outcomes in these conditions. In utero surgery for myelomeningocele also leads to better outcomes for babies born with this condition. The vast majority of prenatally diagnosed conditions cannot be treated in utero. The real hope of fetal medicine centers today is that they will improve outcomes by allowing better planning for perinatal interventions. In some cases, that may lead to changing the timing or mode of delivery. In other cases, it may mean that a team of pediatric subspecialists will be prepared and immediately available to treat the baby in the minutes after birth. These sorts of efforts may improve outcomes.

Expanded Newborn Screening

In recent years, the number of diseases and conditions that can be diagnosed through newborn screening has expanded dramatically. Such screening is under the purview of states, rather than the federal government, and there is wide variation in the number of tests that are performed. In 1995 the average number of tests per state was five (range: zero to eight disorders). Between 1995 and 2005 most states added tests so that the average number of screening tests done by 2005 was 24 (Tarini et al., 2006). Today, there is a panel of 29 tests that has been recommended by the Department of Health and Human Services and has been adopted in all states (Kemper et al., 2014).

The expansion of newborn screening raises three problems. First, even the most accurate test has false positives. For rare conditions, the percentage of positive tests that are false positives is increased. Thus the more rare conditions that are added to a newborn screening panel, the more false positives there will be. False positives are associated with considerable parental anxiety and can lead to potentially dangerous and unnecessary diagnostic procedures or treatments. Second, expanded newborn screening costs money. Most of the cost is not for testing itself. Instead, it is for the follow-up counseling and testing after positive tests. Such follow-up is essential or the screening programs will not work. The Centers for Disease Control and Prevention (2008) has recently expressed concern about these costs. Finally, there is the potential for discrimination against patients for whom documented heterozygous carrier status conveys no recognized medical infirmity, but social or psychological stigma may be real. There is little funding available to assist or counsel these patients.

Recently, some centers have started to offer whole-genome sequencing (WGS) for newborns. Such testing raises all the issues

noted above for newborn screening. However, like newborn screening, it also offers the tantalizing prospect of better diagnosis leading to better treatment for some newborns whose conditions have been difficult to diagnose using more conventional methods. WGS is much more difficult to interpret than traditional newborn screening because every baby – and every adult – has many genetic variations of unknown significance.

Variants can only be interpreted after a good clinical history, family history, and physical examination have been performed. Data from these preliminary steps allow physicians to assess whether there are similar or related phenotypes in other family members; if so, the inheritance pattern can then be evaluated and assessed. Physical examination findings allow physicians to begin a search for potentially relevant genes. Mode of inheritance and a comprehensive phenotype can then be used to classify the patient's genomic variants. WGS may lead to the discovery of a known pathogenic variant, a novel pathogenic variant that is likely to be disease-causing, or a variant of unknown clinical significance in a gene known to cause human disease.

Clinical validity is a complicated and challenging aspect of WGS. Evidence is required to prove that a specific rare variant in a particular gene, detected by WGS, is indeed pathogenic and responsible for a particular clinical phenotype (Thiffault and Lantos, 2016).

Should Policy Dictate Resuscitation Practices?

Many professional societies in many countries have policies about resuscitation for babies born at the borderline of viability. Guillén et al. (2015) showed that among guidelines in 31 countries, 21 (68%) supported comfort care at 22 weeks' gestation and 20 (65%) supported active care at 25 weeks' gestation. Between 23 and 24 weeks' gestation, there was even greater variation.

These policies have practical effects. They lead to very different survival rates for seemingly similar babies in different countries. For example, in the Netherlands and Switzerland, survival at 25 weeks' gestation is as good as in the United States. Over 75% of infants born at 25 weeks' gestation will survive to discharge (Stoll et al., 2015). Before 25 weeks, however, outcomes in different countries vary widely. In the Netherlands and Switzerland, virtually no infant born at 24 weeks' gestation survives because there are policies not to provide intubation, resuscitation, or neonatal intensive care to such babies. (This has started to change at some centers in these countries – see, e.g., Morgillo et al., 2014.) By contrast, survival rates in the United States are 62% at 24 weeks' gestation and

37% at 23 weeks' gestation. Japan reports survival rates of 30% for babies born at 22 weeks' gestation and 70% for babies born at 23 weeks' gestation (Kusuda et al., 2006).

More interestingly, the policies shape the outcomes statistics that then become the evidence upon which future policies are based. It makes sense to not resuscitate babies if they have a 100% mortality rate. But, if they only have 100% mortality rate because they are not resuscitated, then the policies become a self-fulfilling prophecy.

Such policies may reflect a resources allocation decision. In the Netherlands, there is a limited budget and a communitarian ethic. There is a certain rationale behind spending money on all pregnant women, instead of 1% of micro-preemies. The United States appears ambivalent – we value individuals over community, are fascinated with high technology, and claim to prize our children. On the other hand, we will not spend money to prevent unwanted teen pregnancy, expand maternity leave, or provide visiting nurses for new mothers.

Finally, the concept of generational conflict must be considered. Many doctors appear to be quite comfortable calling delivery-room resuscitation of 24-weekers “optional,” based on GA alone (Janvier and Lantos, 2016). Given what we know about the factors other than GA that influence outcomes, this seems to be a curiously oversimplified approach to clinical decisions. It is not an approach that is used in other areas of medicine. For example, professional societies do not recommend the allocation of intensive care unit resources based on age alone. Such a practice might be called “discriminatory ageism.” However, with regard to premature babies, it ought, perhaps, to be considered equally discriminatory and labeled as “gestational ageism” (Wilkinson, 2012). Some suggest that the reason to limit such treatment is because it is too expensive, but studies show that it is remarkably cost-effective compared with the use of intensive care resources for elderly patients with respiratory failure (Lantos and Meadow, 2011). The relative cost-effectiveness of NICUs compared with medical intensive care units (MICUs) is based on the natural history of illnesses that lead to intensive care unit admissions. Most babies who are admitted to the NICU either die relatively quickly or survive. Thus most of the resources in NICUs are expended on patients who survive to leave the hospital. For adults, the opposite is true. With each passing day in the intensive care unit, the chances that an elderly patient will survive to leave the hospital go down. Thus a much higher percentage of the resources expended in adult intensive care units are used by patients who will not leave the hospital alive.

Summary

Ethical philosophy is a place to start, not a place to finish. Data are relatively easy to acquire and agree on. Policy is intriguingly insensitive to data, but that may reflect social and political realities that exist beyond the NICU – perceptions of disability, abortion

politics, individual versus communitarian emphasis, fascination with technology, discrimination, publicity, financial constraints – so that an ethical course of action in one country, one city, or one family might seem perverse elsewhere.

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4

Global Neonatal Health

CYRIL ENGMANN AND MANEESH BATRA

KEY POINTS

- In many parts of the world, childbirth remains a risky, life-threatening process.
- Over the past 25 years, decreases in neonatal mortality rates (NMRs) have been slower than those for maternal and child mortality.
- Intrapartum birth asphyxia, sepsis, and prematurity are the most common causes of global neonatal mortality.
- Lower NMRs are strongly associated with increased gross national income, decreased total fertility rates, higher female literacy, at least one antenatal care visit, presence of a skilled attendance at birth, and a higher percentage of birth registrations.

Global Newborn Health – History

It is essential for all those who work with mothers and their babies to understand the global health milieu and the historical factors that have shaped the field as it stands today. More importantly, as neonatologists look to the future, a contextualizing of their work in neonatal and perinatal health and an appreciation of what levers need to be pulled to affect the future of the world's newborns become even more important. Finally, for those who want to influence these processes and contribute to moving the field forward, a deeper understanding becomes necessary (Darmstadt et al., 2014). Today more than ever, the field of global newborn health is much broader and quite distinct from the technologically infused might of neonatal intensive care units (NICUs) in high-income countries. This chapter will discuss the policies, programs, research, advocacy, and common underlying public health themes that affect the newborn globally (Lawn et al., 2014). Armed with this knowledge, the healthcare provider working with mothers and newborns in today's multicultural environments will be better able to understand and provide the culturally and contextually sensitive care necessary to be effective and will have the tools to springboard the future of global newborn health (Engmann, 2011; Engmann et al., 2013a).

Childbirth is a risky, life-threatening process with good maternal and neonatal outcomes far from assured. It is therefore no surprise that newborns have been given special attention (either because they lived, died, or were maimed during birth) throughout history. Some of the earliest recordings of newborns in ancient history date back to the second century AD and are ascribed to the Greek physician and gynecologist Soranus of Ephesus (Dunn, 1995). Other artists from late antiquity and the middle ages depict the

process of childbirth with a mother in the squatting position, a doula/helpmate supporting her, a midwife, and perhaps in the most important role, someone praying, invoking all the extra help possible from the deities (Fig. 4.1).

The *Boke of Chyldren*, written by Thomas Phaïre in 1545, was one of the earliest books published in English (Phaïre, 1545) while the 17th century treatise by William Harvey remains among the most elegant descriptions of the transition from fetal to extrauterine life (Dunn, 1990). The Talmud and the Old Testament are replete with stories concerning babies. For example, one story describes the birth and early life of Moses, who at 3 months of age was placed in a basket, floated down the River Nile, and subsequently adopted by the Pharaoh's daughter. Another story, perhaps one of the earliest recorded cases of sudden infant death syndrome and baby-snatching, describes two women each with newborn babies who live in the same house. One baby dies in the night, and its mother is accused by the other of waking up and switching her dead baby with the live one. This dispute is brought to King Solomon for adjudication. He recommends that the baby be sawn in two with one-half given to each woman. One woman, the mother of the dead baby, agrees to this proposal, while the mother of the live baby, appalled that her baby will be killed, says its life should be spared and agrees to relinquish her claim to being the mother. This act convinces King Solomon that she is the rightful mother, and he adjudicates the baby should be given to her (Bible: 1 Kings 3:16–28). By AD 315, the Roman Empire had established laws supporting the enslavement of “foundlings” or abandoned babies, in a bid to counter a faltering population growth and infanticide and to promote the adoption or child-rearing of orphans (Spaulding and Welch, 1991). During the 17th century a number of countries started to build “Foundling Hospitals” and by the late 19th century, preterm babies, known as “hatchlings,” were an important draw at exhibitions and public fairs where they were displayed as they lay in incubators known as “hatcheries” (Silverman, 1979).

Various societies have evolved cultural practices such as delayed naming of a child, perhaps in response to high perinatal and neonatal mortality rates (NMRs). In certain Asian cultures, full naming ceremonies are not held until the infant is several months old, while in Jewish tradition, full mourning for the entire year is not required for a neonatal death (Ginzberg et al., 2003). In some African populations, the mother and her newborn baby are not allowed to be seen for the first 7 days after birth except by very close family and friends to ensure the “evil eye” is not put on either the mother or her baby. In other settings, a “naming ceremony”



• **Fig. 4.1** Classical Greek Image of Woman Giving Birth, Depicting Doulas, Midwife, and a Woman Praying for the Safety of Mother and Baby. (From <http://visualizingbirth.org/classical-greek-image-of-woman-giving-birth>.)

where a baby is provided a name and then publicly introduced to the community does not occur for the first 7 days or sometimes longer. This is to ensure that the baby has decided to stay on earth and thus differentiated itself from being a “spirit child” who will die (Denham et al., 2010). To counter the death of a baby in the family, certain societies in Africa and Asia give the next child a coarse or earthy name to ward away spirits who might otherwise also take the new arrival.

Early in the 20th century, an obstetrician advocated in the *British Medical Journal* for the specialized training of doctors and nurses in neonatal medicine and for the construction of specialized newborn health facilities. He went on to predict that within a short period of time, the specialist in neonatal diseases and the nurse intensively trained and expert in the management of the delicate newborn would be commonplace (Ballantyne, 1923). By the mid-20th century, there was a deepening realization among certain pediatric groups, boards, and societies that the study and practice of pediatrics during the first month of life were distinct and specialized enough to require further training. Furthermore, the need for an in-depth understanding of embryonic, fetal, and neonatal pathophysiologic processes, the role of technology, including ventilators, and the complexities of the medical management of a sick baby led to the creation of neonatology as a distinct subspecialty in the United States. A subboard of the American Board of Pediatrics was created to oversee and shape the current and future knowledge and understanding of the field. The first subboard examination in neonatal–perinatal medicine was offered in 1975 (Philip, 2005).

By the close of the 20th century, there was emerging appreciation in global health policy and programmatic circles that the newborn period provided unusual challenges, with drivers influencing survival that were different to the more traditional child health programs. Another realization was that the newborn was absent from maternal and child health programs. This was because national and international maternal health programs had hitherto assumed that child health programs encompassed close attention to newborn care as newborns grow to become children, while child health programs, believing the mother and neonate to be inextricably linked at

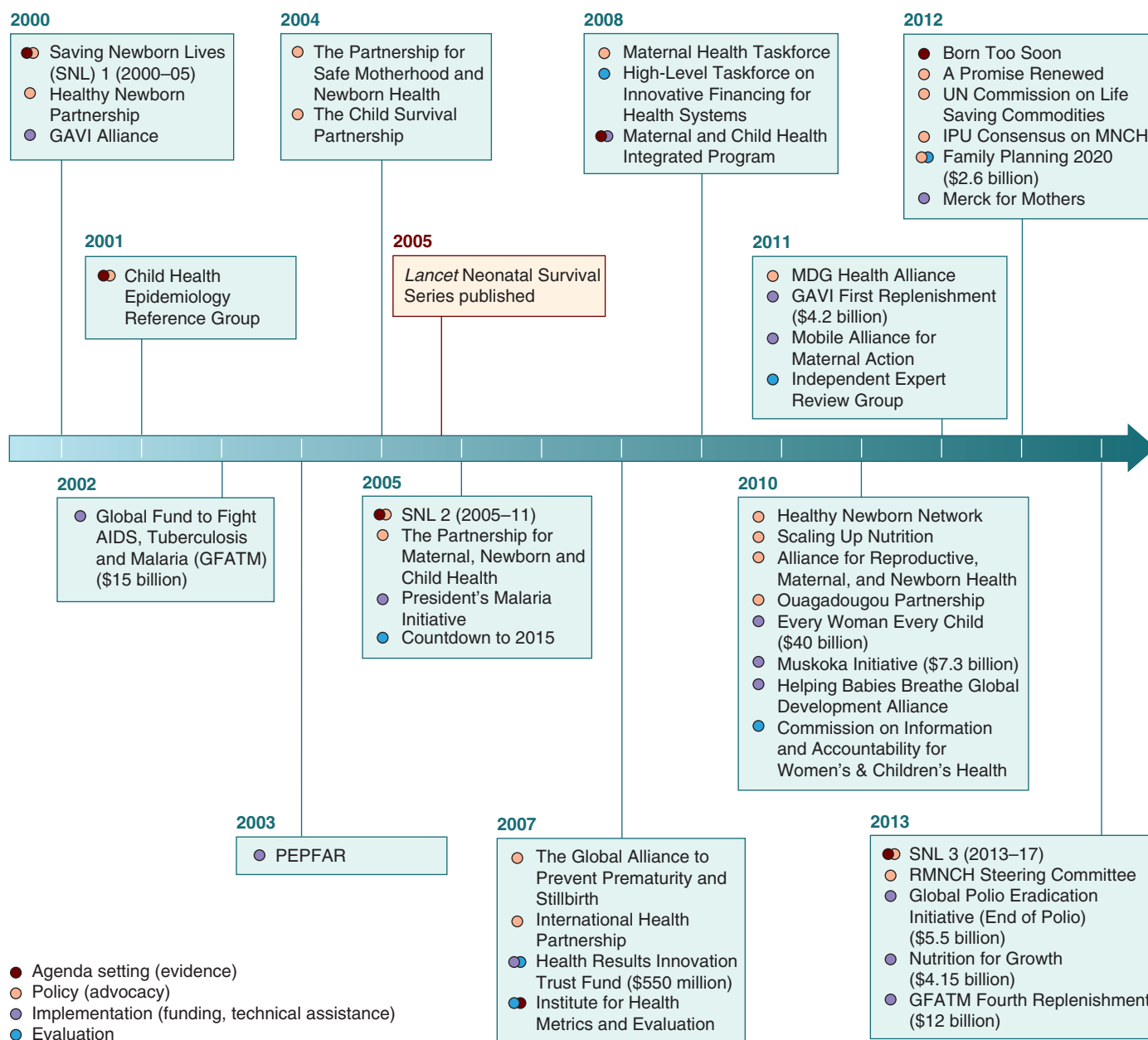
birth, had subsequently assumed that maternal programs included a strong focus on the neonate (Tinker et al., 2010). As a result, there was a paucity of routine newborn surveillance and epidemiologic data collected globally and nationally (The World Health Report, 2005). Programmers, policymakers, and funders started increasing their focus on the neonate. Abhay Bang and colleagues from the Gadchiroli district of India published a landmark series on the effects of a home-based newborn care program and compared these against control village clusters who had no intervention. The program involved a package of interventions that included training of village health workers in neonatal care who then made home visits and managed birth asphyxia, premature birth or low birth weight, hypothermia, breastfeeding problems, and neonatal sepsis (septicemia, meningitis, and pneumonia). In addition, assistance by trained traditional attendants, a health promotion program, and fortnightly supervisory visits were provided. The authors reported a decline in NMR from 62 to 25 per 1000 live births in the intervention clusters, whereas in control clusters, they reported an *increase* in NMRs from 58 to 64 per 1000 live births (Bang et al., 2005).

In 2000, the Bill and Melinda Gates Foundation provided funding to the nongovernmental organization (NGO) Save the Children in order to create a special entity called “Saving Newborn Lives.” This group’s remit was to focus and harness newborn stakeholder energies on moving the global newborn agenda forward through research and program work, which would inform advocacy and policy (Tinker et al., 2010). Soon afterwards, Black and colleagues published a child survival series with the first paper provocatively titled “Where and why are 10 million children dying every year?” (Black et al., 2003). This was followed by another seminal publication from the *Lancet* Neonatal Series – which asked “4 million neonatal deaths: When? Where? Why?” (Lawn et al., 2005). These publications highlighted the following: (1) sepsis, intrapartum birth asphyxia, and prematurity were the major drivers of 4 million newborn deaths; (2) most neonatal births and deaths were occurring in low- and middle-income countries, outside of healthcare facilities, and in the home; and (3) there was an absence of district, regional, national, or global data on newborn survival. These seminal papers and the strong advocacy by a handful of newborn champions spurred national governments, United Nations (UN) agencies, NGOs, academic institutions, funding agencies, and other newborn stakeholders to begin developing programs, policies, and research agendas that were newborn focused (Martines et al., 2005) (Fig. 4.2).

Global Newborn Health 2000–2015: Addressing Cause-Specific Neonatal Mortality

Coinciding with increased understanding from seminal publications and with the support of newborn champions, the turn of the 21st century heralded the launch of the Millennium Development Goals (MDGs) (Fig. 4.3).

The MDGs were seen as a focusing and galvanizing effort through which the global community would channel its collective resources and efforts to address major global development issues (*The Millennium Development Goals Report 2015*, 2015). The goals, set in 2000, included eradicating extreme poverty and hunger, achieving universal education, promoting gender equality and empowerment of women, reducing child mortality (death in children under the



• **Fig. 4.2** Key Stakeholders, Events, and Timeline Pertaining to Global Newborn Health From January 2000 to December 2013. *AIDS*, Acquired immunodeficiency syndrome; *GAVI*, Global Alliance for Vaccines and Immunization; *IPU*, Inter-Parliamentary Union; *MDG*, millennium development goals; *MNCH*, maternal, newborn, and child health; *PEPFAR*, President's Emergency Plan for AIDS Relief; *RMNCH*, reproductive, maternal, newborn, and child health and nutrition; *SNL*, Saving Newborn Lives; *UN*, United Nations. (From Darmstadt GL, Kinney MV, Chopra M, et al. Who has been caring for the baby? *Lancet*. 2014;384(9938):179.)

age of 5 years) by two-thirds, reducing maternal mortality by three-quarters, combating HIV/AIDS, malaria, and other diseases, ensuring environmental sustainability, and establishing global partnerships for development (*United Nations Millennium Declaration, Resolution Adopted by the General Assembly*, 2000).

Although these ambitious goals were not all reached by the target date of 2015, the MDG era has arguably seen the greatest improvements in health and development indicators in the history of humankind (*The Millennium Development Goals Report 2015*, 2015). For example, the number of people living in extreme poverty has decreased from 1.9 billion to 836 million; maternal and child mortality have been halved; net school enrolment has reached

91% with sub-Saharan Africa seeing a 20% increase in average literacy rates of women; and in Southern Asia the number of girls enrolled for every 100 boys had increased from 70 to 103 by 2015. Approximately 91% of the world's population is now using improved drinking water sources, compared to 70% in 1990 (*The Millennium Development Goals Report 2015*, 2015).

One of the main themes that the various newborn publications articulated was that most newborn births, and 99% of newborn deaths, occurred in community-based settings such as the home and not in hospitals and health facilities (Lawn et al., 2005; Darmstadt et al., 2009). Furthermore, country case studies showed that a handful of low- and middle-income countries had seen

The Millennium Development Goals (MDGs) are a set of global development objectives to be achieved by 2015 that were unanimously adopted at the United Nations Millennium Summit in September 2000. Attainment of the eight individual goals is to be measured by progress against 18 associated targets.

| | | |
|---|--|--|
| 1 | Eradicate extreme poverty and hunger | <ul style="list-style-type: none"> • Reduce by half the proportion of people living on less than a dollar a day • Reduce by half the proportion of people who suffer from hunger |
| 2 | Achieve universal primary education | <ul style="list-style-type: none"> • Ensure that all boys and girls complete a full course of primary schooling |
| 3 | Promote gender equality and empower women | <ul style="list-style-type: none"> • Eliminate gender disparity in primary and secondary education at all levels by 2015 |
| 4 | Reduce child mortality | <ul style="list-style-type: none"> • Reduce by two-thirds the mortality rate among children under five |
| 5 | Improve maternal health | <ul style="list-style-type: none"> • Reduce the maternal mortality ratio by three quarters |
| 6 | Combat HIV/AIDS, malaria, and other diseases | <ul style="list-style-type: none"> • Halt and begin to reverse the spread of HIV/AIDS • Halt and begin to reverse the incidence of malaria and other major diseases |
| 7 | Ensure environmental sustainability | <ul style="list-style-type: none"> • Integrate the principles of sustainable development into country policies and programmes; reverse loss of environmental resources • Reduce by half the proportion of people without sustainable access to safe drinking water and basic sanitation • Achieve significant improvement in lives of at least 100 million slum dwellers, by 2020 |
| 8 | Develop a global partnership for development | <ul style="list-style-type: none"> • Targets cover: trading and financial systems, the special development needs of disadvantaged states, debt sustainability, youth employment, affordable access to essential drugs, and access to information and communications technologies |

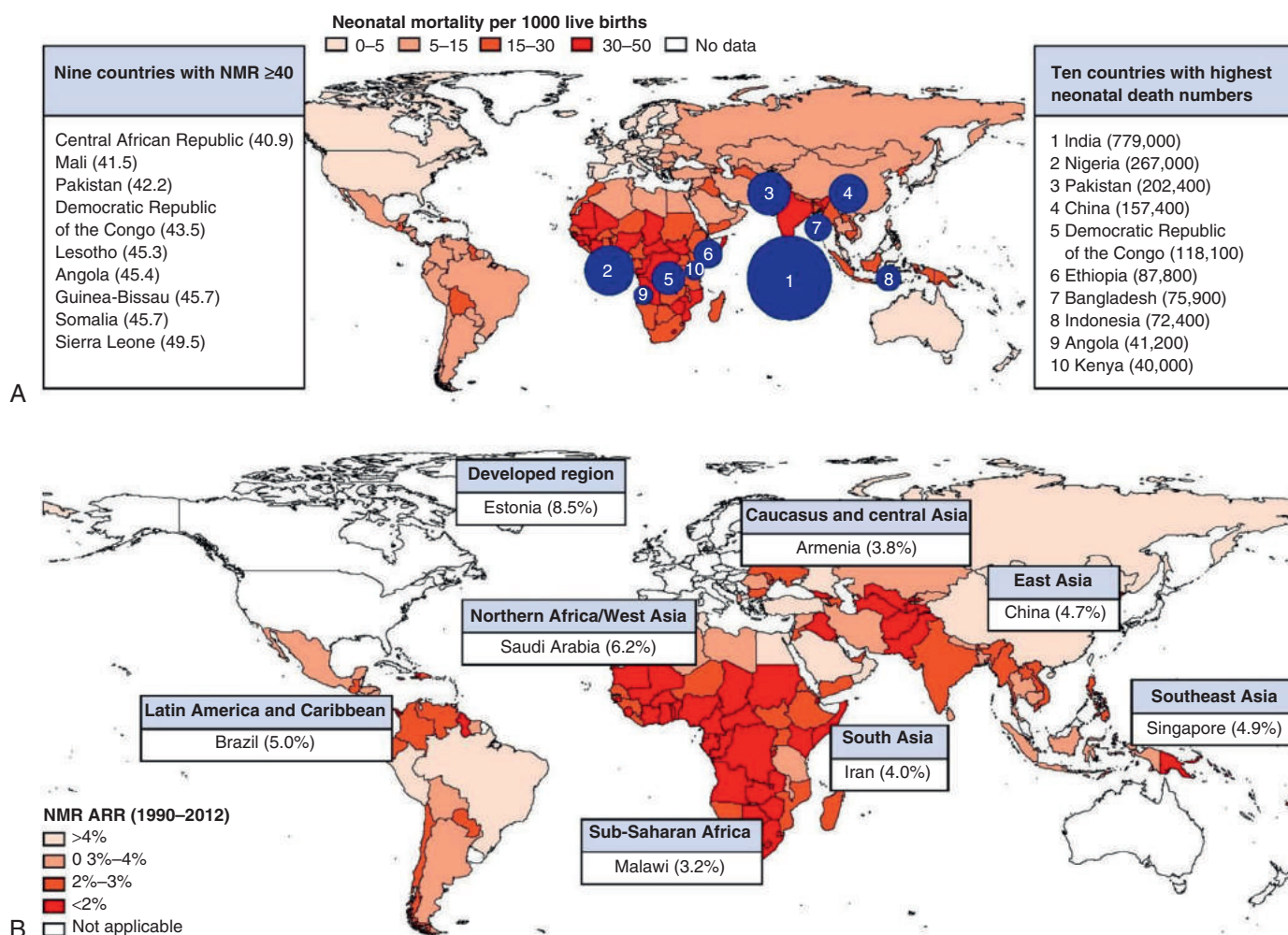
• **Fig. 4.3** The Millennium Development Goals. *AIDS*, Acquired immunodeficiency syndrome; *HIV*, human immunodeficiency virus. (From http://www.budget.gov.au/2008-09/content/ministerial_statements/image/ausaid-5.gif.)

impressive reductions in national NMRs *before* the creation of the first neonatal intensive units in the country. Sri Lanka, for example, had experienced reductions in mortality from over 50 deaths per 1000 live births in the 1950s to less than 20 deaths per 1000 live births by 1985 when the first NICU in the country was developed (Martines et al., 2005). The Indian state of Kerala also had seen similar impressive reductions in neonatal mortality. Further analysis suggested that in both Sri Lanka and Kerala, sustained inputs and utilization of primary care and government facilities had played major roles in the decrease in their NMRs, not the introduction

of NICUs. In Sri Lanka, for example, by 1996 there was a midwife to patient ratio of 1:3000–5000, close to 100% antenatal care coverage, and an 86% facility-based delivery rate. There was no charge to women for their medical care and the average distance from home to a health facility was 1.4 kilometers, with 5 kilometers being the furthest distance of advanced hospitals from those health facilities. Higher maternal educational levels, effective antenatal care provision, and higher contraception prevalence rates were perceived to have contributed significantly to these impressive reductions in NMRs (Lawn et al., 2005).

Thus the prevailing approaches of most newborn stakeholders came to focus primarily outside formal health facilities, since that was where the burden of births and newborn deaths occurred. In contrast with other MDG indicators, including child and maternal mortality that halved between 1990 and 2015, newborn mortality has not achieved the same degree of success. Global NMRs decreased by 37%, from 33 to 21 deaths per 1000, representing a decrease in number of newborn deaths from 4.4 million in 1990 to 2.9 million in 2014 (Lawn et al., 2014). As a result, as child mortality has fallen, the proportion of childhood deaths attributable to neonatal mortality is *increasing* (Lawn et al., 2014 citing [Levels and Trends in Child Mortality Report 2013, 2013]). There is also wide variation in NMRs, absolute neonatal deaths per year, and in annual rates of reduction for all regions of the world, except for high-income countries. Asia has the largest absolute number of neonatal deaths when the top 10 countries with highest neonatal deaths in the world are considered. However, where NMRs are considered, Africa contributes 8 out of the 9 countries with the highest NMRs (Fig. 4.4).

Throughout history, verbal autopsy (VA) has been a commonly used technique to determine neonatal cause of death in settings without strong health systems. Estimates are that virtually every low- and middle-income country uses some aspect of VA for its vital registration activities (Black et al., 2010). VA involves the administration of a systematic, structured questionnaire to the primary caregiver (usually the mother) of the deceased, in this case the baby, by trained questioners. During this process, the signs, symptoms, and circumstances surrounding the death are ascertained. Afterwards, a panel, typically consisting of two or three physicians, individually codes and assigns a cause of death, which is sometimes based on an a priori agreed-upon algorithm (Fottrell and Byass, 2010). When there are discrepancies in assignment of cause of death, the physicians come together to discuss and reach a final agreement (Engmann et al., 2011a). Many studies have been conducted to compare the results of these community-based VA assignments of cause of death to hospital-based assignments, and the results have been favorable for the VA (Engmann et al., 2011a).



• **Fig. 4.4** Variation between countries in neonatal mortality in 2012 (A) and variation in average annual rates of reduction of neonatal mortality rate for all regions apart from developed regions (1990–2012), showing the fastest progressing country according to Millennium Development Goal region (B). Data from UN Interagency Group for Child Mortality Estimation estimates for NMR in 1990–2012. NMR, neonatal mortality rate per 1000 live births; NMR ARR, 1990–2012 average annual rate of reduction in neonatal mortality rate, based on calculation. (From Lawn JE, Blencowe H, Oza S, You D, et al. Every Newborn: progress, priorities, and potential beyond survival, *Lancet*. 2014; 384:189–205. Copyright 2014.)

There are many variations of the execution of VA. Some authors have examined the optimal use of VA interviewers, others the use of VA coders, and others algorithms and neural networks that may help to determine cause of death (Byass et al., 2015). Investigators from the Eunice Kennedy Shriver National Institute of Child Health and Human Development's Global Network for Women and Children's Health probed the technique of perinatal VA to assess the correlation of single versus multiple VA coders, non-physicians versus physicians in assigning cause of death, and the use of birth attendants as VA respondents (Engmann et al., 2009, 2011a, 2011b). After a training period, individual physicians made determinations of cause of stillbirth and early neonatal death that were comparable to physician panels, and non-physician respondents had cognitive and applied knowledge scores comparable to physician panels. Perhaps most promising and with strong programmatic potential, birth attendant responses (when compared to those of primary caregivers of the deceased) showed strong concordance across all questions on early neonatal deaths (94%) and stillbirth (93%) (Engmann et al., 2012b). For as long as there remains a large number of newborn births and deaths that occur outside of formal or weak health systems, VA is likely to be one of the most commonly used methods to inform global, national, and regional programs and policies and to conduct research activities.

Major Causes of Neonatal Mortality

Birth Asphyxia

Birth asphyxia is defined by the World Health Organization (WHO) as the failure to initiate and sustain spontaneous breathing at the time of birth. More recently, there have been calls for more precision in terminology that is focused on marking the timing of the insults such as antepartum, intrapartum, and postpartum asphyxia (de Bernis et al., 2016). Of the 136 million babies born every year, approximately 10% require some form of resuscitation at birth (Kattwinkel et al., 2011). Approximately 14 million of these babies will require only stimulation at birth to establish regular respirations, while approximately 4 million will require stimulation plus basic resuscitation with a self-inflating resuscitation bag and mask and some postresuscitative care. Unfortunately, a further 700,000 newborn babies die from intrapartum-related conditions. A variety of research studies have focused on improving newborn resuscitation skills (Carlo et al., 2010a, 2010b; Wall et al., 2010; Lee et al., 2011; Pasha et al., 2013; Bang et al., 2014). The large, multicenter FIRST BREATH trial—conducted in the Democratic Republic of the Congo, Zambia, Guatemala, Pakistan, India, and Argentina and involving over 50,000 babies—reported a one-third reduction in perinatal deaths after a newborn resuscitation program was instituted. Curiously, the majority of this reduction was in stillbirths. The authors concluded that improved resuscitation skills also reduced the amount of misclassification between stillbirths and early neonatal deaths (Carlo et al., 2010a).

A number of studies have examined the skill level of healthcare providers and the ability of resuscitation training programs to improve resuscitation skills. In one study of resuscitation training conducted among nurses, midwives, nurse anesthetists, and physicians in Ghana, West Africa, nurses had the lowest scores for effective resuscitation prior to training; however, afterwards their scores nearly doubled and in some cases exceeded those of physicians. (Enweronu-Laryea et al., 2009). More recently, the Helping Babies Breathe (HBB) program was developed by the American Academy of Pediatrics. HBB focuses on resuscitation in low- and middle-income settings and emphasizes the first “golden minute.” An

evaluation of this program in India and Tanzania suggested a significant reduction in neonatal deaths (0.53 relative risk [RR], 95 confidence interval [CI] 0.43–0.65) and intrapartum stillbirth rates (0.76 RR, 95% CI 0.64–0.9) (Msemo et al., 2013). As with most research trials, it remains to be seen whether incorporation of HBB into national and regional programs will result in similarly impressive gains.

There is increasing appreciation that in order for a program to be successful, it requires a functioning healthcare system and incorporation of all elements across the continuum of care, including recognition of danger signs (Aborigo et al., 2014). This has led a number of authors to adapt the “three delays” conceptual model proposed for maternal health to newborn health. This model comprises (1) delay in recognition of illness in the home, (2) delay in transportation from home to facility, and (3) delay in appropriate and effective management at the facility (Waiswa et al., 2010; Engmann et al., 2013a). At the policy level, it is imperative that global and national policies exist for the management of intrapartum birth asphyxia and that funding mechanisms are in place to support implementation. Currently one pernicious challenge in many low-income settings where standard neonatal resuscitative practices are absent or weak is that well-meaning international groups of neonatal experts teach a variety of neonatal resuscitation programs, sometimes several different programs within the same hospital. Thus standardization and mastery of practice of one method or program are absent, and overall goals of improving resuscitation skills are not efficiently met. A solution might be for the countries or hospitals involved to agree on one standard program, ensure mastery, and make that the standard of care. Another challenge is that nursing staff that are trained in resuscitation programs are frequently transferred from obstetric and newborn units to other parts of a hospital, which further limits practice and expertise in optimal neonatal resuscitative practices. Recognizing this, many facilities are beginning to change their policies and procedures such that staff trained in neonatal resuscitation remain in one department to ensure opportunities to practice and apply their learnings.

Neonatal Sepsis

Neonatal sepsis has long been one of the major causes of newborn morbidity and mortality, and over the past decade there have been a large number of research and program approaches to this problem (Ganatra and Zaidi, 2010; Seale et al., 2014). Box 4.1 outlines the common research and programming directions undertaken to decrease global neonatal sepsis rates over the past decade.

While the gold standard for treatment of presumed serious bacterial infections in high-income settings is parenteral antibiotic therapy delivered in a health facility, evidence suggests that non-medical providers can decrease mortality from neonatal infections in community settings, by using specific tools to guide the assessment, management, and referral process (Ganatra and Zaidi, 2010; Baqui et al., 2015). Examples of successful community-based intervention trials for sepsis include the Society for Education, Action, and Research in Community Health (SEARCH) trial in India where the sepsis-specific NMR decreased by 90% after the intervention (Bang et al., 2005) and the Projahnmo Study Group trial in Bangladesh where overall NMR declined by 34% (Baqui et al., 2008). In 2015, several trials of simplified antibiotic regimens for neonatal sepsis were published. This cluster of studies conducted in the Democratic Republic of Congo, Nigeria, and Kenya, and similar trials in Bangladesh and Pakistan, all showed that simplified antibiotic regimens for serious bacterial infections showed

• BOX 4.1 Common Research and Programming Directions to Decrease Global Neonatal Sepsis Rates

- Clean delivery kits
- Recognition of danger signs of neonatal sepsis
- Strength of association of different signs and symptoms in the community-based management of severe sepsis through the infant and young child study
- Prevention of neonatal sepsis
- Responsible bacteria for presumed serious bacterial sepsis
- Treatment of neonatal sepsis in both community and facility settings
- Chlorhexidine application to the umbilical cord, the entire baby, and the vagina

Data from Ganatra HA, Zaidi AK. Neonatal infections in the developing world, *Semin Perinatol.* 2010; 34: 416–425, Edmond K, Zaidi A. New approaches to preventing, diagnosing, and treating neonatal sepsis, *PLoS Med.* 2010; 7, pp. e1000213. doi: 10.1371/journal.pmed.1000213, Sinha A, Sazawal S, Pradhan A, Ramji S, Opiyo N. Chlorhexidine skin or cord care for prevention of mortality and infections in neonates, *Cochrane Database Syst Rev.* 2015; 3: doi: 10.1002/14651858.CD007835.pub2

noninferiority when compared to the WHO-recommended standard of care (at least 7–10 days' worth of parenteral antibiotics, at least twice a day) ([African Neonatal Sepsis Trial – AFRINEST, 2015](#)).

Similarly, many studies have examined the use of chlorhexidine for preventing and reducing omphalitis/sepsis-specific neonatal mortality. Cleaning the vagina with chlorhexidine, wiping the entire baby, and cleaning the cord are three approaches that have been investigated ([Sinha et al., 2015](#)). While vaginal wipes and cleaning the entire baby resulted in modest differences in neonatal mortality, more recent metaanalyses suggest that 4% chlorhexidine used to clean the umbilical cord results in overall reductions in NMRs of up to 12% ([Sinha et al., 2015](#)).

At the policy level, the rate of publication of these studies has, in some cases, outrun global policymakers. Currently, the WHO recommends daily application of 7.1% chlorhexidine to the umbilical stump of babies who are born at home in high neonatal mortality settings (greater than 30 deaths per 1000 live births). However, since these recommendations were made based on the available evidence, and since there are a number of more recent studies nearing completion, these recommendations will likely be modified.

There have been some policy breakthroughs in neonatal sepsis management at global and national levels that are worth noting. For example, in India, auxiliary nurse midwives are now allowed to administer injectable antibiotics, and similarly, in Ethiopia, community health workers are now able to provide community management of neonatal sepsis. At the global level, policy groups have also accelerated the speed of policy process-making, and thus there are now clear and timely policy recommendations by the WHO for the use of simplified regimens. These developments have paved the way for program implementation by governments, private practitioners, and other stakeholders and appear poised to contribute to future reductions in sepsis-specific neonatal mortality.

A highly successful approach to the prevention of neonatal sepsis in high-income countries has been the development and implementation of strategies aimed at reducing maternal colonization with group B streptococcus (GBS). In these settings, early-onset GBS disease was a leading cause of neonatal infections, and high maternal colonization rates were contributory. Given the successes of risk-screening and screening-culture based identification of

women whose newborns may benefit from intrapartum antibiotic prophylaxis, one might expect that similar approaches in resource-limited settings may also be effective in reducing early-onset neonatal infections. However, it appears that the causative microbiologic agents in neonatal sepsis in Africa and Asia are quite different from those in North America and Europe. [Table 4.1](#) outlines the causative agents of sepsis in young infants up to 90 days of age in low- and middle-income countries, by age of onset. Although large-scale population-based data on maternal colonization and etiologies of neonatal sepsis do not exist, there have been several reports of causative agents identified through robust culture methods from health facilities in low-income settings. In a recent review, Gram-negative organisms accounted for twice the number of neonatal infections as those caused by all gram-positive organisms (including GBS) ([Zaidi et al., 2009](#)). Because the efficacy of GBS prophylaxis programs in low-income countries have yet to be widely and rigorously evaluated, it will be premature to promote the practice of GBS prophylaxis in such settings.

Preterm Birth

In 2012, over 100 stakeholders from the global newborn community including representation from high, middle, and low-income countries aligned efforts to design, develop, and disseminate the World Prematurity Report, which outlined more precisely than ever before the burden of newborn mortality attributable to prematurity (*Born Too Soon: The Global Action Report on Preterm Birth, 2012*). Estimates are that 15 million babies annually are born preterm, and one million of these babies die from direct preterm birth complications, making this the most common cause of neonatal mortality. Of cause-specific neonatal mortality, the smallest reductions have occurred for preterm birth complications (<20%) globally; thus the authors state, “addressing preterm birth is fundamental to acceleration of NMR reduction” ([Lawn et al., 2014](#)). Preterm rates are rising in most countries, and 85% of these births are between 32 and 36 weeks. The top 10 countries with the highest numbers of premature babies born each year are India, China, Nigeria, Pakistan, Indonesia, United States, Bangladesh, Philippines, Democratic Republic of Congo, and Brazil. While *prevention* of premature delivery by a mother remains somewhat elusive and continues to attract ongoing research attention primarily in high-income countries, *management* of premature babies in all settings, particularly low- and middle-income countries, has received increasing attention over the past 15 years. An overarching challenge and opportunity around the area of prematurity remain a coordinated response by funders, policymakers, researchers, and programmers to systematically address the entire field. To begin this process, a consortium of researchers and funders has proposed a “preterm solution pathway” that maps out the discovery, development, and delivery elements of preterm birth with the prediction, prevention, and clinical care management functions, as a blueprint for this effort ([Lackritz et al., 2013](#)). It is instructive to consider some of the issues that arise in the execution of the following preterm management modalities.

Antenatal Corticosteroids

Liggins and Howie published their seminal work on antenatal corticosteroids in 1972, and, since then, widespread use in health facilities in high-income countries has resulted in marked reductions in the NMR ([Liggins and Howie, 1972](#); [Roberts et al., 2006](#); [Mwansa-Kambafwile et al., 2010](#)). Recently, Althabe and colleagues from the National Institute of Child Health and Human

TABLE 4.1**Etiology of Sepsis in Young Infants up to 90 Days of Age in Developing Countries by Age of Onset**

| Organism Isolated | ≤3 DAYS OF LIFE | | ≤57 DAYS OF LIFE | | 7–59 DAYS OF LIFE ^a | | 29–90 DAYS OF LIFE ^b | |
|--|-----------------|-------|------------------|-------|--------------------------------|-------|---------------------------------|-------|
| | n | % | n | % | n | % | n | % |
| Total | 834 | 100.0 | 3209 | 100.0 | 835 | 100.0 | 141 | 100.0 |
| <i>Staphylococcus aureus</i> | 144 | 17.3 | 560 | 17.5 | 114 | 13.7 | 18 | 12.8 |
| <i>Streptococcus pyogenes</i> | 3 | 0.4 | 33 | 1 | 81 | 9.7 | 16 | 11.3 |
| Group B streptococci | 109 | 13.1 | 207 | 6.5 | 96 | 11.5 | | |
| Group D streptococci/ <i>Enterococcus</i> | 44 | 5.3 | 80 | 2.5 | 7 | 0.8 | | 0.7 |
| Group G streptococci | | | 1 | 0.03 | 1 | 0.1 | | |
| Viridans streptococci | 3 | 0.4 | 5 | 0.2 | 1 | 0.1 | 1 | 0.7 |
| <i>Streptococcus pneumoniae</i> | 9 | 1.1 | 49 | 1.5 | 103 | 12.3 | 38 | 27 |
| Other <i>Streptococcus</i> species/unspecified | 19 | 2.3 | 32 | 1.0 | 3 | 0.4 | 2 | 1.4 |
| <i>Listeria monocytogenes</i> | 4 | 0.5 | 5 | 0.2 | | | | |
| Other Gram positives | | | 69 | 2.2 | 27 | 3.2 | | |
| All Gram positives | 335 | 40.2 | 1041 | 32.4 | 433 | 51.8 | 76 | 53.9 |
| <i>Klebsiella</i> species | 220 | 26.4 | 813 | 25.3 | 47 | 5.6 | 6 | 4.3 |
| <i>Escherichia coli</i> | 105 | 12.6 | 490 | 15.3 | 78 | 9.3 | 13 | 9.2 |
| <i>Pseudomonas</i> species | 49 | 5.9 | 224 | 7.0 | 15 | 1.8 | 4 | 2.8 |
| <i>Enterobacter</i> species | 30 | 3.6 | 141 | 4.4 | 10 | 1.2 | 5 | 3.5 |
| <i>Serratia</i> species | 4 | 0.5 | 10 | 0.3 | 1 | 0.1 | 1 | 0.7 |
| <i>Proteus</i> species | 5 | 0.6 | 27 | 0.8 | 4 | 0.5 | 1 | 0.7 |
| <i>Salmonella</i> species | 6 | 0.7 | 37 | 1.2 | 111 | 13.3 | 11 | 7.8 |
| <i>Citrobacter</i> species | 3 | 0.4 | 43 | 1.3 | | | | |
| <i>Moraxella</i> species | | | | | 2 | 0.2 | 2 | 1.4 |
| <i>Haemophilus influenzae</i> | | 0.1 | 5 | 0.2 | 17 | 2.0 | 11 | 7.8 |
| <i>Neisseria meningitidis</i> | | | 1 | 0.03 | 5 | 0.6 | | |
| <i>Acinetobacter</i> species | 18 | 2.2 | 153 | 4.8 | 15 | 1.8 | 4 | 2.8 |
| Other Gram negatives | 37 | 4.4 | 167 | 5.2 | 63 | 7.5 | 3 | 2.1 |
| All Gram negatives | 478 | 57.4 | 2111 | 65.8 | 368 | 43.9 | 61 | 43.1 |
| Other | 21 | 2.5 | 57 | 1.8 | 34 | 4.1 | 4 | 2.8 |

^aIncludes some data for infants under 2 months of age. Also includes studies reporting 8–28 and 29–59 days' etiology.

^bIncludes infants 29–60 days of age.

From Zaidi AK, Thaver D, Ali SA, Khan TA. Pathogens associated with sepsis in newborns and young infants in developing countries, *Pediatr Infect Dis J*. 2009; 28: S10–18.

Developments Global Network sought to assess the feasibility, effectiveness, and safety of a population-based, multifaceted strategy to implement antenatal treatment of women at risk of preterm labor with corticosteroids compared with standard of care, on neonatal mortality attributable to preterm birth in low- and middle-income settings (Althabe et al., 2015). In this 18-month, cluster-randomized trial conducted in rural and semi-urban clusters within six countries (Argentina, Guatemala, India, Kenya, Pakistan, and Zambia), 28-day neonatal mortality among newborns whose birthweight was less than the 5th percentile (as a proxy for preterm birth) was examined. There were 47,394 live births, with 2520

(5%) less than the 5th percentile for birthweight in the intervention clusters and 50,743 live births and 2258 (4%) less than the 5th percentile who completed follow-up. A total of 2361 women in the intervention group received steroids compared to 215 women in the control group ($P < .001$). Among those less-than-5th-percentile infants, 28-day mortality was 225 per 1000 live births for the intervention group and 232 per 1000 live births for the control group. Among the entire population, 28-day mortality was 27.4 per 1000 live births in the intervention group and 23.9 in the control group (RR 1.12, 1.02–1.22; $P = .0127$). Suspected maternal infection was reported in 1207 (3%) of the intervention

group of women and 2% of the control group with an odds ratio (OR) 1.45, 1.33–1.58, $P < .0001$. The authors concluded that despite increased use of antenatal corticosteroids in low birth weight infants in the intervention group, neonatal mortality increased in the population as a whole: for every 1000 women exposed in this strategy, an excess of 3.5 neonatal deaths occurred, and the risk of maternal sepsis seemed to have increased (Althabe et al., 2015).

Azad and Costello reflected on these results and recommended three questions that should be answered before roll-out of modalities in low- and middle-income countries occurs:

1. Is there biologic plausibility? Does the modality *work* in mothers and infants in poor populations, for which there is limited care available? (For example, in this study there were few level two or intermediate neonatal centers.)
2. Do safety issues exist such that if the modality is applied at scale, in this case with corticosteroids, will there be no significant increase in maternal sepsis, perinatal death, or childhood disability?
3. Is there any evidence of benefit for the great majority of preterm infants born at more than 33 weeks?

Clearly these questions need to be considered carefully a priori, before rolling out any corticosteroid interventions (Azad and Costello, 2014).

Continuous Positive Airway Pressure

In high-income settings, continuous positive airway pressure (CPAP) has long been used as a treatment modality for respiratory distress syndrome, and many studies have shown a decrease in preterm-specific neonatal mortality. Feasibility of CPAP, particularly bubble CPAP, is increasingly being reported from single sites in various countries, including Malawi, Uganda, India, and Bangladesh (van den Heuvel et al., 2011; Brown et al., 2013; Daga et al., 2014; Chisti et al., 2015; McAdams et al., 2015). Although CPAP continues to enjoy widespread use and development, it is only recently that a systematic review examined its safety in low- and middle-income settings (Martin et al., 2014). When compared to oxygen therapy followed by mechanical ventilation if needed, the authors reported a 30%–50% decrease in need for mechanical ventilation. They concluded that bubble CPAP is safe and reduces the need for mechanical ventilation, although they recommended further research into the efficacy of bubble CPAP in low- and middle-income settings. Recent efforts are focused on building and testing different CPAP devices in order to find one that is efficient, durable, affordable, and sustainable in low-income settings. Investigators at Rice University, Houston, TX, Program for Appropriate Technology in Health (PATH), University of Washington, Seattle, WA, and Seattle Children's Hospital, Seattle, WA, are among those working closely with colleagues in countries such as Malawi, Uganda, and Kenya. Other organizations such as the *East Meets West Foundation* have a programmatic focus that develops invasive and noninvasive low-cost, custom-designed, durable equipment including ventilators and CPAP machines that are deployed primarily in Asia at present. A notable feature of this latter program is that it provides equipment maintenance, timely repairs, and support – both on site and remotely.

Surfactant

The widespread use of this treatment to reduce surface tension of the alveoli, especially in respiratory distress syndrome, is well known. However, the cost of implementing widespread use in low- and

middle-income settings has thus far been prohibitive. With a single treatment cost ranging from US\$450–900 in US hospitals, surfactant remains prohibitively expensive in resource-constrained countries and is thus only occasionally seen in the private sector, where patients must purchase it themselves. While there has been interest in developing cheaper alternatives, this has not materialized into tangible outcomes.

Kangaroo Mother Care

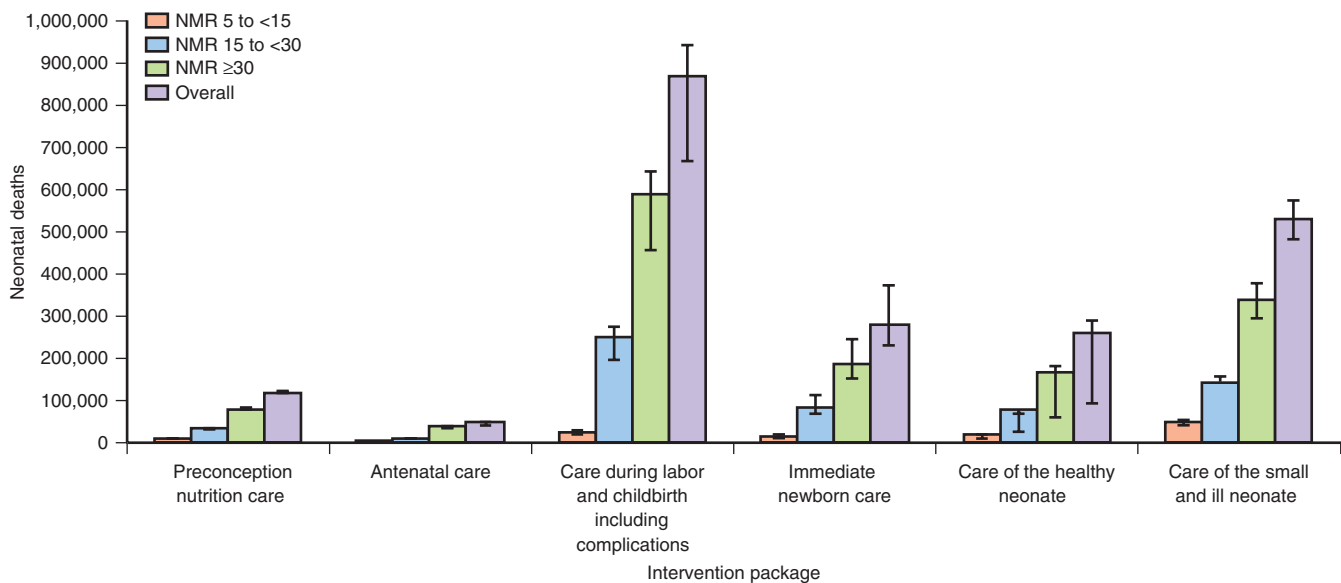
Kangaroo mother care (KMC) is defined as skin-to-skin care, exclusive breastfeeding, supportive care of the mother–baby unit, and close follow-up after discharge (Lawn et al., 2011; Engmann et al., 2013b). This technique was first described in Colombia in the 1970s and was designed to combat the phenomenon of NICU overcrowding. KMC has been shown to have multiple benefits for preterm infants. One analysis estimated that up to 450,000 preterm deaths could be averted each year if near-universal coverage is instituted (Lawn et al., 2010). There are many additional benefits of KMC, including strengthened immunity for the baby, increased psychological benefits, longer-term neurodevelopmental benefits, and low cost. Yet, global uptake of this practice has been very poor, estimated in 2013 to be less than 5%. Reasons postulated for this include:

1. KMC is seen as a poor-persons practice, only to be utilized as a next-best alternative.
2. Many healthcare workers either do not know about or believe in the benefits of KMC and lack skills necessary for successful implementation.
3. Cultural and social norms related to the mother and newborn practices make uptake of KMC challenging.
4. Human resources necessary for the effective implementation of KMC are missing and the role of mothers and communities has been overlooked.
5. KMC has not been included in many country-level government newborn agendas. (Engmann et al., 2013b).

A systematic review of barriers and enablers of KMC practice found that four of the top five ranked barriers were resource related, and all ranked above low awareness of KMC. Thus issues with the facility environment/resources, negative impressions of staff attitudes or interactions, lack of help with KMC practice, other obligations, and low awareness of KMC infant health are significant barriers. Top enablers included “mother–infant attachment” and support from mothers, families, friends, and mentors. The authors conclude that since KMC implementation is so context-specific, it is critical that focused ethnographic and qualitative research need to be done, while at the same time, the effectiveness of various user-centric designs for promoting KMC and ensuring support for the mother must be studied (Seidman et al., 2015). A global KMC Acceleration Group has recently been formed to help drive this agenda (Vesel et al., 2015).

Global Newborn Health at the End of the Millennium Development Goals Era

The newborn is at the nexus of many intersections across the health sectors, and numerous newborn-sensitive and newborn-specific facets affect morbidity and mortality. The conceptual framework for interventions to improve the health of every mother and newborn, as described by Bhutta and colleagues, is useful to consider (Fig. 4.5). With many of these interventions, improvements in



| | Preconception nutrition care | Antenatal care | Care during labor and childbirth including complications | Immediate newborn care | Care of the healthy neonate | Care of the small and ill neonate |
|--|------------------------------|------------------------------|--|------------------------------|-----------------------------|-----------------------------------|
| Estimated maternal lives saved by 2025 | 0 | 7500 (6700–9100) | 150,000 (137,100–158,900) | NA | NA | NA |
| Estimated stillbirths prevented by 2025 | 23,000 (17,800–26,500) | 240,000 (150,800–374,500) | 550,000 (432,500–531,400) | NA | NA | NA |
| Estimated neonatal lives saved by 2025 | 110,000 (111,800–118,300) | 43,000 (36,500–46,900) | 790,000 (588,500–865,000) | 190,000 (136,300–280,400) | 230,000 (64,300–261,500) | 580,000 (531,000–621,000) |
| Estimated additional child lives (postneonatal) saved by 2025 | 7500 | 1200 | 0 | 0 | 1900 | 0 |
| Costs by 2025 (in billion US\$) | 1.88 | 0.4 | 2.29 | 0.035 | 0.11 | 0.96 |
| Costs (billion US\$) per 100,000 maternal and newborn lives and stillbirths saved | 1.38 | 0.16 | 0.15 | 0.02 | 0.05 | 0.17 |
| Costs (billion US\$) per 100,000 newborn babies saved | 1.65 | 0.92 | 0.29 | 0.02 | 0.05 | 0.17 |

• **Fig. 4.6** Estimated Effect of the Intervention Packages on Numbers of Neonatal Lives Saved and Costs According to Levels of Care by the Year 2025. Error bars represent ranges. NA, not applicable; NMR, neonatal mortality rate.

Understanding the milieu, the knowledge, attitudes, beliefs, and interpretations of families and communities is a critical first step in any intervention design (Moyer et al., 2014). It is also important for individualized care of the patient, especially for care occurring in the home. This perspective has led to the development of a technique known as social autopsy at the community level. Social autopsy provides the rich contextual tapestry of social factors that frames the biomedical model of the VA (Kallander et al., 2011).

The end of the MDG period saw the launch of a coordinated global effort involving all 194 countries signatory to the UN that was entitled “Every Newborn: An Action Plan to End Preventable Deaths.” Box 4.2 outlines the vision, targets, goals, and strategies of the Every Newborn Action Plan. This effort brought together a large array of countries and their newborn stakeholders, including

Ministries of Health and Finance, academics, programmers, NGOs, funders, policymakers, clinicians, and, most importantly, civil society groups (*Every Newborn: An Action Plan to End Preventable Death*, 2016). A key differentiator of this Every Newborn Action Plan (ENAP) from other previous action plans remains the key demand-generation role contributed by civil society groups, including engaged parents, families, and communities. Additionally, ENAP has a strong focus on accountability and transparency. All countries are required to make public annual updates to the UN on how they have implemented the evidence-based recommendations noted in their country’s action plan.

One of the major gains of the ENAP was harnessing the entire newborn community of stakeholders and more. Certain groups, not hitherto typically part of the global newborn community of

• BOX 4.2 The Every Newborn Action Plan

Vision

A world where there are no preventable deaths of newborns or stillbirths, where every pregnancy is wanted, every birth celebrated, and women, babies, and children survive, thrive, and reach their full potential.

Every Newborn Action Plan Goals & Targets

1. End preventable newborn death: by 2035, all countries should have a neonatal mortality rate of 10 or less per 1000 live births.
2. End preventable stillbirth: by 2035, all countries should have stillbirth rates of 10 or less per 1000 total births.
3. A global neonatal mortality rate of 7 deaths per 1000 live births by 2035.

Strategic Objectives

Strengthen and invest in care during labor, childbirth, and the first day and week of life

Improve the quality of maternal and newborn care

Reach every woman and every newborn to reduce inequities

Harness the power of parents, families, and communities

Count every newborn: measurement, program tracking, and accountability

Guiding Principles

Country leadership

Integration

Equity

Accountability

Innovation

stakeholders, were actively sought out and engaged as key stakeholders, including mothers' groups, as well as other "communities" such as the maternal, child, and nutrition communities. Furthermore, politicians and high-profile individuals were sought out to act as newborn champions. It is worth emphasizing that in many low- and middle-income countries, more funding is increasingly being generated internally. Thus country-led advocates, policymakers, funders, programmers, and researchers are forming a growing number of key newborn stakeholders. Collaborations have formed around newborn health to develop research infrastructures that function within and across countries and liaise with multinational agencies to evaluate best practices and scale-up. The African Newborn Network, for example, includes Ethiopia, Ghana, Malawi, Mali, Mozambique, South Africa, Tanzania, and Uganda and is funded by the Bill and Melinda Gates Foundation via Save the Children's Saving Newborn Lives Initiative. Another example is the Eunice Kennedy Shriver National Institute of Child Health and Development Global Network for Women and Children's Health. These collaborations include ministries of health, UN agencies, and researchers across many continents and have resulted in networks that have conducted and evaluated community and facility-based interventions to improve maternal and newborn health with the goal of delineating those that may be effective and scalable in low- and middle-income settings (Koso-Thomas et al., 2015). Table 4.2 outlines examples of some of the key stakeholders in global newborn health.

In addition to providing a "north star" for health and development until 2015, the MDGs were successful at leveraging the attention, political will, and resources of the global community, nations, regions, researchers, aspiring innovators, and initiative leaders, all working toward a common goal. The Sustainable Development Goals (SDGs) have been built on the successes and lessons learned from the MDGs and represent a new era of global health and

development. While the SDGs are much more comprehensive, holistic, and ambitious and extend beyond the health and development sectors into a more integrated whole, some critics are challenged by the differences in their design, context, concept, scope, significance, targets, and approach (Fig. 4.7). It remains to be seen how the implementation of these goals will be rolled out, what the financing mechanisms involved will look like, and perhaps, most importantly, how sharp and clear the 17 targets and subtargets will be for policy coherence and how specific, measureable, attainable, relevant, and time-bound (SMART) they are.

The Global Strategy for Women's and Children's Health was launched by the Secretary General of the UN in 2010 when it became increasingly clear that progress toward achieving MDG 4, 5, and 6 was lagging the furthest behind. The overarching goal of the global strategy was to bring together and mobilize a wide array of partners from all sectors to join a global effort to reach the MDGs and improve the health and well-being of women and children in the 49 poorest countries of the world. The Global Strategy has helped to strengthen political commitment, mobilize resources (over US\$40 billion increase in women and children's health between September 2010 and May 2014), focus attention, coordinate, and consolidate efforts, bringing together partners and initiatives such as A Promise Renewed, Family Planning 2020, Every Newborn Action Plan, UN Commission on Life-Saving Commodities, and Ending Preventable Maternal Mortality. Together with working groups on innovations, commodities accountability, and financing, these partnerships are driving maternal, newborn, and child health interventions to scale. The Global Strategy stands on three pillars – partnerships, accountability, and innovation (Temmerman et al., 2015).

Despite increasing global consensus around essential interventions and programs to improve newborn outcome, substantial gaps remain in ensuring that the majority of neonates have access and high uptake (coverage) of these interventions and programs (Fig. 4.8). Lack of resources, including human resources, is a contributor to these gaps, though the complete picture of the causes underlying these inequities is more complicated and remains somewhat controversial. Despite the fact that evidence exists to support the implementation of many low-cost, high-impact interventions for newborns, challenges remain at local and national levels to scale-up sustainability. Differences in context from one low-resource setting to another hinder uniform implementation and scale-up of interventions. The keys to implementation at scale for proven interventions include a deep understanding of the local context, provision of an enabling environment, empowerment of local champions, and engagement of key stakeholders.

There are numerous inequities to combat in the global struggle to improve newborn health. In general, regions that have the highest NMRs also have the lowest healthcare worker density (Lawn et al., 2010). Three-quarters of the world's physicians are working in high-income countries serving approximately one-third of the world's population. Further analysis suggests that in Africa, 3% of the world's healthcare workforce has 1% of the world's expenditure on health to tackle approximately 24% of the global burden of disease (Serour, 2009). In addition to the shortage of the healthcare workforce, most hospitals in sub-Saharan Africa are not equipped to provide even basic neonatal resuscitation (Howie et al., 2008).

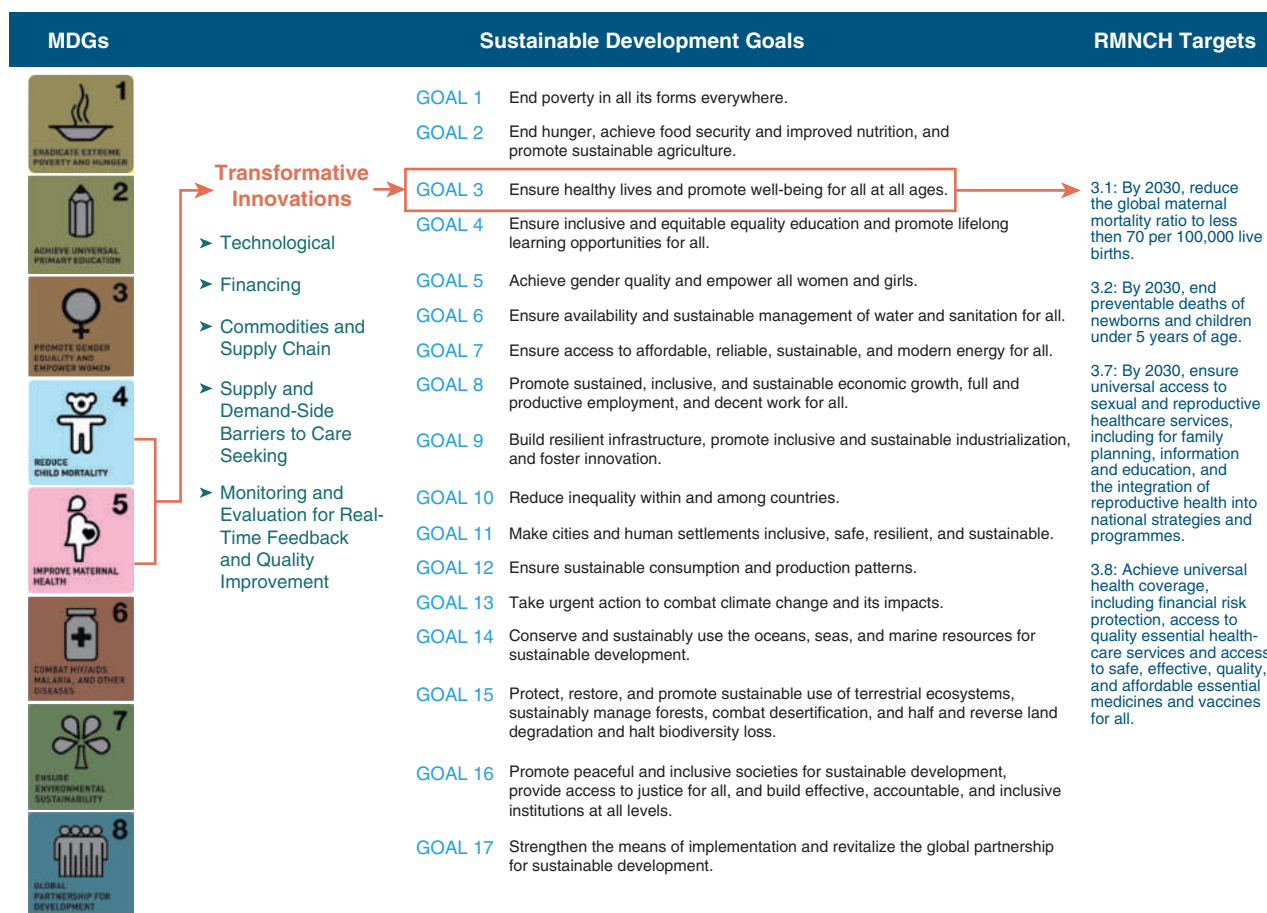
Toward a "Grand Convergence"

A number of authors have modeled analyses that indicate that by making today's medicines, vaccines, and other health tools

TABLE 4.2 Examples of Some of the Key Categories of Stakeholders in Global Newborn Health

| National Governments | Private Foundations | United Nations Agencies | Academic Institutions and Networks | Nongovernmental Organizations | Civil Society Organizations | Other Communities of Practice | Newborn Champions | Bilaterals | Professional Bodies |
|--|---------------------------------------|---|---|--|-----------------------------|-------------------------------|--|---|---|
| Indian Ministry of Health and Family Welfare | Bill and Melinda Gates Foundation | World Health Organization | INDEPTH Network | Save the Children – Saving Newborn Lives | Social Good Moms | Nutrition community | Mrs. Graca Machel – wife of the late President of South Africa, Nelson Mandela | United States Agency for International Development | International Pediatric Association |
| Kenya Ministry of Health | Children's Investment Foundation Fund | United Nations Children's Fund | Healthy Newborn Network | PATH | Kybele, Inc. | Child health community | Secretary Hilary Clinton | United Kingdom Department for International Development | International Federation of Gynecology and Obstetrics |
| Ethiopian Ministry of Health | March of Dimes | United Nations Population Fund | NICHD Global Network for Women and Children's Health | Johns Hopkins Program for International Education in Gynecology and Obstetrics | | Pneumonia community | Prime Minister Erna Solberg of Norway | Norwegian Agency for Development Cooperation | International Confederation of Midwives |
| South Africa Ministry of Health | Welcome Trust | H4 Partnership (WHO, UNFPA, UNAIDS, & UN Women) | Schools of Public Health and Medicine Building Resources Across Communities | World Vision | | Breastfeeding community | Ex-President Banda of Malawi | Danish International Development Agency | American Academy of Pediatrics |

NICHD, National Institute of Child Health and Human Development; *UNFPA*, United Nations Population Fund; *UNAIDS*, Joint United Nations Programme on HIV and AIDS; *UN*, United Nations; *WHO*, World Health Organization.



• **Fig. 4.7** Conceptual Framework for How Transformative Innovations in Reproductive, Maternal, Newborn, and Child Health That Were Started in the Millennium Development Goals Era and Continued Through the Sustainability Development Goals Era Might Help Achieve Reproductive Maternal, Newborn Child Health Targets by 2030. *AIDS*, Acquired immunodeficiency syndrome; *HIV*, human immunodeficiency virus; *MDG*, millennium development goal; *RMNCH*, reproductive, maternal, newborn, and child health. (Courtesy of Dr. Cheryl Moyer, University of Michigan, Ann Arbor, MI.)

universally available, and by stepping up tomorrow's innovation efforts, the health gap between high- and low-income countries could be closed within a generation (Jamison et al., 2013). Estimates are that by 2035 such a “grand convergence” in global health is achievable, reducing preventable maternal and child deaths to unprecedented low levels. The authors report that with aggressively scaled-up investments, 10 million lives can be saved annually with huge economic benefits: every dollar invested in low- and middle-income countries would yield US\$9–20. What innovations are necessary to achieve this grand convergence by 2035?

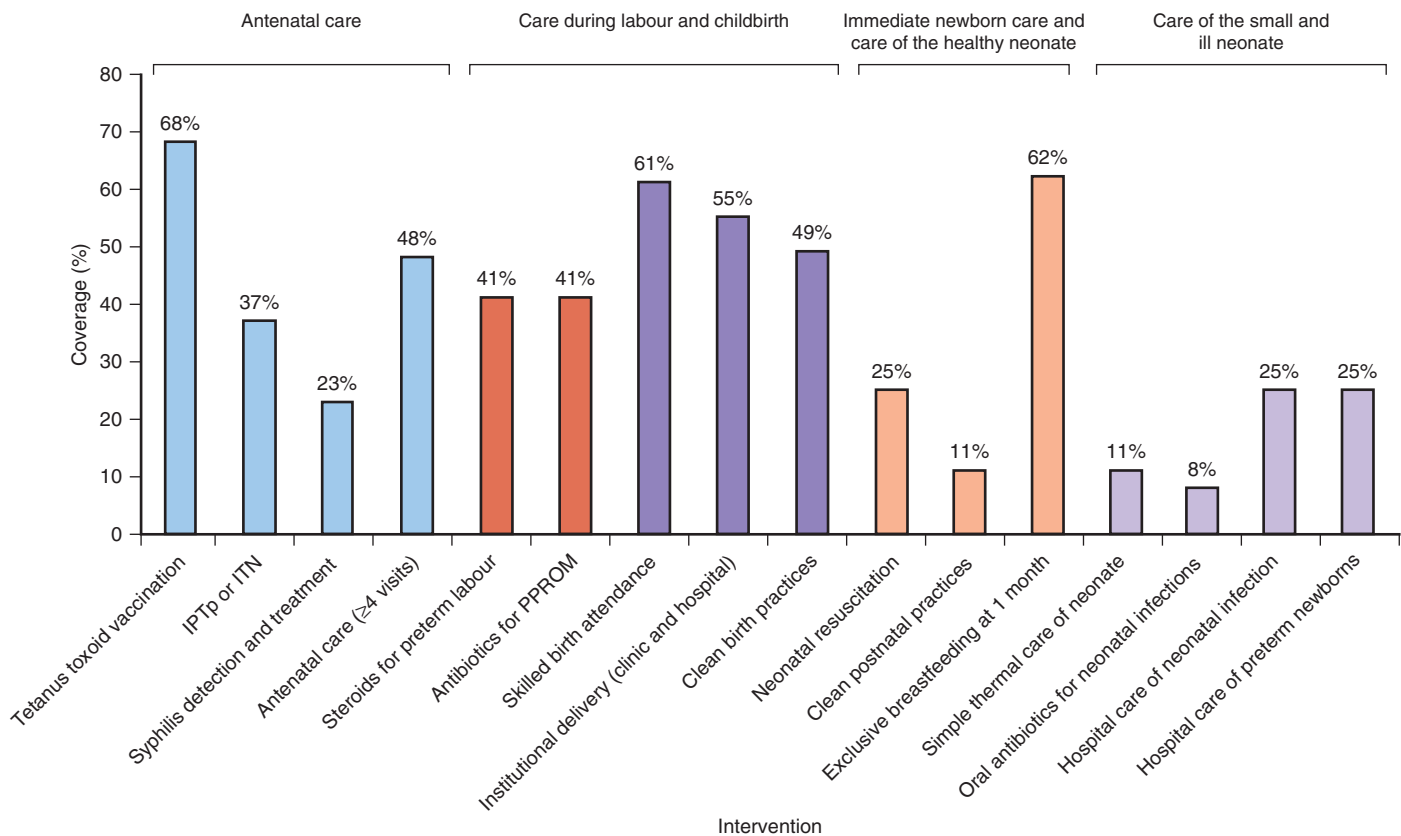
Summary

Global newborn health has gained momentum over the past 15 years. Set against the backdrop of increasing national and governmental awareness coupled with an impressive array of global initiatives, strategies, and action plans, many countries have responded and are beginning to actively invest time and effort toward developing newborn policies, research, and programs. Still, there remains an unfinished agenda. Although the last 25 years represented the period of the greatest health and development improvements in human history, some of the original MDG targets, especially the maternal and child ones, were not achieved. Newborn mortality, especially, is a high global priority.

The conceptual framework utilized by a group of authors is useful to consider. In this model, several innovations are described. These include innovations in technology, commodities, demand-side barriers to care seeking, provision and supply-side issues especially to marginalized populations, financing, better monitoring and measurement, and real-time feedback (Engmann et al., 2016). While we are still in the early days of the SDG era, the previous 15 years and concerted thrust of the global community provide promise that such an accelerated grand convergence can occur.

In addition to ongoing work to improve global newborn survival, the future directions, trends, and themes across newborn health include the following:

1. **Survive and thrive:** incorporating a thrive lens into the current mortality lens is a strategic direction. This may include effective implementation of newborn and developmental milestone screening procedures, promotion of exclusive access and intake of human milk, early referral and treatment when developmental milestones are not attained, and provision of anticipatory guidance and counseling for parents and caregivers.



• **Fig. 4.8** Coverage of Selected Interventions to Improve Neonatal Health in Countdown Countries. IPTp, intermittent preventative treatment in pregnancy; ITN, insecticide-treated bednets. (From Bhutta ZA, Das JK, Bahl R, et al. Can available interventions end preventable deaths in mothers, newborn babies, and stillbirths, and at what cost? *Lancet*. 2014; 384: 347–370.)

2. Quality of care, especially at birth: leveraging the triple return on improved maternal and neonatal mortality and reduced stillbirth.
3. Implementation science: utilizing implementation science to effectively implement newborn programs at scale.
4. Integrating newborn health into the reproductive, maternal, newborn, child, and adolescent health and nutrition spectrum.

5. Newborn health among special populations such as the urban poor, displaced populations, those in emergency settings, and the adolescent.

All who work with mothers and newborns, wherever we are in the world – in high-, middle-, and low-income settings – can play a pivotal role in improving global conditions for the baby. The question is, will we?

Suggested Readings

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Complete references used in this text can be found online at www.expertconsult.com

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5

Development, Function, and Pathology of the Placenta

EMIN MALTEPE AND ANNA A. PENN

KEY POINTS

- The placenta is the first organ to form in mammals and is required for establishment of a maternal–fetal vascular interface capable of supplying the bioenergetic needs of the developing conceptus.
- Multiple placental cell types engage in highly varied functions, from attachment, invasion, and vascular remodeling to cell fusion, hormone production, and nutrient transport.
- Multiple mechanisms allow transport of waste and nutrients across the placenta, including diffusion, transporter protein-mediated (facilitated diffusion and active transporters), and receptor-mediated mechanisms.
- The placenta is not an inert transport interface. It consumes 40%–60% of the oxygen and glucose delivered to the uterus at term. Thus conditions that alter placental metabolism can indirectly affect nutrient transport to the fetus.
- Maternal and fetal health alter placental function, which in turn influences fetal adaptations and contributes to in utero fetal programming.
- Abnormal placentation and placental infections can lead to preeclampsia (PE), fetal growth retardation, or preterm birth, which can have lifelong bearing on health.
- In the United States, iatrogenic delivery is responsible for almost half the births that occur between 28 and 37 weeks of gestation, primarily caused by placental pathologies such as PE or intrauterine growth restriction.
- Efforts to standardize placental examination after delivery are in progress so that connections between specific placental problems and poor outcomes can be better defined. In parallel, new advanced imaging techniques and biomarkers for placental function are being developed.

The placenta is a remarkable organ. Its brief existence enables the mammalian fetus to survive and thrive within the otherwise inhospitable confines of the intrauterine environment. To accomplish this, the placenta plays a range of roles, from anchoring the conceptus and preventing its rejection by the maternal immune system to enabling the transport of nutrients and wastes between the mother and the embryo/fetus. As with all organs, multiple specialized cell types derived from lineage-committed precursors are responsible for these functions. Genetic, epigenetic, and physiologic cues direct placental development across gestational stages (Maltepe et al., 2010). Impairments in

these processes due to intrinsic or extrinsic insults can lead to placental dysfunction and result in long-lasting increases in disease susceptibility, a process known as fetal programming (Murphy et al., 2006; Eichenwald and Stark, 2008). Both preterm and term infants are at risk from poor placental function, particularly those that are extremely low birth weight (<1 kg). Preterm infants in particular may suffer from placental dysfunction in utero followed by early loss of placental support, including nutrition, hormones, and immune protection. Preterm delivery rates continue to rise while the survival of preterm infants has increased due to numerous advances in medical management and technology (Saigal and Doyle, 2008). This convergence has generated an expanded population of patients admitted to and graduating from intensive care nurseries. Not only are these infants more likely to develop complications such as bronchopulmonary dysplasia, failure to thrive, pulmonary hypertension, cerebral palsy, and blindness, but they are also more likely to develop chronic adult ailments such as diabetes and heart disease. Although further improvements in neonatal care are critical for diminishing the long-term consequences of prematurity, prevention or delay of preterm delivery will have the greatest healthcare impact for this at-risk population. A better understanding of the most common placental pathologies is therefore critically important for advancing maternal, fetal, and adult medicine.

Placental Origin

The mammalian lineage began approximately 200 million years ago with monotremes, or egg-laying mammals, e.g., the echidna and platypus. Echidnas lay their eggs into an egg pouch, whereas platypuses lay their eggs into a burrow, where the mother curls around them to provide them with warmth and protection. Lacking a true placenta, these embryos rely on a yolk sac to provide them with nourishment. The duck-billed platypus, on the other hand, develops a primitive allantoic vitelline placenta from trophoblast (TE)-like cells called vitellocytes. These support the embryo to the 19-somite stage, indicating that the molecular mechanisms enabling segregation of extraembryonic TE from embryonic tissues may have predated evolution of a true placenta (Hughes and Hall, 1998; Selwood and Johnson, 2006). Marsupial embryos also have yolk sacs but are born live. Although the primitive TE cells

surrounding the early marsupial embryo produce a single cell-layered transport interface that may represent a primitive placenta, it can only provide for the metabolic demands of embryonic/fetal development for a short while, accounting for the short internal gestation period of marsupials (Renfree, 2010).

Eutherian, or true placental, mammals diverged from marsupials approximately 140 million years ago. The placenta is a tremendously successful evolutionary adaptation that has enabled the creation of around 18 taxonomic orders grouped into four superorders. These groups gave rise to many lineages, each of which evolved independently in different geographic locations and in response to differing environmental challenges. There is thus a vast variety of placental forms, and identifying what the original placenta looked like is therefore quite difficult, but it may have been hemochorial, discoid, and labyrinthine (Wildman et al., 2006), similar to that observed in rodents. The placenta is likely the most mutable of all organs, with at least 20 identifiable types, depending on the manner of classification (Benirschke and Kaufmann, 1995). Perhaps not surprisingly, divergence among placental types may have developed a large number of species-specific pathologies, leading to potential limitations in cross-species investigations (Gutmacher et al., 2014).

Internal development supported by a placenta provides many benefits including protection from environmental fluctuations in temperature, oxygen, and osmolarity, as well as protection from predation (Shine, 1989; Clutton-Brock, 1991; Wourms and Lombardi, 1992). It also allows females to produce larger offspring with a higher rate of survival due to enhanced feeding, digestion, movement, or behavior (Amoroso, 1968; Baylis, 1981; Wourms, 1994). However, this also comes with high energetic costs to the mother (Boehlert et al., 1991; Qualls and Shine, 1995). In addition to limiting maternal mobility, increasing time between births, and decreasing frequency of reproduction (Thibault and Schultz, 1978; Goodwin et al., 2002), internal, placenta-supported reproduction demands adaptation of multiple maternal organ systems during pregnancy. The complexity of this benefit–risk ratio may also add to the many placental variations that exist.

Development of the Placenta

Trophoblast Lineage Allocation

The placenta is the first organ to form in mammals. This is because it is required for establishment of a functional maternal–fetal vascular interface capable of supplying the bioenergetic needs of the developing conceptus (Maltepe et al., 2010). The fertilized embryo undergoes a series of cell divisions before implantation, to produce up to eight seemingly identical cells called blastomeres. Three further sets of divisions generate the blastocyst, consisting of two distinct cell populations. Surrounding the blastocyst is the TE, which gives rise to the placenta. The inner cell mass (ICM), located inside the blastocyst, gives rise to the embryo and visceral endoderm (Nishioka et al., 2009; Sasaki, 2010). In mice, each blastomere is able to generate either ICM or TE derivatives and is thus totipotent. This also occurs in humans (Zdravkovic et al., 2015). Once TE or ICM commitment occurs, however, it is considered largely irreversible. Importantly, however, individual blastomeres are found to harbor intrinsic biases regarding which lineage they adopt as early as the four-cell stage (Piotrowska-Nitsche et al., 2005; Tabansky et al., 2013) and which appear to depend on positional cues (Nishioka et al., 2009).

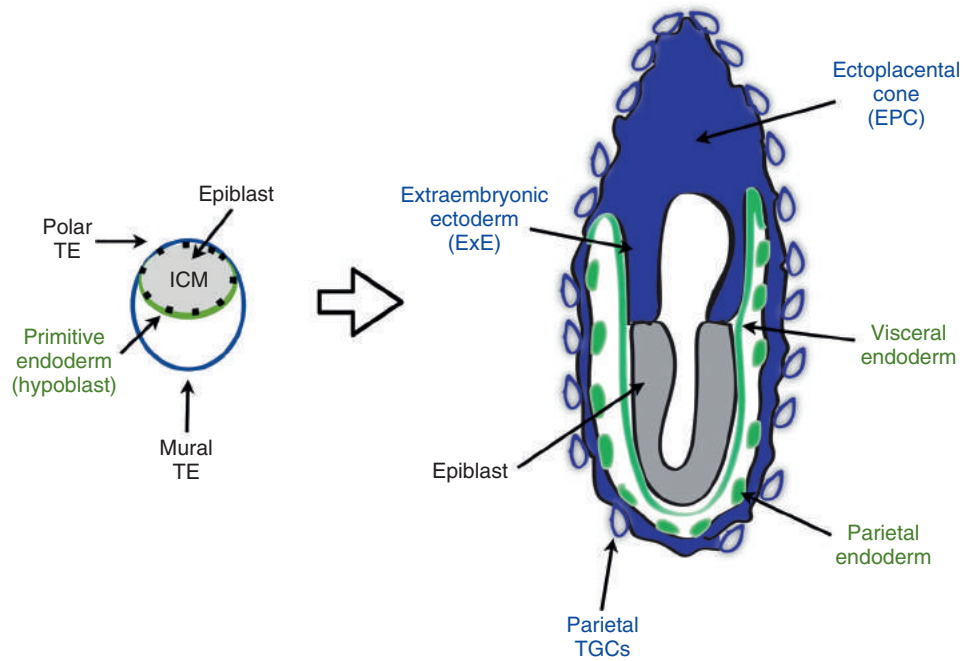
Multiple factors govern lineage allocation. One major determinant includes differences in polarity and adhesion between inner and outer cells of the blastocyst that are associated with differential

activation of the Hippo signaling cascade (Nishioka et al., 2009; Hirate et al., 2013). Hippo helps restrict expression of key lineage regulatory genes such as *Cdx2* that are stochastically expressed as early as the eight-cell stage in mice but restricted thereafter to the TE (Dietrich and Hiiragi, 2008). The Hippo pathway is inactivated in outer TE cells, resulting in nuclear localization of the transcriptional coactivator Yes associated protein (YAP), which interacts with TEA domain transcription factor 4 (TEAD4) to drive Caudal type homeobox 2 (*Cdx2*) gene expression (Nishioka et al., 2008). Notch signaling also acts in parallel with Hippo to promote *Cdx2* expression in this process (Rayon et al., 2014). Positional cues governed by E-cadherin expression help regulate Hippo signaling. Cell–cell contact within inside cells of the ICM activates Hippo and suppresses nuclear YAP activity. Signaling cascades implicated in this include the LATS2 kinase. For example, Hippo can be suppressed by a dominant negative form of the LATS2 kinase or by combined LATS1/2 deficiency. This results in ICM cells exhibiting nuclear YAP localization, *Cdx2* expression, and TE fates (Nishioka et al., 2009), placing LATS1/2 downstream of E-cadherin-dependent signaling. In mice, CDX2 helps repress genes critical for ICM identity, such as *Oct4* and *Nanog*. Its absence in mouse embryos results in the lack of TE differentiation, and all cells of the blastocyst stage embryo express the typically ICM-restricted OCT4 protein (Niwa et al., 2005). This ability is species dependent, however, and is not observed in bovine (Berg et al., 2011) or human blastocysts (Niakan and Eggan, 2013). Amazingly, in mice its expression is sufficient to convert embryonic stem cells (ESCs) into trophoblast stem cells (TSCs) that can contribute to all lineages found within the placenta (Tanaka et al., 1998; Niwa et al., 2005). *Cdx2* expression is further maintained in mice via a positive feedback loop driven by the combinatorial activities of the transcription factors Eomesodermin (EOMES), ETS-related transcription factor 5 (ELF5), ETS proto-oncogene 2 (ETS2), and transcription factor AP-2, gamma (TCFAP2c) that help maintain the TE lineage (Yamamoto et al., 1998; Russ et al., 2000; Auman et al., 2002; Werling and Schorle, 2002; Donnison et al., 2005; Georgiades and Rossant, 2006; Ng et al., 2008; Weber et al., 2010). Interestingly, ELF5, CDX2, and EOMES can collaborate to regulate hundreds of TSC genes by binding to enhancer elements that harbor endogenous retrovirus-derived sequences, indicating that these serve as trophoblast-specific enhancer elements in mice (Chuong et al., 2013). This opens the exciting possibility that differences in the incorporation of these foreign viral elements across different placental mammals may have contributed to the diversity of placental structures seen across the animal kingdom.

Uterine evolution paralleled placental evolution in eutherian mammals. This was recently found to involve large-scale and rapid changes in endometrial gene regulatory networks mediated by ancient transposable elements. These modulate responses to pregnancy hormones as well as other pathways to ensure pregnancy success (Lynch et al., 2011; Lynch et al., 2015). Thus a set of genetic tricks coupled with host–virus interactions enabled the rapid evolution of the placenta–uterus axis in mammals and helped contribute to the diversity of mammalian life forms and modes of procreation observed today.

Trophoblast Differentiation

The placenta is comprised of multiple different cell types that engage in highly varied functions, ranging from attachment, invasion, and vascular remodeling to cell fusion, hormone production, and nutrient transport (Maltepe et al., 2010). Thus trophoblast-specific



• **Fig. 5.1** Embryonic Development in the Mouse Conceptus. At the blastocyst stage, the conceptus is comprised of an inner cell mass (ICM) that gives rise to the embryo proper (epiblast), overlaid by a layer of primitive endoderm (hypoblast) that gives rise to the visceral and parietal endoderm. The outer cells comprise the trophoctoderm (TE) that gives rise to the placenta. TE cells near the ICM are referred to as polar TE, while TE cells not in contact with the ICM are referred to as mural TE. The mural TE gives rise directly to parietal trophoblast giant cells in the initial wave of trophoblast differentiation. These cells aid the initial attachment and invasion process. The polar TE differentiates into the ectoplacental cone and extraembryonic ectoderm. The ectoplacental cone resides closest to the implantation site and also gives rise to lineages that further help incorporate the conceptus into the receptive uterus. The extraembryonic ectoderm resides closest to the developing epiblast and gives rise to lineages comprising the placental transport interface, such as the syncytiotrophoblast. TGCs, Trophoblast giant cells.

progenitors need to enact a complex set of lineage restriction decisions to help form a functioning placenta.

The isolation of rodent TSCs, the extraembryonic equivalent of ESCs, has dramatically increased our understanding of cell fate decisions in the placenta (Tanaka et al., 1998). Combined with genetic approaches in mice and analyses of resultant placental phenotypes in vivo, derivation of “knock-out” TSC lines has enabled dissection of lineage commitment decisions within the placenta with great precision. For the human placenta, in vitro experiments with primary cytotrophoblasts (CTBs) as well as ex vivo culture of placental explants have yielded a great deal of information regarding placental development. Human ESCs can also be induced to differentiate into the trophoblast lineage by activating bone morphogenetic protein 4 (BMP4)-dependent signaling pathways (Li and Parast, 2014). Additionally, the recent derivation of single blastomere-derived cell lines from human embryos that can differentiate along the trophoblast lineage is aiding our understanding of human placental development (Zdravkovic et al., 2015). These have been combined with pathologic analyses of placentas following delivery or pregnancy terminations. While primary human trophoblast progenitor cell lines have recently been derived (Genbacev et al., 2011), the exact equivalents of TSCs have not yet been isolated in humans.

TSCs can be derived from mouse blastocysts on fibroblast feeder cells with the addition of fibroblast growth factor 4 (FGF4). Feeder cells produce growth factors such as the TGF- β family member Nodal (Erlebacher et al., 2004; Guzman-Ayala et al., 2004), along

with extracellular matrix-dependent cues (Choi et al., 2013) that maintain TSC proliferation and help prevent their differentiation. In utero, this environment is only maintained for approximately 3 days following implantation, as TSCs cannot be derived from mouse embryos past embryonic day 6.5 (Uy et al., 2002). Removal of growth factor, feeders, or feeder-conditioned medium triggers loss of TSC proliferation as well as markers associated with “stemness” (Roberts and Fisher, 2011). Depending on environmental or culture conditions, these cells then differentiate into the various cells that comprise the placenta. In the mouse, the extraembryonic ectoderm differentiates into cells that comprise the chorion and labyrinth, which perform the transport functions of the placenta, whereas the ectoplacental cone, located nearer to the uterine implantation site, differentiates into the spongiotrophoblast layer as well as glycogen trophoblasts and trophoblast giant cells (TGCs) (Fig. 5.1).

To function as a transport organ, the placenta must establish an extensive vascular interface between the maternal and fetal circulatory systems. Humans and rodents have a hemochorial placenta, which means that the maternal vascular space comes in direct contact with differentiated trophoblasts, not endothelial cells (Benirschke and Kaufmann, 1995). In humans, trophoblasts lining maternal arteriolar spaces are relatively well characterized (Red-Horse et al., 2004), but very little is known about the cells associated with the draining vascular bed, for example. In mice, there appear to be at least five distinct populations of TGCs that lie at various positions within these maternal vascular spaces and are defined by their

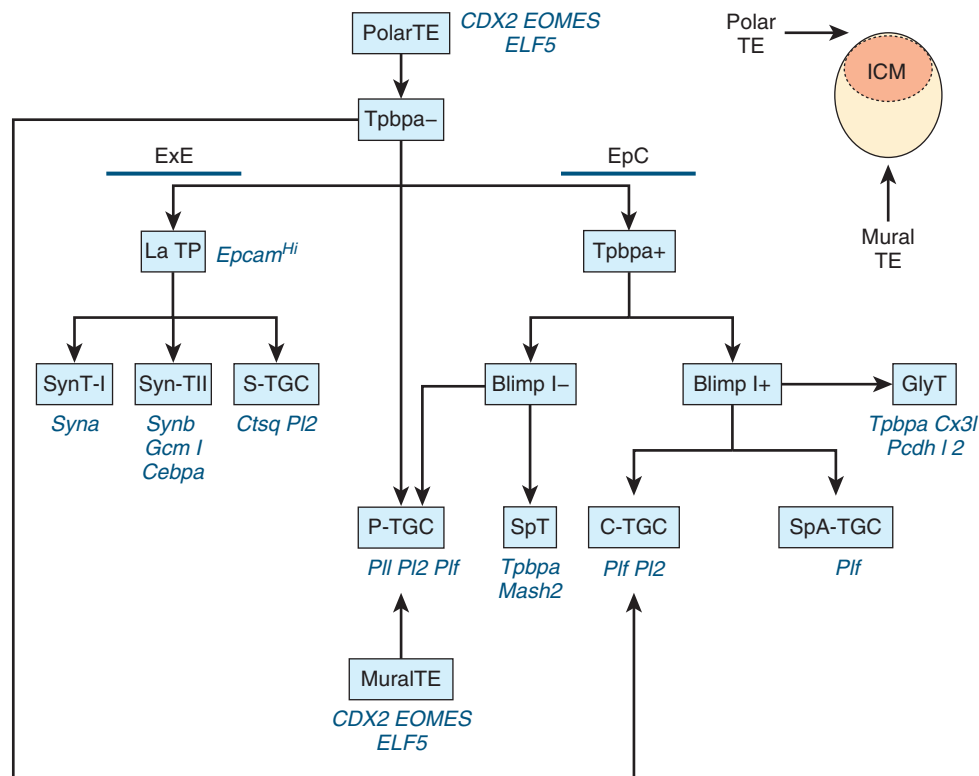
location and lineage-specific gene expression (Simmons et al., 2007). These arise from various sources (see Fig. 5.1). Their “giant” size is, in part, a reflection of their DNA content, which continuously replicates without engaging in cell division via a process called endoreplication (Edgar et al., 2014). Endocycles are thought to be used by cell types that need to be very large or that are highly metabolically active. Consistent with this, TGCs in the mouse placenta are responsible for the bulk of placental hormone production (Soares et al., 2007). In humans, placental hormones are produced by the syncytiotrophoblast (SynT) layer, potentially accounting for the reduced ploidy of invasive trophoblast subtypes in this species compared with rodents, although they still appear to exhibit a significant amount of aneuploidy (Weier et al., 2005).

Trophoblast Invasion

Primary TGCs differentiate from the mural TE, and this represents the first terminally differentiated cell type in mice to aid in implantation. In humans, the initial wave of invasion following implantation is thought to occur via formation of a primitive syncytium, through

which invasive CTBs push following approximately day 13 or 14 of gestation (Cantor and Ginsberg, 2012). This early wave of syncytialization does not occur in rodents. In mice, the remaining secondary TGCs differentiate either from trophoblast specific protein alpha (Tpbpa)/4311+ outer ectoplacental cone cells or from Tpbpa/4311– chorionic progenitors (Simmons et al., 2007) (Fig. 5.2). Secondary TGCs come in various forms that have differing locations as well as characteristics. As the name implies, spiral artery (SpA) TGCs invade the spiral arteries and displace the smooth muscle and endothelial cells to remodel them, canal TGCs line the large vascular spaces delivering maternal blood to the base of the labyrinth, sinusoidal TGCs sit within the small vascular spaces of the labyrinth, and parietal TGCs surround large pools of deoxygenated blood that ultimately drain into the maternal uterine veins.

In addition to their location, these cells can be identified based on lineage-specific gene expression (see Fig. 5.2), although very few unique identifiers are known at this time, and all can be derived in vitro following TSC differentiation (Simmons et al., 2007). A growing list of transcription factors and signaling molecules are

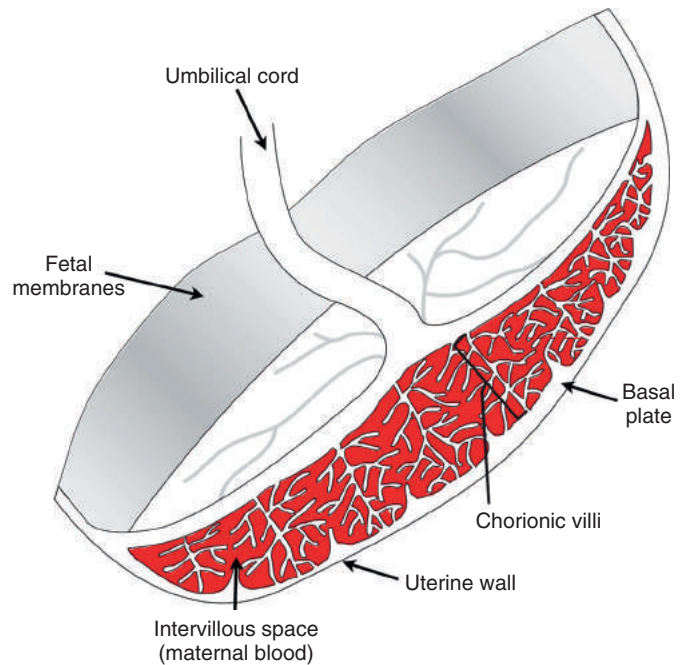


• **Fig. 5.2** Trophoblast Lineage Determination in the Mouse Placenta. Cell type-specific genes are indicated in blue. Mural trophoblast (TE) cells give rise directly to parietal trophoblast giant cells (TGCs). Parietal TE cells, from which trophoblast stem cells (TSCs) are derived, are defined by their expression of key transcription factors including CDX2, EOMES, and ELF5 and can be maintained in vitro by exogenous fibroblast growth factor 4 as well as Nodal signaling. These give rise to all cell types within the mature placenta. Polar TE cells can also give rise directly to parietal TGCs via passage through a trophoblast specific protein alpha (Tpbpa)/4311 negative (–) state. Alternatively, Tpbpa– progenitors can give rise to labyrinth trophoblast progenitors (La TP) characterized by high EpcAM expression that gives rise to lineages that populate the exchange interface. These are the two syncytiotrophoblast (SynT) lineages—SynT-I and SynT-II, as well as the sinusoidal TGCs (S-TGC). Tpbpa+ progenitors, on the other hand, further differentiate through either a Blimp1+ or – state into spongiotrophoblasts (SpT), glycogen trophoblasts (GlyT), or multiple additional types of TGCs, including spiral artery-associated TGCs (SpA-TGCs) and canal TGCs (C-TGCs). Each terminally differentiated cell type is associated with a unique combination of lineage-specific genes that enable their identification in vitro and in vivo (indicated in blue). Epc, Ecto-placental cone; ExE, extraembryonic ectoderm; ICM, inner cell mass; P-TGC, parietal TGC.

known to be critical for these differentiation events and have been reviewed extensively (Hu and Cross, 2010; Maltepe et al., 2010; Pfeffer and Pearton, 2012; Soares et al., 2012; Knofler and Pollheimer, 2013; Knott and Paul, 2014; Latos and Hemberger, 2014; Soncin et al., 2015). An organizational chart depicting the cell fate regulatory hierarchy within the placenta can be made based on our current understanding but still remains poorly characterized when compared with other systems such as hematopoiesis. CDX2-, EOMES-, and ELF5-positive TSCs sit at the apex of this hierarchy (see Fig. 5.2).

Trophoblasts that invade and line blood vessels appear to do so via two different mechanisms: (1) vascular invasion with endothelial mimicry and (2) vasculogenic mimicry (Rai and Cross, 2014). In the former, trophoblasts invade into and displace maternal endothelial cells from within maternal arterioles and include SpA-TGCs in mice or endovascular trophoblasts (EVTs, also known as extravillous trophoblasts) in humans. During vasculogenic mimicry, however, trophoblasts undergo morphogenesis to create vascular tubes de novo. Sinusoidal TGCs perform this function in mice. Whether this also occurs in human placentation is not clear. Transplanting trophoblasts subcutaneously in mice or culturing them as 3-dimensional trophospheres in vitro allows one to visualize them from de novo vascular spaces, where they generate tumors harboring large blood sinuses surrounded by trophoblasts, as opposed to host endothelium (Kibschull and Winterhager, 2006; Rai and Cross, 2014). Many pathways known to regulate endothelial development also drive trophoblast differentiation and formation of the maternal–fetal vascular interface (reviewed in Rai and Cross, 2014). For example, TGCs produce both vascular endothelial growth factor (VEGF) and placenta growth factor (PlGF) and express VEGF receptors -1 and -2, suggesting both autocrine and paracrine roles (Achen et al., 1997; Abbott and Buckalew, 2000; Hirashima et al., 2003). Additionally, Notch signaling, known to be critically important for vascular endothelium, is also important for differentiation of SpA-TGCs and Canal (C)-TGCs in the mouse placenta (Hunkapiller et al., 2011; Gasperowicz et al., 2013). These same factors are also expressed in homologous cells within the human placenta (Zhou et al., 2003a, 2003b; Hunkapiller et al., 2011). Furthermore, the endothelium and trophoblast are primary regulators of hemostasis in the adult and fetal circulation. Trophoblasts can regulate the coagulation cascade like endothelial cells and produce such molecules as thrombomodulin, tissue factor, tissue factor pathway inhibitor, annexin V, and endothelial protein C receptor (Wang et al., 1999; Lanir et al., 2003; Sood et al., 2006). These factors are critical for preventing thrombotic or hemorrhagic events from occurring in the developing placenta. Consistent with this, tissue factor deficiency in mice results in early embryonic lethality associated with massive hemorrhage at this extremely vascular interface (Erlich et al., 1999). Thus mammalian placentas have solved the problem of hemochorial placentation by having trophoblasts take over functions typically performed by endothelial cells.

Remodeling of uterine vasculature is critical for successful pregnancy in humans and mice (Pijnenborg et al., 2006; Maltepe et al., 2010; Soares et al., 2012; Rai and Cross, 2014). The equivalents of TGCs in humans, invasive EVT, are derived from column CTB progenitors located at the tips of anchoring villi (Fig. 5.3). They migrate through the uterine parenchyma via interstitial invasion, in search of maternal spiral arterioles and veins. This invasion peaks early in pregnancy, around 9 to 12 weeks of gestation (Pijnenborg et al., 1981). EVTs then breach the spiral arterioles, via a process termed endovascular invasion, and replace



• **Fig. 5.3** Human Placenta at Mid Gestation. The human (and mouse) placenta is disc shaped (discoid) and hemochorial; i.e., fetal trophoblasts are in direct contact with maternal blood. In contrast to the labyrinthine placenta in mice, the human placenta is comprised of chorionic villi that project into the intervillous space bathed by maternal blood. Fetal blood vessels coursing through the umbilical cord branch when they reach the placenta and dive into the chorionic villi where gas, nutrient, and waste exchange occurs. Tips of anchoring villi attach to the uterine wall (basal plate) and send out waves of invasive cytotrophoblasts (also known as extravillous trophoblasts) to both anchor the placenta and establish blood flow to it. (Adapted from Hunkapiller NM and Fisher SJ. *Methods Enzymol.* 2008;445:281–302.)

resident endothelial and smooth muscle cells (Red-Horse et al., 2006). This results in these high resistance vessels being remodeled into low resistance/high capacitance conduits necessary for proper fetal perfusion as well as modulation of maternal hemodynamics (Red-Horse et al., 2004). While EVT interactions with veins are largely confined to the inner surface of the uterus, they migrate along much of the intrauterine course of maternal arterioles. Although endovascular invasion begins quite early, and typically begins within the center of the placental bed, uterine arterial blood only begins to flow into the intervillous space by the end of the first trimester. Before this point, EVTs paradoxically plug these vessels, preventing blood flow to the placenta. As a result, all of first trimester placental development occurs in a highly hypoxic environment with the bulk of placental nutrients being provided by endometrial secretions (i.e., histiotrophic nutrition) (Burton, 2009). Only about one-third of the uterine SpAs are actually invaded by 18 weeks' gestational age (Pijnenborg et al., 1983), indicating that the more lateral arteries are only invaded throughout the second and third trimesters in a progressive manner (Brosens et al., 2011; Pijnenborg et al., 2011), because most are completely remodeled when examined following delivery at term.

Following unplugging of these vessels, maternal blood begins to bathe floating chorionic villi that are covered by a layer of multinucleated SynTs. SynTs form as a result of the fusion of lineage-committed progenitors. The need for multinucleated syncytium formation is not clear but may have been driven

evolutionarily by a response to viral infections that may help minimize pathogen transmission to the fetus (Tsurudome and Ito, 2000). A combination of fusogenic protein expression, particularly syncytins (Blond et al., 2000; Mi et al., 2000; Malassine et al., 2007; Chen et al., 2008; Esnault et al., 2008; Simmons et al., 2008; Dupressoir et al., 2009; Dupressoir et al., 2011), and dramatic cytoskeletal rearrangement appears to be essential for this trophoblast fusion. Cytoskeletal rearrangement is a common theme in trophoblast differentiation in general (Parast et al., 2001). For example, calponin 3-mediated actin rearrangement can promote SynT fusion (Shibukawa et al., 2010), while caspases can remodel the fodrin cytoskeleton during this process (Gauster et al., 2010), and stathmin expression, a microtubule regulatory protein, is associated with invasive trophoblast migration (Yoshie et al., 2008). Along these lines, invasive TGCs in mice and EVT_s in humans exhibit robust microtubule and actin cytoskeletons, whereas the cytoskeletal network in multinucleated SynT_s found in both species is severely disrupted (Choi et al., 2003; Zhou et al., 2014). Consistent with this, microtubule or actin disrupting agents direct block TGC formation and direct TSC differentiation along the SynT lineage (Choi et al., 2013). Additionally, caspase 8 activity, frequently implicated in apoptosis, aids this process during human SynT formation (Huppertz et al., 1999). These cytoskeletal changes are frequently accompanied by another apoptosis-associated process—externalization of phosphatidylserine to the outer leaflet of the plasma membrane. Typically acting as an “eat me” signal for the clearance of apoptotic cells, phosphatidylserine externalization is associated with SynT fusion (Lyden et al., 1993; Huppertz and Gauster, 2011). Apoptosis is not completed during SynT formation, however, and the syncytium is maintained in this “preapoptotic” state until being sloughed off into the maternal circulation. Other molecules such as connexins are also known to be critical for SynT formation (Kibschull et al., 2008). CD98, an amino acid transporter, also plays a role (Kudo et al., 2003; Kudo and Boyd, 2004; Dalton et al., 2007), and its actions can be opposed by placental protein 13, a galectin family member (Than et al., 2004). In addition to regulating amino acid transport, CD98 can also interact with cell surface integrins to regulate cell morphology and invasion (Cantor and Ginsberg, 2012), playing a unique dual role in the nutrient transport and fusion capabilities of SynT_s. As a result of the dramatic cytoskeletal changes required for cell fusion, in addition to changes in the composition of the membrane lipid bilayer, the biophysical properties of the SynT_s change to become much more rigid, possibly aiding the infection barrier properties of the placenta (Zeldovich et al., 2013).

Analysis of human EVT invasion *in situ* and *in vitro* established that a unique epithelial-to-endothelial switch is a vital component of this process (reviewed in Red-Horse et al., 2004). Initially, CTB progenitors in floating villi express E-cadherin (epithelial cadherin) and integrin $\alpha 6 \beta 4$. Upon differentiation, these cells repress these molecules and upregulate other adhesion molecules such as vascular endothelial cadherin, $\alpha 5 \beta 1$, $\alpha V \beta 3$, PECAM-1, and VCAM-1 as well as the matrix metalloproteinase MMP-9. Other data suggest that CTB differentiation/invasion also entails a switch from a venous to an arterial phenotype in terms of the cells' expression of Eph and ephrin molecules (Red-Horse et al., 2005) and the modulation of Notch family members (Hunkapiller et al., 2011). This change is accompanied by an induction of several growth factors and receptors (e.g., VEGF and angiopoietin family members) that function during conventional vasculogenesis and angiogenesis, as well as placental development in other species (Andraweera et al., 2012; Chen and Zheng, 2014).

Placental Functions

Transport

The transport functions of the placenta are performed by the multinucleated SynT_s that sit at the maternal–fetal interface. Multiple mechanisms allow transport of waste and nutrients across the placenta (Dilworth and Sibley, 2013). The simplest is diffusion. The high surface area of the placental transport interface, along with the hemochorial nature of the rodent and human placentas, enables efficient diffusion across the placenta. The rate of diffusion depends on the molecular properties and concentrations of the solute, however, in addition to the composition of the exchange barrier (Sibley and Boyd, 1988). In the human placenta at term, a single SynT layer separates maternal blood from fetal capillary endothelium, whereas in the mouse, two SynT layers as well as an sinusoidal (S)-TGC layer, surprisingly, separate the vascular spaces (Rossant and Cross, 2001). These layers are progressively thinned out to minimize their barrier properties and increase the surface area for exchange (Simmons et al., 2008). Oxygen is transported across the placenta via passive diffusion, aided by the high affinity of fetal hemoglobin and the concentration differential across the maternal–fetal vascular beds. The orientation of the maternal and fetal vascular blood spaces produces a countercurrent exchange mechanism in mice. This maximizes transport efficiency in rodents, whereas the human placenta has a less efficient multivillous arrangement, which necessitate a larger placental size relative to the mouse (Benirschke and Kaufmann, 1995).

Hydrophilic molecules do not readily cross plasma membranes. Transporter protein-mediated mechanisms are typically required for transporting hydrophilic molecules. Classic transporter proteins include facilitated diffusion transporters, i.e., the glucose transporter (GLUT) family (Jansson et al., 1993) as well as active transporters, i.e., transporters associated with calcium transport (Belkacemi et al., 2005) and the amino acid transporters (Desforages and Sibley, 2010). Transport can occur down a concentration gradient, as is the case with GLUT1-mediated glucose transport (Desforages and Sibley, 2010), or against a concentration gradient, as is the case with calcium (Dilworth et al., 2010) and amino acid transport (Battaglia, 2007). Interestingly, nearly all amino acids in the fetal circulation are found at higher levels than in the maternal circulation, indicating active uptake and/or synthesis of these nutrients via the placenta or fetus.

The fetal–placental unit is both physically and metabolically interconnected with each other and the maternal circulation. Ultimately, all fetal–placental metabolism is constrained by the nutrients delivered from the maternal circulation. However, the placenta and fetal liver are both capable of producing and metabolizing various nutrients that impact their levels in the fetal–placental circulation largely independent of placental transport mechanisms (Cetin, 2001). This has been well described in ovine species, wherein the fetal liver of sheep *in utero* actively produces large quantities of serine and glutamate that are consumed by its placenta (Cetin et al., 1992; Chung et al., 1998). In the placenta, serine is converted to glycine via a process that contributes to one-carbon metabolism-dependent DNA methylation pathways that play important roles in cell fate regulation and growth mechanisms (Amelio et al., 2014). Additionally, nonglucose carbohydrates such as fructose, mannose, inositol, and sorbitol are also either transported by or synthesized in the placenta (Jauniaux et al., 2005; Battaglia, 2007) and play important roles in regulating fetal growth as well as in redox regulation.

Finally, antibody-mediated immunity is transferred from mother to fetus across the placenta via receptor-mediated mechanisms (Saji et al., 1999; Schneider and Miller, 2010). Immunoglobulin G (IgG) transport across the human placenta, for example, begins at approximately 16 weeks' gestation, and fetal serum IgG levels reach maternal levels by 26 weeks' gestational age. This process is highly efficient, enabling fetal concentrations to exceed maternal values at term.

Metabolism

The placenta is not an inert transport interface. It consumes 40%–60% of the oxygen and glucose delivered to the uterus at term, despite only comprising approximately 10%–20% of the total mass of the uterus at that time (Bell et al., 1986; Carter, 2000). Changes in this metabolism can regulate placental biology. Mitochondrial fusion, a process that enables greater mitochondrial bioenergetic capacity, is critical for invasive TGC formation in mice, triggering placental failure when compromised (Chen et al., 2003; Alavi et al., 2007). Interestingly, mitochondrial fusion can promote cardiomyocyte differentiation as well, highlighting conserved mechanisms between cellular metabolism and cell fate determination (Kasahara et al., 2013). Thus alterations of placental metabolic function can impact oxygen and nutrient delivery to the fetus, both by altering placental metabolic demand intrinsically, as well as by impacting cell fate regulatory pathways. Primary culture of human CTBs indicates that they exhibit high rates of aerobic glycolysis when compared with other terminally differentiated adult cells (Bax and Bloxam, 1997), much like rapidly proliferating cancer cells that rely on high glycolytic flux rates to augment biosynthetic precursor production. Aerobic lactate production, i.e., the Warburg effect, also allows the placenta to produce and transfer large amounts of lactate to the fetus, which can readily oxidize it. Interestingly, the placenta also appears able to metabolize lactate during mid gestation but loses this ability by term (Carter et al., 1993). These studies additionally suggest that glycogen breakdown (glycogenolysis) helps supply the high rates of glucose consumption in proliferating trophoblasts before differentiation into terminally differentiated SynTs. Interestingly, excess glycogen accumulation has been noted within the SynT layer of some human preeclampsia (PE) placentas (Arkwright et al., 1993), consistent with their altered turnover and suggesting a potential link to altered glucose metabolism in the setting of this pregnancy complication. Importantly, epidemiologic studies confirm that derangements in glucose and fatty acid metabolism may drive pregnancy complications. For example, maternal gestational diabetes mellitus (GDM) and obesity are independently, and additively, associated with elevated rates of PE (Catalano et al., 2012) as well as spontaneous preterm birth (Shaw et al., 2014). There may be shared mechanisms involving impaired trophoblast invasion contributing to PE and preterm labor (PTL) pathogenesis. For example, up to 30% of patients with spontaneous PTL have placental lesions consistent with impaired SpA remodeling typically observed during PE (Kim et al., 2002; Kim et al., 2003; Romero et al., 2014). Thus improving our understanding of the links between placental metabolism and placental development may shed light on the growing epidemic of preterm birth.

Metabolic stressors such as hypoxia at high altitude, or placental underperfusion associated with intrauterine growth restriction (IUGR), alter placental metabolism in particular ways. During isolated hypoxia induced at high altitude, for example, the human placenta appears to decrease its consumption of oxygen in favor

of glycolysis to maintain its bioenergetic needs (Postigo et al., 2009), which preserves placental growth. While preserving fetal oxygen (O_2) delivery, this comes at the expense of glucose, however (Illsley et al., 2010). Given that fetal hypoglycemia is strongly associated with fetal growth restriction, this limits fetal growth. With maternal undernutrition, however, where uterine O_2 delivery is relatively spared, glucose delivery to the fetus is compromised (Coan et al., 2010; Sandovici et al., 2012) in a manner associated with restricted placental growth. Given that the placenta is a complex organ with multiple different cell types (Simmons et al., 2007, 2008), it is likely that changes in placental cellular composition due to alterations in cell fate regulatory pathways play important roles in the reallocation of placental metabolic flux patterns. Consistent with this, maternal calorie restriction leads to a loss of glycogen trophoblasts in the mouse (Coan et al., 2010). Importantly, isolated hypoxia results in increased Hypoxia-inducible Factor-1 (HIF-1) levels and target gene expression in the human placenta (Nevo et al., 2006; Ietta et al., 2007; Zamudio et al., 2007), and HIF activity regulates trophoblast cell fate decisions, suggesting a potential contribution to these pathologic changes (Cowden Dahl et al., 2005; Maltepe et al., 2005; Choi et al., 2013; Zhou et al., 2014). HIF activity can mediate metabolic adaptation to hypoxia via numerous ways (Semenza, 2010), including repressing mitochondrial O_2 consumption while increasing glycolysis and modulating glucose transport as well as amino acid metabolism.

Endocrine Function

In addition to performing the essential transport functions of the placenta in humans, SynTs secrete numerous pregnancy-related hormones (Maltepe et al., 2010). Mammalian placenta produces a greater diversity of hormones in greater quantity than any other single endocrine tissue. Near term, steroid hormones (primarily estrogens and progestins) are being made at the rate of 0.5 grams per day, and protein hormones (lactogens, growth factors, and other hormones similar to those of the hypothalamic–pituitary–adrenal [HPA] axis) are being made at more than twice this rate. Many of these hormones are species specific, but the categories of hormones (i.e., steroids, pituitary-like, hypothalamic-like etc.) and their endocrine, paracrine, and autocrine functions in pregnancy are frequently conserved (Table 5.1).

Steroid Hormones

The placenta is an “incomplete” steroidogenic organ and does not express a complete set of enzymes for de novo production of estrogens and progestins. Steroid hormone synthesis in the placenta is dependent on precursors from mother and fetus, leading to the concept of an integrated *maternal–fetal–placental unit* (Ryan, 1980). Fig. 5.4 diagrams the tissues and enzymes that participate in the biosynthesis of progestins and estrogens (Kallen, 2004). The concentration of steroid hormones in the maternal circulation increases dramatically throughout gestation (Tulchinsky et al., 1972).

Progesterone

Maternal cholesterol, derived from low-density lipoprotein, is transported to the placenta and bound to low-density lipoprotein receptors on SynTs, where it is incorporated by endocytosis and hydrolyzed to free cholesterol in lysosomes (Hussa, 1980). There is no significant 3-hydroxy-3-methylglutaryl coenzyme A activity in human placenta and thus maternal cholesterol must be used

TABLE 5.1 Placental Classification (Incorporating the 2014 Amsterdam Placental Workshop Group Criteria)

1. Placental Vascular Processes
 - a. Maternal stromal-vascular lesions
 - Developmental
 - Superficial implantation/decidual arteriopathy
 - Increased immature extravillous trophoblast
 - Malperfusion
 - Global/partial
 - Early: distal villous hypoplasia
 - Late: accelerated villous maturation
 - Segmental/complete
 - Villous infarct(s)
 - Loss of integrity
 - Abruptio placenta (atrial)
 - Marginal abruption (venous)
 - Acute
 - Chronic
 - b. Fetal stromal-vascular lesion
 - Developmental
 - Villous capillary lesions
 - Delayed villous maturation (maturation defect)
 - Dysmorphic villi
 - Malperfusion
 - Global/partial
 - Obstructive lesions of umbilical cord
 - Recent intramural fibrin in large fetoplacental vessels
 - Small foci of avascular or karyorhectic villi
 - Segmental/complete
 - Chorionic plate or stem villous thrombi
 - Large foci of avascular or karyorhectic villi
 - Loss of integrity
 - Large vessel rupture (fetal hemorrhage)
 - Small vessel rupture (fetomaternal hemorrhage)
 - Villous edema
2. Placental Inflammatory Immune Processes
 - a. Infectious inflammatory lesions
 - Acute
 - Maternal inflammatory response: chorioamnionitis, subchorionitis
 - Fetal inflammatory response: chorionic/umbilical vasculitis
 - Chronic
 - Villitis (CMV, others)
 - Intervillositis (malaria, others)
 - b. Immune/idiopathic inflammatory lesions
 - Villitis of unknown etiology and related/associated lesions
 - Chronic villitis
 - Chronic chorioamnionitis
 - Lymphoplasmacytic deciduitis
 - Eosinophil T-cell fetal vasculitis
 - Chronic histiocytic intervillositis
3. Other Placental Processes
 - Massive perivillous fibrin(oid) deposition (maternal floor infarction)
 - Abnormal placental shape or umbilical insertion site
 - Morbidly adherent placentas (accreta)
 - Meconium-associated changes
 - Increased circulating nucleated red blood cells

CMV, Cytomegalovirus.

From Redline, R.W. Classification of placental lesions. *Am J Obstet Gynecol*. 2015 Oct;213(4 Suppl):S21-8.

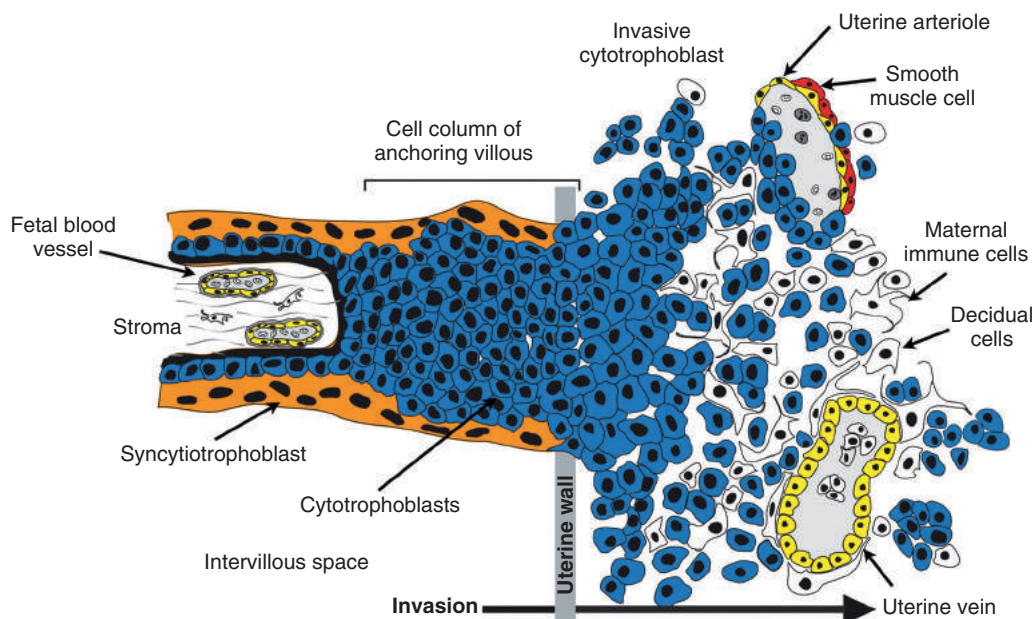
for production of pregnenolone, the first step in steroid synthesis. Cholesterol is converted to pregnenolone in the mitochondria by cytochrome P450 cholesterol side-chain cleavage enzyme. After transfer to the cytosol, progesterone is produced from pregnenolone by type-1 3β -hydroxysteroid dehydrogenase (Osathanondh and Tulchinsky, 1980; O'Connor et al., 1998). Before the ovarian-placental shift, the corpus luteum of pregnancy is the primary source of progesterone, but by 35 to 47 days postovulation the placenta produces enough progesterone to maintain pregnancy (Csapo et al., 1973). The majority (>90%) of progesterone goes to the mother and the rest to the fetus. A limited amount of pregnenolone is also released into the circulation. The fetus has the enzyme activity needed for pregnenolone synthesis but has minimal ability to produce progesterone. High levels of circulating fetal progesterone are of placental origin so that circulating progesterone levels thus reflect placental function, not fetal wellbeing.

Progesterone can be metabolized to 17-hydroxyprogesterone (17-OHP), but relative efficiency of the enzymes favors progesterone production. 17-OHP levels do rise in the third trimester as progesterone levels peak. Additional progesterone metabolites, particularly 5-dihydroprogesterone (5-DHP) and its metabolite allopregnanolone, are also produced in the SynT at increased levels during gestation (Dombroski et al., 1997). These steroids have been hypothesized to play an endocrine role in fetal brain development and provide neuroprotection in the face of hypoxia (Pasca and Penn, 2010; Hirst et al., 2014).

Progesterone (da Fonseca et al., 2003) or a synthetic form of 17-OHP (Meis et al., 2003) is used therapeutically in gestation, as an adjunct for pregnancy maintenance after in vitro fertilization or in the second half of gestation for prevention of preterm delivery in women with a prior history of preterm birth (Di Renzo et al., 2012). Progesterone is required for the maintenance of pregnancy in part by means of its suppressant effect on uterine contractions (Csapo et al., 1973; Kallen, 2004; Ragusa et al., 2004). Progesterone inhibits genes that promote contractility (Mesiano et al., 2002) and has immunosuppressive activity that may promote uterine quiescence (Ragusa et al., 2004; Hardy et al., 2006). Progesterone also counteracts uterine estrogen effects. Unlike the drop in progesterone levels prior to labor seen in most mammals, there is no progesterone withdrawal per se that occurs before labor in women; however, modulation of progesterone receptor expression in combination with a shift in the progesterone to estrogen balance is presumed to play the same biological role. The relationship of therapeutic response to normal physiologic mechanisms at work in the maternal-fetal-placental unit is not yet understood.

Estrogens

Unlike the requirement for maternal precursors for progesterone production, estrogen production relies on fetal precursors. In pregnancy, estrogens are synthesized from C19 steroids (Siiteri and MacDonald, 1966), primarily from dehydroepiandrosterone sulfate (DHEA-S) made in the fetal adrenals. The fetal adrenals rapidly inactivate steroids through sulfatization. Pregnenolone is sulfated and converted to DHEA-S (Benirschke et al., 1956), which then may be hydroxylated in the fetal liver. These biologically inactive androgens are then transferred back to the placenta. Placental sulfatases rapidly cleave the sulfate, and placental 3β -hydroxysteroid dehydrogenase converts DHEA or hydroxylated DHEA to androstenedione or hydroxylated androstenediones, respectively. These androgens are then aromatized to estrone (E1),



• **Fig. 5.4** Basal Plate of the Human Placenta at Mid Gestation. A multinucleated layer of syncytiotrophoblasts (SynTs) covers the finger-like projections of chorionic villi. SynTs perform all of the transport functions of the placenta. A single cell layer of cytotrophoblasts (CTBs) sits just below the SynT layer and is referred to as villous CTB. These cells fuse with each other as they differentiate into SynTs. At the tips of anchoring villi sits a column of CTBs that are proliferative and that put out waves of invasive CTBs (extravillous trophoblasts) that help anchor the placenta to the uterus. Importantly, these also invade maternal spiral arterioles, where they replace the endothelial lining, induce the apoptotic death of surrounding smooth muscle cells, and transdifferentiate into an endothelialized cell type. This invasion typically courses through the extent of the uterine myometrium. CTBs can also invade veins but do not appear to remodel them. Additionally, maternal uterine immune cells, such as natural killer cells, appear to play an important role in enabling CTBs to invade the uterus and its vasculature. Finally, establishment of a receptive deciduum is also of critical importance for proper placental development and pregnancy success. (Adapted from Genbacev O, et al. *Dev Biol.* 2001 May 15;233[2]:526-36.)

16 α -OH estrone, or 15 α -OH estrone and then converted to estradiol (E2), estriol (E3), or estetrol (E4) respectively by placental 17 β -hydroxylation (Jameson et al., 1986; Morrish et al., 1987; Shi and Zhuang, 1993). E3 is the major estrogen of pregnancy with the majority secreted into the maternal compartment; E1 is the only estrogen preferentially secreted into the fetal compartment. Although maternal DHEA-S serves as 40% of the precursor for E2 synthesis, E3 and E4 are formed predominantly from fetal precursors because the maternal liver has limited 5 α -hydroxylation or 16 α -hydroxylation capabilities (Madden et al., 1978). E3 and E4 are thus indicators of fetal function (Tulchinsky et al., 1972), although neither is a clinically useful marker because of rapid shifts in circulating levels. The primary function of high E3 levels remains unclear, but it does increase uteroplacental blood flow (Resnik et al., 1974).

Estrogens influence uterine growth, blood flow, contractility, metabolism, and breast development (Branchaud et al., 1983). However, high estrogen levels are not apparently needed for pregnancy. Parturition can proceed in the absence of fetal and placental sulfatase (Bradshaw and Carr, 1986) or aromatase (Harada, 1993), although in the latter case both fetus and mother are virilized. In such pregnancies, there is still circulating estradiol. There are no reports of pregnancy without detectable estrogen levels, suggesting that a basal level of estrogen is likely required. Before parturition, an increase in the estrogen to progesterone ratio occurs within the intrauterine tissues and may increase prostaglandin (PG) and oxytocin (OT) activity. Steroid hormone production is

altered by trophic hormones and other factors, including hypothalamic-like releasing or inhibiting hormones. In turn, estrogens affect other endocrine systems (i.e., renin-angiotensin system) (Carr and Gant, 1983) and support organ maturation such as surfactant production in the lung (Parker et al., 1987).

Glucocorticoids

In addition to the sex steroids, circulating levels of glucocorticoids and mineralocorticoids are increased in pregnancy (Dorr et al., 1989). The placenta has the ability to produce cortisol and to convert it to inactive cortisone via 11 β -hydroxysteroid dehydrogenase type 2. This enzyme also converts maternal cortisol to cortisone at the placental interface. The primary role of this system appears to be to protect the fetus from elevated cortisol exposure, which may play a role in long-term reprogramming of the fetal HPA axis (Challis et al., 2001). Dual oxidative and reductive enzymatic activity regulates the balance between cortisol and cortisone (Pepe and Albrecht, 1995). In the placenta, oxidation of cortisol to cortisone predominates, whereas in the decidua, the reverse reaction dominates, potentially providing localized hormone exposure.

Pituitary-Like Hormones

A combination of pituitary-like growth hormones are required to support fetal growth while maintaining maternal metabolic homeostasis.

Human Chorionic Gonadotropin

Human chorionic gonadotropin (hCG) is one of the first hormones of pregnancy, produced by trophoblasts even before placenta formation (Hay and Lopata, 1988) and is unique to human pregnancy (Maston and Ruvalo, 2002). After placentation, hCG is synthesized primarily by the SynT (Midgley, 1962) and passes into the maternal circulation via secretion into the intervillous space. hCG is a glycoprotein heterodimer (36 to 40 kDa) composed of α and β subunits encoded by genes on chromosome 6 and 19, respectively (Hussa, 1980; Fournier et al., 2015). The α subunit is homologous to pituitary thyroid-stimulating hormone (TSH), luteinizing hormone (LH), and follicle-stimulating hormone (FSH), while the β subunit is homologous to LH. Intact hCG (i.e., having both α and β subunits) is required for hCG endocrine activities. Since it shares a receptor with LH, the LH chorionic gonadotrophin receptor (LHCGR), hCG mimics the function of LH, but the functions of LH and hCG are quantitatively different due to the longer half-life of hCG and its relative stability compared with the pulsatile release of pituitary LH (Muyan and Boime, 1997).

hCG maintains corpus luteal progesterone production until this function shifts to the maturing placenta. hCG peaks approximately 2 weeks after the shift of progesterone production from ovary to placenta, potentially minimizing the chance of loss of the progestational environment. hCG can be detected in human serum or urine within a week of conception and is the most frequently used biochemical marker for pregnancy. hCG doubling time may be used in early gestation to predict general pregnancy outcome. After hCG can first be detected, it increases with a doubling time averaging 2.11 days. It reaches peak levels of approximately 50 international units (IU)/mL at 9 to 10 weeks from the date of the last menstrual period, declining to 1 IU/mL by mid gestation (Osathanondh and Tulchinsky, 1980). An abnormally slow doubling time of hCG is considered to be a sign of a poor prognosis for pregnancy outcome, while rising hCG without detection of an intrauterine embryo suggests an ectopic pregnancy (Fritz and Guo, 1987).

In addition to corpus luteum maintenance, hCG has multiple additional activities that regulate placental structure and function. hCG acts as an autocrine signal in trophoblasts expressing LHCGR promoting the differentiation of SynTs, thus amplifying its own production since it is made primarily by these cells (Shi and Zhuang, 1993). Phosphorylation of the receptors via this pathway also decreases LHCGR expression in differentiating SynTs, thus completing a feedback loop (Pidoux et al., 2007). hCG may also have roles in endometrial angiogenesis, uterine quiescence, and immunotolerance to the fetus (Osathanondh and Tulchinsky, 1980; Fritz and Guo, 1987; Fournier et al., 2015). In addition, hCG can alter maternal TSH levels, elevating free thyroxine (T₄), although this increase does not appear to cause maternal hyperthyroidism (Challis et al., 2009).

Glycosylation state and subunit availability regulate hCG activity. A hyperglycosylated form (hCG-H) has been detected in early pregnancy, as well as in choriocarcinoma cells. hCG-H appears to enhance trophoblast invasion (Fournier et al., 2015) and thus may be a very early biomarker of placental invasion of the endometrium. A decreased level of hCG glycosylation in very early pregnancy has been correlated with early pregnancy loss (O'Connor et al., 1998; Kovalevskaya et al., 1999; Fournier et al., 2015). Isoform production may also regulate activity. β -Subunit production exceeds α -subunit production in early pregnancy, but this ratio rapidly shifts to α -subunit excess, increasing as gestation progresses; circulating hCG is mostly intact hCG or free α -hCG. It has been proposed

that ratios of hCG isoforms (intact hCG, independent subunits, and nicked breakdown products) present in maternal blood and urine might be useful for detection of pregnancy-related disorders since only intact hCG is fully active (Montagnana et al., 2011).

Local and systemic factors influence hCG production. Locally, its expression is regulated by a releasing factor, gonadotropin-releasing hormone (GnRH) 1 and 2 (Khodr and Siler-Khodr, 1978; Khodr and Siler-Khodr, 1980; Siler-Khodr and Grayson, 2001). Neurotransmitters (Shi and Zhuang, 1993), cyclic adenosine monophosphate (Jameson et al., 1986), epidermal growth factor (EGF) (Morrish et al., 1987), activin (Steele et al., 1993), cytokine (Wegmann and Guilbert, 1992), and PGs (Licht et al., 1993) regulate hCG production, as does hCG itself as noted previously. Each of these factors is produced by the placenta, as well as by other extraembryonic tissues. hCG alters placental steroidogenesis by stimulating both progesterone and estrogen formation. Estrogens inhibit GnRH stimulation of hCG (Branchaud et al., 1983), thereby completing a feedback axis in the placenta.

Human Chorionic Somatomammotropin

Human chorionic somatomammotropin (hCS), originally known as human placental lactogen (Higashi, 1961; Josimovich and Maclaren, 1962), has both growth hormone-like and lactogenic activity. hCS is detectable in extraembryonic tissues within 10 days of conception and in maternal serum by the third to fourth week of gestation. It is a single 191 amino acid nonglycosylated peptide chain with considerable homology to growth hormone (GH) (96%) and PRL (67%); it is transcribed from a gene cluster on chromosome 17 containing two genes for hCS, one hCS pseudogene and two GH genes (Rygaard et al., 1998; Handwerger and Freemark, 2000). SynTs produce hCS at a constant rate during gestation, thus hCS levels reflect total placental mass and gross placental function. By term, hCS is made at 1 gram per day, representing 10% of total placental protein synthesis.

hCS is considered one of the major diabetogenic factors of pregnancy, along with placental steroids, placental GH variant (hGH-V), and maternal cortisol. It is almost exclusively found in maternal rather than fetal circulation. This has led to the hypothesis that the primary role of hCS is to ensure adequate fetal nutrition because, in maternal circulation, it induces metabolic changes such as mobilization of fatty acids, insulin resistance, decreased utilization of glucose, and increased availability of amino acids through decreased maternal use of protein (Grumbach et al., 1968). Circulating maternal glucose and free fatty acids are thus increased. While glucose readily crosses the placenta, fatty acids cross slowly, thus biasing glucose delivery toward the fetus and use of fatty acids for maternal energy, especially during maternal fasting. Within the placenta, hCS may regulate insulin-like growth factor (IGF-1) (Kanda et al., 1998) and alter fetal growth through direct action on placental nutrient transport systems. In addition to its metabolic activity, the lactogenic activity of hCS may prepare the breast for lactation, working synergistically with PRL and steroids (Alsatt et al., 1998). Most recently, a role for hCS as a placental angiogenic factor has been suggested (Corbacho et al., 2002).

Placental Growth Hormone Variant

hGH-V is encoded in the same chromosome 17 gene cluster as hCS and pituitary GH. Two transcripts are generated from the hGH-V gene, a major form and an alternatively spliced version. Secreted hGH-V is translated from the major version and is produced in a highly bioactive 22 kD nonglycosylated form and to a lesser degree in a 25 kD glycosylated form (Alsatt et al., 1998).

Early in pregnancy, maternal pituitary GH is produced, but from 15 to 20 weeks' gestation to term hGH-V secretion increases, suppressing maternal GH. hGH-V peaks about a month before term delivery and disappears from maternal circulation immediately after delivery (Newbern and Freemark, 2011). hGH-V is not detected in the fetal circulation. Much like hCS, hGH-V modifies maternal metabolism to meet fetal needs. hGH-V primarily appears to control maternal IGF-1 production (Newbern and Freemark, 2011). In mice overexpressing hGH-V (not normally found in rodents), body weight was increased, IGF-1 levels were elevated, and insulin resistance developed, suggesting that hGH-V strongly contributes to the insulin resistance of pregnancy (Barbour et al., 2002) and increases the risk of gestational diabetes and other pregnancy-related pathologies. This risk is counterbalanced by placental lactogens, hCS and PRL, which induce increased insulin secretion by pancreatic β -cell expansion. hGH-V secretion is tonic, in contrast to pulsatile pituitary GH, and is not regulated by hypothalamic releasing factors (Newbern and Freemark, 2011). Secretion is inhibited by elevated glucose and mildly increased by hypoglycemia, creating a feedback loop that may ensure constant delivery of nutrients to the developing fetus.

Insulin-Like Growth Factors

IGF1 and IGF2, are the primary somatotrophs in gestation. IGFs are highly homologous single chain polypeptides with similarities to pro-insulin made in human placental tissues (Han and Carter, 2000). The majority of the components of the insulin/IGF system are found in the placenta (IGF1, IGF2, and the IGF-binding proteins [IGFBP] 1–6) (Forbes and Westwood, 2008) except insulin itself, although maternal insulin has profound indirect effects on fetal growth and wellbeing. hGH-V levels regulate placental IGF levels (Kanda et al., 1998), and IGFBPs are carrier proteins expressed in the human placenta that prevent IGF from degradation while blocking bioactivity (Hill et al., 1993; Han and Carter, 2000; Forbes et al., 2008). IGFBP1 is also produced by the decidua in large amounts. IGFBPs are themselves regulated by protease activity and through posttranslational modifications, adding a further layer of regulatory complexity.

IGF1 is expressed predominantly in SynT throughout gestation, with some CTB expression, while IGF2 is expressed only in CTB with a declining level across gestation (Hill et al., 1993; Han and Carter, 2000; Forbes and Westwood, 2008; Newbern and Freemark, 2011). At physiologic concentrations, both IGF1 and IGF2 bind to the IGF1 receptor (IGF1R). The localization of IGF1R shifts during gestation; initially it is predominantly expressed on SynTs (closer to the maternal circulation), and by term it is mainly expressed on the fetal CTB side, reflecting the shifting activity from maternal to fetal growth control (Forbes and Westwood, 2008). The IGF2 receptor (IGF2R; also known as the cation-independent mannose-6-phosphate receptor) controls extracellular IGF2 concentrations by mediating the endocytosis and degradation of IGF2, rather than by direct signaling via the receptor (Gicquel and Le Bouc, 2006). An additional receptor, possibly a variant of the insulin receptor, may mediate some of the fetal growth effects of IGF2 (Baker et al., 1993).

Information on the role of IGFs in fetal growth comes from genetic manipulation in mouse models as well as examination of human tissues, especially from fetal growth restricted pregnancies (Gicquel and Le Bouc, 2006). Disruption of mouse Insulin-like growth factor (*Igf1*), *Igf2*, or insulin-like growth factor 1 receptor (*Igf1r*) genes retards fetal growth (Baker et al., 1993), while disruption of *Igf2r* or overexpression of IGF2 enhances fetal growth

(Eggenschwiler et al., 1997). In humans, mutations in the *IGF1* or *IGF1R* genes are extremely rare, and no *IGF2* gene deletions have been reported (Gicquel and Le Bouc, 2006). However, *IGF2* is an imprinted gene normally expressed exclusively from the paternal allele in placenta and fetal tissues. Changes in *IGF2* expression because of abnormal imprinting have been linked to both overgrowth (Beckwith–Wiedemann syndrome) and growth retardation (Russel–Silver syndrome) (Gicquel and Le Bouc, 2006). Whether placentally derived IGFs, versus fetal IGFs, directly contribute to these fetal growth changes is uncertain since these factors also have paracrine effects in the placenta that determine nutrient transport and placental growth.

IGF1 can promote SynT differentiation, while IGF2 does not appear to have this function despite its very early placental expression. In vitro experiments suggest that placental mass is regulated directly by placental IGFs (Forbes et al., 2008); in vivo, loss of IGF2 reduces the placental surface area available for gas and nutrient exchange more than IGF1 loss. Both IGFs increase nutrient transport, especially of amino acids, which may be reflected in elevated fetal amino acids associated with gestational diabetes (Cetin et al., 2005; Forbes et al., 2008). IGFs may alter fetal growth through additional mechanisms since they potentiate EGF activity (Bhaumick et al., 1992), increase prolactin and progesterone production (Nestler, 1987; Kubota et al., 1991), and inhibit placental thromboxane production (Siler-Khodr et al., 1995).

Other Secreted Growth Factors

Platelet-derived growth factor A, transforming growth factor (TGF)- α , and TGF- β (Rappolee et al., 1988) expression in blastocysts appear to be involved in implantation. Other growth factors, including EGF, basic FGF, nerve growth factor, granulocyte colony-stimulating factor, and hepatocyte growth factor as well as growth factor receptors are expressed by the placenta and membranes at later gestation stages (Chegini and Rao, 1985; Stewart, 1996; Uehara and Kitamura, 1996; Morrish et al., 1998). The actions of many of these growth factors may be nonclassical autocrine actions. For example, EGF is made in SynTs, and EGF receptors on SynTs correlate with trophoblast differentiation rather than proliferation (Maruo and Mochizuki, 1987; Mitchell, 1987; Marzoni et al., 2005). Additional growth factor actions on placental development are under intensive investigation.

Inhibin and Activin

Inhibin and activin, an antagonist and agonist of pituitary FSH, respectively, are expressed by CTBs and fetal membranes (Petraglia et al., 1987b; Petraglia, 1997) while activin receptors are expressed in SynT (Florio et al., 2004a). Inhibin inhibits hCG and reduces progesterone production (Petraglia et al., 1987b), while activin has the opposite effect (Petraglia et al., 1989). Inhibin elevation is associated with fetal trisomy 21, while elevated activin is associated with PE and gestational diabetes (Florio et al., 2004a). Thus during pregnancy these hormones may serve as potential biomarkers of placental pathologies.

Proopiomelanocortin Hormones

Pituitary-like peptides derived from proopiomelanocortin (POMC), including adrenocorticotrophic hormone (ACTH), melanocyte-stimulating hormone, β -endorphins, and β -lipoproteins, as well as full length POMC itself, are found in the human placenta (Krieger, 1982; Raffin-Sanson et al., 1999). The processing of POMC in the placenta is different from in the pituitary: POMC is released largely intact from the placenta, while it is cleaved into

several peptide hormones in the nonpregnant state. While pituitary POMC-derived peptides respond to and regulate physiologic stress, placental POMC is not inhibited by glucocorticoids nor do circulating levels correlate with ACTH or cortisol levels, although they do correlate with corticotrophin-releasing hormone (CRH) levels (Raffin-Sanson et al., 1999). Chorionic CRH is produced by the placenta and stimulates the release of chorionic ACTH (see later) (Reis et al., 1999). The physiologic role of chorionic ACTH has not been defined, but it may affect placental cortisol production or maternal resistance of ACTH suppression by glucocorticoids.

Hypothalamic-Like Hormones

Every known hypothalamic releasing or inhibiting hormone has a placental analogue (Khodr and Siler-Khodr, 1978, 1980; Siler-Khodr, 1993; Siler-Khodr and Grayson, 2001). These hormones act in placental paracrine–autocrine regulatory networks that control release of placental endocrine hormones.

Gonadotropin-Releasing Hormone

In the placenta, chorionic GnRH, which regulates the paracrine axis, is important for early pregnancy maintenance, as well as regulating gonadal steroid production through stimulation of pituitary LH and FSH (Pawson et al., 2003). Two isoforms of GnRH (GnRH1 and GnRH2) are produced (Seeburg and Adelman, 1984; Siler-Khodr and Grayson, 2001; Sasaki and Norwitz, 2011). GnRH1 is encoded on chromosome 8 as a precursor protein that includes a signal sequence, the GnRH decapeptide, a processing sequence, and a GnRH-associated peptide (Cheng and Leung, 2005). GnRH2 is encoded on chromosome 20 and has 70% homology to GnRH1. GnRH1 and GnRH2 signal through the same G protein coupled receptor, GnRHR1, expressed in SynTs, but may activate different intracellular signaling pathways (Haning et al., 1982; Chou et al., 2003; Sasaki and Norwitz, 2011). Blocking GnRHs or GnRHR1 activity can lead to pregnancy failure (Das and Talwar, 1983; Jagannadha Rao et al., 1985; Sridaran, 1986; Kang et al., 1989), possibly through alteration of hCG and placental steroids, whose production and release they modulate (Siler-Khodr et al., 1986b). The release of placental GnRH1 is affected by cyclic adenosine monophosphate, PGs, epinephrine (Petraglia et al., 1987a), and inhibin (Higashi, 1961), while the expression of GnRHR1 is regulated by GnRH1, activin, and inhibin, creating a feedback loop (Sasaki and Norwitz, 2011).

Corticotrophin-Releasing Hormone and Urocortins

Chorionic CRH and CRH receptors are expressed in placenta and fetal membranes (Shibasaki et al., 1982; Frim et al., 1988; Riley and Challis, 1991; Florio et al., 2000). Urocortins, members of the CRH-hormone family, are also produced and bind to CRH receptors as well (Florio et al., 2004b). Early in gestation, CRH family members may promote immune tolerance (Makrigiannakis et al., 2004). As gestation progresses, CRH and urocortin levels rise peaking at term with delivery (Florio et al., 2004b). These hormones stimulate POMC-derived hormones, including ACTH and β -endorphinins, (Margioris et al., 1988; Florio et al., 2004b), as well as PG release, suggesting roles in parturition (Jones and Challis, 1989). CRH can also stimulate fetal adrenal estrogen and glucocorticoid production (Mesiano and Jaffe, 1997), which may contribute to the timing of parturition. Glucocorticoids can increase placental CRH expression (Jones et al., 1989), in contrast to glucocorticoid inhibition of CRH in the hypothalamus, creating a positive feedback loop that amplifies CRH activity (Nicholson and King, 2001). Because of its tight

association with delivery timing, CRH is often viewed as a *placental clock* (McLean and Smith, 2001) and may be biomarker of pregnancy pathology. In pregnancies complicated by hypertension, the maternal circulating levels of CRH are already elevated by 28 weeks of pregnancy whereas local urocortin levels may be decreased (Petraglia et al., 1996; Florio et al., 2004b). CRH has been proposed as a predictor for preterm delivery (Holzman et al., 2001; McLean and Smith, 2001), but significant clinical utility has not yet been demonstrated (McGrath and Smith, 2002; Smith and Nicholson, 2007).

Thyrotropin-Releasing Hormone

Chorionic thyrotropin-releasing hormone (Shambaugh et al., 1979) is made by the placenta and fetal membranes, but a clear role for either the mother or the fetus has not been identified. Pituitary TSH does not cross the placenta, nor does the placenta make thyroid hormone itself, but maternal T4 and triiodothyronine (T3) cross the placenta carried by placentally produced transthyretin (Landers et al., 2009; Li et al., 2012; Forhead and Fowden, 2014). The placental role in thyroid metabolism has been of considerable recent interest since thyroid disease is common in women of childbearing age and impacts pregnancy outcomes (Nathan and Sullivan, 2014). Early maternal hypothyroidism appears to be associated with lower intelligence quotient in offspring, but conflicting reports exist on the impact of maternal hypothyroidism after onset of fetal thyroid function in mid gestation (Chan et al., 2009; Nathan and Sullivan, 2014). Maternal T4 continues to cross from maternal to fetal circulation in the second and third trimesters; even fetuses with complete thyroid dysgenesis have 30%–50% normal T4 levels in cord blood (Vulsma et al., 1989). Placenta regulation of thyroid hormone transport and metabolism may play a critical role in fetal wellbeing, but the regulatory pathways remain to be defined.

Growth Hormone-Releasing Hormone, Somatostatin, and Ghrelin

Additional releasing factors are made in the CTB, including growth hormone-releasing hormone (Berry et al., 1992), somatostatin (Nishihira and Yagihashi, 1978), and ghrelin (Gualillo et al., 2001) and may regulate hGH-V production as well as placental differentiation (Fuglsang et al., 2005).

Leptin

Leptin is normally secreted by adipocytes and decreases food intake through hypothalamic actions, but in pregnancy, the placenta is the primary leptin source (Ashworth et al., 2000; Linnemann et al., 2001). The precise roles of leptin in the placenta, the mother, or the fetus are not known but may differ significantly from the nonpregnant state, as leptin levels in pregnancy do not correlate with body mass nor produce satiety (Henson and Castracane, 2000; Hauguel-de Mouzon et al., 2006). Increased leptin levels are seen in PE and gestational diabetes (Miehle et al., 2012; Tessier et al., 2013).

Oxytocin

OT is another hypothalamic hormone produced in the placenta and membranes (Gimpl and Fahrenholz, 2001). OT is a potent uterotonic hormone, used clinically to induce or speed labor. However, neither circulating maternal OT nor locally produced OT appears to increase markedly before labor; rather uterine response to OT is increased through increases in OT receptor (OTR) expression and function (Fuchs et al., 1995; Mitchell and

Chibbar, 1995). Progesterone suppresses OTR signaling during gestation (Gimpl and Fahrenholz, 2001), but a decline in progesterone activity at term (although not absolute progesterone levels in humans) increases OTR expression making the uterus more responsive to OT.

Additional Placental Secreted Factors

Vasoactive Peptides

The angiotensin–renin system has been described in the placenta and is thought to be a factor in the regulation of vascular tone in the placental bed. Multiple vasoactive peptides—VEGF, endothelin, angiotensin, arginine vasopressin, and atrial natriuretic peptide—and their receptors are placentally expressed (Myatt et al., 1992; Chao et al., 1993; Kingdom et al., 2000; VanWijk et al., 2000; Kaufmann et al., 2004). A balance of these factors is likely required for appropriate fetoplacental perfusion. For example, atrial natriuretic peptide inhibits the vasoconstrictive action of endothelin and angiotensin and induces vasodilatation in the uterus and the placenta.

Endogenous Opioid Peptides

Opioid peptides, enkephalins (Tan and Yu, 1981) and dynorphin (Lemaire et al., 1983), and their receptors are expressed in placenta, with an increase in placental receptors at term.

Cytokines

Cytokines—interferons, tumor necrosis factor- α (TNF- α), leukemia inhibitory factor, and interleukins (Chaouat et al., 2002; Kimber, 2005; Paulesu et al., 2005; Piccinni, 2005; Hauguel-de Mouzon and Guerre-Millo, 2006)—and their receptors are produced by the placenta, as well as by uterine endothelial cells and invading macrophages (Jokhi et al., 1997; Sel'kov et al., 2000; Piccinni, 2005; Varla-Leftherioti, 2005). Successful implantation requires a proinflammatory cytokine environment (Dealtry et al., 2000; Loke and King, 2000), while pregnancy maintenance requires cytokine expression that suppresses the maternal immune response (Keelan et al., 1999; Challis et al., 2009). Before parturition, this balance again shifts back to proinflammatory cytokines (Jokhi et al., 1997; Keelan et al., 1999; Sel'kov et al., 2000; Varla-Leftherioti, 2005). The balance of cytokines and related factors, either proinflammatory or antiinflammatory, may be a key trigger for preterm labor caused by intrauterine infection or other types of inflammation (Sel'kov et al., 2000; Challis et al., 2009). Cytokine expression also regulates trophoblastic and vascular placental function (Saito, 2001). Cytokines affect these activities by regulation of other cytokines, growth factors, hormones, and prostanoid production (Lundin-Schiller and Mitchell, 1991; Laham et al., 1997; Mohan et al., 2001).

Eicosanoids

Eicosanoids, such as thromboxanes (TXAs), PGs, and leukotrienes, are inflammatory mediators expressed in placenta that are derived from arachidonic acid (Harper et al., 1983; Majed and Khalil, 2012). Human term placentas convert arachidonic acid primarily to TXAs and the PGs PGE₂, PGF₂ α , and PGD₂ (Harper et al., 1983; Siler-Khodr et al., 1986a). Much like cytokines, they play a role in trophoblast implantation (Lewis, 1982) and in parturition (Challis and Patrick, 1980; Casey and MacDonald, 1988). After implantation, these factors, particularly prostacyclin (PGI₂) and PGE₂, appear to be vasoregulators of the fetal–placental unit (Challis and Patrick, 1980; Ylikorkala et al., 1983; Sorem and Siler-Khodr,

1995). PGI₂ is a potent vasodilator in placental vessels, an inhibitor of platelet aggregation, and a uterine relaxing factor; its loss has been implicated in PE. TXA₂ opposes PGI₂, and production is increased in PE; low-dose aspirin preferentially inhibits TXAs in the placenta and may decrease development of PE.

Immunologic Function

Throughout pregnancy the risk of fetal infection must be balanced against fetal rejection. This balance is maintained on both the maternal and fetal sides of the placenta. Unique features of the cells at the placental interface are required to allow the genetically distinct fetal “graft” to inhabit the maternal host. Placental trophoblast cells directly encounter maternal immune cells: SynT_s covering the placental villi are bathed in maternal blood and the invading trophoblasts exposed to the maternal decidua. Different strategies appear to be used at these sites to prevent destruction by cytotoxic maternal immune cells. For example, neither SynT_s nor invading trophoblasts express classic human leukocyte antigen (HLA)-A or HLA-B class Ia major histocompatibility complex antigens nor HLA class II antigens. However, invading trophoblasts do express nonclassic HLA-G and HLA-C, HLA types that can actually suppress immune responses, especially through leukocyte inhibitory receptors on uterine natural killer (NK) cells and macrophages. A balance of innate immunity and modulation of adaptive immune responses is required, and this balance shifts throughout gestation (Hunt et al., 2010; Christiansen, 2013).

The decidua is replete with innate immune cells including T cells, regulatory T cells, macrophages, dendritic cells, and uterine NK (uNK) cells. The best-studied subtype is the NK population. NK cells peak and constitute the largest leukocyte population in the early pregnant uterus, accounting for 60%–70% of total lymphocytes. These cells diminish in proportion as pregnancy proceeds. Despite being replete with cytotoxic perforin, granzymes A and B, and the natural cytotoxicity receptors (NKp30, NKp44, NKp46, NKG2D, NGK2B4, and LFA-1), these NK cells are tolerant cytokine-producing cells at the maternal–fetal interface (Kalkunte et al., 2008). The temporal occurrence around the SpAs and timed amplification of these specialized NK cells observed during the first trimester implicate their role in SpA remodeling. NK cell-deficient mice display abnormalities in decidual artery remodeling and trophoblast invasion, possibly because of a lack of uNK cell-derived interferon γ (Ashkar et al., 2000). Other studies have shown that uNK cells are a major source of VEGF-C, angiopoietins 1 and 2, and TGF- β 1 within the placental bed that decrease with gestational age (Lash et al., 2006). These observations implicate uNK cells in promoting angiogenesis. Recent studies suggest that VEGF-C may induce the noncytotoxic activity in maternal immune cells as well (Kalkunte et al., 2009). Additional molecules expressed on trophoblasts, such as members of the B7 family that alter lymphocyte activity and FasL, which interacts with Fas leukocyte receptors, may also modulate cytotoxicity in the placenta.

Both maternal macrophages and Hofbauer cells (macrophages in the villi that are derived from the fetus) are present in the placenta during pregnancy. These cells may prevent uterine infections or facilitate vascular remodeling and immune suppression (Nagamatsu and Schust, 2010b). Much like NK populations, alterations in macrophage activation, both maternal and fetal, have been linked to pregnancy complications such as IUGR, preterm birth, and PE (Nagamatsu and Schust, 2010a).

Maternal tolerance to fetal alloantigens was initially explored in the context of T-helper cells (Th)1/Th2 balance in mice, with

Th2 cells and cytokines proposed to predominate over Th1 cellular immune response under normal pregnancy. In human pregnancy, the role of specialized T lymphocytes, termed *regulatory T cells* (Tregs), in producing immune tolerance has emerged. Tregs are potent suppressors of T-cell-mediated inflammatory immune responses and prevent autoimmunity and allograft rejection. CD4+CD25+ Tregs are found in the decidua throughout pregnancy. Fetal-specific Tregs persist between pregnancies, and they accumulate and reexpand their population rapidly in subsequent pregnancies, potentially providing a persistent protective regulatory memory to fetal antigen (Rowe et al., 2012). The specific role of these Tregs in human pregnancy loss remains to be defined.

Immunosuppressive immune modulators are also highly expressed at the placental interface. Many of the endocrine factors produced by the placenta (progesterone, PGE2, and interleukins) as described above appear critical to maternal immune modulation. For example, the antiinflammatory cytokine interleukin (IL)-10 is expressed by human trophoblasts and Tregs, increasing across the first two trimesters and then declining before delivery. Low IL-10 expression has been linked to pregnancy loss and preterm delivery, as well as PE. However, how IL-10 protects the fetus is poorly understood. IL-10^{-/-} mice are fertile if maintained pathogen-free but are highly susceptible to complications from infection suggesting that IL-10 deficiency plus a “second hit” such as infection, environmental factors, or hormonal dysregulation may contribute to poor pregnancy outcomes (Thaxton and Sharma, 2010).

There is no generalized immunosuppression in pregnant women. Rather, there is a balance struck between specific types of immune suppression and activation. Indeed, cytokine production capacity is higher in pregnant than in nonpregnant women. It is the balance of proinflammatory to antiinflammatory cytokines that may determine outcome. When this balance is altered early in gestation, implantation could be affected, while immune alterations in late gestation may contribute to susceptibility to preterm birth, particularly in the face of an immune challenge. Similar shifts in the fetal immune response from tolerance to activation are being investigated across normal gestation (Mold et al., 2010). The role of placental immune activation in poor neonatal outcome, particularly in neurologic complications, has become an area of active study in the past decade (Kim et al., 2015a; Kim et al., 2015b). Defining and manipulating placental immune responses are key components of current efforts to improve pregnancy outcomes.

Fetal Programming

Complex yet intricate interactions between maternal and fetal systems promote fetal growth and normal pregnancy outcomes, interactions that must occur via the placenta. The placenta does not play a passive role as the interface between mother and fetus. Rather, it adapts to maternal status—nutrition, stress, environmental exposures—with altered vascularity and cellular composition and changes in endocrine and transport functions. Fetal genotype and fetal metabolic demands can also alter placental nutrient transfer and possibly other placental functions. Epidemiologic evidence first suggested that these complex interacting pathways alter the in utero environment in ways that lead to long-term changes in health and disease, particularly cardiovascular and metabolic disease (Barker and Osmond, 1986). How these interactions occur, as well as specific links between the in utero environment and later adult morbidities, is referred to as “fetal programming” or the “developmental origins of health and disease” (Fig. 5.5) (Burton et al., 2016).

Nutrition is the best-studied mechanism of fetal programming, although many other maternal and fetal programming events likely have long-term impacts (Fig. 5.6). Undernutrition can elicit placental and fetal adaptive responses that lead to local ischemia and metabolic, hormonal, and immune reprogramming, resulting in small for gestational age (SGA) fetuses. Maternal health and dietary status, exposure to environmental factors, uteroplacental blood flow, placental transfer, and fetal genetic and epigenetic responses likely all contribute to in utero fetal programming. Adult diseases such as coronary heart disorders, hypertension, atherosclerosis, type 2 diabetes, insulin resistance, respiratory distress, altered cell-mediated immunity, cancer, and even psychiatric disorders are being linked to fetal programming in utero (Sallout and Walker, 2003). In addition to maternal predisposing factors, cytokines, hormones, growth factors, and the intrauterine immune milieu also contribute to in utero programming. Therefore a healthy mother with healthy placentation is critical to healthy fetal outcomes.

Regulation of Placental Function

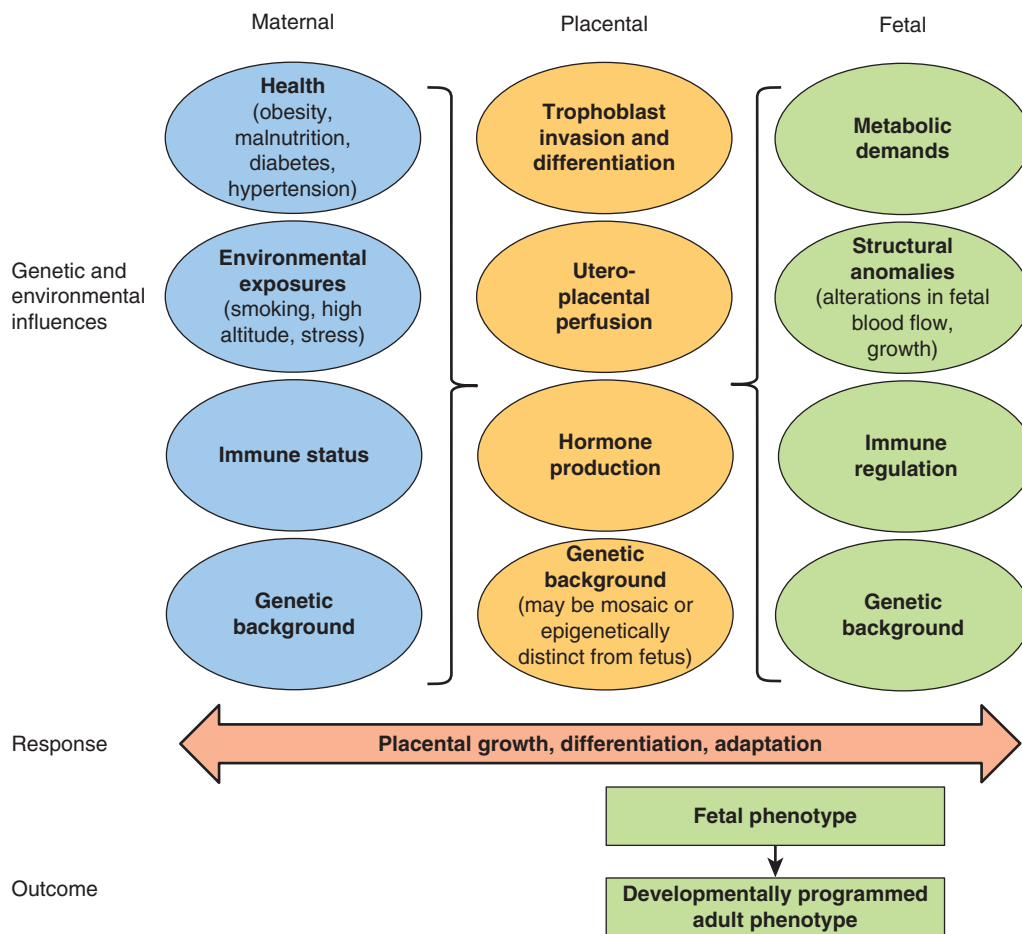
While it is now recognized that placental function can have lifelong impacts on health, the molecular mechanisms that underlie this complex signal integration and regulation are not yet well understood but are critically important to human disease. An understanding of the molecular mechanisms underlying the placental integration of maternal and fetal information that results in fetal programming may suggest new ways to manage pregnancy complications that lead to fetal growth failure and long-term alterations to health. To date, the two best documented forms of placental regulation are (1) signal integration thorough specific signaling cascades and (2) epigenetic modifications of placental gene expression.

Nutrient-Sensing Signaling Pathways

Multiple signaling molecules are expressed in SynT cells that are responsive to nutrient supply, including mechanistic target of rapamycin (mTOR), adenosine monophosphate-activated protein kinase, and glycogen synthase-3 (see Fig. 5.6). Emerging evidence suggests that mTOR may be a key component of the placental nutrient-sensing signaling pathway (Jansson and Powell, 2013; Dimasuay et al., 2016). mTOR is a serine/threonine-specific protein kinase belonging to the family of phosphatidylinositol-3 kinase (PI3K)-related kinases. mTOR regulates cellular metabolism, growth, and proliferation by targeting and signaling through two protein complexes, mammalian target of rapamycin complex (mTORC1) and mTORC2. Both hypoxia and limited nutrient supply result in the mTORC1 inhibition seen in placentas of IUGR fetuses (Roos et al., 2007; Kavitha et al., 2014). In the case of maternal obesity and enhanced nutrition, mTORC1 appears to be activated. mTOR expression in turn regulates nutrient transporter trafficking, particularly amino acid transporters. Recent experiments in mice suggest that the maternal and the fetal genotype of the upstream regulator of mTOR, PI3K, both influence placental function, nutrient delivery, and maternal physiology (Sferruzzi-Perri et al., 2016). While the regulatory details of this pathway need to be elucidated in humans, regulating components of the mTOR pathway might provide novel treatments for disorders of fetal growth (Jansson and Powell, 2007).

Epigenetic Regulation in the Placenta

The placenta has unique epigenetic features that may make it particularly responsive to its environment. Epigenetic regulation



• **Fig. 5.5** Fetal Programming. Maternal and fetal health alter placental function, which in turn influences fetal adaptations. Dietary status, exposure to environmental factors, uteroplacental blood flow, placental transfer, and genetic and epigenetic changes contribute to in utero fetal programming.

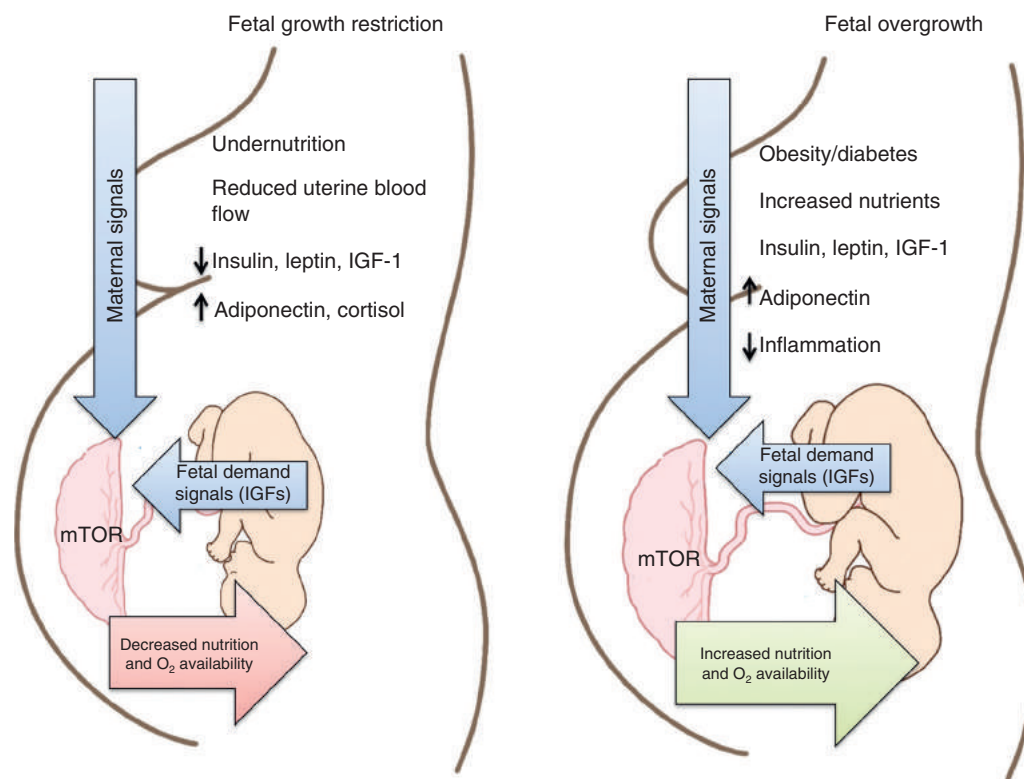
of gene expression allows for heritable changes that are mediated independently of changes in DNA sequences themselves. Gene expression is largely determined by the accessibility of DNA to transcription factors, accessibility that is controlled by a variety of epigenetic mechanisms: methylation of DNA promoter regions, histone modifications, genomic imprinting, and expression of noncoding ribonucleic acids (RNAs) such as microRNA (miRNA). Since these alterations are not encoded in DNA, epigenetic changes can occur in response to the cellular environment. In utero changes in the epigenome have been linked to poor pregnancy outcomes, although the causal relationships remain unclear (Januar et al., 2015).

Massive epigenetic reprogramming with loss of DNA methylation occurs just after fertilization and before implantation. The placenta and other extraembryonic tissue remain hypomethylated, even though methylation increases with cellular differentiation so that even at term, the placenta is the most hypomethylated human tissue (Bianco-Miotto et al., 2016). Within the placenta, the various cell types have distinct methylation profiles. Nutritional components, such as folate, can alter placental and fetal methylation patterns (Jansson and Powell, 2007). Placental methylation changes have been linked to PE, IUGR, and preterm birth (Januar et al., 2015; Bianco-Miotto et al., 2016).

Methylation changes also contribute to genomic imprinting, in which an allele is silenced in a specific parent-of-origin manner.

Placental genomic imprinting is distinct from somatic imprinting, with most imprinted placental genes impacting fetal and/or placental growth or maternal preparation for care of offspring. Most of what is known about placental gene imprinting comes from studies on the IGF pathway in mouse, described above. Paternally expressed imprinted genes, such as *IGF2*, promote placental and fetal growth while maternally expressed ones, such as *IGF-2R*, suppress growth (Gicquel and Le Bouc, 2006). However, recent human studies suggest significant species specificity of imprinting, emphasizing the need for large-scale human placental studies to better define the role of this epigenetic mechanism in human pregnancy (Monk, 2015).

An additional layer of epigenetic regulation may be added by miRNAs, the small noncoding RNA gene products that regulate gene expression through repression of messenger RNA (mRNA) translation or mRNA decay (Mouillet et al., 2015). At least 500 miRNA species are expressed in the placenta, and those expressed in trophoblasts can be detected in maternal circulation (Chim et al., 2008) (Fig. 5.7). The functions of some of these miRNAs are starting to be decoded. For example, miR-675 is an miRNA embedded in the first exon of H19, a highly expressed large intergenic noncoding RNA. miR-675 is expressed only in the placenta when placental growth is slowing in the second half of gestation. Loss of H19 and thus miR-675 results in placental overgrowth, while overexpression of the miRNA results in reduced proliferation (Keniry et al., 2012).



• **Fig. 5.6** Placenta as a Nutrient Sensor. The role of the placenta in maternal–fetal resource allocation has been hypothesized to be a critical element of fetal programming (Jansson et al., 2013; Sferruzzi-Perri and Camm, 2016). Maternal and fetal genotype influence signaling pathways (i.e., the phosphoinositol 3-kinase/mechanistic target of rapamycin [*mTOR*] pathway) in the placenta that balance maternal environmental status and fetal demands. When the maternal nutrient supply is altered, the placenta responds to maternal signals to match fetal growth to the nutrient supply. The placental alterations are thus central to the long-term health of the fetus. *IGFs*, Insulin-like growth factors; *IGF-1*, insulin-like growth factor-1.

It is notable that epigenetic mechanisms used by the placenta are most similar to those seen in tumorigenesis, reflecting their invasive similarities. Large-scale patterns of hypomethylation, as well as site-specific hypermethylation of tumor suppressor genes often seen in cancers, occur in the placenta. In addition, some miRNAs that have been implicated in regulating uterine invasion are associated with malignancies when reactivated outside of the placental context (Mouillet et al., 2015). Given the limited invasive potential of the placenta under normal circumstances, there is likely to be an additional layer of regulation controlling the placental epigenetic response to both intrinsic and environmental cues.

Placental Diseases

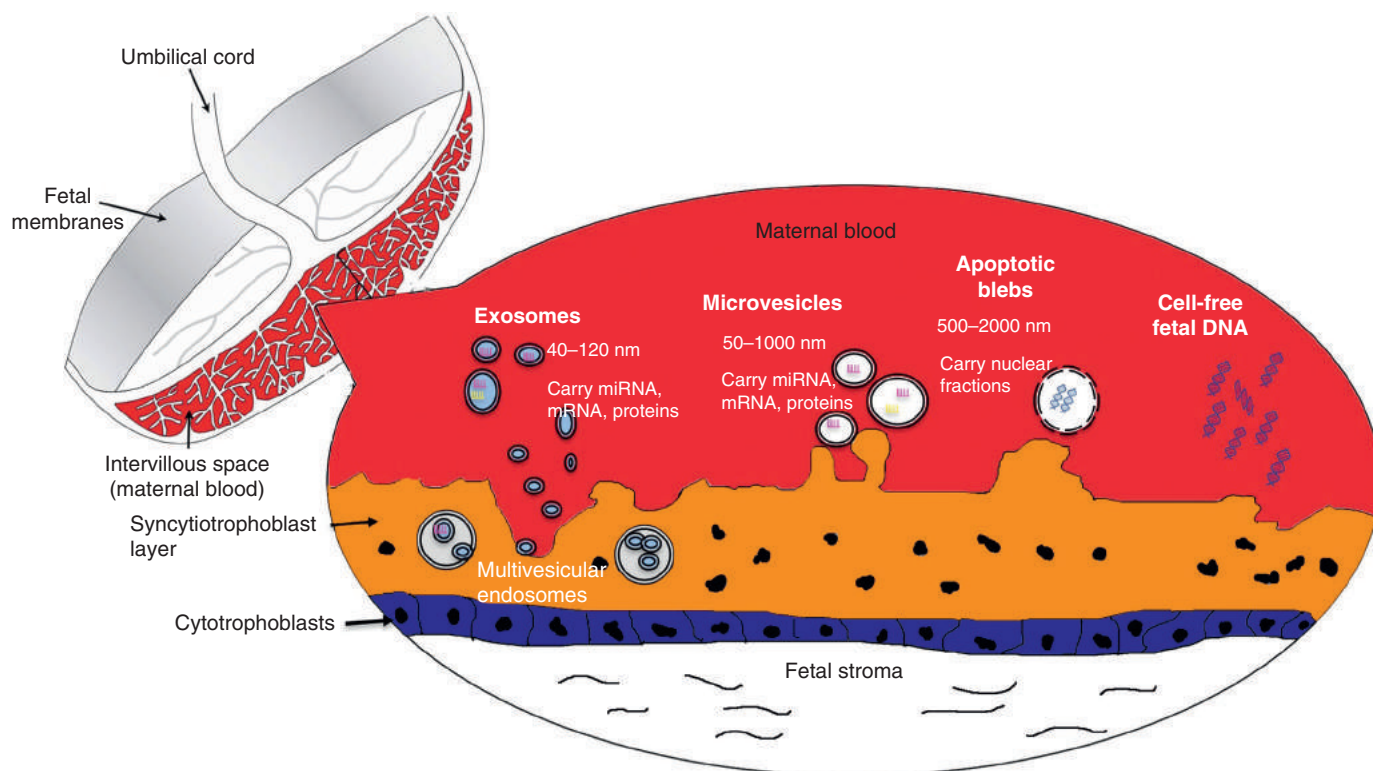
Understanding placental dysfunction and disease during pregnancy is critical to improving neonatal outcomes. Healthy development of the placenta requires efficient metabolic, immune, hormonal, and vascular adaptation by the maternal system as well as the fetus. Abnormal placentation and placental infections can lead to PE, growth retardation, or preterm birth, which can have a lifelong bearing on health. Most major obstetric syndromes originate in early gestation because of abnormal trophoblast invasion or immune dysregulation but present clinically in late gestation. Maternal factors such as ascending infections, obesity, hypertension, diabetes, and environmental exposures also contribute to placental dysfunction.

Placental Disorders of Pregnancy

Preeclampsia

Maternal hypertension affects 5%–10% of human pregnancies, mainly due to PE (Ghulmiyyah and Sibai, 2012). PE is clinically associated with maternal symptoms of hypertension, proteinuria, and glomeruloendotheliosis; it can progress to eclampsia resulting in seizure, coma, and maternal death. The most common fetal complication is growth restriction (Srinivas et al., 2009). Early onset (<24 weeks' gestation) carries a greatly increased risk of IUGR. PE resolves only with delivery of the fetus and placenta. The maternal symptoms are mediated mainly by secreted placental factors, while the fetal symptoms result from impaired placental perfusion. Abnormal remodeling of SpAs and shallow trophoblast invasion are two hallmark features of PE (Brosens et al., 2011), but the etiology of this failure to invade remains unclear; endothelial dysfunction is a key component, and both fetal and maternal factors are thought to contribute. Poor placental perfusion may cause release of circulating factors leading to maternal symptoms, which then exacerbate placental failure and impaired fetal growth.

Despite limited mechanistic understanding of placental pathology leading to PE, several pathways are consistently implicated in this disease. Angiogenic and antiangiogenic factors, excessive complement cascade activation, and immune intolerance may all play a role. In normal pregnancy, angiogenic factors including VEGF and circulating PlGF steadily increase in the first and second trimesters,



• **Fig. 5.7** Placental Products Circulate in Maternal Blood During Gestation. The placental syncytiotrophoblasts produce and secrete extracellular vesicles of various sizes, shapes, and functions into the maternal circulation in both healthy and compromised pregnancies. The function and cargo of these vesicles is not yet completely defined but likely plays a role in maternal–fetal cross talk between cells during pregnancy. Circulating placental products may also provide biomarkers for placental function and pathologies. Exosomes are constitutively secreted products of the endosomal pathway that carry proteins, micro ribonucleic acid (RNA) and messenger RNA that can modulate maternal cell functions, including immune and endothelial responses. Microvesicles and apoptotic bodies are larger vesicles produced by direct budding of the plasma membrane following alterations in cellular conditions (oxygen tension, glucose, or calcium level changes) or as part of apoptosis, respectively. These vesicles may carry unsorted protein and nucleic acid cargo picked up from the cell cytosol. They may have a proinflammatory role in pregnancy. Circulatory cell-free DNA can be up to 13% of cell-free DNA in maternal circulation. Circulatory cell-free DNA is fragmented and significantly smaller than maternal fragments, allowing fragment size and sequence to be used to distinguish fetal from maternal origin. *mRNA*, Messenger ribonucleic acid; *miRNA*, micro RNA; *nm*, nanometers.

peak at 29 to 32 weeks, and decline thereafter. However, free VEGF remains low and unchanged during this window. Reduced placental expression of VEGF and PlGF is consistently observed in PE. Furthermore, PE is frequently accompanied by enhanced placental expression and free circulation of the soluble fms-like tyrosine kinase-1 (sFlt-1), which binds to and inactivates VEGF and PlGF (Levine et al., 2004a; Romero et al., 2008). Soluble endoglin, which enhances sFlt-1 activity, is also elevated in PE (Levine et al., 2004a; Venkatesha et al., 2006). Excessive complement cascade activation and immune activation may also contribute to PE, although whether it is a cause or effect of the pathology is unclear (Lynch et al., 2008). Altered regulatory T-cell function may alter trophoblast interaction with the uterine lining, and increased villous turnover from placental damage may increase maternal immune system response to circulating placental debris (Laresgoiti-Servitje et al., 2010).

Intrauterine Growth Restriction

IUGR or fetal growth restriction designates a fetus that has not reached its growth potential; it can be caused by fetal, placental, or maternal factors. Disparities between fetal nutritional or

respiratory demands and placental supply can result in impaired fetal growth. PE is a frequent cause of IUGR, but placental surface area reduction with decreased villi and fetal capillaries can be seen in IUGR alone, suggesting distinctive disease etiologies (Daayana et al., 2004; Srinivas et al., 2009). Chromosomal abnormalities (aneuploidy, partial deletions, and gene mutation, particularly on the gene for IGFs), congenital abnormalities, multiple gestation, and infections can also result in IUGR. IUGR may result in an SGA newborn. Mortality and morbidity are increased in SGA infants compared with those who are appropriate for gestational age. At birth, SGA infants may have impaired thermoregulation; poor cardiopulmonary transition with perinatal asphyxia, pulmonary hypertension, hypoglycemia, polycythemia, and hyperviscosity; impaired cellular immune function; and increased risk for perinatal mortality. In childhood, having been SGA increases the risk of neurodevelopmental impairments, and in adulthood, cardiovascular and metabolic disease risks are elevated (Sallout and Walker, 2003).

Preterm Birth

Infants born before the 37th week of gestation are considered premature, and their care places an enormous burden on the

healthcare infrastructure. Preterm infants face an increased risk of lifelong disabilities such as mental retardation, learning and behavioral problems, autism, cerebral palsy, chronic lung diseases, vision and hearing loss, and an increased risk for diabetes, hypertension, and heart disease in adulthood. In countries such as the United States, preterm birth accounts for approximately 10%–13% of all deliveries (Goldenberg et al., 2008). Despite improvements in our understanding of the risk factors associated with preterm delivery, the rate of prematurity has risen over the past two decades, due in large part to an increase in the rate of indicated preterm deliveries (Goldenberg et al., 2008). In the United States, iatrogenic delivery is responsible for almost half the births that occur between 28 and 37 weeks of gestation, primarily because of placental pathologies such as PE or IUGR. The majority of spontaneous preterm deliveries are due to preterm labor. Other factors leading to spontaneous premature birth are preterm premature rupture of membranes, cervical incompetence, and antepartum bleeding. Additional risk factors for preterm birth include stress, occupational fatigue, uterine distention by polyhydramnios or multifetal gestation, systemic infection such as periodontal disease, intrauterine placental pathology such as abruption, vaginal bleeding, smoking, substance abuse, maternal age (<18 or >40 years), obesity, diabetes, thrombophilia, ethnicity, anemia, and fetal factors such as congenital anomalies and growth restriction.

There is increasing evidence that approximately 50% of preterm births are associated with infection of the decidua, amnion, or chorion and amniotic fluid caused by either systemic or ascending genital tract infection (Goldenberg et al., 2008). Both clinical and subclinical chorioamnionitis are implicated in preterm birth. Maternal or fetal inflammatory responses to chorioamnionitic infection can trigger preterm birth (Romero et al., 2014). Activated neutrophils and macrophages and the release of cytokines IL-1 β , IL-6, IL-8, TNF- α , and G-CSF can lead to an enhanced cascade of signaling activity, causing release of PGs and expression of various MMPs of fetal membranes and the cervix. Elevated levels of TNF- α and apoptosis are associated with term premature rupture of membranes. Noninfection-related inflammation caused by placental insufficiency and apoptosis can also cause preterm birth. In addition to augmented inflammatory responses to infections, pathogenic microbes (e.g., *Staphylococcus*, *Streptococcus*, *Bacteroides*, and *Pseudomonas* spp.) are thought to directly degrade fetal membranes by releasing proteases, collagenases, and elastases, producing phospholipase A2, and releasing endotoxin that stimulates uterine contractions and causes preterm birth (Slattery and Morrison, 2002).

Interaction between the maternal HPA axis as a result of major maternal physical or psychological stress may also alter the normal placental production of HPA-like hormones, including increased production of CRH, which leads to increased fetal ACTH and cortisol production. Premature activation of the fetal HPA axis can eventually stimulate PG production, ultimately resulting in parturition. In addition, activation of the HPA axis promotes increases in estrogens, decreased progesterone, and OTR expression, further enhancing myometrial activation and preterm birth (Jones and Challis, 1989; Mesiano and Jaffe, 1997).

Gestational Diabetes

Impaired maternal glucose tolerance during pregnancy results in GDM. Impaired placental function may alter hCS production, and in turn, hyperglycemia can impair placental development, growth, and nutrient transport (Araujo et al., 2015). Remarkably, GDM can be associated with either IUGR or, more commonly, with fetal overgrowth (Sibley et al., 2005; Jansson et al., 2006). Placental integration of multiple signals including glucose levels,

intrinsic genetic susceptibility, and fetal demands may be a critical regulator of this fetal growth outcome (Sibley et al., 2005).

Central Nervous System Injury

Both acute and chronic placental dysfunction are linked to adverse neurologic outcomes in fetuses and neonates. Acute mechanical disruptions of placental function (umbilical cord occlusion or placental abruption) can result in severe neurologic hypoxic–ischemic damage before or during delivery but are relatively rare events. More commonly, chronic placental vascular lesions (chronic villitis, fetal thrombotic vasculopathy, or infection-associated fetal vasculitis) are correlated with neurologic injury, including cerebral palsy (Redline, 2005). Almost 90% of term infants with neonatal encephalopathy and brain injury have placental lesions noted postdelivery (Wintermark et al., 2010), although the high frequency of placental lesions in apparently neurologically intact newborns makes interpretation complex (Lachapelle et al., 2015; Redline, 2015). Preterm birth is an independent risk factor for neurologic injury, but chorioamnionitis, which frequently causes preterm delivery, is also associated with brain injury and increased risk of cerebral palsy, particularly when funisitis or severe fetal inflammation is present (Leviton et al., 1999; Romero et al., 2014; Kim et al., 2015a). IUGR infants are also at significantly increased risk of neurodevelopmental impairments (Redline et al., 2007; Apel-Sarid et al., 2010).

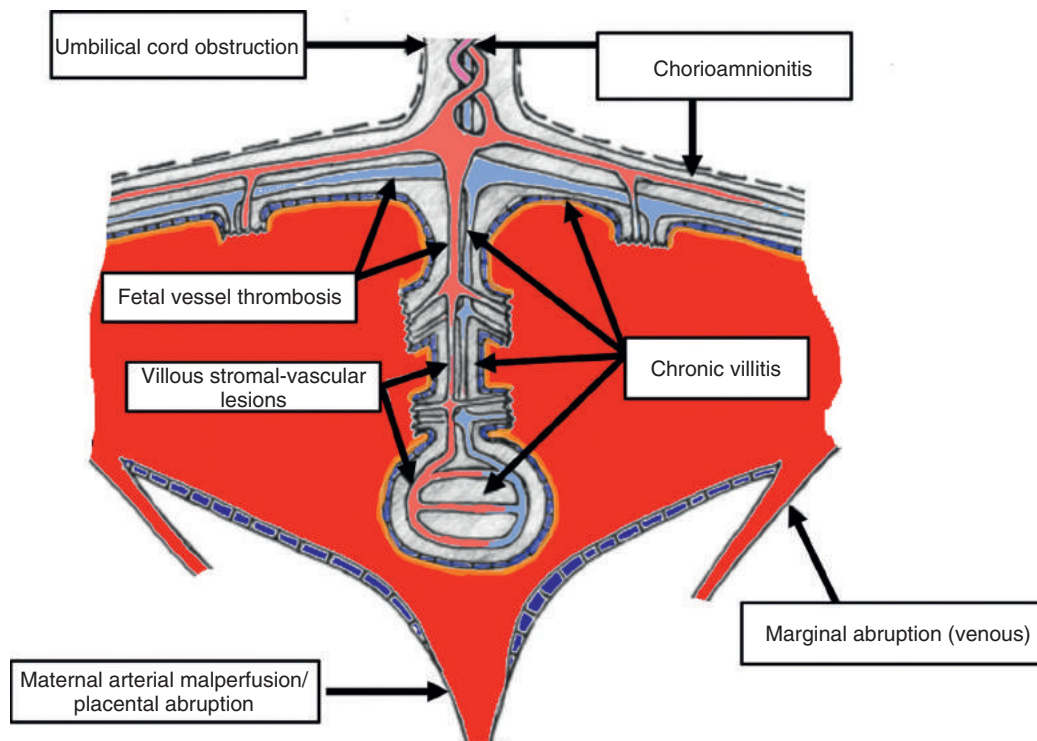
Evaluation of Placental Dysfunction

Until recently, placental assessment was performed almost exclusively after delivery, using traditional anatomic pathology techniques. While significant correlations have been described between specific pathologic lesions and neonatal outcomes, our understanding of how and when these lesions develop during gestation and ultimately lead to poor outcomes remains limited. There has been a recent resurgence of interest in developing advanced tools to investigate placental function during gestation, particularly following the launch of the “Human Placenta Project” by the Eunice Kennedy Shriver National Institutes of Child Health and Disease (Guttmacher et al., 2014; Guttmacher and Spong, 2015). In addition to promoting the development of new advanced imaging techniques (Andescavage et al., 2015; Siauve et al., 2015) and biomarker methods for measuring placental function (Cuffe et al., 2017), there has been a renewed interest in standardizing placental histopathologic classification and diagnosis. In 2014 an international group of placental pathologists met in Amsterdam to establish consensus guidelines for placental examination and classification of lesions (Khong et al., 2016), and these are summarized in Table 5.1.

Placental Histopathology

Direct examination of the placenta after birth can give some clues to the timing and extent of important adverse prenatal or neonatal events. Some disorders are readily apparent in the delivery room (listeria lesions, placental abruption associated with large clots, abnormal cord insertion), and others require more detailed gross and microscopic examination. In a high-risk delivery service, approximately 50% of placentas qualify for pathologic examination (Curtin et al., 2007).

The majority of placental lesions described by pathologists involve either vascular or immunologic/infectious processes (Table 5.1). Vascular processes can be further localized as maternal or fetal lesions (Fig. 5.8, villous maternal stromal-vascular lesions versus fetal-vascular lesions), potentially providing clues to both the underlying etiology of the lesion and its implications for maternal



• **Fig. 5.8** Histopathologic Sites of Action of Major Placental Disease Processes. (Modified from Redline RW. The clinical implications of placental diagnoses. *Semin Perinatol.* 2015;39:2–8.)

or fetal death. Immune processes likewise can be subdivided into chronic or acute infections and also distinguished from inflammatory processes associated with immune activation without infection (see Fig. 5.8, chronic villitis). Links have been made between each major pathologic lesion type and different pregnancy complications (Table 5.2).

Placental Imaging

Ultrasound (US) imaging remains the standard imaging method used for placental evaluation during pregnancy because of its availability, safety, and relatively low cost. However, US evaluation of placental anatomy can be limited by placental implantation site, maternal body habitus, and amniotic fluid volume, and US-detected lesions correlate poorly with postnatal histopathology (Moran et al., 2011). As a primary screening tool, placental US has proven very useful, but its use for functional placental assessment that can predict which pregnancies are at risk of later placental compromise is limited.

The placenta may be visible on US as early as 5 weeks' gestation (Wong et al., 2009). It becomes readily visible by 15 weeks' gestation by US and undergoes progressive increases in thickness and diameter as well as changes in echogenicity and shape (Moran et al., 2011). Both increased and decreased placental size are associated with abnormal development and risk of fetal complications. Many placental lesions can be seen by US as pregnancy progresses. Some, such as placental lakes, which are anechoic regions of low maternal blood flow, are very common but appear to be of limited clinical significance. Others, including echogenic areas of infarct, may be significant when large or centrally located. Placental calcifications increase across gestation. There appears to be an association between early development of calcifications and poor placental function, but use of early, high-grade calcification to predict pregnancy outcome has proved unreliable (Moran et al., 2011). Doppler

velocimetry is used in conjunction with anatomic US to functionally assess placental blood flow (Abramowicz and Sheiner, 2008). Fetal villous vascular damage results in high resistance in the umbilical artery (UA) circulation, and chronic fetal hypoxia decreases umbilical venous flow. Loss of UA end-diastolic flow is associated with severe IUGR and indicative of significant fetal compromise. Monitoring of placental blood flow allows detection of high resistance and poor circulation within the placenta but is usually apparent only when significant fetal compromise has already occurred. Additional US measures that may be more predictive of placental compromise, such as 3-dimensional placental volumes and vascularization indices (Moran et al., 2011), as well as new elastography and higher resolution US techniques, are under investigation.

Magnetic resonance imaging (MRI) is increasingly being used for anatomic placental evaluation, and advanced functional techniques may yield information on oxygenation, vascularization, and metabolism (Andescavage et al., 2015; Siauve et al., 2015). MRI benefits from having multiplanar images in a wider field of view, as well as having higher spatial and temporal resolution when compared with US. It is currently used primarily to assess fetal structural anomalies and, more recently, for improved detection of placenta accreta and other invasive placental anomalies (Lam et al., 2002). Precise quantitation of placental volume is more readily performed using MRI than US and may allow earlier prediction of fetal growth retardation based on small placental size (Derwig et al., 2011; Andescavage et al., 2015). Use of standard MRI gadolinium-based contrast agents has been limited due to fetal safety concerns, but new agents are being developed. Placental application of noncontrast functional MRI methods, including diffusion-weighted imaging and diffusion tensor imaging, are being investigated along with methods that rely on endogenous contrast agents, such as hemoglobin-based detection of oxygen level changes measured by blood-oxygen level-dependent MRI (Andescavage

TABLE 5.2 Common Placental Causes of Specific Adverse Pregnancy Outcomes

1. Fetal Growth Restriction
Maternal stromal-vascular lesions: global/partial vascular malperfusion with accelerated villous maturation
Fetal stromal-vascular lesions: developmental lesions (superficial implantation), global/partial vascular malperfusion (fetal thrombotic vasculopathy)
Villitis of unknown etiology
2. Spontaneous Preterm Birth
Maternal inflammatory response: acute chorioamnionitis
Maternal vascular lesions: mild global/partial vascular malperfusion with accelerated villous maturation
Acute marginal placental abruption
3. Preterm Fetal Death
Maternal stromal-vascular lesions: global/partial vascular malperfusion with accelerated villous maturation
Fetal global/partial vascular malperfusion (cord accidents/obstruction)
Placental abruption
4. Term Fetal Death
Fetal stromal-vascular lesions: developmental lesions (delayed villous maturation), global/partial vascular malperfusion (cord accidents/obstruction)
Placental abruption
Fetomaternal hemorrhage
5. Term Neurologic Injury
Fetal stromal-vascular lesions: global/partial vascular malperfusion (fetal thrombotic vasculopathy or cord occlusion)
Chronic villitis of unknown etiology with obliterative fetal vasculopathy
Acute chorioamnionitis with severe fetal inflammatory response
Presence of multiple placental lesions

Modified from Redline RW. Classification of placental lesions. *Am J Obstet Gynecol*. 2015 Oct;213(4 Suppl):S21-8.

et al., 2015; Siauve et al., 2015). Use of placental MRI for real-time assessments is likely to provide new information about placental vascular development and function, as well as new diagnostic tools for use in high-risk pregnancies.

Serum Biomarkers of Placental Disease

A major goal of pregnancy screening is to identify women early in gestation who will go on to develop placenta-mediated complications that threaten either fetal or maternal health so that targeted early therapies can be provided. Detection of factors released by the placenta into maternal circulation that predict disease is a longstanding area of investigation. In addition to physiologic secretion of placental factors into maternal circulation, cellular stress (i.e., oxidative, hypoxic, or inflammatory stress) can lead to increased villous trophoblast turnover with release of placental vesicles and cellular debris into the circulation (Cuffe et al., 2017). Maternal serum analytes, circulating cell-free DNA, and extracellular vesicle contents derived from the placenta are under investigation as potential biomarkers of placental dysfunction.

Serum Analytes

Maternal serum screening has been applied successfully in the identification of fetuses at increased risk of aneuploidy or structural anomalies (open neural tube defects, abdominal wall defects). The association is less clear between pregnancies at risk of placental dysfunction and abnormal values for the most common first and

second trimester serum screening markers: alpha fetoprotein, hCG, unconjugated estriol (uE₃), inhibin-A, and pregnancy-associated plasma protein-A (PAPP-A). Both single serum analyte abnormalities and combinations have been assessed for their value as biomarkers of specific pregnancy complications, with limited success (Gagnon et al., 2008). For example, higher levels of second trimester inhibin-A levels are associated with PE, although no predictive cutoff level has been identified. Elevated inhibin-A in combination with UA Doppler abnormality, however, may be strongly predictive of PE (Ay et al., 2005). Likewise, extensive studies of low PPAP-A and UA Doppler changes suggest an association with fetal growth restriction and PE (Pilalis et al., 2007). Some alterations of these maternal serum markers have been associated with specific pathologies linked to poor placental function, but the strongest associations are serum marker abnormalities and a generalized increased risk of third trimester fetal death (Gagnon et al., 2008). Increased surveillance or treatment of these pregnancies has not yet shown clinical benefit.

Additional maternal serum biomarkers of placental dysfunction, particularly in PE, include elevated circulating sFlt-1 and reduced PlGF. Syncytial stress leads to placental secretion of these and other angiogenic factors (Cuffe et al., 2017). A recent metaanalysis suggested that the diagnostic accuracy of maternal sFlt-1/PlGF for early onset PE is high, but false positives and false negatives are both greater than 15%, limiting the utility of this ratio as a broad clinical screening tool (Liu et al., 2015).

Circulating Cell-Free Fetal DNA

Direct measurement of DNA found in maternal serum has become possible in the past decade. Continuous turnover of placental villous trophoblasts releases placental microparticles and freely circulating nucleic acids (see Fig. 5.7). These fragments are called fetal but actually originate from the placenta (Taglauer et al., 2014). Noninvasive prenatal screening for aneuploidies and genetic mutations using circulating cell-free fetal DNA (cffDNA) screening from maternal serum has rapidly gained popularity in the past 5 years. Confined placental mosaicism with a normal fetal karyotype can confound these screening results, but the high sensitivity and specificity of these tests combined with their limited risk compared with chorionic villous sampling or amniocentesis have lead to their rapid clinical adoption. Total cffDNA levels, rather than specific cffDNAs, may also be useful biomarkers for placental health and function. cffDNA is increased in PE, both before and during the development of clinical symptoms, likely because of increased trophoblast apoptosis associated with oxidative stress (Levine et al., 2004b). High concentrations of cffDNA are also associated with increased preterm birth risk (Farina et al., 2005). Cell-free RNA and miRNAs have also been found in maternal circulation, and their utility as biomarkers is actively being assessed.

Extracellular Vesicles

Multiple types and sizes of vesicles are shed by the placenta in both normal and compromised pregnancies (see Fig. 5.7), but the amounts and contents vary with placental health (Cuffe et al., 2017). Exosomes are a subtype of extracellular vesicle derived from endosomes that carry proteins and RNAs and that are released by exocytosis into the extracellular space. Exosomes play a significant role in intercellular signaling in multiple systems, and their role in pregnancy has garnered intense interest in the past few years (Mitchell et al., 2015). Placentally derived exosomes carry SynT specific proteins, including placental alkaline phosphatase and the miRNAs from H19 described previously, allowing their identification as placental vesicles in maternal circulation. Their release is regulated

by multiple environmental factors including oxygen tension and glucose concentrations, making them particularly appealing as reporters of placental function. Exosomes mediate communication between the placenta and maternal immune cells, and widespread placental–maternal cellular communication using this mechanism has been proposed. There is a general increase in placental exosomes

in maternal circulation across gestation, and these levels may vary with placental pathology; the vesicle contents may also reflect pathology, such as decreased cell fusion proteins in exosomes from preeclamptic placentas (Mitchell et al., 2015). As placental exosome biology becomes better defined, use of exosomes as biomarkers of placental function is an exciting possibility.

Summary

The placenta is a complex organ that develops from many cell types to form a sophisticated interface between mother and fetus that integrates intrinsic and extrinsic signals to optimize fetal development. The consequences of impaired placental function have

lifelong impact not only on individual offspring but potentially on multiple generations, effected by epigenetic changes. Understanding and optimizing placental health is critical, not just to newborns but also to improving health outcomes for the entire population.

Suggested Readings

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6

Abnormalities of Fetal Growth

REBECCA SIMMONS

KEY POINTS

- Customized fetal growth charts remain controversial.
- The major determinant of intrauterine growth is the placental supply of nutrients to the fetus.
- Fetal factors restricting intrauterine growth include fetal gender, familial genetic inheritance, and chromosomal abnormalities or dysmorphic syndromes.
- Fetal growth differs for women of diverse ethnicities, with Latina and white women having higher rates of large for gestational age (LGA) infants and African-American and South Asian women having a higher incidence of small for gestational age (SGA) infants.
- Impaired fetal growth is linked to the later development of diseases such as type 2 diabetes and heart disease.

Normal fetal growth is determined by a number of factors, including genetic potential, the ability of the mother to provide sufficient nutrients, the ability of the placenta to transfer nutrients, and intrauterine hormones and growth factors. The pattern of normal fetal growth involves rapid increases in fetal weight, length, and head circumference during the last half of gestation. During the last trimester, the human fetus accumulates significant amounts of fat. The birthweight for gestational measurements among populations has been shown to increase over time, and thus standards for normal fetal growth require periodic re-evaluation for clinical relevance. These increases in birthweight for gestational age over time are attributed to improvements in living conditions and maternal nutrition and changes in obstetric management. Variations in fetal growth have been identified in diverse populations and are associated with geographic locations (sea level versus high altitude), populations (white, African-American, Latino), maternal constitutional factors, parity, maternal nutrition, fetal gender, and multiple gestations. In this chapter, we discuss these factors in greater detail and critically review the long-term effects of abnormal fetal growth.

Defining Fetal Growth

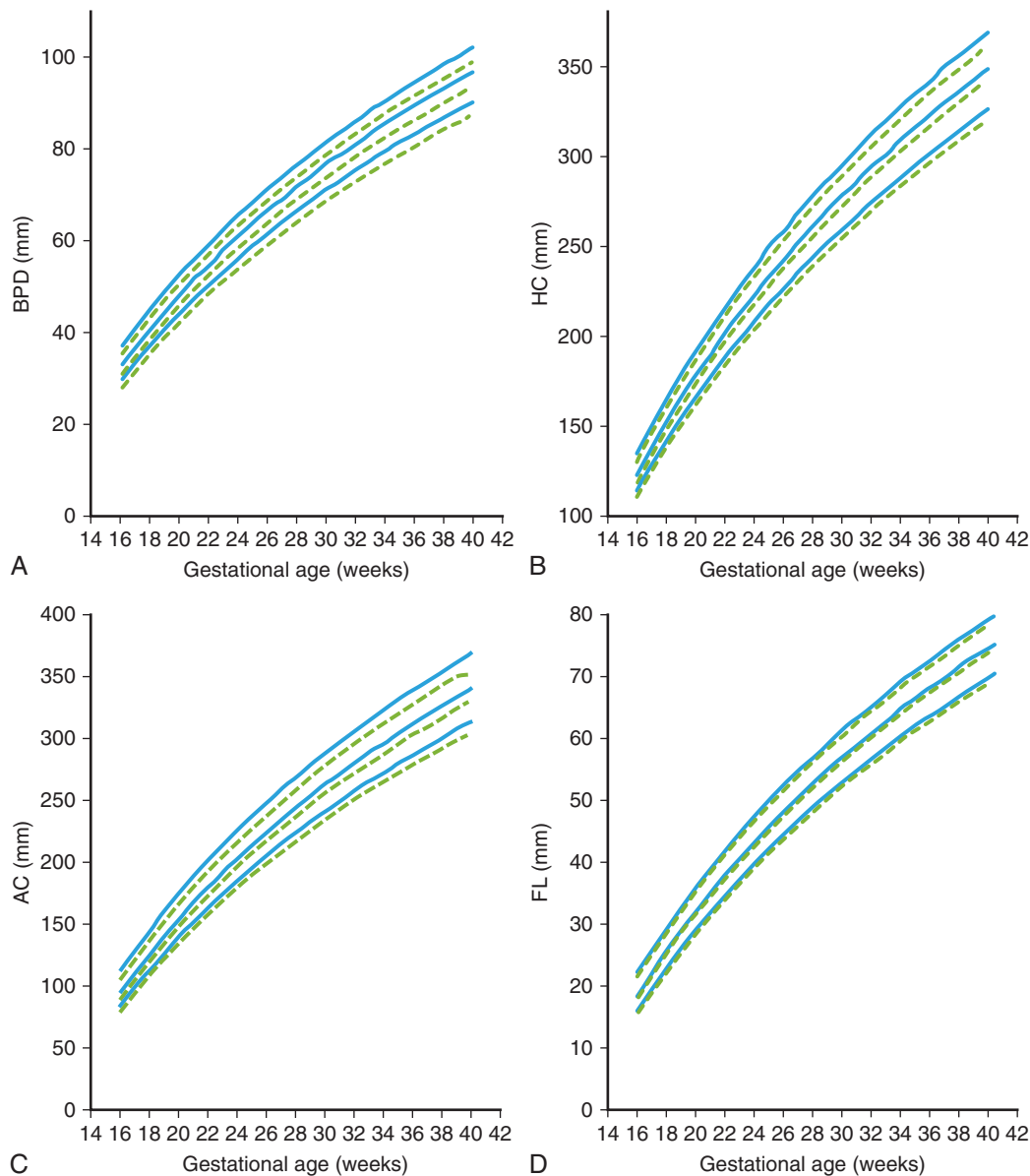
The duration of pregnancy is an integral component of prenatal growth assessment, and all currently prevailing definitions of fetal growth are gestational age (GA) specific. Assessing the GA accurately, however, can be challenging, and any error in dating will lead to misclassification of the infant, which can have significant clinical implications. In many instances, the method of GA determination has contributed to variations in the GA specific reference growth

curves. For example, some nomograms are based on approximating the GA to the nearest week, whereas others use the completed weeks. The birthweight charts are also affected by other variables that may limit their reliability. Many of these factors, such as fetal gender, race, parity, birth order, parental size, and altitude, contribute to the normal biologic variations in human fetal growth.

There is continuing controversy on whether the reference growth charts should be customized by multiple variables or developed from the whole population. The customized approach predicts the optimal growth in an individual pregnancy and therefore specifically defines suboptimal growth for that pregnancy. However, it has been argued that such an approach may lead to a profusion of standards and may not contribute to improving the outcome of small for gestational age (SGA) infants. In recognition of the utility of a national standard, a population-based reference chart for fetal growth was developed from all the singleton births (over 3 million) in the United States in 1991 (Alexander et al., 1999). More recently, a similar national population-based fetal growth chart, which is also sex specific, has been developed in Italy. In a multicenter cross-sectional study, 8070 ultrasonographic examinations from low-risk singleton pregnancies between 16 and 40 weeks' gestation were used to develop growth curves based on biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC), and femur length (FL). Quantile regression was used to examine the impact of fetal sex across the biometric percentiles of the fetal measurements considered together with parents' height, weight, parity, and race (Rizzo et al., 2016) (Fig. 6.1).

There is insufficient evidence about whether one approach is superior to the other in improving the perinatal outcome.

There is no universal agreement on the classification of a SGA infant. Various definitions appear in the medical literature, making comparisons between studies difficult. Additionally, investigators have shown that the prevalence of fetal growth restriction varies according to the fetal growth curve used (Alexander et al., 1996). The most common definition of SGA refers to a weight below the 10th percentile for GA or birthweight less than two standard deviations from the mean. Some investigators use measurements below the third percentile to define SGA. However, these definitions do not make a distinction between those infants who are constitutionally small, those who are growth restricted and small, and those who are not small but growth restricted relative to their potential. For example, as many as 70 percent of fetuses who weigh below the 10th percentile for GA at birth are small simply because of constitutional factors such as female sex or maternal ethnicity, parity, or body mass index (BMI); they are not at high risk of perinatal mortality or morbidity. In contrast, true fetal growth



• **Fig. 6.1** Comparison of the 5th (bottom line), 50th (middle line), and 95th (top line) centiles of the biometric measurements between male (blue line) and female sex (green dotted line) in a fetus with a paternal height of 180 cm, maternal height of 160 cm, maternal weight of 60 kg, mother nulliparous, and of Caucasian race. (A) Biparietal diameter. (B) Head circumference. (C) Abdominal circumference. (D) Femur length. AC, Abdominal circumference; BPD, biparietal diameter; FL, femur length; HC, head circumference. (Data from Rizzo G, Prefumo F, Ferrazzi E, et al. The effect of fetal sex on customized fetal growth charts. *J Matern Fetal Neonatal Med.* 2016;29:3768–3775.)

restriction is associated with numerous perinatal morbidities. This has clinical relevance to perinatologists and neonatologists, as many of the tiniest premature neonates in the neonatal intensive care units are probably growth restricted. McIntire and colleagues (1999) reported a “threshold” of increased adverse outcomes in infants born with measurements below the third percentile and suggested that this level of restriction represents a clinically relevant measurement. Other researchers have found higher rates of neonatal complications when the 15th percentile of birthweight is used as a cutoff level (Seeds and Peng, 1998).

There is an important distinction in identifying the fetus that suffers from intrauterine growth restriction (IUGR) and the fetus that is constitutionally small (SGA). IUGR refers to a condition

in which a fetus is unable to achieve its genetically determined potential size and represents a deviation and a reduction in the expected fetal growth pattern. IUGR complicates about 5%–8% of all pregnancies and 38%–80% of all low birth weight (LBW) neonates. This discrepancy underscores the fact that no uniform definition of IUGR exists. Even when a normal intrauterine growth pattern is established for a population, somewhat arbitrary criteria are used to define growth restriction.

Patterns of Altered Growth

Neonates with IUGR can be classified as demonstrating either symmetric or asymmetric growth. Infants with symmetric IUGR

have reduced weight, length, and head circumference at birth. Weight is affected (and then length) in infants with asymmetric growth restriction, with a relatively normal head circumference or “head-sparing” growth pattern. In general, factors intrinsic to the fetus cause symmetric IUGR, whereas maternal medical conditions such as preeclampsia, chronic hypertension, and uterine anomalies cause asymmetric IUGR. Asymmetric fetal growth patterns generally develop during the third trimester, a period of rapid fetal growth. However, now that fetal surveillance is more common, asymmetric growth restriction is often diagnosed in the second trimester.

Fetal Causes of Growth Restriction

Factors that are well recognized to limit the growth of both the fetal brain and body include chromosomal anomalies (e.g., trisomies), congenital infections (toxoplasmosis, rubella, cytomegalovirus, and herpes simplex [TORCH]), malaria, HIV, and parvovirus, skeletal dysplasias, and some inborn errors of metabolism. Cardiac and renal structural anomalies are common conditions associated with SGA. These conditions restrict fetal growth primarily by impaired cell proliferation. Recognized causes of IUGR are listed in Table 6.1. In one large population-based study, the frequency of IUGR among infants with congenital malformations was 22%. The majority of the infants affected had chromosomal abnormalities. Other studies have similarly found that fetal growth restriction is more common among infants with malformations. Fetal gender also influences size, with male infants showing greater intrauterine growth than female infants (Glinianaia et al., 2000; Skjaerven et al., 2000; Thomas et al., 2000).

TABLE 6.1 Causes of Intrauterine Growth Restriction

| | |
|---|--|
| Genetic | Inheritance, chromosomal abnormalities, fetal gender |
| Maternal constitutional effects | Low maternal prepregnancy weight, low pregnancy weight gain, ethnicity, socioeconomic status, history of IUGR |
| Nutrition | Low prepregnancy weight (body mass index), low pregnancy weight gain, malnutrition (macronutrients, micronutrients), maternal anemia |
| Infections | TORCH infections |
| Decreased O ₂ -carrying capacity | High altitude, maternal congenital heart disease, hemoglobinopathies, chronic anemia, maternal asthma |
| Uterine/placental anatomy | Abnormal uterine anatomy, uterine fibroid, vascular abnormalities (single umbilical artery, velamentous umbilical cord insertion, twin-twin transfusion), placenta previa, placental abruption |
| Uterine/placental function | Maternal vasculitis (system lupus erythematosus), decreased uteroplacental perfusion, maternal illness (preeclampsia, chronic hypertension, diabetes, renal disease) |
| Toxins | Tobacco, ethanol, lead, arsenic |

IUGR, Intrauterine growth restriction; TORCH, toxoplasmosis, rubella, cytomegalovirus, syphilis.

Placental Causes of Growth Restriction

In mammals, the major determinant of intrauterine growth is the placental supply of nutrients to the fetus (Fowden et al., 2006). Indeed, in many species, fetal weight near term is positively correlated to placental weight, as a proxy measure of the surface area for maternofetal transport of nutrients. Fetal weight near term is positively correlated to placental weight, and the nutrient transfer capacity of the placenta depends on its size, morphology, blood flow, and transporter abundance (Fowden et al., 2006). In addition, placental synthesis and metabolism of key nutrients and hormones influence the rate of fetal growth (Fowden and Forhead, 2004). Changes in any of these placental factors can, therefore, affect intrauterine growth. However, the fetus is not just a passive recipient of nutrients from the placenta. The fetal genome exerts a significant acquisitive drive for maternal nutrients through adaptations in the placenta, particularly when the potential for fetoplacental growth is compromised.

Placental maturation at the end of pregnancy is associated with an increase in substrate transfer, a slowing (but not cessation) of placental growth, and a plateau in fetal growth near term (Fox, 1997). Fetal size and placental growth are directly related, and placentas from pregnancies yielding growth-restricted infants demonstrate a higher incidence of smallness and abnormality than those from pregnancies with appropriately grown infants. The difference in size is seen even in a comparison of placentas associated with growth-restricted infants and those associated with appropriate for gestational age (AGA) infants of the same birthweight (Heinonen et al., 2001). Clinical conditions associated with reduced placental size (and subsequent reduced fetal weight) include maternal vascular disease, uterine anomalies (fibroids, abnormal uterine anatomy), placental infarctions, unusual cord insertions, and abnormalities of placentation.

Multiple gestations are associated with greater risk for fetal growth restriction. The higher risk stems from crowding and from abnormalities with placentation, vascular communications, and umbilical cord insertions. Divergence in fetal growth appears from about 30 to 32 weeks in twin gestation compared with singleton pregnancies (Alexander et al., 1996; Glinianaia et al., 2000; Skjaerven et al., 2000), although this may occur earlier in gestation (Devoe and Ware, 1995). Abnormalities in placentation are also more common with multiple gestations (Benirschke, 1995). Monochorionic twins can share placental vascular communication (twin-twin transfusion), leading to fetal growth restriction during gestation. Fetal “competition” for placental transfer of nutrients raises the incidence of growth restriction and discordance in growth between fetuses. The rate of birthweights less than the fifth percentile is higher in monochorionic twins. Placental growth is restricted in utero because of limitation in space, leading to a higher incidence of placenta previa in multiple-gestation pregnancies. Additionally, abnormalities in cord insertions (marginal and velamentous cord insertions) and occurrence of a single umbilical artery are more frequently found in multiple gestations.

Investigators have shown an effect of altitude on fetal growth, with infants born at high altitudes having lower birthweights (Moore et al., 2011). Differences in fetal growth are detected from about 25 weeks’ gestation, when pregnant women reside at altitudes greater than 4000 meters. In these high-altitude pregnancies, the AC is most affected (Krampl et al., 2000). Interestingly, investigators have shown that adaptation to high altitude during pregnancy is also possible. Tibetan infants have higher birthweights than infants

of more recent ethnic Chinese immigrants living at the same high-altitude (2700–4700 meters) region of Tibet (Moore et al., 2001). Tibetan infants also have less IUGR than infants born to more recent immigrants to the area.

Maternal Causes of Growth Restriction

Maternal health conditions associated with chronic decreases in uteroplacental blood flow (maternal vascular diseases, preeclampsia, hypertension, maternal smoking) are associated with poor fetal growth and nutrition. Preeclampsia has been shown to be associated with fetal growth restriction (Spinillo et al., 1994; Xiong et al., 1999; Ødegård et al., 2000). Investigators have shown that the extent of growth restriction correlates with the severity and the onset during pregnancy of the preeclampsia. Ødegård et al. (2000) showed that fetuses exposed to preeclampsia from early in pregnancy had the most serious growth restriction, and more than half of these infants were born SGA. Chronic maternal diseases (cardiac, renal) may decrease the normal uteroplacental blood flow to the fetus and thus may also be associated with poor fetal growth (Spinillo et al., 1994).

Maternal constitutional factors have a significant effect on fetal growth. Maternal weight (prepregnancy), maternal stature, and maternal weight gain during pregnancy are directly associated with maternal nutrition and correlate with fetal growth (Goldenberg et al., 1997; Clausson et al., 1998; Mongelli and Gardosi, 2000; Doctor et al., 2001). Numerous studies show that these findings are often confounded by highly associated cultural and socioeconomic factors. The woman with a previous SGA infant has a higher risk of a subsequent small infant (Robinson et al., 2000). Investigators have shown a higher incidence of SGA infants to be associated with lower levels of maternal education (Clausson et al., 1998). Parity of the mother also affects fetal size, nulliparous women having a higher incidence of SGA infants (Cnattingius et al., 1998). A large population-based study in Sweden found that women who were older than 30 years, were nulliparous, or had hypertensive disease were at increased risk of preterm and term growth-restricted infants.

Studies have shown differential fetal growth for women of diverse ethnicities, with Latina and white women having higher rates of large for gestational age (LGA) infants and African-American and South Asian women having a higher incidence of SGA infants (Fuentes-Afflick et al., 1998; Alexander et al., 1999; Collins and David, 2009). These gender and ethnic differences in birthweight become pronounced after 30 weeks' gestation (Thomas et al., 2000). Investigators in California have shown that US-born black women have higher rates of prematurity and LBW infants than foreign-born black women. Other researchers have found that even among women with very low risk of LBW infants (married, age 20–34 years, 13 or more years of education, adequate prenatal care, and absence of maternal health risk factors and of tobacco or alcohol use), the risk of delivering an SGA infant is still higher for African-American women than for white women (Collins and David, 2009; Alexander et al., 1999). It is unclear whether these differences in fetal growth are due to inherent differences or to differential exposure to environmental factors, including stress.

Maternal nutrition significantly impacts fetal growth, primarily in developing countries (Godfrey et al., 1996; Neggers et al., 1997; Robinson et al., 2000; Doctor et al., 2001; Zeitlin et al., 2001). Although numerous factors interact with and affect fetal development, maternal malnutrition is assumed to be a major cause of IUGR in developing countries.

Teen pregnancy represents a special condition in which fetal weight is highly influenced by maternal nutrition. Teen mothers (<15 years) have been shown to have a higher risk for delivering a growth-restricted infant (Ghidini, 1996). Teen pregnancies are complicated by the additional nutritional needs of a pregnant mother, who is still actively growing, as well as by socioeconomic status of pregnant teens in developed countries (Scholl and Hediger, 1995).

The effects of micronutrients on pregnancy outcomes and fetal growth have been less well studied. Maternal intake of certain micronutrients has been found to affect fetal growth. Zinc deficiency has been associated with fetal growth restriction as well as other abnormalities, such as infertility and spontaneous abortion (Jameson, 1993; Shah and Sachdev, 2001). Additionally, dietary intake of vitamin C during early pregnancy has been shown to be associated with an increase in birthweight (Mathews et al., 1999). Other clinicians have shown strong associations between maternal intake of folate and iron and infant and placental weights (Godfrey et al., 1996). In developing countries, the effects of nutritional deficiencies during pregnancy are more prevalent and easier to detect. Rao and colleagues (2001) have estimated that one-third of infants in India are born weighing less than 2500 g, mainly because of maternal malnutrition. These investigators have shown significant associations between infant birthweight and maternal intake of milk, leafy greens, fruits, and folate during pregnancy.

Although toxins such as cigarette smoke and alcohol have a direct effect on placental function, they may also affect fetal growth through an associated compromise in maternal nutrition. Other environmental toxins (lead, arsenic, mercury) are associated with IUGR and are believed to affect fetal growth by entering the food chain and depleting body stores of iron, vitamin C, and possibly other nutrients (Iyengar and Nair, 2000; Srivastava et al., 2001).

Numerous studies have shown associations between birthweight and maternal intake of macronutrients and micronutrients, but the effects of nutritional supplements used during pregnancy on fetal growth are equivocal (de Onis et al., 1998; Jackson and Robinson, 2001; Rush, 2001; Say et al., 2003). This is underscored by the results of a large double-blind, randomized controlled trial including 1426 pregnancies that was carried out in rural Burkina Faso (Roberfroid, 2008). Pregnant women were randomly assigned to receive either iron and folic acid or the UNICEF/WHO/UNU international multiple micronutrient preparation (UNIMMAP) daily until 3 months after delivery. Birthweight was only increased by 52 grams and birth length by 3.6 mm. Unexpectedly, the risk of perinatal death was marginally, though significantly, increased in the UNIMMAP group (odds ratio: 1.78; 95% confidence interval: 0.95, 3.32; $P = .07$).

Maternal socioeconomic status and ethnicity have also been identified as risk factors for IUGR and poor health outcomes in infants, in both developing and developed countries (Wilcox et al., 1995). In the United States, low levels of maternal and paternal education, certain maternal and paternal occupations, and low family income are associated with lower birthweights in children of African-American, Hispanic, and white women (Balcazar, 1994; Parker et al., 1994). In a large population-based study from Sweden, investigators have similarly shown a higher incidence of fetal growth restriction in association with low maternal education (Clausson et al., 1998). The incidence of IUGR is also higher in women without medical insurance (Collins and Butler, 1997; Frisbie et al., 1997; Thomas et al., 2000). Interestingly, Mexican-born immigrants in California have better perinatal outcomes (including birthweight) than both African Americans and US-born women of Mexican

descent (Fuentes-Afflick et al., 1998). The reasons for this apparent paradox are unclear, but one postulate is the tendency of recent immigrants to maintain the favorable nutritional and behavioral characteristics of their country of origin (Guendelman and English, 1995). These studies support the speculation that the differences in fetal growth between groups do not reflect inherent differences in fetal growth but rather stem from inequalities in nutrition, health care, and other environmental factors (Keirse, 2000; Kramer et al., 2000).

Finally, multiple studies have shown an association between assisted reproductive technology (ART) and low birth weight (Hansen and Bower, 2014). Recent metaanalyses of infants born following ART compared with non-ART singletons show increases in low birth weight, preterm birth, and small for gestational age (SGA). Although there have been small reductions in recent data, odds associated with these outcomes are still higher for ART singletons. It is generally thought that both ART procedures and underlying infertility contribute to these increased risks.

Maternal Smoking

Cigarette smoking is consistently found to adversely affect intrauterine growth in all studies in which this factor is considered. In developed countries, cigarette smoking is the single most important cause of poor fetal growth (Kramer et al., 2000). The incidence of IUGR in smokers is estimated to be 3–4.5 times higher than in nonsmokers (Nordentoft et al., 1996; Bernstein et al., 2000). Lieberman and associates (1994) report that cigarette smoking also appears to have a dose-dependent effect on the incidence of IUGR, with this effect being seen especially with heavy smoking and smoking during the third trimester. These investigators have shown that if women stop smoking during the third trimester, their infants' birthweights are indistinguishable from those of infants born to the normal population. Other researchers have shown that even a reduction in smoking is associated with improved fetal growth (Li et al., 1993; Walsh et al., 2001). Numerous potential causes of the effects of smoking on fetal growth have been suggested, including direct effects of nicotine on placental vasoconstriction, decreased uterine blood flow, higher levels of fetal carboxyhemoglobin, fetal hypoxia, adverse maternal nutritional intake, and altered maternal and placental metabolism (Pastrakuljic et al., 1999; Andres and Day, 2000).

Short-Term Outcomes of Fetal Growth Restriction

IUGR alters many physiologic and metabolic functions in the fetus and neonate that result in a number of morbidities. A large cohort study of 37,377 pregnancies found a fivefold to sixfold greater risk of perinatal death for both preterm and term fetuses with IUGR (Cnattingius et al., 1998; Mongelli and Gardosi, 2000; Lackman et al., 2001). Regarding predictive factors for perinatal mortality in preterm IUGR fetuses, of all the antenatal factors examined, only oligohydramnios and abnormal umbilical artery Dopplers with absent or reversed diastolic flow were predictive of perinatal mortality (Scifres et al., 2009). Although the growth-restricted fetus may show symmetric or asymmetric growth at birth, it is unclear whether the proportionality of the fetus with IUGR truly affects outcomes or is related to the timing or the severity of the insult. Lin and colleagues (1991) found that symmetric IUGR resulted in higher levels of prematurity and higher

rates of neonatal morbidity. In contrast, Villar and associates (1990) have shown that infants with asymmetric IUGR have higher morbidity rates at birth. They found that infants with low ponderal index measurements (which they defined as weight/length³) had higher risk for low Apgar scores, long hospitalization, hypoglycemia, and asphyxia at birth than infants with symmetric IUGR. Other investigators propose that IUGR represents a continuum, with asymmetric IUGR occurring as the severity of the growth retardation increases. Data also suggest that the more severe the growth restriction, the worse the neonatal outcomes, including risk of stillbirth, fetal distress, neonatal hypoglycemia, hypocalcemia, polycythemia, low Apgar scores, and mortality (Kramer et al., 1990; Spinillo et al., 1995).

Fetal growth restriction is associated with intrauterine demise. Almost 40% of term stillbirths and 63% of preterm stillbirths are SGA (Mongelli and Gardosi, 2000). Both short-term and long-term effects of abnormalities in SGA fetuses have been described. Perinatal mortality for intrauterine SGA infants is higher overall than that for appropriately grown term and preterm infants (Clausson et al., 1998). The risk of perinatal death is estimated to be fivefold to sixfold greater for both preterm and term fetuses with IUGR (Lackman et al., 2001). Overall, intrauterine death, perinatal asphyxia, and congenital anomalies are the main contributing factors to the higher mortality rate in SGA infants. The effects of acute fetal hypoxia may be superimposed on chronic fetal hypoxia, and placental insufficiency may be an important etiologic factor in these outcomes. Investigators have described higher incidences of low Apgar scores, umbilical artery acidosis, need for intubation at delivery, seizures on the first day of life, and sepsis in SGA infants (Villar et al., 1990; McIntire et al., 1999). The incidence of adverse perinatal effects correlates with the severity of the growth restriction, the highest rates of respiratory distress syndrome, metabolic abnormalities, and sepsis being found in the most severely growth-restricted infants (Spinillo et al., 1995).

Preterm infants with growth abnormalities have a much higher risk of adverse outcomes. Preterm SGA infants have a higher incidence of a number of complications, including sepsis, severe intraventricular hemorrhage, respiratory distress syndrome, necrotizing enterocolitis, cholestatic jaundice, and death, than normally grown preterm infants (Gortner et al., 1999; McIntire et al., 1999; Simchen et al., 2000; Robinson and Ehrenkranz, 2008). Additionally, SGA infants have higher incidence of chronic lung disease at corrected gestational ages of 28 days and 36 weeks (Bose et al., 2009; Mestan and Steinhorn, 2011; Morsing et al., 2012; Briana and Malamitsi-Puchner, 2013).

Neonatal hypoglycemia and hypothermia occur more frequently in growth-restricted infants (Doctor et al., 2001). These metabolic abnormalities presumably result from decreased glycogen stores, inadequate lipid stores, and impaired gluconeogenesis in the growth-restricted neonate. Growth-restricted neonates have inadequate fuel stores and are at increased risk for hypoglycemia during fasting, and these risks are increased in preterm SGA infants. Infants with IUGR also have a higher incidence of hypocalcemia, the incidence correlating strongly with the severity of the growth restriction (Spinillo et al., 1995).

Developmental Outcomes of Fetal Growth Restriction: Early Childhood

Neurologic outcomes, including intellectual and neurologic function, are affected by growth restriction. Overall, neurologic morbidity

is higher for SGA infants than for AGA infants. Even without identified perinatal events, SGA infants have a higher incidence of long-term neurologic or developmental handicaps (Walker and Marlow, 2008; Batalle et al., 2012; Llurba et al., 2013; Lohaugen et al., 2013; Longo et al., 2013; Von Beckerath et al., 2013). SGA infants born at term appear to have double or triple the risk for cerebral palsy, between 1–2 per 1000 live births and 2–6 per 1000 live births (Goldenberg et al., 1998). The rate of cerebral palsy is also higher in preterm growth-restricted infants than in preterm infants with appropriate fetal growth (Gray et al., 2001). At 7 years of age, children whose birth was associated with hypoxia-related factors had a higher risk for adverse neurologic outcomes. Infants with symmetric IUGR (or perhaps more severe restriction) were at higher risk than infants with asymmetric IUGR. Other researchers have shown higher rates of learning deficits, lower IQ scores, and increased behavioral problems in children with a history of fetal growth restriction, even at 9–11 years of age (Low et al., 1992).

Long-Term Consequences of Fetal Growth Restriction: the Developmental Origins of Adult Disease

Programming

The period from conception to birth is a time of rapid growth, cellular replication and differentiation, and functional maturation of organ systems. These processes are very sensitive to alterations in the intrauterine milieu. *Programming* describes the mechanisms whereby a stimulus or insult at a critical period of development has lasting or lifelong effects. The “thrifty phenotype” hypothesis proposes that the fetus adapts to an adverse intrauterine milieu by optimizing the use of a reduced nutrient supply to ensure survival, but because this adaptation favors the development of certain organs over that of others, it leads to persistent alterations in the growth and function of developing tissues (Hales and Barker, 1992). Also, although the adaptations may aid in survival of the fetus, they become a liability in situations of nutritional abundance.

Epidemiology

It has been recognized for over 80 years that the early environment in which a child grows and develops can have long-term effects on subsequent health and survival (Kermack, 1934). The landmark cohort study of 300,000 men by Ravelli and colleagues (1976) showed that men who were exposed in utero to the effects of the Dutch famine of 1944 and 1945 during the first half of gestation had significantly higher obesity rates at age 19 years. Subsequent studies demonstrated a relation between low birth weight and the later development of cardiovascular disease (Barker et al., 1989) and impaired glucose tolerance (Fall et al., 1995) in men in England. Those men who were smallest at birth (2500 g) were nearly seven times more likely to have impaired glucose tolerance or type 2 diabetes than those who were largest at birth. Barker and colleagues (1993) also found a similar relationship between lower birthweight and higher systolic blood pressure and triglyceride levels.

Valdez and associates (1994) observed a similar association between birthweight and subsequent glucose intolerance, hypertension, and hyperlipidemia in a study of young adult Mexican-American and non-Hispanic white men and women participants

in the San Antonio Heart Study. Normotensive, nondiabetic individuals whose birthweights were in the lowest tertile had significantly higher rates of insulin resistance, obesity, and hypertension than subjects whose birthweights were normal. In the Pima Indians, a population with extraordinarily high rates of type 2 diabetes, McCance and coworkers (1994) found that the development of diabetes in the offspring was related to both extremes of birthweight. In their study, the prevalence of diabetes in subjects 20–39 years old was 30% for those weighing less than 2500 g at birth, 17% for those weighing 2500–4499 g, and 32% for those weighing 4500 g or more. The risk for development of type 2 diabetes was nearly fourfold higher for those whose birthweight was less than 2500 g. Other studies of populations in the United States (Curhan et al., 1996), Sweden (Lithell et al., 1996; McKeigue et al., 1998), France (Leger et al., 1997; Jaquet et al., 2000), Norway (Egeland et al., 2000), and Finland (Forsen et al., 2000) have all demonstrated a significant correlation between low birth weight and the later development of adult diseases.

Studies controlling for the confounding factors of socioeconomic status and lifestyle have further strengthened the association between low birth weight and higher risk of coronary heart disease, stroke, and type 2 diabetes in adulthood. In 1976, the Nurses' Health Study was initiated, and a large cohort of American women born from 1921 to 1946 was established. The association between low birth weight and increased risks of coronary heart disease, stroke, and type 2 diabetes remained strong even after adjustment for lifestyle factors such as smoking, physical activity, occupation, income, dietary habits, and childhood socioeconomic status (Rich-Edwards et al., 1999).

The Role of Catch-Up Growth

Many studies have suggested that the associations between birth size with later disease can be modified by BMI in childhood. The highest risk for the development of type 2 diabetes is among adults who were born small and become overweight during childhood (Eriksson et al., 2000). Insulin resistance is most prominent in Indian children who were SGA at birth but had a high fat mass at 8 years of age (Bavdekar et al., 1999). Similar findings were reported in 10-year-old children in the United Kingdom (Whincup et al., 1997). In a Finnish cohort, adult hypertension was associated with both lower birthweight and accelerated growth in the first 7 years of life. In contrast, in two preliminary studies from the United Kingdom, catch-up growth in the first 6 months of life was not clearly related to blood pressure in young adulthood, although birthweight was (McCarthy et al., 2001).

Interpretation of the findings of these studies is complicated by the vague definitions of *catch-up growth*. The term, which can encompass either the first 6–12 months only or as much as the first 2 years after birth, usually refers to realignment of one's genetic growth potential after IUGR. This definition allows for fetal growth restriction at any birthweight; even large fetuses can be growth restricted in relation to their genetic potential. However, postnatal factors can obviously affect infant growth in the first few months of life. For example, breastfeeding appears to protect against obesity later in childhood, yet breastfed infants usually exhibit higher body mass during the first year of life than formula-fed infants. Although it is likely that accelerated growth confers an additional risk to the growth-retarded fetus, these conflicting results demonstrate the need for additional, carefully designed studies to determine just how childhood growth rates affect the later development of cardiovascular disease and type 2 diabetes.

Size at Birth, Insulin Secretion, and Insulin Action

The mechanisms underlying the association between size at birth and impaired glucose tolerance or type 2 diabetes are unclear. A number of studies in children and adults have shown that nondiabetic or prediabetic subjects with low birth weight are insulin resistant and thus are predisposed to development of type 2 diabetes (Phillips et al., 1994; Yajnik et al., 1995; Lithell et al., 1996; Clausen et al., 1997; Hoffman et al., 1997; Leger et al., 1997; McKeigue et al., 1998; Bavdekar et al., 1999; Flanagan et al., 2000; Li et al., 2001). IUGR is known to alter the fetal development of adipose tissue, which is closely linked to the development of insulin resistance (Widdowson et al., 1979; Lapillonne et al., 1997). In a well-designed case-control study of 25-year-old adults, Jaquet and colleagues (2000) demonstrated that individuals who were born SGA at 37 weeks or later had a significantly higher percentage of body fat (15%). Insulin sensitivity, even after adjustment for BMI or total fat mass, was markedly impaired in these SGA subjects. There were no significant differences between the SGA and control groups with respect to parental history of type 2 diabetes, cardiovascular disease, hypertension, or dyslipidemia. Of importance to generalization of the findings to other populations, the causes of IUGR in these subjects were gestational hypertension (50%), smoking (30%), maternal short stature (7%), congenital anomalies (7%), and unknown (6%).

The adverse effect of IUGR on glucose homeostasis was originally thought to be mediated through programming of the fetal endocrine pancreas. Growth-restricted fetuses and newborns have been shown to have a reduced population of pancreatic β -cells (Van Assche et al., 1977). Jensen and colleagues (2002) measured insulin secretion and insulin sensitivity in a well-matched population of 19-year-old, glucose-tolerant white men whose birthweights were either below the 10th percentile (SGA) or between the 50th and 75th percentiles (controls). To eliminate the major confounding factors, such as “diabetes genes,” the researchers ensured that none of the participants had a family history of diabetes, hypertension, or ischemic heart disease. They found no differences between the groups with regard to current weight, BMI, body composition, and lipid profile. When data were controlled for insulin sensitivity, insulin secretion was found to be lower by 30%. However, insulin sensitivity was normal in the SGA subjects. These investigators hypothesized that defects in insulin secretion may precede defects in insulin action and that once SGA individuals accumulate body fat, they do demonstrate insulin resistance.

Epidemiologic Challenges

The data described in the preceding section suggest that low birth weight is associated with glucose intolerance, type 2 diabetes, and cardiovascular disease. However, the question remains whether these associations reflect fetal nutrition or other factors that contribute to birthweight and the observed glucose intolerance. Because of the retrospective nature of the cohort identification, many confounding variables were not always recorded, such as lifestyle, socioeconomic status, education, maternal age, parental build, birth order, obstetric complications, smoking, and maternal health. Maternal nutritional status, either directly in the form of diet histories or indirectly in the form of BMI, height, and pregnancy weight gain, were usually not recorded. Instead, birth anthropometric measures were used as proxies for presumed undernutrition in pregnancy.

Size at Birth Cannot Be Used as a Proxy for Fetal Growth

Birthweight is determined by the sum of multiple known and unknown factors, including GA, maternal age, birth order, genetics, maternal prepregnancy BMI, and pregnancy weight gain, plus multiple environmental factors, such as smoking, drug use, infection, and maternal hypertension. Some of these determinants may be related to susceptibility to adult disease, and others may not. Conversely, some prenatal determinants of adult outcomes may not be related to fetal growth. A good example of how size at birth may potentially be a proxy for an underlying causal pathway is the hypothesis that essential hypertension in the adult is due to a congenital nephron deficit (Brenner et al., 1993). A study has now shown that kidney volume is smaller in adults who were thinner at birth, after adjustment for current body size.

Genetics versus Environment

Several epidemiologic and metabolic studies of twins and first-degree relatives of patients with type 2 diabetes have demonstrated an important genetic component of diabetes (Vaag et al., 1995). The association between low birth weight and risk of type 2 diabetes in some studies could theoretically be explained by a genetically determined reduced fetal growth rate. In other words, the genotype responsible for type 2 diabetes may itself restrict fetal growth. This possibility forms the basis for the *fetal insulin hypothesis*, which suggests that genetically determined insulin resistance could result in insulin-mediated low growth rate in utero as well as insulin resistance in childhood and adulthood (Hattersley and Tooke, 1999). Insulin is one of the major growth factors in fetal life, and monogenic disorders that affect the fetus's insulin secretion or insulin resistance also affect its growth (Elsas et al., 1985; Froguel et al., 1993; Stoffers et al., 1997; Hattersley et al., 1998). Mutations in the gene encoding glucokinase have been identified that result in low birth weight and maturity-onset diabetes of the young. Although such mutations are rare, and no analogous common allelic variation has yet been discovered, it is likely that some variations exist that, once identified, will explain a proportion of the cases of diabetes in LBW subjects.

There is obviously a close relationship between genes and the environment. Not only can maternal gene expression alter the fetal environment but also the maternal intrauterine environment affects fetal gene expression. An adverse intrauterine milieu is likely to have profound long-term effects on the developing organism that may not be reflected in birthweight.

Cellular Mechanisms of Developmental Programming

Fetal malnutrition has two main causes: poor maternal nutrition and placental insufficiency. In the extensive literature about the fetal origins hypothesis, these two concepts have not been clearly discerned. Such a distinction is necessary, however, because maternal nutrition has probably been adequate in the majority of populations in which the hypothesis has been tested. Only extreme maternal undernutrition, such as occurred in the Dutch famine, reduces the birthweight to an extent that could be expected to raise the risk of adult disease (Lumey et al., 1995). Thus it is reasonable that placental insufficiency has been a main cause of low birth weight in these populations. The oxygen and nutrients that support

fetal growth and development rely on the entire nutrient supply line, beginning with maternal consumption and body size but extending also to uterine perfusion, placental function, and fetal metabolism. Interruptions of the supply line at any point could result in programming of the fetus for the future risk of adult diseases.

The intrauterine environment influences development of the fetus by modifying gene expression in both pluripotent cells and terminally differentiated, poorly replicating cells. The long-range effects on the offspring (into adulthood) are determined by which cells are undergoing differentiation, proliferation, or functional maturation at the time of the disturbance in maternal fuel economy. The fetus also adapts to an inadequate supply of substrates (such as glucose, amino acids, fatty acids, and oxygen) through metabolic changes, redistribution of blood flow, and changes in the production of fetal and placental hormones that control growth.

The fetus's immediate metabolic response to placental insufficiency is catabolism, consuming its own substrates to provide energy. A more prolonged reduction in availability of substrates leads to a slowing in growth. This enhances the fetus's ability to survive by reducing the use of substrates and lowering the metabolic rate. Slowing of growth in late gestation leads to disproportion in organ size, because the organs and tissues that are growing rapidly at the time are affected the most. Placental insufficiency in late gestation may, for example, lead to reduced growth of the kidney, which is developing rapidly at that time. Reduced replication of kidney cells may permanently reduce cell numbers, because there seems to be no capacity for renal cell division to catch up after birth.

Substrate availability has profound effects on fetal hormones and on the hormonal and metabolic interactions between the fetus, placenta, and mother. These effects are most apparent in the fetus of the mother with diabetes. Higher maternal concentrations of glucose and amino acids stimulate the fetal pancreas to secrete exaggerated amounts of insulin and the fetal liver to produce higher levels of insulin-like growth factors. Fetal hyperinsulinism stimulates the growth of adipose tissue and of other insulin-responsive tissues in the fetus, often leading to macrosomia. However, many offspring of diabetic mothers with fetal hyperinsulinism are not overgrown by usual standards, and many with later obesity and glucose intolerance were not macrosomic at birth (Pettitt et al., 1987; Silverman et al., 1995). These observations suggest that birthweight is not a good indication of intrauterine nutrition.

Molecular Mechanisms of Developmental Programming: Epigenetics

Epigenetics is the study of heritable changes in gene expression or phenotype occurring without changes in DNA sequence and is critically important for embryogenesis and normal fetal development. The best example of how epigenetic mechanisms can influence fetal growth are imprinting disorders such as Angelman syndrome and Beckwith–Wiedemann syndrome. Epigenetic modifications of the genome provide a mechanism that allows the stable propagation of gene activity states from one generation of cells to the next. There are at least two distinct classes of epigenetic information that can be inherited with chromosomes. One class of epigenetic control of gene expression involves changes in chromatin proteins, usually involving modifications of histone tails. In eukaryotes, DNA is assembled with histones to form the nucleosome, in which

DNA is wrapped approximately two turns around an octameric complex composed of two molecules of each of the four histones H2A, H2B, H3, and H4. The amino termini of histones can be modified by acetylation, methylation, sumoylation, phosphorylation, glycosylation, and ADP ribosylation.

The second class of epigenetic regulation is DNA methylation, in which a cytosine base is modified by a DNA methyltransferase at the C5 position of cytosine, a reaction that is carried out by various members of a single family of enzymes. Approximately 70% of CpG dinucleotides in human DNA are constitutively methylated, whereas most of the unmethylated CpGs are located in CpG islands. CpG islands are CG-rich sequences located near coding sequences and serve as promoters for the associated genes. Approximately half of mammalian genes have CpG islands. DNA methylation is commonly associated with gene silencing and contributes to X-chromosomal inactivation, genomic imprinting, as well as transcriptional regulation of tissue-specific genes during cellular differentiation (Gopalakrishnan et al., 2008). The methylation status of CpG islands within promoter sequences works as an essential regulatory element by modifying the binding affinity of transcription factors to DNA binding sites.

Most CpG islands remain unmethylated in normal cells; however, under some circumstances such as cancer (Klutstein et al., 2016) and oxidative stress, they can become methylated *de novo*. It is not known why particular CpGs are susceptible to aberrant methylation. A study by Feltus et al. (2003) suggests that there is a “sequence signature associated with aberrant methylation.”

The metabolic or nutritional state of the organism directly influences epigenetic modifications, as essentially all known epigenetic modifications rely upon substrates derived from intermediary metabolism such as S-adenosyl methionine, acetyl CoA, α -ketoglutarate, and nicotinamide adenine dinucleotide (Kaelin and McKnight, 2013).

There are numerous studies in humans examining the relationship between decreased fetal nutrient availability and epigenetic modifications in the offspring (Rakyan et al., 2011). Many of these are confounded by small sample size, cellular heterogeneity of tissues examined, and lack of validation. Moreover, most DNA methylation assays are performed in total peripheral blood monocytes, where the unique methylation profiles of the various cellular lineages complicate interpretation of the data. Furthermore, most changes appear to be stochastic and not reproducible in other cohorts. Despite these issues, multiple studies in diverse populations repeatedly show changes in DNA methylation associated with low birth weight or altered nutrient availability. Thus it is likely that an adverse *in utero* milieu does indeed induce epigenetic modifications in the offspring, but whether these modifications have biologic relevance remains to be determined. The field of “epigenetic epidemiology” remains an active and growing field of investigation.

Macrosomia

Excessive fetal growth (macrosomia, being LGA) is found in 9%–13% of all deliveries and can lead to significant complications in the perinatal period (Gregory et al., 1998; Wollschlaeger et al., 1999). Maternal factors associated with macrosomia during pregnancy include increasing parity, higher maternal age, and maternal height. Additionally, the previous delivery of a macrosomic infant, prolonged pregnancy, maternal glucose intolerance, high prepregnancy weight or obesity, and large pregnancy weight gain have all been found to raise the risk of delivering a macrosomic

infant (Mocanu et al., 2000; Weissmann-Brenner et al., 2012; Rossi et al., 2013).

Maternal complications of macrosomia include morbidities related to labor and delivery, such as prolonged labor, arrest of labor, and higher rates of cesarean section and instrumentation during labor. Also, the risks of maternal lacerations and trauma, delayed placental detachment, and postpartum hemorrhage are higher for the woman delivering a macrosomic infant (Lipscomb et al., 1995; Perlow et al., 1996). Complications of labor are more pronounced in primiparous women than in multiparous women (Mocanu et al., 2000). The neonatal complications of macrosomia include traumatic events such as shoulder dystocia, brachial nerve palsy, birth trauma, and associated perinatal asphyxia. Other complications for the neonate are elevated insulin levels and metabolic derangements, such as hypoglycemia and hypocalcemia (Wollschlaeger et al., 1999). In a large population-based study in the United States, macrosomia (defined as birthweight greater than 4000 g) was detected in 13% of births. Of these, shoulder dystocia was noted in 11% (Gregory et al., 1998).

Macrosomia is often not detected during pregnancy and labor. The clinical estimation of fetal size is difficult and has significant false-positive and false-negative rates. Ultrasonography estimates of fetal weight are not always accurate, and the literature reports a wide range of sensitivity estimates for the ultrasound detection of macrosomia. Additionally, there is controversy in how to define macrosomia and which ultrasound measurement is most sensitive in its prediction. Smith and colleagues (1997) have demonstrated a linear relation between AC and birthweight. They showed that the equations commonly used for estimated fetal weight have a median error rate of 7%, with greater errors seen with larger infants. Using receiver operating characteristic curves to measure the diagnostic accuracy of ultrasound, O'Reilly-Green and Divon (1997) reported sensitivity and specificity rates of 85% and 72%, respectively, for estimation of birthweight exceeding 4000 g. In their study, the positive predictive value (i.e., a positive test result

represents a truly macrosomic infant) is about 49%. Chauhan and associates (2000) found lower sensitivity for the use of ultrasound measurement of AC, HC, and FL (72% sensitivity), similar to the sensitivity of using clinical measurements alone (73%). Other investigators have shown that clinical estimation of fetal weight (43% sensitivity) had higher sensitivity and specificity than ultrasound evaluation in predicting macrosomia (Gonen et al., 1996). In a retrospective study, Jazayeri and coworkers (1999) showed that an ultrasound measurement of AC of greater than 35 cm predicts macrosomia in 93% of cases and is superior to measurements of BD or FL. Other researchers have reported that an AC of either more than 37 cm or more than 38 cm is a better predictor (Gilby et al., 2000; Al-Inany et al., 2001).

Numerous investigators have also questioned whether antenatal diagnosis improves birth outcomes in macrosomic infants. Investigators point to the low rates of specificity of antenatal tests resulting in high rates of false-positive results (O'Reilly-Green and Divon, 1997; Bryant et al., 1998). Antenatal identification of macrosomia (or possible macrosomia) may lead to a higher rate of cesarean section performed for infants with normal birthweights (Gonen et al., 2000; Mocanu et al., 2000; Parry et al., 2000). Macrosomia is a risk factor for shoulder dystocia, but the majority of cases of shoulder dystocia and birth trauma occur in nonmacrosomic infants (Gonen et al., 1996). A retrospective study of infants weighing more than 4200 g at birth showed a cesarean section rate of 52% in infants predicted antenatally to have macrosomia, compared with 30% in infants without such an antenatal prediction. The antenatal prediction of fetal macrosomia is also associated with a higher incidence of failed induction of labor and no reduction in the rate of shoulder dystocia (Zamorski and Biggs, 2001). Using retrospective data from a 12-year period, Bryant and colleagues (1998) estimated that a policy of routine cesarean section for all infants with estimated fetal weight greater than 4500 g would require between 155 and 588 cesarean sections to prevent a single case of permanent brachial nerve palsy.

Summary

There are many identified biologic and genetic factors associated with normal and abnormal fetal growth. Physicians are limited in their ability to identify a causative agent in every case. Modification of fetal growth is possible and occurs from such diverse influences as socioeconomic status, maternal nutrition, and maternal constitutional factors. Abnormal fetal growth influences

not only acute perinatal outcomes but also health during infancy, childhood, and, intriguingly, adulthood. In schools of public health, students are taught to search “up river” for solutions to health problems. Solutions for ill health in adulthood may lie in the identification of methods to improve the health of the fetus.

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Multiple Gestations and Assisted Reproductive Technology

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KEY POINTS

- The multiple birth rate has increased by 76% in the past three decades.
- Increased utilization of assisted reproductive technologies and women delaying childbearing are the main contributors to the increase in multiples.
- Multiple gestations are at high risk for both maternal and fetal morbidity and mortality, which increase as fetal number increases.
- Zygosity and chorionicity are important predictors of perinatal morbidity, with monochorionic diamniotic and monochorionic monoamniotic twins at higher risk.
- The risk of monozygotic twinning is increased in in vitro fertilization (IVF); etiologies may include zona manipulation and extended in vitro culture.
- Most neonatal complications in multiple gestations are sequelae of prematurity, including low birthweight, respiratory distress syndrome, neonatal intensive care unit (NICU) admission, intraventricular hemorrhage, and necrotizing enterocolitis.
- Up to 80% of women with multiple gestations experience antepartum complications, which include preterm labor, preterm premature rupture of membranes (PPROM), and placental abruption.
- Women carrying multiples are at increased risk for the three major causes of maternal mortality: postpartum hemorrhage, venous thromboembolism, and hypertensive disorders.
- Multiple gestations are associated with increased financial and psychosocial costs.
- Strategies for decreasing the rate of multiples resulting from assisted reproductive technology (ART) include increasing the number of single embryo transfers performed in IVF and using “low and slow” protocols for superovulation cycles with gonadotropins.
- Multifetal pregnancy reduction can be performed to decrease the fetal number and lower the risk of morbidity, although the procedure does involve some medical and psychological risk.

Epidemiology of Multiples

There are multiple factors influencing the increase in multiple births in the United States in the past half-century. The incidence of multiples was stable through the 1970s at 2% of all births ([Martin et al., 2012](#)). We then began to see an “epidemic of multiples,” with the twin birth rate increasing by 76% from 1980 to 2009 ([Martin et al., 2012](#)). The overall proportion of total

national births that were multiples increased from 1.8% in 1971 to 3.5% in 2011 ([Kulkarni et al., 2013](#)). Twins continue to account for the vast majority of multiple pregnancies, comprising 96% in 2009 ([Martin et al., 2012](#)). The growing use of assisted reproductive technology (ART), in addition to delayed childbearing until age-related fertility issues become apparent, has contributed greatly to multiple birth rates. The rate of women delaying childbearing has dramatically increased, with women over the age of 30 accounting for 20% of births in 1980, compared with more than 35% in 2009 ([Martin et al., 2012](#)). Between 1980 and 2009, the twin birth rate increased by 100% in women 30–35 years old and by more than 200% in women aged 40 and older ([Martin et al., 2012](#)). In 2009, 7% of all births to women aged 40 or older were twins ([Martin et al., 2012](#)). Spontaneous twinning is more common as women age, perhaps related to higher follicle-stimulating hormone (FSH) levels in the follicular phase leading to ovulation of more than one oocyte, with a peak at age 37 ([Hall, 2003](#)). However, the increasing age of childbearing women is estimated to account for only one-third of the rise in twinning, with ART responsible for the remainder ([Martin et al., 2012](#)). Since the birth of the first in vitro fertilization (IVF) baby in 1978, the numbers of IVF clinics, ovarian stimulation cycles, and live births from ART have all steadily increased. In 2013, ART contributed to the births of 1.6% of total infants, 18.7% of multiple birth infants, and 25.2% of triplet and higher order multiple (HOM, 4 or more) infants ([Sunderam et al., 2015](#)). About 3.5% of all US births are multiples, yet in 2013 the ART twin birth rate was 39% and the ART HOM birth rate was 2% ([Sunderam et al., 2015](#)).

It is important to note that, since 1997, IVF has *not* been the leading contributor to the multiple birth rate, contributing to only 16% of multiple births in 2013 ([Adashi, 2016](#)). Non-IVF treatment options including ovulation induction with oral medications and superovulation with injectable gonadotropins were responsible for 19% of twins and 45% of triplet or HOMs in 2011 ([Kulkarni et al., 2013](#)). These modalities involve less monitoring and are subject to less control than IVF and are thus less susceptible to efforts to decrease the multiples rate. While the twin birth rate has continued to increase, the proportion of triplet or HOM births arising from medically assisted conceptions has been declining in recent years, from 84% in 1998 to 77% in 2011 ([Kulkarni et al., 2013](#)). The incidence of triplet and HOM births increased by a factor of 6.7 from 1971 to 1998 but has since decreased by 29% from 1998 to present ([Kulkarni et al., 2013](#)). This is largely due

to a decrease in the number of embryos transferred per cycle of IVF and to the reticence of providers to use superovulation with injectable gonadotropins.

Given the maternal, perinatal, and neonatal complications associated with multiples, the goal of infertility treatment is one healthy child. Multifetal pregnancies drastically affect individuals, families, and public health systems. Of particular importance in both maternal and fetal outcomes are fetal number and placentation.

Diagnosing Zygosity and Chorionicity

Determining zygosity and chorionicity is important medically, genetically, and psychosocially for the individual and family. More immediately, the chorion–amnion arrangement is crucial to antepartum management, as it determines risks of complications such as twin–twin transfusion syndrome (TTTS), growth discordance, intrauterine growth restriction (IUGR), congenital anomalies, and cord accidents. Chorionicity also guides next steps in cases of one fetal demise or desired selective reduction. Zygosity refers to the number of oocytes and spermatozoa involved in conception. Dizygotic twins result from fertilization of two separate ova by two spermatozoa, and these comprise 67% of spontaneous twins (Gibbs and Danforth, 2008). Monozygotic twins (MZTs) are identical and result from fertilization of a single ovum with one spermatozoa and subsequent postzygotic division (McNamara et al., 2016). The timing of this division, or splitting, determines the number of fetuses, chorionic plates, and amniotic sacs.

To determine zygosity we can use prenatal determination of fetal gender or blood typing and DNA analysis in the postnatal period (Hall, 2003; Ohm et al., 2006). Diagnosing chorionicity is possible using ultrasound markers including the number of placental sites, thickness of dividing membrane, and the lambda sign. The lambda sign indicates dichorionicity and is a triangular projection of tissue that extends beyond the chorionic surface of the placenta (Finberg, 1992).

A first trimester screening ultrasound is essential in diagnosing multiple pregnancy. In a large randomized trial, in the cohort of women that did not have a screening ultrasound, 37% were not diagnosed as having a twin pregnancy until 26 weeks, and 13% were not diagnosed until the time of delivery (LeFevre et al., 1993). Current ultrasonographic technology is very effective at diagnosing chorionicity and amnionicity. In one study, the reported sensitivity, specificity, and positive and negative predictive values for ultrasonography at less than 14 weeks' gestation were 89.8%, 99.5%, 97.8%, and 97.5%, respectively. Overall, chorionicity was correctly diagnosed in 95% of cases (Lee et al., 2006) (Table 7.1).

The Effect of Chorionicity

Chorionicity and placentation greatly affect fetal morbidity and mortality in multifetal pregnancies. Dizygotic twins (DZTs), with few exceptions, lead to a dichorionic diamniotic (DCDA) arrangement in which the placenta can be separate or fused. Rare cases of dizygotic monochorionic diamniotic (MCDA) twins have been reported (Souter et al., 2003). Theories as to the etiology of this include fusion of two genetically distinct zygotes or fertilization of a binovular follicle (McNamara et al., 2016). The highest rates of spontaneous twinning are in Nigeria, where 1 out of every 12 persons is a member of a twin pair, and the lowest twinning rates are seen in China, where 1 in every 70 persons is a member of a twin pair. North American and European countries are considered

TABLE 7.1

Twinning Rates per 1000 Births by Zygosity

| Country | Monozygotic | Dizygotic | Total |
|-------------------|-------------|-----------|-------|
| Nigeria | 5.0 | 49 | 54 |
| United States | | | |
| African American | 4.7 | 11.1 | 15.8 |
| Caucasian | 4.2 | 7.1 | 11.3 |
| England and Wales | 3.5 | 8.8 | 12.3 |
| India | 3.3 | 8.1 | 11.4 |
| Japan | 3.0 | 1.3 | 4.3 |

From MacGillivray I. Epidemiology of twin pregnancy. *Semin Perinatol.* 1986;10:4–8; and Cunningham FG, Hauth JC, Wenstrom KD, et al. (eds). *Williams Obstetrics*, ed. 22, New York: McGraw-Hill; 2005.





to have an intermediate prevalence of spontaneous dizygotic twinning (Hoekstra et al., 2008). Risk factors for DZT include advancing maternal age, increased parity, female relatives with DZT, taller height, and higher body mass index (MacGillivray, 1986; Hoekstra et al., 2008). Historically, a seasonal trend in DZT has been seen, with higher rates in the summer and autumn months (Hoekstra et al., 2008).

The true incidence of MZTs is difficult to ascertain because of its rarity, inaccuracies in diagnosis, and lack of confirmatory studies at birth, but spontaneous MZT rates are estimated to occur in 0.3%–0.5% of all pregnancies and in less than 30% of all twins (Bulmer, 1970; MacGillivray, 1986; Hall, 2003). This rate was geographically constant prior to the advent of ART (Hall, 2003). Unlike DZT, it is unclear whether MZT is related to genetics or environment (Bortolus et al., 1999; Hoekstra et al., 2008). Familial association has been seen but is very rare (Hall, 2003; Hamamy et al., 2004).

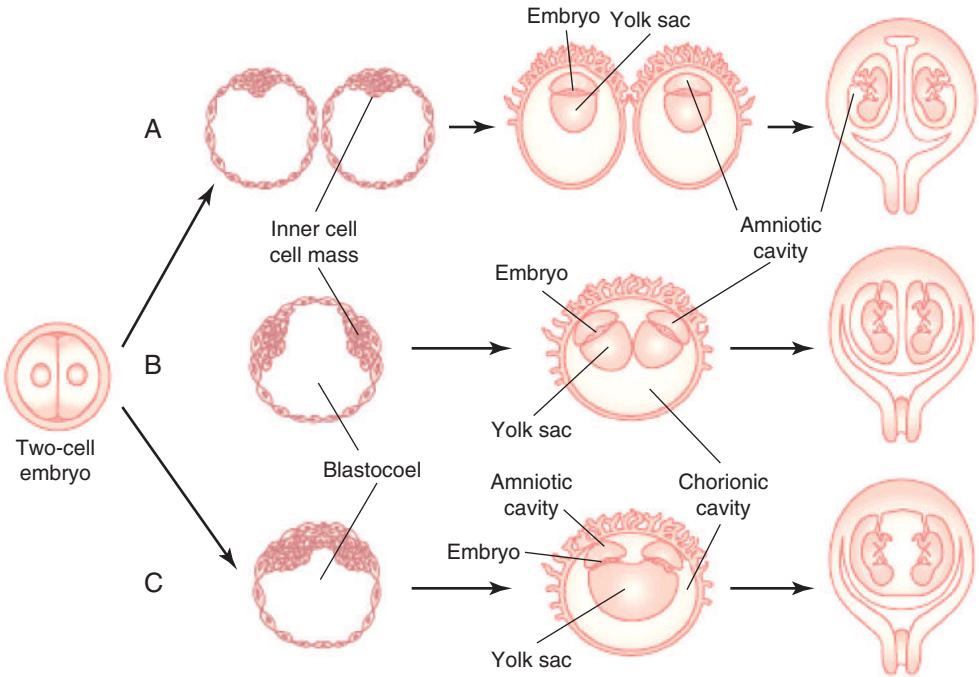
Chorionicity in monozygotic gestations is determined by the timing of the embryonic division (Figs. 7.1 and 7.2). In 18%–36% of MZTs, the zygote divides within 72 hours of fertilization, resulting in a DCDA gestation (the placenta can be separate or fused); 60%–75% split between days 4 and 8, leading to a MCDA unit; and 1%–2% separate between days 8 and 13, leading to an monochorionic monoamniotic (MCMA) pregnancy. Embryonic division after day 13 results in conjoined twins that are MCMA (Hall, 2003; Gibbs and Danforth, 2008; Cunningham and Williams, 2010; McNamara et al., 2016).

There are no naturally occurring animal models of MZT with the exception of armadillos. Models of MZT can be induced in laboratory animals by exposure to toxins, hypoxia, or manipulation of the zona pellucida (Hall, 2003; Knopman et al., 2014). The cause of spontaneous monozygotic twinning in humans is unknown. An uneven sex ratio has been noted, with 0.484 male:female pairs in all monozygotic twins and 0.231 in monoamniotic twins (Derom et al., 1988; Hall, 2003), leading to the theory that skewed X inactivation may play a role. The development of two inner cell masses (ICM) at the blastocyst stage can lead to MZT, either from damage or through immune-mediated cell-to-cell crosstalk (Hall, 2003).

Although the majority of ART MZTs are MCDA, any of the three monozygotic placental arrangements can transpire after ART, implying that the timing and mechanism of embryonic splitting

| Zygosity | Dizygotic or Monozygotic | | Monozygotic | |
|-----------------|---|---|--|---|
| Day of division | 0–3 days | 0–3 days | 4–8 days | 8–13 days |
| |  |  |  |  |
| Fetal membranes | 2 Amnions, 2 Chorions, 2 Placentas | 2 Amnions, 2 Chorions, 1 Placenta | 2 Amnions, 1 Chorion, 1 Placenta | 1 Amnion, 1 Chorion, 1 Placenta |
| | A | B | C | D |

• **Fig. 7.1** Placentation and Membranes Based on Timing of Embryonic Division. (A) Two amnions, two chorions, and separate placentas from the division of either a dizygotic or monozygotic embryo within 3 days of fertilization. (B) Two amnions, two chorions, and one fused placenta from the division of either a dizygotic or monozygotic embryo within 3 days of fertilization. (C) Two amnions, one chorion, and one placenta from a monozygotic embryonic cleavage, days 4–8 after fertilization. (D) One amnion, one chorion, and one placenta from a monozygotic embryo splitting, days 8–13 after fertilization. (Modified from Gibbs R, Karlan B, Haney A, et al. *Danforth's Obstetrics & Gynecology*, ed. 10, Philadelphia; Lippincott, Williams & Wilkins, 2008.)



• **Fig. 7.2** Types of Monozygotic Placentation. (A) Dichorionic diamniotic pregnancy. (B) Monochorionic diamniotic pregnancy. (C) Monochorionic monoamniotic pregnancy. (Adapted from Hall JG. Twinning. *Lancet*. 2003;362:735–743; and Benirschke K, Kim CK. Multiple pregnancy. 1. *N Engl J Med*. 1973; 288:1276–1284.)

are variable (Aston et al., 2008; Knopman et al., 2010). To date, embryonic splitting has not been observed in vitro (Vithala et al., 2009).

Increase in Monozygotic Twins With Assisted Reproductive Technology

The first reported association between ART and MZT (Yovich et al., 1984) preceded numerous accounts of similar findings. The

majority (>90%) of ART twins are dizygotic (Gibbs et al., 2008) secondary to transferring multiple embryos; however, the rate of MZTs per pregnancy after fertility treatment is higher (0.7%–13%) (Sills et al., 2000; Schachter et al., 2001; Alikani et al., 2003; Aston et al., 2008; Vithala et al., 2009; Knopman et al., 2010) versus the general population (0.3%–0.5%) (Bulmer, 1970; MacGillivray, 1986). It is also suspected that the incidence of MZT in ART may be underestimated because DCDA gestations after transfer of more than one embryo are often assumed to be dizygotic, and genetic analysis is rarely performed postnatally

(Knopman et al., 2014). Several theories to explain the mechanism responsible for elevated MZTs with ART have been proposed. Following is a discussion of those theories.

Age

As mentioned previously, spontaneous dizygotic twinning increases with advancing maternal age (Bulmer, 1970; Bortolus et al., 1999; MacGillivray, 1986), but the connection between age and MZTs is controversial. Some studies report trends toward elevated MZT rates in older women (Bulmer, 1970; Abusheikha et al., 2000; Alikani et al., 2003), whereas others found no association with increasing maternal age and MZT (Bortolus et al., 1999; Skiadas et al., 2008), and one study found that the MZT risk doubles in women younger than 35 years (Knopman et al., 2010). Overall, the correlation between age and MZT in ART remains unclear.

Zona Pellucida Manipulation

The zona pellucida (ZP), an acellular protein surrounding the ovum, provides a species-specific sperm barrier and decreases polyploidy by inhibiting penetration by multiple sperm (Fritz and Speroff, 2011). It has been shown that the thickness and hardness of the ZP can vary in relation to stimulation protocol, elevated FSH or estradiol (E2) levels, and culture conditions. There is debate as to whether ZP manipulations performed during IVF affect MZT risk. Manipulation of the ZP in IVF occurs via both intracytoplasmic sperm injection (ICSI) and assisted hatching (AH). The injection of one sperm into a mature oocyte (i.e., ICSI) is most commonly performed for male factor infertility. AH is achieved with an artificial breach in the ZP by laser, chemical, or mechanical methods and has been shown to increase clinical pregnancy rates, although not live birth rates, in patients with a poor prognosis (Practice Committee of the American Society for Reproductive Medicine; Practice Committee of the Society for Assisted Reproductive Technology, 2014). A recent Cochrane Review demonstrated that AH is associated with an increase in multiple pregnancy, with an odds ratio of 1.39 (Carney et al., 2012). However, the increase in MZT with AH was not statistically significant, and this was confirmed in other large studies (Sills et al., 2000; Valojerdi et al., 2008; Knopman et al., 2010; Carney et al., 2012). However, the data are conflicting, with a retrospective review of over 35,000 IVF cycles showing a threefold risk of MZT with AH (Schieve et al., 2000), a finding also demonstrated in many smaller studies (Alikani et al., 2003; Skiadas et al., 2008). Similarly, there are mixed data to support that ICSI increases the risk of MZT. The most recent committee opinion from the American Society for Reproductive Medicine endorses that the data confirm an association (Abusheikha et al., 2000; Vitthala et al., 2009; Practice Committee of the American Society for Reproductive Medicine, 2012). However, more recent data have called this into question, with a recent study examining twinning after single embryo transfer finding a decrease in rates of MZT with ICSI (Kanter et al., 2015). Interestingly, the defect created in the ZP for ICSI is much smaller than for AH, lending plausibility to other recent studies that have shown no association (Nakasuji et al., 2014; Sobek et al., 2015; Vaughan et al., 2016).

Blastocyst Transfer

After oocyte retrieval and insemination, embryos undergo intra-uterine transfer at either the cleavage stage (day 2–3 after retrieval)

or the blastocyst stage (day 5–6 after retrieval). Blastocyst-stage embryo transfer yields higher live birth rates and lowers overall multiple rates because of an increase in single embryo transfer at the blastocyst stage (Frattarelli et al., 2003; Glujovsky et al., 2012). However, evidence has supported the increased incidence of MZTs with extended culture to the blastocyst stage (Behr et al., 2000; Milki et al., 2003; Toledo, 2005; Skiadas et al., 2008; Knopman et al., 2010; Sharara and Abdo, 2010; Kanter et al., 2015). The theory is that extended culture may impact the integrity of the ZP, causing herniation and splitting of the blastomeres (Alikani et al., 1994; Carrillo-Vadillo et al., 2007). One institution initially noted increased MZT after blastocyst transfer (5.6% vs 2%; Milki et al., 2003), but a follow-up study 3 years later demonstrated similar MZT rates between blastocyst and day 3 embryos (2.3% vs 1.8%), indicating that changes in culture media and an experienced embryology team may affect the rate of MZTs (Moayeri et al., 2007). Another recent study from a large IVF center showed a higher MZT rate with blastocyst transfer, but this association disappeared after controlling for patient age, embryo quality, and availability of supernumerary embryos (Franasiak et al., 2015). Higher-quality embryos are more likely to be cultured out to the blastocyst stage, which may be a confounder when comparing day of transfer. Recent data from this study and others also indicate that younger oocyte age, another marker of embryo quality, is related to higher incidence of MZT (Knopman et al., 2010, 2014; Franasiak et al., 2015). The role that culture media may play remains to be determined. Elevated glucose levels in extended culture and subsequent glucose-induced apoptotic remodeling of the ICM and ICM splitting in extended culture in murine and human embryos may explain the phenomenon of MZTs in blastocysts (Menezo and Sakkas 2002; Cassuto et al., 2003). Another environmental factor that may affect MZTs is temperature variation in frozen–thawed embryo cycles. Although minute evidence links frozen embryo transfers and temperature fluctuations to MZTs (Toledo, 2005; Faraj et al., 2008), most studies show no difference between fresh and frozen–thawed embryos and multiple rates or MZT rates (Alikani et al., 2003; Knopman et al., 2010).

Ovulation Induction and Superovulation

Human studies of ovarian stimulation with clomiphene citrate and gonadotropins reveal a twofold increased risk of MZTs compared with the general population. MZT incidences of 1.5% after ovulation induction with gonadotropins, 0.72% after IVF, and 0.87% with IVF with ICSI and AH suggest that elevated levels of steroid hormones may elevate MZTs regardless of ZP manipulation (Schachter et al., 2001). However, this finding may be confounded by a higher baseline risk of MZT in patients with infertility (Zhu et al., 2007).

Fetal Complications Associated With Multiples

Multiple pregnancies account for a small percentage of overall live births but are responsible for a disproportionate amount of morbidity and mortality. This is largely attributable to complications of prematurity, as women with multiple pregnancies are six times more likely to deliver preterm and 13 times more likely to deliver before 32 weeks than those with a singleton. Of multiple pregnancies, 11% are delivered before 32 weeks and 59% before 37 weeks, compared with 9.6% of singletons delivered preterm (Hamilton

TABLE 7.2 Morbidity and Mortality by Fetal Number

| Characteristic | Twins | Triplets | Quadruplets |
|--|--------------------------------|---------------------------------|-------------|
| Average birthweight | 2347 g | 1687 g | 1309 g |
| Average gestational age at delivery | 35.3 weeks | 32.2 weeks | 29.9 weeks |
| Percentage with growth restriction | 14%–25% | 50%–60% | 50%–60% |
| Percentage requiring admission to NICU | 25% | 75% | 100% |
| Average length of stay in NICU | 18 days | 30 days | 58 days |
| Percentage with major handicap | — | 20% | 50% |
| Risk of cerebral palsy | 4 times more than singletons | 17 times more than singletons | — |
| Risk of death by age 1 year | 7 times higher than singletons | 20 times higher than singletons | — |

NICU, Neonatal intensive care unit.

From American College of Obstetricians and Gynecologists Committee on Practice Bulletins—Obstetrics; Society for Maternal-Fetal Medicine; ACOG Joint Editorial Committee. ACOG Practice Bulletin #56: Multiple gestation: complicated twin, triplet, and high-order multifetal pregnancy. *Obstet Gynecol.* 2004;104:869–883.

et al., 2015). As a consequence, multiples have a fivefold increased risk of stillbirth and a sevenfold increased risk of neonatal death (Scher et al., 2002). The risk of prematurity increases with fetal number, with 9 out of every 10 triplets being born preterm or low birthweight (Hamilton et al., 2015). The average gestational ages at delivery for twins, triplets, and quadruplets are 35.3, 31.9, and 29.5 weeks, respectively (American College of Obstetricians and Gynecologists; Society for Maternal-Fetal Medicine, 2014), corresponding to neonatal intensive care unit (NICU) admission rates fivefold higher in twins and 17-fold higher in triplets and HOMs (Ross et al., 1999).

Fetal and maternal complications are more common in twin pregnancies, and this risk increases with triplets and HOMs (Table 7.2). Multiples have increased risks of intraventricular hemorrhage, periventricular leukomalacia, cerebral palsy, sepsis, necrotizing enterocolitis, and respiratory distress syndrome (Blondel and Kaminski, 2002; Rettwitz-Volk et al., 2003; Salihu et al., 2003; Luke and Brown, 2008). Surviving infants of preterm multifetal pregnancies have higher rates of developmental handicap (Yokoyama, et al., 1995; Rettwitz-Volk et al., 2003; Luke et al., 2006). Higher fetal number correlates with increased risk of growth restriction, earlier delivery, low birth weight (LBW), NICU admission, length of stay, risk of major handicap and cerebral palsy, and death in first year of life (Garite et al., 2004; Luke and Brown, 2008). A review of 100 triplet gestations (88 with assisted conception) revealed

that 78% experienced preterm labor (PTL), 14% delivered before 28 weeks, 5% had congenital anomalies, and 9.7% died in the perinatal period (Devine et al., 2001).

It is important to note that singleton pregnancies after assisted conception have increased complications, including preterm delivery, LBW, prolonged hospital stay, cesarean deliveries, blood transfusion, NICU admission, and mortality compared with spontaneous singletons (Helmerhorst et al., 2004; Jackson et al., 2004; Van Voorhis, 2006; Schieve et al., 2007; Martin et al., 2016). A common phenomenon in ART is the “vanishing twin,” an arrest of development and subsequent absorption of one or more fetuses of a multiple gestation in the first trimester (Practice Committee of the American Society for Reproductive Medicine, 2012). Estimates of the incidence of a vanishing twin after ART range from 10%–30% and are increased in HOMs (Landy and Keith 1998; Tummers et al., 2003; McNamara et al., 2016). Recent evidence suggests that the surviving twin(s) is at increased risk for LBW, preterm birth, and possibly cerebral impairment (Pinborg et al., 2005, 2007; Anand et al., 2007a, 2007b; Luke et al., 2009). The risk of LBW is related to the gestational age at the time of a twin demise, with later gestations conferring more risk (Pinborg et al., 2007). The majority of vanishing twins (80%) occur prior to 9 weeks (McNamara et al., 2016). Demise of a twin after the first trimester is more common in monozygotic gestations (Burke, 1990).

Similar to ART singleton versus spontaneous singleton outcomes, ART multiples may have higher morbidity compared with spontaneous multiples. Assisted-conception twins are at increased risk for LBW, preterm delivery, cesarean delivery, NICU admission, longer length of stay, respiratory distress syndrome, and birthweight discordance versus spontaneously conceived twins (Moise et al., 1998; Nassar et al., 2003; Smithers et al., 2003; Helmerhorst et al., 2004; McDonald et al., 2005). In contrast, other studies suggest comparable outcomes in assisted conception and spontaneous multiples. Rates of pregnancy-induced hypertension, gestational diabetes mellitus, preterm premature rupture of membranes (PPROM), placenta previa, placental abruption, congenital malformations, and mortality were similar in IVF twins versus spontaneous twins (Nassar et al., 2003; Pinborg et al., 2004; McDonald et al., 2005).

Besides fetal number, another critical factor in pregnancy outcome is placental architecture. Monochorionic multiples experience higher rates of morbidity and mortality, largely because of placental factors (Table 7.3) (Hack et al., 2008; Cunningham and Williams, 2010; Glinianaia et al., 2011). Negative outcomes such as stillbirth, neonatal mortality, congenital malformations, and cerebral palsy are higher in monochorionic twins compared with dichorionic twins (Lewi et al., 2010; Glinianaia et al., 2011; Burgess et al., 2014). These complications are thought to be related to placental sharing and the vascular anastomoses that may cause unequal distribution of placental blood flow, causing TTTS. TTTS affects 10% of MCDA gestations and usually presents in the second trimester with oligohydramnios of the donor twin and polyhydramnios in the recipient twin (Society for Maternal-Fetal Medicine and Simpson, 2013; American College of Obstetricians and Gynecologists; Society for Maternal-Fetal Medicine, 2014).

The severity of TTTS ranges from stage I to stage V, and sequelae can include hydrops and fetal death. The incidence of progression from stage I to more severe disease is 15%; the survival rate in those that don't progress is 86% (Bebbington et al., 2010; Rossi and D'Addario, 2013). The prognosis for TTTS that is stage III or higher is bleak, with a loss rate of 70%–100% (Berghella and Kaufmann, 2001; Hack et al., 2008). Overall, TTTS accounts for

TABLE 7.3 Incidence of Twin Pregnancy Zygosity and Chorionicity With Corresponding Complications

| Type of Twinning | Incidence (%) | COMPLICATION (%) | | |
|----------------------------|---------------|------------------|-------------------------------|---------------------|
| | | IUGR | Delivery <37 Weeks' Gestation | Perinatal Mortality |
| Dizygotic | 67–70 | 25 | 40 | 10–12 |
| Monozygotic | 30–33 | 40 | 50 | 15–18 |
| Diamniotic dichorionic | 18–36 | 30 | 40 | 18–20 |
| Diamniotic monochorionic | 60–75 | 50 | 60 | 30–40 |
| Monoamniotic monochorionic | 1–2 | 40 | 60–70 | 30–70 |

IUGR, Intrauterine growth restriction.

From Cunningham FG, Hauth JC, Wenstrom KD, et al. (eds). *Williams Obstetrics*, ed. 22, New York: McGraw-Hill; 2005. Originally from Manning FA. Fetal biophysical profile scoring. In *Fetal Medicine: Principles and Practices*, Norwalk, CT: Appleton & Lange, 1995.

half of perinatal deaths in MCDA twins (Lewi et al., 2008, 2013), and surviving infants are at risk for long-standing adverse neurologic outcomes (Cleary-Goldman and D'Alton, 2008).

MCMA twins are very rare, occurring in 1 in 10,000 pregnancies, but they suffer the highest risk of perinatal morbidity and mortality (American College of Obstetricians and Gynecologists; Society for Maternal-Fetal Medicine, 2014). Similar to MCDA twins, MCMA twins are susceptible to TTTS, growth discordance, IUGR, preterm delivery, and congenital anomalies, but they also face the unique complication of cord entanglement (Cordero et al., 2006). These factors historically account for perinatal mortality rates of up to 80% (American College of Obstetricians and Gynecologists; Society for Maternal-Fetal Medicine, 2014). However, lower morbidity and mortality rates (2%–25%) reported in recent articles may be attributed to increased prenatal diagnosis and fetal surveillance (Cordero et al., 2006; Baxi and Walsh 2010; Murata et al., 2013).

Maternal Complications

Approximately 80% of women pregnant with multiples experience antepartum complications versus 25% of those with singletons, and hospitalization for hypertensive disorders, PTL, PPROM, placental abruption, and hyperemesis gravidarum are elevated sixfold (American College of Obstetricians and Gynecologists; Society for Maternal-Fetal Medicine, 2014; Norwitz et al., 2005). The risk of severe maternal morbidity, including intensive care unit admission, uterine rupture, eclampsia, major obstetric hemorrhage, and peripartum hysterectomy, is elevated fourfold (Witteveen et al., 2016). Mothers with two or more fetuses are at increased risk for the three major causes of maternal mortality: postpartum hemorrhage, venous thromboembolism, and hypertensive disorders (Conde-Agudelo et al., 2000; Walker et al., 2004). Hypertensive disorders occur in 20% of multiples compared with 6.5% of singletons (Day et al., 2005).

Plurality correlates with maternal morbidity, with triplets and HOMs experiencing significantly increased maternal morbidity

compared to twins. They have higher rates of PPROM, postpartum hemorrhage, gestational diabetes, placental abruption, cesarean delivery, and hypertensive disorders (Wen et al., 2004; Luke and Brown, 2008). Results of a triplet cohort showed that 96% had maternal complications, 96% required antenatal hospitalization, one in four were diagnosed with preeclampsia, and 44% encountered postpartum complications (Devine et al., 2001).

Psychosocial Factors

As would be expected, psychosocial stressors increase with pregnancy and delivery of multiples. As fetal number increases, parents report decreased quality of life, increased social stigma, increased financial stress, and decreased marital satisfaction (Glazebrook et al., 2004; Ellison et al., 2005; Golombok et al., 2007; Sheard et al., 2007; Roca de Bes et al., 2009; van den Akker et al., 2016). Studies have also demonstrated an increased risk of postpartum depression in mothers of multiples (Choi et al., 2009; Vilska et al., 2009; Ross et al., 2011). It is estimated that adequately caring for 6-month-old triplets and performing household duties would require 197.5 hours per week, clearly exceeding the 168 hours available (Bryan, 2003). Additionally, many multiple pregnancy infants are born preterm and have ongoing health issues, further exacerbating parental stress. Most studies have not demonstrated a psychosocial difference between naturally conceived multiples and multiples conceived with ART (Sydsjo et al., 2008; Vilska et al., 2009; Roca-de Bes et al., 2011; van den Akker et al., 2016).

Cost

Multiple gestations have a large economic impact, both for individual families and society. As previously discussed, multiples have increased risks of preterm delivery and LBW, which are the major drivers of short-term and long-term costs (Cuevas et al., 2005). The average first-year medical costs for preterm infants are 10 times higher than for term infants (American College of Obstetricians and Gynecologists; Society for Maternal-Fetal Medicine, 2014). According to gestational age, the mean initial hospital charge for infants born between 26 and 28 weeks' gestation is approximately \$240,000 compared with approximately \$4800 for a term infant. By birthweight, infants weighing less than 1250 g cost approximately \$250,000 compared with infants weighing more than 2500 g, who cost \$5800 (Cuevas et al., 2005). Overall, the cost of a twin gestation is fourfold to fivefold that of a singleton, and this increases to 10–20-fold for triplets and HOMs (Collins, 2007; Lemos et al., 2013).

ART multiples and their associated comorbidities have a significant role in healthcare expenditures. The estimated financial cost of ART-associated preterm deliveries in the United States is \$1 billion annually (Bromer et al., 2011). Women with ART pregnancies have higher rates of antenatal hospitalization with longer length of stay than women who conceived naturally, contributing to increased costs (Martin et al., 2016). A recent Dutch study showed that multiples conceived with IVF were 3.3 times more costly than singletons conceived with IVF through 5 years of life (van Heesch et al., 2015). If iatrogenic multiples were singletons in future, the estimated cost savings would be \$6.3 billion in the first year of life (Allen et al., 2014). Data also indicate that ART-conceived infants, especially in the case of multiples, have higher risks of hospital admission in the first 1–2 years of life, incurring additional costs (Pinborg et al., 2004; Hansen et al., 2009).

Decreasing the Risk of Multiples

Primary forms of preventing multiples include canceling ovulation induction cycles in which multifollicular development occurs, or converting those cycles to IVF, and limiting the number of embryos transferred in IVF cycles. Embryo transfer policies vary significantly on the global stage, ranging from strict legislation to recommendations from professional societies that are not binding. In some countries single embryo transfer (SET) is mandatory with rare exceptions (European IVF-Monitoring Consortium; European Society of Human Reproduction and Embryology, [Kupka et al., 2016](#)). The American Society for Reproductive Medicine (ASRM) and the Society for Assisted Reproductive Technology established transfer guidelines in the United States, beginning in 1998, to assist in determining an appropriate number of embryos to transfer and help decrease the rate of multiples. Recommended limits are based on age, prognosis, and embryo stage and further differentiate between a favorable prognosis (first IVF cycle, good-quality embryos, number of embryos for potential cryopreservation, successful past IVF cycles) and less favorable prognosis.

In the most recent 2013 guidelines, ASRM highly recommends consideration of SET in patients younger than 35 with a favorable prognosis ([Practice Committee of the American Society for Reproductive Medicine; Practice Committee of the Society for Assisted Reproductive Technology, 2013](#)). Recommendations state that the number of embryos transferred in a frozen cycle should not exceed the recommended limits in a fresh cycle. These transfer guidelines are not legally binding, and they are subject to interpretation or adjustment based on clinical experience and unique patient characteristics ([Practice Committee of the American Society for Reproductive Medicine; Practice Committee of the Society for Assisted Reproductive Technology, 2013](#)).

The rates of SET in the United States have been increasing in response to the epidemic of multiples, with the proportion of cycles in which one embryo was transferred increasing from 6% in 1971 to 21% in 2011. Similarly, the proportion in which three or more embryos were transferred decreased from 79% to 24% in that same time period ([Kulkarni et al., 2013](#)). The term “elective SET” (eSET) refers to couples who have more than one quality embryo available for transfer but elect to transfer one. The rate of eSET in the United States has historically been quite low but increased from 3.3% in 2008 to 9% in 2011 ([Practice Committee of the American Society for Reproductive Medicine, 2012; Kulkarni et al., 2013](#)). These efforts have resulted in an increase in the singleton birth rate after IVF, which increased by 29% from 1998 to 2011, with 2005 marking the first year that more than half of IVF deliveries were singletons ([Kulkarni et al., 2013](#)).

There are many factors contributing to the increase in SET and stabilization of the multiple birth rate. These include revised guidelines from ASRM that lowered the recommended number of embryos to transfer, improved technology leading to more blastocyst transfers, and expanded insurance coverage for ART ([Kulkarni et al., 2013](#)). The overall number of embryos transferred each year has steadily declined since publication of the initial ASRM guidelines in 1998, and it is estimated that between 13,500 and 16,300 HOM deliveries have been prevented, which is a cost savings of more than \$6 billion ([Stern et al., 2007; Lee et al., 2016](#)).

A recent metaanalysis compared the effectiveness of eSET to double embryo transfer (DET). Overall live birth rates (LBR) were lower after SET (27%) than DET (42%), but the cumulative LBR after one additional frozen single embryo transfer was not

significantly different. The likelihood of a singleton LBR was five times higher in the SET group ([Thurin et al., 2004; McLernon et al., 2010](#)). Birth rates with SET and DET seem to be more comparable with blastocyst transfers than with cleavage stage transfers. One program in the United States mandates SET in patients less than 38 years old with more than seven zygotes and at least one quality blastocyst. After implementation of this mandate in 2004, they demonstrated an overall increase in LBR (51.1% to 55.9%) and a decrease in the rate of multiples (34.8% to 17.5%). The LBR in the mandated single embryo transfer (mSET) group was impressive at 64.6% with only 3.4% multiples (monochorionic gestations) ([Kresowik et al., 2011](#)). A study comparing SET at the blastocyst versus the cleavage stage found significantly higher pregnancy rates in the blastocyst group, and this is reflected in the ASRM transfer guidelines ([Papanikolaou et al., 2006](#)).

Expanding insurance coverage for ART is another strategy that may help to decrease multiple births. Currently, 15 states have enacted infertility insurance mandates that vary in the ART services covered. Studies have shown that women with insurance coverage are more likely to elect for SET, likely with the goal of accelerating the process of family building and avoiding the cost of future cycles ([Griffin et al., 2012; Adashi, 2016; Styer et al., 2016](#)).

Patient education is a powerful tool to increase the acceptability of eSET. Many patients are undereducated as to the risks and complications associated with multiples and elect to have more than one embryo transferred because of a desire for twins. It is incumbent upon providers to explain the multitude of reasons that one healthy liveborn infant is the goal of every IVF cycle. Educational programs targeted to improve attitudes toward SET have shown some success ([Newton et al., 2007; Ryan et al., 2007; Hope and Rombauts, 2010; Griffin et al., 2012](#)).

Strategies for reducing multiples caused by non-IVF infertility therapy should also be considered. As previously discussed, ovulation induction and superovulation contribute up to 20% of twin births in the United States ([Adashi, 2016](#)). During superovulation cycles with gonadotropin stimulation, follicular growth is monitored with ultrasound, and E2 levels are serially measured in an attempt to minimize multifollicular development. Overall the risk of multiples correlates with increasing E2 levels and the size and number of follicles ([Dickey, 2009; Practice Committee of the American Society for Reproductive Medicine, 2012](#)). However, an attempt to define thresholds at which couples are at low risk for multiples has been unsuccessful ([Ragni et al., 1999; Gleicher et al., 2000; Ghesquiere et al., 2007; Rosen et al., 2008](#)). A strategy that has shown promise is the use of “low and slow” protocols, using low-dose gonadotropin stimulation over a longer period of time. Studies examining these protocols have documented multiple rates of 1.0% or less ([Papageorgiou et al., 2004; Ragni et al., 2006](#)). Lastly, some have advocated for the elimination of superovulation as an intermediary step between oral ovulation induction and IVF, given the associated high rate of multiples and HOMs, and this has been adopted as a cost-effective strategy by some health plans ([McClamrock et al., 2012; Adashi, 2016](#)).

Multifetal Pregnancy Reduction

As the rates of ART procedures, multiple pregnancies, and prematurity-related sequelae have increased, so has the use of selective reduction. Primary prevention of multiple gestations is optimal; however, in reality, multifetal pregnancies continue to occur. Multifetal pregnancy reduction (MFPR) provides an option to reduce the risk of fetal or neonatal morbidity and mortality

(Practice Committee of the American Society for Reproductive Medicine, 2012; American College of Obstetricians and Gynecologists; Society for Maternal-Fetal Medicine, 2014). First developed in the 1980s, termination of one or more fetuses is performed to reduce the final fetal number. The procedure most often involves passage of a needle transabdominally into the thorax of the fetus and subsequent injection of potassium chloride (Evans et al., 2014). Selective termination refers to reduction of an abnormal fetus that is part of a multifetal gestation. The majority of patients choose to reduce a triplet or HOM pregnancy to twins, but the number reducing to singletons is increasing with heightened awareness of the morbidity associated with twins (Stone et al., 2008; Evans et al., 2014). Improvements in MFPR techniques have enhanced

success rates such that quadruplet or triplets reduced to twins have comparable outcomes to natural twins (Evans et al., 2014). Studies have shown that reduction of triplet pregnancies to twins reduces the risk of preterm birth, LBW, cesarean delivery, and neonatal death (Dodd and Crowther, 2004). The average loss rate currently quoted as a complication of MFPR is about 4%, down from 13% in the 1990s (Stone et al., 2008; Evans et al., 2014). It is important to consider that MFPR is not without medical and psychological risks and that the ethical framework of each individual will guide decision making (Evans and Britt, 2010). A discussion regarding the ethical and medical implications of MFPR should be undertaken with all patients prior to undergoing ART (American College of Obstetricians and Gynecologists, 2013).

Summary

Over the last three decades, advances in ART have helped countless infertile couples achieve parenthood. This has had the unintended consequence of causing an epidemic of multiple births, which are associated with maternal, fetal, and neonatal morbidity and mortality. The subsequent psychosocial and economic costs are significant.

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Suggested Readings

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Births attributable to ART are likely to continue to increase with improvements in both effectiveness and accessibility, and strategies aimed at primary prevention of multiples should be our focus going forward.

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Prematurity and Stillbirth

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KEY POINTS

- Preterm birth, defined as birth prior to 37 weeks' gestation, is the leading cause of under-five child mortality worldwide.
- Preterm birth can be spontaneous or medically indicated, in which cesarean delivery or induction of labor is performed to avoid the risks of continued pregnancy.
- Preterm birth and stillbirth have been associated with multiple risk factors, including demographic, environment, genetic, placental, nutritional, microbial, and infectious factors.
- Further study of these factors and their associated pathways may lead to discovery of more direct ways to control the biology of pregnancy and, ultimately, the prevention of preterm birth.

Preterm birth, defined as birth prior to 37 weeks' gestation, affects 15 million newborns every year (Blencowe et al., 2012). It is the leading cause of under-five child mortality worldwide and accounts for one million deaths annually (Liu et al., 2015). More than 11% percent of newborns in the United States are born preterm, the annual cost of which exceeds \$26 billion (Behrman and Butler, 2007). Among surviving infants, preterm birth is also a major cause of disability-adjusted life years due to lifelong neurologic and developmental sequelae (Murray et al., 2012). The rate of preterm birth increased dramatically in the late 20th century, from less than 7% in the 1960s to a peak of 12.8% in 2006. However, there has been a recent decline to 11.4% in 2013, likely caused by a reduction in indicated late preterm deliveries (Frey and Klebanoff, 2016).

Preterm birth can be spontaneous, in which labor starts too soon, or medically indicated, in which cesarean delivery or induction of labor is performed to avoid the risks of continued pregnancy to the mother or fetus, e.g., due to preeclampsia or intrauterine growth restriction. Another subtype of spontaneous preterm birth is associated with premature preterm rupture of membranes (PPROM), in which rupture of amniotic membranes occurs both prior to 37 weeks' gestation and prior to the onset of labor (Fig. 8.1). Approximately 45% of preterm births result from spontaneous preterm labor, 30% from medically indicated delivery, and 25% from PPRM (Ananth et al., 2005; Goldenberg et al., 2008b). The extent to which the etiologic pathways leading to each type of preterm delivery are distinct versus overlapping is uncertain. Etiologic differences also exist between early preterm birth (e.g., <28 weeks' gestation) and late preterm birth, although the definition

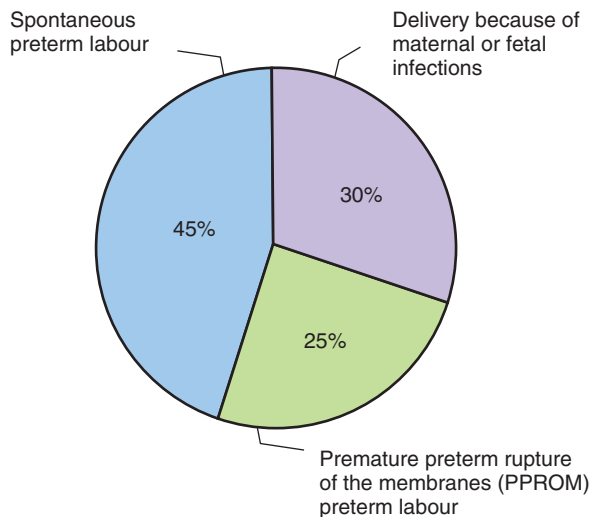
of “early” versus “late” preterm birth is somewhat arbitrary in the context of continuous gestational progression.

Spontaneous preterm birth is an exceedingly complex phenomenon that is not well understood. Preterm labor and labor at term are both characterized by uterine contractions that eventually lead to delivery of a human newborn but the underlying factors that initiate them may differ (Romero et al., 2006, 2014a). Physiologic or disease processes that induce uterine contractility, cervical dilatation, or rupture of amniotic membranes prior to 37 weeks' gestation can lead to preterm birth (Fig. 8.2). There is evidence that spontaneous preterm birth may be related to perturbations in the maternal–fetal environment, such as abnormal placental development, infection, uterine overdistension, and inflammation. The biology underlying these disease states is just now being addressed (Schober et al., 2012; Chatenoud, 2014; Gaudilliere, 2015; Schumacher and Zenclussen, 2014). However, the factors associated with the majority of preterm births remain unexplained (Muglia and Katz, 2010; Wallenstein et al., 2016a).

In most mammalian species, progesterone plays a vital role in maternal–fetal tolerance during pregnancy and functions to prevent pathologic (early) onset of labor. Humans, however, have evolved to retain an elevated progesterone level until after delivery, meaning that a functional withdrawal of progesterone (i.e., loss of sensitivity to progesterone) appears to play an important role in the onset of parturition at term (Druckmann and Druckmann, 2005). Progesterone also plays an important role in modulation of the maternal immune system via alteration in the production of inflammatory and anti-inflammatory cytokines (e.g., tumor necrosis factor- α and interleukin-10) and inhibition of natural killer cell activity (Druckmann and Druckmann, 2005). These immunologic cascades are vital for maternal tolerance of the “semi-allograft” fetus (Trowsdale and Betz, 2006), as is also demonstrated by the prevention of preterm birth by exogenous progesterone (da Fonseca et al., 2003). Studies of biologic markers in blood and amniotic/vaginal fluid have identified several unique biologic signatures, such as inflammatory cytokines, that reflect these pathways related to preterm birth (Moawad et al., 2002; Goldenberg et al., 2005; Jelliffe-Pawlowski et al., 2013, 2014; Wallenstein et al., 2016b), but a complete understanding of the immunologic and inflammatory pathways of pregnancy remains elusive.

Spontaneous preterm birth is a predominantly human phenomenon, which is the result of evolutionary pressures to maintain the advantages of bipedal ambulation and high intelligence (Wittman and Wall, 2007). Human head size at birth is near the dimensional limit of what is physically possible for delivery through the maternal pelvis. The competing interests of intelligence (i.e.,

head size) and bipedal ambulation (i.e., narrow pelvis) probably led to adjustments in gene expression and biologic signaling pathways that steered the earlier timing of parturition for humans, leading to the programmed delivery of relatively immature fetuses, compared with other nonhuman mammals. This alteration in timing of delivery has left us with a more easily perturbed physiology of parturition and, as a result, preterm birth. Over the course of human evolution, the risk of lethal immaturity was of little consequence relative to the gains of easy ambulation and high intelligence for the majority of the human species, which is likely why the risk of preterm birth has persisted and now remains a global problem of epidemic proportions.

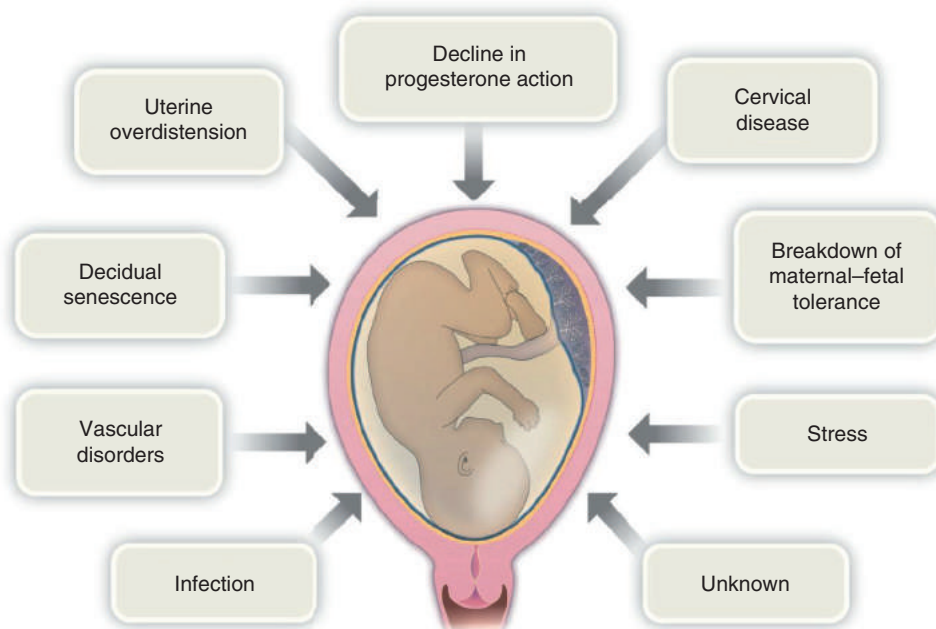


• **Fig. 8.1** Obstetric Precursors of Preterm Birth. (From Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet*. 2008;371:75–84.)

Demographic Factors and Disparities

Multiple studies have demonstrated elevated risk of preterm birth among black women. The rate of preterm birth among blacks is as high as 18%, compared with 11% in the general US population. It is even more disparate for early preterm birth (<28 weeks' gestation), which is up to 3–4 times higher (Goldenberg et al., 1996a; Messer et al., 2008). Elevated risk remains after controlling for socioeconomic status and psychosocial risk factors, which themselves are associated with preterm birth (Lobel et al., 1992; Copper et al., 1996; Ananth et al., 2001; Braveman et al., 2015). Disparities are more modest to nonexistent for Hispanic women, despite social disadvantage within this group (Goldenberg et al., 2008b). Other studies have found that the rate of preterm birth varies greatly based on geographic location and is driven by socioeconomic and demographic characteristics of those locales but possibly less so for blacks than whites (Hamilton et al., 2013; Carmichael et al., 2014; Byrnes et al., 2015). Other factors associated with lower socioeconomic status, including educational background, extremes of maternal age, short interval between pregnancies (<6 months), and single marital status, have been independently associated with preterm birth (Brett et al., 1997; Smith et al., 2003, 2007; Conde-Agudelo et al., 2006; Thompson et al., 2006; Wong et al., 2016).

Some portion of the racial/ethnic and socioeconomic disparities can be explained by increased psychosocial stress among these populations (Kramer et al., 2011). Several studies have found that maternal stress, as measured by a survey of life events and/or biomarkers of activation of the hypothalamic–pituitary–adrenal axis, is also strongly associated with preterm birth (Stillerman et al., 2008; Holzman et al., 2009; Shaw et al., 2014b). However, interventions to reduce stress have had limited success in preventing preterm birth (Khianman et al., 2012). Other factors proposed to contribute to disparities include nutrient deficiencies and infection, although further research is needed to clarify their roles (Dunlop et al., 2011; Menon et al., 2011).



• **Fig. 8.2** Proposed Mechanisms of Disease Implicated in Spontaneous Preterm Labor. (From Romero R, Hassan SS, Gajer P, et al. The vaginal microbiota of pregnant women who subsequently have spontaneous preterm labor and delivery and those with a normal delivery at term. *Microbiome*. 2014;2:18. doi: 10.1186/2049-2618-2-18.)

Environmental Factors

Exposure to toxins – both self-inflicted and environmental – has been shown to increase the risk of preterm birth. Tobacco smoking during pregnancy is one of the most significant of those toxins (Andres and Day, 2000; Cnattingius, 2004). The mechanism is unclear but may involve systemic inflammation (leading to spontaneous preterm birth) or placental vasoconstriction from nicotine causing fetal growth restriction, which, in turn, may lead to an indicated preterm birth (Goldenberg et al., 2008b). Alcohol has not been consistently related to risk of preterm birth, although cocaine and other illicit substances increase preterm birth risk, likely via alteration of placental blood flow and/or placental abruption (Janisse et al., 2014).

Environmental air pollution has also been associated with increased risk of preterm birth. There are a myriad of air pollutants that have been investigated, including ozone, carbon monoxide, sulfur dioxide, and lead, among others (Padula et al., 2014a, 2014b; Stillerman et al., 2008).

Exposure to other toxicants, including various pesticides and heavy metals, has also been associated with preterm birth (Harley et al., 2011; Shachar et al., 2013).

Nutrition and Maternal Body Weight

Obesity, defined as body mass index (BMI) greater than or equal to 30 kg/m², is a major global health problem, affecting hundreds of millions of people worldwide. Prepregnancy obesity is a known risk factor for preterm birth (Wise et al., 2010; Djelantik et al., 2012; Gould et al., 2014; Shaw et al., 2014a). Comorbidities associated with obesity, such as diabetes and hypertension, may contribute to this obesity–preterm birth association (Sibai et al., 2000; Hedderson et al., 2003; Metzger et al., 2008). The biologic mechanisms behind the association may involve pathologic inflammation and altered vascular development (Mohamed-Ali et al., 1999; Visser et al., 1999; Royblat et al., 2000; Wallenstein et al., 2016b).

Low maternal BMI is also a risk factor for spontaneous preterm birth (Hendler et al., 2005). Poor nutrient intake is associated with both low and high BMI and serves as one potential explanation for their association with preterm birth. Iron deficiency anemia, lower intakes of nutrients such as folic acid and other B vitamins, zinc, calcium, magnesium, and polyunsaturated fatty acids, and higher dietary glycemic index have all also been associated with an increased risk (Kramer et al., 2009; Dunlop et al., 2011; Carmichael et al., 2013).

Infection and Microbiota

More than one-fourth of preterm births can be attributed to intra-amniotic infection, in which microorganisms perturb maternal–fetal homeostasis and trigger a cascade of biologic signals that initiate spontaneous preterm labor, PPROM, or fetal distress that leads to indicated preterm delivery (Romero et al., 2001; Elovitz et al., 2003; Menon and Furtunato, 2007; Moore et al., 2007; Agrawal and Hirsch, 2012). Microorganisms and their immunogenic cellular components activate toll-like receptors, chemokines, and cytokines, which tips the balance of maternal–fetal tolerance and leads to uterine contractility, rupture of amniotic membranes, and preterm delivery (Goldenberg et al., 2000; Romero et al., 2006). This may represent an evolutionary defense, as expulsion of an infected fetal–placental unit may allow the mother to survive and reproduce at a later time. Spontaneous preterm

births associated with chorioamnionitis are more common in women who deliver at earlier gestational periods (Hendler et al., 2005; Scholl, 2005).

The role of microorganisms in preterm birth is often subclinical, in which there are no signs or symptoms of overt maternal infection. One study showed that more than one-fifth of preterm newborns have umbilical cords colonized by *Mycoplasma* species (Goldenberg et al., 2008a). Bacterial vaginosis, which may affect up to one-third of pregnancies, has also been associated with preterm birth, particularly among black women (Hillier et al., 1995; Meis et al., 1995; Goldenberg et al., 1996b), as have sexually transmitted infections (Donders et al., 1993). Other infectious processes, such as periodontal disease and urinary tract infections, may also increase the risk of preterm birth (Offenbacher et al., 1996; Goldenberg et al., 2005). Unfortunately, however, treatment of these infections has not reduced the rate of preterm birth, including treatment for periodontal disease and bacterial vaginosis, among others (Kekki et al., 2001; Boggess, 2010).

There is also evidence that the microbiome, and perturbations therein, plays an important role in maternal–fetal tolerance and preterm birth (Romero et al., 2014b; DiGiulio et al., 2015; Gaudilliere, 2015). Recent technologic advances have enabled sequencing and amplification of DNA from myriad microbial communities, including the placental microbiome, revealing complex potential interactions between these microbial communities and clinical pregnancy outcomes (Aagaard et al., 2014). Study of microbial communities during pregnancy, and microbiota in general, represents an important and exciting frontier in the effort to further our understanding of preterm birth.

Genetic Factors

Preterm birth tends to run in families and appears to be inherited across generations (Porter et al., 1997; Winkvist et al., 1998). The heritability of preterm birth has been estimated to be 15%–40% (Muglia and Katz, 2010). Women with a prior history of preterm birth have more than a twofold increased risk of preterm birth in the subsequent pregnancy, although environmental factors may also explain this finding (Mercer et al., 1999).

Advances in our ability to conduct genome-wide association studies – and to do so in a cost-effective manner – have led to increased interest in the study of single-nucleotide polymorphisms. Several large genome-wide association studies were recently conducted and found no definitive evidence of association between preterm birth and any one single nucleotide polymorphism (Uzun et al., 2013; Zhang et al., 2015). However, additional analyses suggest that gene–gene interactions, particularly those related to inflammation and metabolic disorders, may affect the risk of preterm birth (Uzun et al., 2013). Several candidate genes have been identified by other investigators (Crider et al., 2005; Engel et al., 2005; Menon et al., 2006), but there are many inconsistencies in the data and findings (Plunkett and Muglia, 2008).

These inconsistencies may arise from the complex gene–gene and gene–environment interactions related to preterm labor and/or delivery (Ward et al., 2005; Plunkett and Muglia, 2008; Plunkett et al., 2009). One study found an association between a tumor necrosis factor- α allele, bacterial vaginosis, and preterm birth, although neither maternal carrier status nor bacterial vaginosis alone was associated with preterm birth (Macones et al., 2004). Similar interactions with bacterial vaginosis were reported in a recent study on maternal carrier status of an interleukin-6 allele among black women (Engel et al., 2005). As we refine our ability

to conduct genetic and epigenetic research, and to do so more affordably, we will continue to take incremental steps toward solving the puzzle of preterm birth.

Placental and Pregnancy Factors

Several factors associated with disruption of the uterine environment during pregnancy have been associated with preterm birth (Romero et al., 2014a). Abnormal vascular development of the placenta is a frequent occurrence among women who deliver before term (Kim et al., 2003). Perturbations of placentation may also account for many other adverse pregnancy outcomes that are seen in clinical practice, and that often cooccur with preterm birth, such as intrauterine growth restriction and preeclampsia (Cha et al., 2012). Vaginal bleeding, isolated or associated with placental abruption or placenta previa, also carries increased risk of preterm delivery (Krupa et al., 2006).

Overdistension of the uterus, caused by multiple gestation or polyhydramnios, substantially increases the risk of preterm birth (Williams et al., 1991; Kiely, 1998; Han et al., 2001). More than half of twin pregnancies result in delivery prior to 37 weeks' gestation, likely because of mechanical distension from increased fetal mass and other comorbidities associated with multiple gestation. Polyhydramnios and oligohydramnios are both risk factors for PPRM and preterm labor (Goldenberg et al., 2008). Anatomic abnormalities of the uterus and previous uterine or cervical surgical intervention also increase the risk of preterm birth (Jakobsson et al., 2007). Likewise, short cervix and cervical insufficiency carry increased risk, which varies greatly depending on degree of shortening (Copper et al., 1990; Iams et al., 1996; Andrews et al., 2000).

Prevention of Preterm Birth

Efforts to prevent – and to predict – preterm birth have largely failed, although several targeted interventions, including low-dose aspirin and 17-hydroxyprogesterone, have been shown to reduce the risk of preterm birth among women at high risk for adverse pregnancy outcomes, such as women with a personal history of preterm birth or stillbirth (da Fonseca et al., 2003; Meis et al., 2003; Henderson et al., 2014). Use of cervical cerclage among patients with short cervix and a history of preterm birth has effectively reduced the risk of preterm birth among this small subset of women (Iams et al., 2008). Population-level interventions that address underlying disparities, such as improving access to prenatal care, or other public policies related to smoking cessation or environmental pollution, may reduce the overall rate of preterm birth.

Other obstetrical interventions have been successful at mitigating the neonatal morbidity and mortality associated with preterm birth, such as antibiotics, antenatal betamethasone, magnesium sulfate, and tocolytic agents, but these interventions rarely prevent delivery prior to 37 weeks' gestation.

Stillbirth

Stillbirth, defined here as in utero death of a fetus at 20 weeks' gestation or later, is more common than most people realize. In the United States, more babies die in utero than die in the neonatal period. Stillbirths affect 5–6 per 1000 pregnancies in the United States, and the prevalence is several times higher in lower-income countries. For comparison, overall annual noninfant mortality is 8 per 1000 people in the United States (Hoyert et al., 2012). In recent decades, stillbirths have declined, but these gains are largely

attributable to improvements in obstetric care and prevention of intrapartum complications, and they are confined to later stillbirths (>28 weeks) (MacDorman and Kirmeyer, 2009). Despite its huge impact, little research has focused on finding causes of stillbirth, thus severely limiting opportunities for prevention (Lawn et al., 2011).

Over 80% of stillbirths are delivered before 37 weeks' gestation, and at least half of those fetuses are growth-restricted. In addition, at the earliest gestational ages, a large proportion of all deliveries (i.e., live births plus stillbirths) are stillbirths; for example, among deliveries at less than 28 weeks' gestation in California, about one-third are defined as stillbirths. Given these overlaps, we also expect overlaps in the etiologies of stillbirth, preterm delivery, and growth restriction. Indeed, all of these outcomes share similar risk factors, including chronic maternal medical conditions such as obesity, hypertension and diabetes, maternal stress, and a marked black–white disparity (Willinger et al., 2009; Fretts, 2010; Hogue et al., 2013; Warland and Mitchell, 2014; Yao et al., 2014; Carmichael et al., 2015). In addition, some of the most common clinical antecedents of stillbirth are similar to those of preterm delivery, such as spontaneous preterm labor or rupture of membranes, infection, and placental insufficiency. Inflammation may be a shared pathway contributing to stillbirth and preterm delivery (Fretts, 2010; Mullins et al., 2012; Warland and Mitchell, 2014). Further understanding of preterm birth will likely provide clues to help us understand stillbirth. More frequent inclusion of stillbirth in studies of preterm delivery – especially early preterm delivery – will be important to fully understanding either outcome.

Looking Forward

Multiple causal pathways likely lead to preterm birth and stillbirth. Further study of these pathways may lead to discovery of more direct ways to control the biology of pregnancy. Preliminary evidence already exists for effective prevention of preterm birth through interference with some of these pathways (Henderson et al., 2014). Future interventions may take the form of targeted pharmaceuticals, such as low-dose aspirin, or through community-based interventions that indirectly affect the biology, such as implementation of policies related to socioeconomic disparities or nutrition. Understanding when and where we are able to intervene to prevent preterm birth will require collaboration on large and transdisciplinary scales with concomitant integration of data from multiple levels of inquiry (Stevenson et al., 2013).

Suggested Readings

- Goldenberg RL, Culhane JF, Iams JD, et al. Epidemiology and causes of preterm birth. *Lancet*. 2008;371:75-84.
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9

Nonimmune Hydrops

SCOTT A. LORCH AND THOMAS J. MOLLEN

KEY POINTS

- Nonimmune hydrops fetalis results from many etiologies.
- Management involves correcting the underlying cause.
- The condition is associated with significant morbidity and mortality.
- Some etiologies are amenable to treatment in utero.

A newborn with hydrops has an abnormal accumulation of fluid. The condition varies from mild, generalized edema to massive anasarca with effusions in multiple body cavities and with peripheral edema so severe that the extremities are fixed in extension. Fetuses with severe hydrops may die in utero; if liveborn, they may die in the neonatal period from the severity of their underlying disease or from severe cardiorespiratory failure.

The first description of hydrops, in a newborn twin, may have appeared in 1609 (Liley et al., 2009). Ballantyne (1892) suggested that the finding of hydrops was an outcome from many different causes, in contrast to the belief at that time that hydrops was a single entity. Potter (1943) was the first to make the distinction between nonimmune hydrops and hydrops secondary to erythroblastosis fetalis by describing a group of infants with generalized body edema who did not have hepatosplenomegaly or abnormal erythropoiesis. Potter's description of more than 100 cases of hydrops included two sets of twins in which one had hydrops and the other did not, thus presenting the first description of twin–twin transfusion syndrome. With the nearly universal use of anti-D globulin and refinement of the schedule and doses for its administration, the occurrence of immune-mediated hydrops has steadily declined and may be as low as 6%–10% of all cases of hydrops (Bellini et al., 2015).

Incidence

The reported incidence of nonimmune hydrops in the general population has been highly variable, ranging from 6 per 1000 pregnancies in a high-risk referral clinic in the United Kingdom between 1993 and 1999 (Sohan et al., 2001), to 1 in 4000 pregnancies (Norton, 1994); other published rates are 6 per 1000 pregnancies (Santolaya et al., 1992), 1.3 per 1000 pregnancies (Wafelman et al., 1999), and 1 per 1700 pregnancies (Heinonen et al., 2000). However, all the published studies come from single institutions, with the at-risk populations ranging from that of a high-risk

pregnancy clinic to infants in a neonatal intensive care unit and generally predating the more routine use of ultrasound investigation in the late first trimester of pregnancy (Iskaros et al., 1997). There have been no recent studies of the prevalence of this condition in all pregnant women in one geographic area to calculate the true population incidence of nonimmune hydrops, especially monitoring fetuses who died in utero. Geography also affects the incidence; several causes of nonimmune hydrops, such as α -thalassemia, are more common in certain areas of the world.

Etiology

Nonimmune hydrops has been associated with a wide range of conditions (Table 9.1). In many of these conditions, edema formation results from one of the following possible processes:

- Elevated central venous pressure (CVP), in which the cardiac output is less than the rate of venous return
- Anemia, resulting in high-output cardiac failure
- Decreased lymphatic flow
- Capillary leak

The actual pathophysiology of hydrops for many of the conditions in Table 9.1, however, is still not understood.

The most common causes of nonimmune hydrops are chromosomal, cardiovascular, hematologic, thoracic, infectious, and conditions related to twinning (Wilkins, 1999; Abrams et al., 2007; Bellini et al., 2009a). As with reported incidence rates, the relative contribution of these causes varies by study. The studies that focus on early fetal presentation of hydrops (postconceptional age of less than 24 weeks' gestation) have found that chromosomal abnormalities, such as Turner syndrome and trisomies 13, 18, and 21, contribute 32%–78% of all cases of hydrops (Boyd and Keeling, 1992; McCoy et al., 1995; Iskaros et al., 1997; Heinonen et al., 2000; Sohan et al., 2001). For fetuses whose hydrops becomes evident after 24 weeks' gestation, cardiovascular and thoracic causes are most prevalent, with rates ranging between 30% and 50% (Machin, 1989; McCoy et al., 1995). Studies from Asia have noted a higher percentage of cases from hematologic causes, probably because of the higher rates of α -thalassemia in the population (Lin et al., 1991; Nakayama et al., 1999).

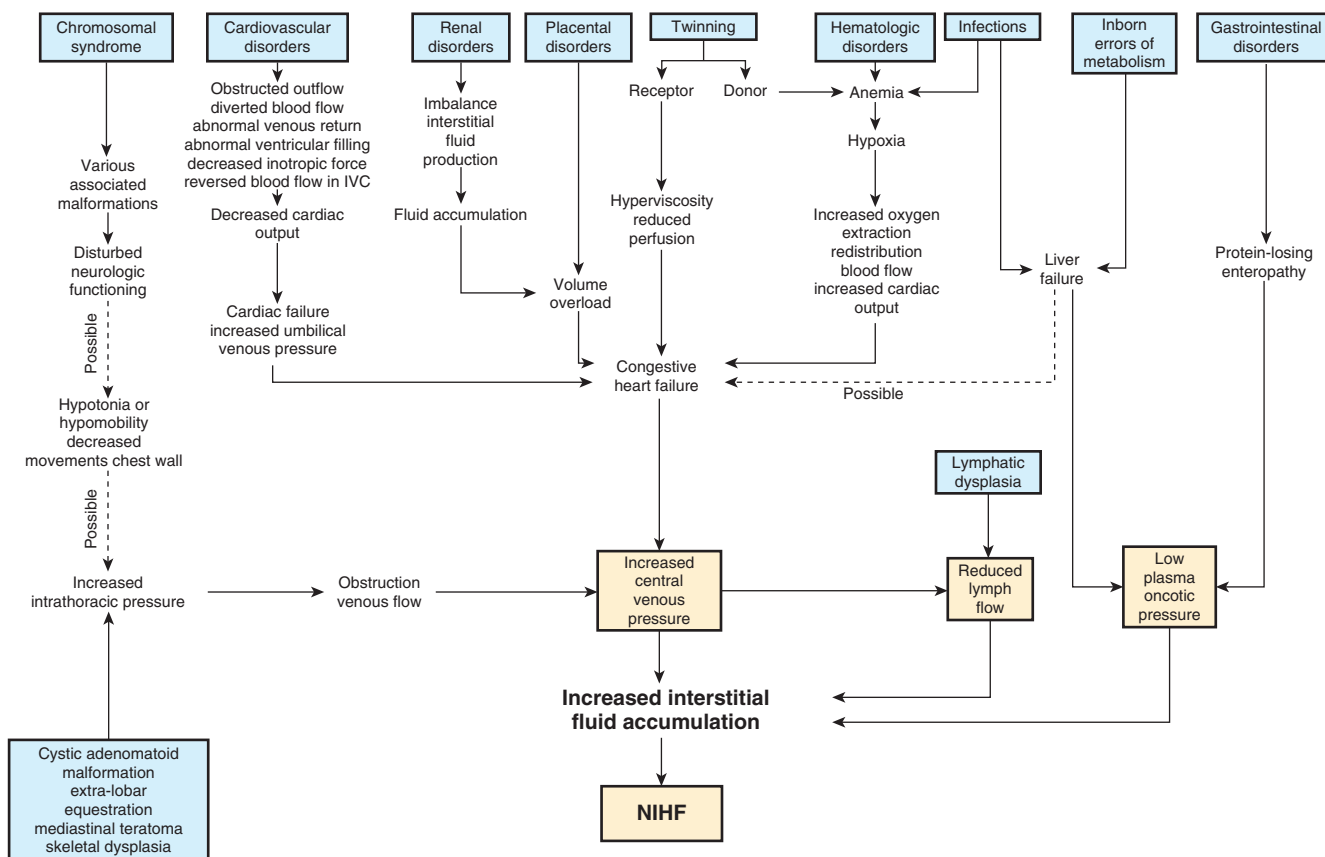
A recent review of studies published between 2008 and 2013 suggests a potential change in the etiologies of nonimmune hydrops, when compared with studies published between 1979 and 2008 (Bellini et al., 2015). These more recent studies reported a higher rate of lymphatic etiologies (15.0% compared with 5.7% in older

TABLE 9.1 Conditions Associated With Hydrops Fetalis

| Condition Type | Specific Conditions | Condition Type | Specific Conditions |
|-------------------------|---|----------------------------------|---|
| Hemolytic anemias | Alloimmune, Rh, Kell, α -chain hemoglobinopathies (homozygous α -thalassemia) Red blood cell enzyme deficiencies (glucose phosphate isomerase deficiency, glucose-6-phosphate dehydrogenase) | Pulmonary conditions | Cystic adenomatoid malformation of the lung Mediastinal teratoma Diaphragmatic hernia Lung sequestration syndrome Lymphangiectasia |
| Other anemias | Fetomaternal hemorrhage Twin–twin transfusion Diamond–Blackfan | Renal conditions | Urinary ascites Congenital nephrosis Renal vein thrombosis Invasive processes and storage disorders Tuberous sclerosis Gaucher disease Mucopolysaccharidosis Mucopolipidosis |
| Cardiac conditions | Premature closure of foramen ovale Ebstein anomaly Hypoplastic left or right heart Subaortic stenosis with fibroelastosis Cardiomyopathy, myocardial fibroelastosis Atrioventricular canal Myocarditis Right atrial hemangioma Intracardiac hamartoma or fibroma Tuberous sclerosis with cardiac rhabdomyoma | Chromosome abnormalities | Trisomy 13, trisomy 18, trisomy 21 Turner syndrome 46, XX/XY chimerism |
| Cardiac arrhythmias | Supraventricular tachycardia Atrial flutter Congenital heart block | Bone diseases | Osteogenesis imperfecta Achondroplasia Asphyxiating thoracic dystrophy |
| Vascular malformations | Hemangioma of the liver Any large arteriovenous malformation Klippel–Trénaunay syndrome Idiopathic infantile arterial calcification | Gastrointestinal conditions | Bowel obstruction with perforation and meconium peritonitis Small bowel volvulus Other intestinal obstructions Prune-belly syndrome |
| Vascular accidents | Thrombosis of umbilical vein or inferior vena cava Recipient in twin–twin transfusion | Tumors | Neuroblastoma Choriocarcinoma Sacrococcygeal teratoma Hemangioma or other hepatic tumors Congenital leukemia Cardiac tumors Renal tumors |
| Infections | Cytomegalovirus, congenital hepatitis, human parvovirus, enterovirus, other viruses Toxoplasmosis, Chagas disease Coxsackie virus Syphilis Leptospirosis | Maternal or placental conditions | Maternal diabetes Maternal therapy with indomethacin Multiple gestation with parasitic fetus Chorioangioma of placenta, chorionic vessels, or umbilical vessels Toxemia Systemic lupus erythematosus |
| Lymphatic abnormalities | Congenital lymphatic dysplasia Lymphatic malformations Lymphangiectasia Cystic hygroma Noonan syndrome Multiple pterygium syndrome Congenital chylothorax Hereditary lymphedema type 1 | Miscellaneous | Neu–Laxova syndrome Myotonic dystrophy Hereditary lymphedema type 1 |
| Nervous system lesions | Absence of corpus callosum Encephalocele Cerebral arteriovenous malformation Intracranial hemorrhage (massive) Holoprosencephaly Fetal akinesia sequence | Idiopathic | |

studies) and a lower rate of thoracic or chromosomal etiologies. Idiopathic hydrops, or hydrops of unknown etiology, remained constant at between 18% and 20% of all cases. As with older studies, papers published between 2008 and 2013 varied widely in the prevalence of specific etiologies depending on the ability of the clinicians to complete their diagnostic evaluation, geography, and the inclusion of fetal deaths in the analysis (Machin, 1989;

Santolaya et al., 1992; McCoy et al., 1995; Iskaros et al., 1997; Yaegashi et al., 1998; Nakayama et al., 1999; Wafelman et al., 1999; Wy et al., 1999; Heinonen et al., 2000; Sohan et al., 2001; Bellini et al., 2009b). It is likely that with increased understanding of (and genetic testing for) many of the conditions listed in Table 9.1, the number of infants diagnosed with idiopathic, nonimmune hydrops will continue to decline.



• **Fig. 9.1** Impact of Various Etiologies for Nonimmune Hydrops on Fluid Homeostasis. IVC, Inferior vena cava; NIHF, nonimmune hydrops fetalis. (Modified from Bellini C, Hennekam RCM. Non-immune hydrops fetalis: a short review of etiology and pathophysiology. *Am J Med Genet A*. 2012;158A: 597–605.)

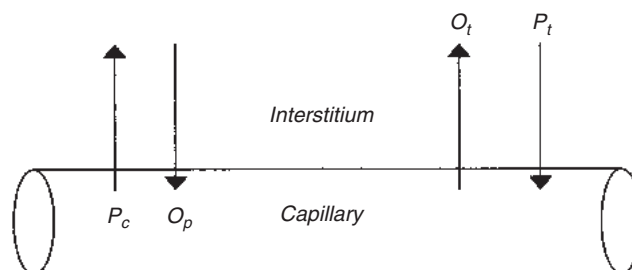
Pathophysiology

Normal Fluid Homeostasis

Abnormal body fluid homeostasis is the underlying cause of edema, whether localized or generalized. To understand the pathogenesis of hydrops, the clinician must consider the forces underlying normal fluid homeostasis that underpin a general pathophysiologic pathway for this disorder (Fig. 9.1) as proposed by Bellini and Hennekam (2012). The regulation of net fluid movement across a capillary membrane depends on the Starling forces, which were first described by E. H. Starling (1896). Flow between intravascular and interstitial fluid compartments is determined by the balance among (1) capillary hydrostatic pressure, (2) serum colloid oncotic pressure, (3) interstitial hydrostatic pressure or tissue turgor pressure, and (4) interstitial osmotic pressure, which depends on lymphatic flow. The Starling equation defines the relationship among these forces and their net effect on net fluid movement, or filtration, across a semipermeable membrane (such as the capillary membrane) as:

$$\text{Filtration} = K[(P_c - P_t) - R(O_p - O_t)]$$

where K = capillary filtration coefficient, representing the extent of permeability of a membrane to water and thus describing capillary integrity; P_c = capillary hydrostatic pressure; P_t = interstitial hydrostatic pressure or tissue turgor pressure; R = reflection coefficient for a solute, representing the extent of permeability of the capillary wall to that solute; O_p = plasma oncotic pressure as



• **Fig. 9.2** Starling Forces and Net Effect on Fluid Homeostasis. Arrows represent net effect of movement of fluid across the capillary membrane for each factor under normal conditions. P_c , Capillary hydrostatic pressure; P_t , interstitial hydrostatic pressure or tissue turgor pressure; O_p , plasma oncotic pressure as determined by plasma proteins and other solutes; O_t , interstitial osmotic pressure.

determined by plasma proteins and other solutes; and O_t = interstitial osmotic pressure (Fig. 9.2).

Although an abnormality of any of the components of this equation may, in theory, result in the accumulation of edema fluid, the fetal-placental unit presents a unique physiologic condition that effectively eliminates two of the factors, assuming unimpeded fetal-placental flow and an appropriately functioning maternal-placental interface. Because approximately 40% of fetal cardiac output is allocated to the placenta, there is rapid transport

of water between the fetus and mother. Any condition resulting in elevated fetal capillary hydrostatic pressure or low plasma colloid oncotic pressure would likely cause the net flow of water from fetal villi in the placenta to the maternal blood stream, where it can be effectively eliminated. This elimination of fluid would counteract the accumulation of interstitial fluid by the fetus. Although the placenta of a fetus with hydrops is also edematous, these changes are believed to occur with, and not before, fetal fluid accumulation.

Derangements in Fluid Homeostasis

Elevated Central Venous Pressure

The most commonly diagnosed causes of nonimmune hydrops that appear in fetuses older than 24 weeks' gestation are cardiac disorders. Any state in which cardiac output is lower than the rate of venous return results in an elevated CVP that raises capillary filtration pressures and, if high enough, restricts lymphatic return. Both of these mechanisms may then contribute to interstitial accumulation of fluid. Structural cardiac causes of elevated CVP include right-sided obstructive lesions and valvular regurgitation. The most common and easily reversible cause of nonimmune hydrops is supraventricular tachycardia (SVT). In general, cardiac output rises with heart rate. At the increasingly high rates seen in SVT, however, cardiac output plateaus and then diminishes. The heart rates observed with SVT are often associated with decreased cardiac output. Impaired cardiac output results in elevated CVP, which can give rise to edema through mechanisms discussed previously (Gest et al., 1990).

Infants with alloimmune hydrops (and several of the nonimmune hydrops conditions as well) have significant anemia that may also result in elevated CVP. It has been proposed that anemia leads to congestive heart failure with increased hydrostatic pressure in the capillaries, causing vascular damage that results in edema. However, the hematocrit values of infants with and without hydrops overlap significantly, suggesting that anemia alone is not the only explanation. A rapidly lowered hemoglobin concentration results in greater cardiac output to maintain adequate oxygen delivery. This increased cardiac output results in higher oxygen demands by the myocardium, which may be difficult to meet because of the anemia. The hypoxic myocardium can become less contractile and less compliant, with ventricular stiffness causing increased afterload to the atria. High-output congestive heart failure may then develop, resulting in elevated CVP. Raised CVP leads to increased capillary filtration pressures and impairment of lymphatic return (Weiner, 1993).

An intrathoracic mass such as a pulmonary malformation may also increase CVP, resulting in impaired filling of the right ventricle and consequent increased capillary hydrostatic pressure. While the underlying reason for the elevated CVP differs between these two conditions, the resulting clinical conditions are similar.

Decreased Lymphatic Flow

If the rate of fluid filtration from plasma to tissues exceeds the rate of lymph return to the central venous system, then edema and effusions may form. A structural impediment or increased CVP that opposes lymphatic return to the heart can impair lymph flow. Recent advances in the radiographic evaluation of the lymphatic system, using magnetic resonance imaging (MRI) and lymphangiography, have improved the assessment of this system. To determine the effects of alterations in CVP on lymphatic return, Gest et al. (1992) inserted a catheter into the thoracic duct of fetal lambs and applied an opposing hydrostatic pressure by varying

the height of the catheter. Thoracic duct flow was nearly constant over the physiologic range of CVP but sharply decreased at elevated pressures; therefore lymphatic flow may be reduced or essentially blocked in pathologic states associated with elevated CVP.

Decreased Oncotic Pressure

A third potential etiology for nonimmune hydrops is hypoalbuminemia. The most convincing evidence for this etiology is in conditions where reduced compliance of a right ventricle may result in flow reversal in the inferior vena cava, which may in turn cause end-organ damage to the liver, with consequent hypoalbuminemia and portal hypertension enhancing formation of both edema and ascites. Hydrops has been produced in fetal lambs (Blair et al., 1994) in which the hemoglobin content was lowered in 12 fetuses through exchange transfusion using cell-free plasma; six became hypoproteinemic. Anemia developed more rapidly with a higher CVP in fetuses with hydrops than in the fetuses without hydrops. In the most severely anemic fetuses, it is probable that decreased oxygen transport causes tissue hypoxia, which in turn increases capillary permeability to both water and protein. These changes in capillary permeability may contribute to the development of hydrops.

However, human fetuses with hypoproteinemia as a result of nephrotic syndrome or analbuminemia rarely experience hydrops, supporting the hypothesis that hypoproteinemia alone is not sufficient to cause hydrops. To elucidate the role of isolated hypoproteinemia in the genesis of hydrops, Moise et al. (1991) induced hypoproteinemia in sets of twin fetal lambs. One twin from each set underwent serum protein reduction through repeated removal of plasma and replacement with normal saline; the other twin served as the control. Over 3 days, plasma protein concentrations were reduced by an average of 41%, with a 44% reduction in colloid osmotic pressure, in experimental subjects. No fetal animals became edematous, and total body water content values were similar in experimental and control animals. Thus hypoproteinemia alone was insufficient to cause hydrops fetalis over the course of the study. Transcapillary filtration probably increased with hypoproteinemia but was compensated for by lymphatic return. Hypoproteinemia may lower the threshold for edema formation in the presence of impaired lymphatic return or increased intravascular hydrostatic pressures.

Increased Capillary Leak

One final potential etiology for hydrops is increased capillary leak. Increased capillary leak is typically identified not as a primary mechanism for hydrops, but as part of the pathway between nonimmune hydrops and other insults, such as infection, hypoxic injury, or increased capillary hydrostatic pressure seen with elevated CVP or decreased lymphatic flow (Bellini and Hennekam, 2012).

Prenatal Diagnosis

Despite the underlying cause of hydrops or the clinical presentation, the prenatal diagnosis is made via the ultrasonographic finding of excess fluid in the form of ascites, pleural or pericardial effusions, skin edema, placental edema, or polyhydramnios. Several definitions for ultrasonographic diagnosis based on quantity and distribution of excess fluid have been proposed. One widely accepted set of criteria consists of the presence of excess fluid in any two of the previously listed compartments. A recent study suggests that the involvement of more body compartments is associated with higher mortality (Kim et al., 2015). Because this definition is based on

TABLE 9.2 Antenatal Investigation of Fetal Hydrops

| Area | Testing |
|----------------------|---|
| Maternal | History, including: Age, parity, gestation Medical and family histories Recent illnesses or exposures Medications Complete blood count and indices Blood typing and indirect Coombs antibody screening Hemoglobin electrophoresis Kleihauer–Betke stain of peripheral blood Syphilis, TORCH, and parvovirus B19 titers Anti-Ro and anti-La antibodies, systemic lupus erythematosus preparation Oral glucose tolerance test Glucose-6-phosphate dehydrogenase, pyruvate kinase deficiency screening |
| Fetal | Serial ultrasound evaluations Middle cerebral artery peak systolic velocity Limb length, fetal movement Echocardiography Fetal MRI |
| Amniocentesis | Karyotype Alpha-fetoprotein Viral cultures; polymerase chain reaction analysis for toxoplasmosis, parvovirus 19 Establishment of culture for appropriate metabolic or DNA testing Lecithin-to-sphingomyelin ratio to assess lung maturity |
| Fetal blood sampling | Genetic testing Complete blood count Hemoglobin analysis Immunoglobulin M test; specific cultures Albumin and total protein measurements Measurement of umbilical venous pressure Metabolic testing |

MRI, Magnetic resonance imaging; TORCH, toxoplasmosis, other agents, rubella, cytomegalovirus, and herpes simplex.

Adapted from Swain S, Cameron AD, McNay MB, Howatson AG. Prenatal diagnosis and management of nonimmune hydrops fetalis. *Aust N Z J Obstet Gynaecol.* 1999;39:285–290 and Bellini C, Hennekam RC, Bonioli E. A diagnostic flow chart for non-immune hydrops fetalis. *Am J Med Genet A.* 2009;149A:852–853.

the presence of excess fluid alone, the degree of severity is generally subjective.

Swain et al. (1999) and Bellini and Hennekam (2012) outline a multidisciplinary approach to the evaluation and management of the mother and fetus with hydrops. Table 9.2 provides recommendations for the investigation of fetal hydrops. Patient history should focus on ethnic background, familial history of consanguinity, genetic or congenital anomalies, and complications of pregnancy, including recent maternal illness and environmental exposures. Maternal disorders such as diabetes, systemic lupus erythematosus, myotonic dystrophy, and any type of liver disease should also be noted. Initial laboratory investigation includes blood typing and a Coombs test to rule out immune-mediated hydrops. Additional studies may include screening for hemoglobinopathies, a Kleihauer–Betke test

to eliminate fetal–maternal hemorrhage, and testing for TORCH (i.e., toxoplasmosis, other agents, rubella, cytomegalovirus, and herpes simplex), including syphilis and parvovirus B19.

Rapid evaluation is necessary to determine whether fetal intervention is possible and to estimate the prognosis for the fetus. Many conditions, such as arrhythmias, twin–twin transfusion, large vascular masses, and congenital diaphragmatic hernias and other chest-occupying lesions, are discovered during the initial ultrasonographic evaluation (Coleman et al., 2002). Middle cerebral artery peak systolic velocity measurement can aid in detecting the presence of fetal anemia (Hernandez-Andrade et al., 2004). If the initial ultrasonic examination is not helpful in identifying a cause, it may be helpful to repeat it at a later date to reassess fetal anatomy, monitor progression of the hydrops, and evaluate well-being of the fetus. Fetal MRI is being employed more commonly in cases of hydrops fetalis where the sonographic examination is unable to present a clear picture regarding etiology or to further delineate the anatomy in cases with a known anatomic etiology.

Fetal echocardiography should also be performed to evaluate for cardiac malformations and arrhythmia. Amniotic fluid can be obtained for fetal DNA analysis, cultures, and lecithin-to-sphingomyelin ratio to assess lung maturity. Fetal blood sampling allows for other tests, such as a complete blood cell count, routine chemical analyses, DNA analysis, bacterial and viral cultures, metabolic studies, and serum immunoglobulin measurements.

Prenatal Management

The goals of antenatal evaluation and management of fetal hydrops depend on the underlying cause. In diagnoses for which therapy is futile, the goal is to avoid unnecessary invasive testing and cesarean section. The prognosis should be discussed frankly with the parents, who should be given the option of terminating the pregnancy. If the underlying cause is amenable to fetal therapy, the risks and benefits of such therapy, as well as the warning that diagnostic error is possible, should be discussed with the family.

SVT is one of the most common known causes of nonimmune hydrops, and it is the most amenable to treatment (Newburger and Keane, 1979; Huhta, 2004). Usually the mother is given antiarrhythmic agents, and the fetus is monitored closely for resolution of the SVT. Digoxin is most commonly administered, although other antiarrhythmics have been used, such as sotalol or flecainide, because transplacental transfer of digoxin may be impaired in the setting of hydrops. In extreme circumstances, such as fetal tachyarrhythmia refractory to maternal treatment, direct fetal administration of antiarrhythmic agents via percutaneous umbilical blood sampling or intramuscular injection, although untested and highly risky, has met with some success.

If anemia is the cause of hydrops, transfusions of packed red blood cells may be administered to the fetus. Often a single transfusion reverses the edema, although serial transfusions may be necessary. Parvovirus B19 (Anand et al., 1987) and fetal–maternal hemorrhage are examples of diagnoses that are amenable to this therapy. Transfusions should be given, with the use of ultrasonographic guidance, into the intraperitoneal space or umbilical vein. Blood instilled into the abdominal cavity is taken up by lymphatics, but elevated CVP present in hydropic fetuses may impair this uptake. If uptake of intraperitoneal blood is incomplete, treatment for the hydrops is less successful. In addition, degeneration of the remaining hemoglobin may create a substantial bilirubin load, necessitating phototherapy or exchange transfusion after the fetus is delivered. Other diagnoses involving anemias that are refractory

to transfusions, such as α -thalassemia, may require neonatal stem cell transplantation.

High morbidity and mortality rates in severe twin–twin transfusion with associated hydrops led to multiple international trials of laser photocoagulation of interfetal vascular connections. A recent systematic review of 34 studies of 3868 patients published between 1990 and 2014 found improved survival comparing the 1990–1995 with the 2011–2014 epoch. Of pregnancies requiring laser photocoagulation, both twins survived in 65% of the pregnancies in the more recent epoch, with an additional 23% of pregnancies reporting a survival of one of the fetuses (Akkermans et al., 2015). However, the current level of evidence is limited in the reported effect on neurodevelopmental outcomes in survivors. The most recent Cochrane Review from 2008, then, recommends considering treatment with laser coagulation at all stages of twin–twin transfusion (Roberts et al., 2008).

Fetal intervention has met with some success in surgical defects and other conditions associated with hydrops (Adzick et al., 1998; Kitano et al., 1999; Azizkhan and Crombleholme, 2008). While fetal lung lesions such as congenital cystic adenomatoid malformation (CCAM) and pulmonary sequestration frequently involute and may disappear before delivery, in the most extreme cases they can result in mediastinal shift, pulmonary hypoplasia, cardiovascular compromise, and hydrops. The highest risk lesions for the development of hydrops include those lesions with a CCAM volume ratio greater than 2, an everted hemidiaphragm or a mass-to-thorax ratio greater than 0.56 (Vu et al., 2007; Cass et al., 2011). Early surgical resection for these most severe lesions has occurred in several centers. The latest therapy involves ex utero intrapartum therapy, or EXIT delivery, where the resection of the CCAM occurs immediately after delivery but before separation of the placenta. Reported outcomes vary widely between centers. Thoracoamniotic shunts for large unicystic lesions and pleuroamniotic shunts for hydrothorax have reportedly enhanced survival in extreme cases. Similarly, in cases of massive urinary ascites, urinary diversion via peritoneal shunts has been reported.

There are complications associated with fetal interventions. Preterm birth can be as high as 80% under 37 weeks' gestation and 15% under 30 weeks' gestation, particularly for open surgical procedures (Adzick et al., 2011; Bennett et al., 2014). Maternal morbidities related to fetal intervention range from spontaneous and/or premature rupture of membranes, oligohydramnios, uterine wound infection with dehiscence to mild postoperative interstitial pulmonary edema, especially in mothers with longer operative times (Adzick et al., 2011; Pedreira et al., 2016; Peranteau and Adzick, 2016).

In cases in which the cause of hydrops can be corrected by appropriate care at the time of delivery, such as elimination of a chorioangioma, as well as those cases in which no cause can be ascertained, close observation for fetal demise is the focus of prenatal management. Many cases of nonimmune hydrops manifest in the third trimester as preterm labor. It is difficult to decide whether to attempt tocolysis and delay delivery so as to allow the potentially beneficial administration of steroids before birth or to deliver the fetus immediately. If tocolysis is possible, expectant management should include usual biophysical testing, although fetal decompensation may be difficult to assess. Abnormal fetal heart tracings, oligohydramnios, decreased fetal movement, and poor fetal tone are all ominous signs. Prolonging the pregnancy beyond attainment of a mature lung profile is not generally recommended, unless available evidence indicates improvement or resolution of the hydrops.

TABLE 9.3 Diagnostic Evaluation of Newborns With Nonimmune Hydrops

| System | Type of Evaluation |
|-----------------------|---|
| Cardiovascular | Echocardiogram, electrocardiogram |
| Pulmonary | Chest radiograph, pleural fluid examination |
| Hematologic | Complete blood cell count, differential platelet count, blood type and Coombs test, blood smear for morphologic analysis |
| Gastrointestinal | Abdominal radiograph, abdominal ultrasonography, liver function tests, peritoneal fluid examination, total protein and albumin levels |
| Renal | Urinalysis, blood urea nitrogen, and creatinine measurements |
| Genetic | Chromosomal analysis, skeletal radiographs, genetic consultation |
| Congenital infections | Viral cultures or serologic testing, including TORCH agents and parvovirus |
| Pathologic | Complete autopsy, placental examination |
| Lymphatic | MRI, lymphoscintigraphy, ICG lymphography |

ICG, Indocyanine green; MRI, magnetic resonance imaging; TORCH, toxoplasmosis, other agents, rubella, cytomegalovirus, and herpes simplex.
Adapted from Carlton DP, McGillivray BC, Schreiber MD. Nonimmune hydrops fetalis: a multidisciplinary approach. *Clin Perinatol*. 1989;16:839–851 and Bellini C, Hennekam RC, Bonioli E. A diagnostic flow chart for non-immune hydrops fetalis. *Am J Med Genet A*. 2009b;149A:852–853.

Neonatal Evaluation

Table 9.3 summarizes the diagnostic evaluations recommended for newborns with nonimmune hydrops of unknown cause. There are a few key points:

- Prenatal results may help guide initial evaluation.
- Early cardiac and hematologic evaluations will determine most known etiologies.
- A multidisciplinary approach is essential.
- Response to interventions may help guide evaluation.

Intensive Care of the Infant With Hydrops Fetalis

After successful resuscitation, including intubation, administration of surfactant, and placement of umbilical catheters, the clinical management can address both the cause and the complications of hydrops. Morbidity and mortality may result from the hydropic state, the underlying conditions giving rise to hydrops, or both. A fetus with hydrops that is delivered prematurely is subject to the additional complications of prematurity. If there is massive ascites or pleural effusions, initial resuscitation may require thoracentesis or peritoneal tap. Because of pulmonary edema, newborns with hydrops are susceptible to pulmonary hemorrhage and may require high levels of positive end-expiratory pressure.

Respiratory Management

Virtually all neonates with hydrops require mechanical ventilation because of pleural and peritoneal effusions, pulmonary hypoplasia,

surfactant deficiency, pulmonary edema, poor chest wall compliance caused by edema, or persistent pulmonary hypertension of the newborn. The presence of persistent pleural effusions may necessitate the placement of chest tubes. Ascites may also compress the diaphragm and impair lung expansion. Breath sounds, chest wall movement, blood gas levels, and radiographs must all be monitored frequently so that ventilator support can be reduced in response to improvements in lung compliance and water clearance. Pneumothoraces and pulmonary interstitial emphysema remain potential complications as long as ventilator support is continued. Neonates who need a prolonged course of ventilation, particularly those born prematurely, may develop bronchopulmonary dysplasia. Chronic lung disease results in a longer and more complicated hospital course and contributes to the late mortality of neonatal hydrops.

Fluid and Electrolyte Management

A primary goal of fluid management is resolution of the hydrops itself. Maintenance fluids should be restricted, with volume boluses given only in response to clear signs of inadequate intravascular volume. The hydropic newborn has an excess of free extracellular water and sodium. Fluids given during resuscitation further increase the amount of water and sodium that must be removed during the immediate neonatal period. Initial maintenance fluids should contain minimal sodium. Serum and urine sodium levels, urine volume, and daily weights should be monitored carefully to guide administration of fluids and electrolytes. Urinary sodium levels may help differentiate between hyponatremia caused by hemodilution and urinary losses.

Cardiovascular Management

Hydropic infants may develop hypovolemia as a result of capillary leakage, poor vascular tone, impaired myocardial contractility from hypoxia or infection, impaired venous return caused by shifting or compression of mediastinal structures, and/or pericardial effusion. Thus these infants may present in shock. Adequate intravascular volume must be maintained, and correctable causes of impaired venous return should be addressed. Peripheral perfusion, heart rate, blood pressure, and acid–base status should be monitored carefully.

Lymphatic Evaluation and Interventions

Recent advances in the use of T2-weighted MRI, contrast-enhanced magnetic resonance lymphangiograms, and lymphangiography have identified a group of infants with nonimmune hydrops who have obstructive lymphatic systems. These infants, most of whom have congenital anomalies of the thoracic duct, may benefit from new procedures to relieve these obstructions (Gray et al., 2014; Schild et al., 2015). There are few studies in neonates, although data from older infants, many of whom had single-ventricle physiology, suggest that the use of percutaneous lymphatic interventions after a bilateral intranodal lymphangiogram may be useful in these infants (Itkin et al., 2011; Dori et al., 2014, 2016). Further work is needed in the neonatal population with nonimmune hydrops to identify those infants who may benefit from such interventions.

Clinical Course and Outcome

Despite improvements in diagnosis and management, mortality from nonimmune hydrops remains high. For example, a recent

study of 92 infants born with nonimmune hydrops between 2000 and 2012 reported a 45% fetal death rate and a 36% survival rate to 1 year (Ota et al., 2016). Other recent studies report survival rates between 20% and 30% (Moreno et al., 2013; Turgal et al., 2015).

The best predictors of survival are the cause of the hydrops, the gestational age of the child at delivery, and the condition of the neonate at birth. Highest survival rates are seen in infants with parvovirus infection, chylothorax, or SVT. The lowest survival rates are for hydrops associated with a chromosomal diagnosis, although the figures may be biased because a significant number of pregnancies in such cases are terminated (Heinonen et al., 2000; Sohan et al., 2001). A recent review of 598 patients with nonimmune hydrops found other risk factors for increased mortality, including younger gestational age, lower 5-minute Apgar score, and the need for increased respiratory support (Abrams et al., 2007). A smaller study from Taiwan also found that lower albumin levels were associated with a higher mortality rate (Huang et al., 2007).

Interventions to improve outcomes in hydrops are limited by the rarity of the disease. Most hydropic neonates lose a minimum of 15% of their birthweight, and some lose as much as 30%. Ordinarily, diuresis begins on the second or third day after birth and continues for a period of 2–4 days. Once the edema has resolved, the neonates have normal levels of circulating protein and eventually recover from their apparent capillary leak syndrome. No specific management strategies during the neonatal period, such as the use of high-frequency oscillatory ventilation, have been shown to improve outcome (Wy et al., 1999). However, extracorporeal membrane oxygenation may be a reasonable option for some infants with nonimmune hydrops, with one study showing a 54% survival rate in 28 cases (Bealer et al., 1997). More recent data have not been published.

For infants who survive the immediate neonatal period, long-term outcomes largely depend on the etiology of the condition. For example, an older study from Japan found that 13 of 19 surviving infants with nonimmune hydrops had normal development at 1–8 years (Nakayama et al., 1999). More recent data, though, found only a 45%–50% survival rate without developmental delay by 1 year (Fukushima et al., 2011; Ota et al., 2016). In both these studies, hydropic infants with mild or severe delays had other morbidities, such as extreme prematurity, structural cardiac lesions, or chromosomal anomalies. Thus similar to mortality, long-term morbidities from nonimmune hydrops appear to result from the underlying cause of the hydrops, gestational age at delivery, and complications arising immediately after delivery.

Suggested Readings

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10

Maternal Diabetes

ZANE BROWN AND JUSTINE CHANG

KEY POINTS

- The incidence of diabetes in pregnancy is steadily rising, likely in parallel with the rising incidence of obesity among women of reproductive age.
- Women with diabetes are at increased risk for fetal complications (such as congenital malformations, fetal growth abnormalities, and stillbirth) and perinatal/neonatal complications (such as prematurity, respiratory distress, and metabolic abnormalities including hypoglycemia and electrolyte derangements).
- Good maternal glycemic control achieved preconception and maintained throughout pregnancy is key to optimizing fetal and neonatal outcomes.
- Breastfeeding should be strongly encouraged and supported as it may reduce some of the possible adverse effects of intrauterine programming in the context of maternal diabetes.

Diabetes mellitus is an increasingly common disorder in the United States and around the world. According to 2012 data from the US Centers for Disease Control and Prevention (CDC), 8.9 million adults aged 20 years old or older had diabetes and approximately half of these were women ([CDC National Diabetes Statistics Report, 2014](#)) ([Table 10.1](#)). Another 86 million adults are expected to have prediabetes, a condition associated with an elevated risk of developing frank diabetes. The rapidly rising rate of diabetes parallels the rising rates of obesity and appears to be notable for a more prominent rise in type 2 diabetes (more typically associated with insulin resistance common with obesity) as compared with type 1 diabetes (characterized by insulin deficiency) ([Figs. 10.1–10.2](#)). The rise in type 2 diabetes diagnoses is noted in adults as well as in children and adolescents ([Dabelea et al., 2014](#)). Similarly, there has been a significant increase in the prevalence of gestational diabetes mellitus (GDM, elevated blood sugars due to insulin resistance arising for the first time in pregnancy); studies suggest that US prevalence rates more than doubled over a 15-year period ([Getahun et al., 2008](#)). Data from 2010 derived from birth certificates as well as the Pregnancy Risk Assessment Monitoring System estimated the prevalence of GDM in the United States to be as high as 9.2% ([DeSisto et al., 2014](#)). Increasing rates of GDM are hypothesized to be due to increasing rates of obesity, which is known risk factor ([Fig. 10.1](#)), older maternal ages at delivery, as well as physical inactivity, smoking, and diets high in saturated fats ([Ferrara, 2007](#)).

Given the statistics noted above, diabetes represents one of the most common medical disorders in pregnancy and can have profound implications on fetal and neonatal health and outcomes. There is good evidence that maternal pregestational diabetes is associated with an increased risk of congenital anomalies, abnormalities in fetal growth, as well as neonatal complications such as hypoglycemia, electrolyte abnormalities, respiratory distress, and cardiomyopathy. There is increasing evidence that the intrauterine environment in the context of maternal diabetes may impact pediatric neurodevelopmental outcomes as well as risks of chronic disorders such as obesity and the metabolic syndrome.

Types of Diabetes

Type 1 Diabetes

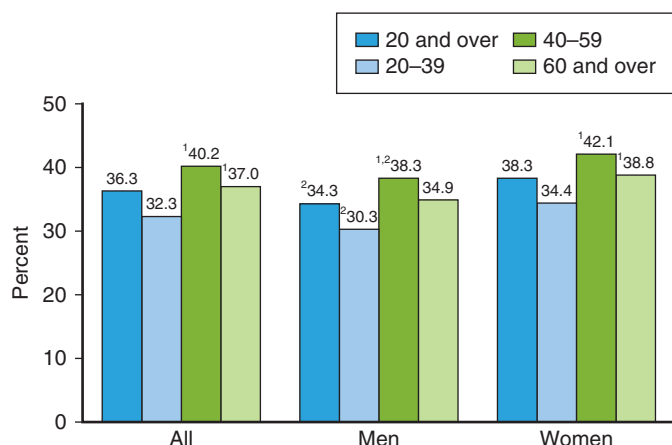
Type 1 diabetes is characterized by insulin deficiency resulting from autoimmune destruction of pancreatic insulin-producing beta cells. Historically, most women with type 1 diabetes will develop clinical signs and symptoms of diabetes in childhood. More recently, adult onset of autoimmune diabetes has also been recognized (latent autoimmune diabetes of adults). These women are dependent on exogenously administered insulin; their underlying pathophysiology places them at elevated risk for diabetic ketoacidosis (DKA), which may be particularly challenging in pregnancy.

Type 2 Diabetes

Type 2 diabetes is characterized by insulin resistance and is therefore more significantly associated with obesity. This form of diabetes is more prevalent in certain ethnicities: Latino, Native American, South Asian, and Pacific Islander populations have particularly high rates of type 2 diabetes. Although type 2 diabetes was historically of adult onset, there have been increasing rates of diagnoses of type 2 diabetes among children and adolescents that appear to parallel the rise in obesity rates in these age groups.

Monogenic Diabetes

Monogenic diabetes, which has also been referred to as maturity-onset diabetes of the young, accounts for approximately 1%–2% of all diabetes cases. Multiple types of monogenic diabetes exist; all of the genes involved share the common feature of affecting beta-cell development, function, or regulation. Clinical presentations



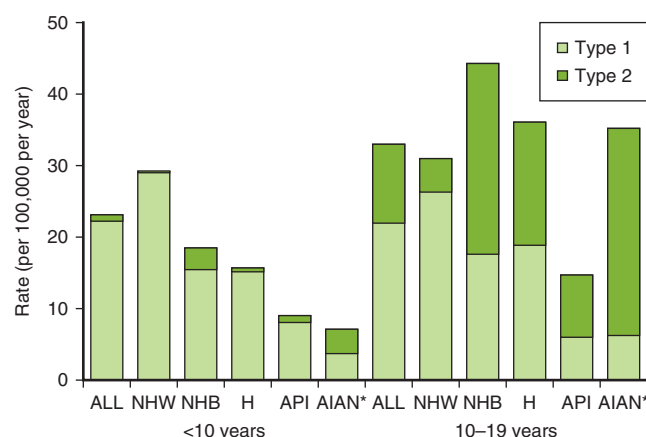
¹Significantly different from those aged 20–39.

²Significantly different from women of the same age group.

NOTES: Totals were age-adjusted by the direct method to the 2000 US census population using the age groups 20–39, 40–59, and 60 and over. Crude estimates are 36.5% for all, 34.5% for men, and 38.5% for women.

SOURCE: CDC/NCHS, National Health and Nutrition Examination Survey, 2011–2014.

• **Fig. 10.1** Prevalence of Obesity Among Adults Aged Greater Than 20 Years Old, by Gender and Age, United States, 2011–2014. (From Ogden CL, Carroll MD, Fryar CD, Fleegal KM. Prevalence of obesity among adults and youth: United States, 2011–2014. *NCHS Data Brief*. 2015; 219:1–8.)



NHW = non-Hispanic whites; NHB = non-Hispanic blacks; H = Hispanics;

API = Asians/Pacific Islanders; AIAN = American Indians/Alaska Natives.

*The American Indian/Alaska Native (AI/AN) youth who participated in the SEARCH study are not representative of all AI/AN youth in the United States. Thus these rates cannot be generalized to all AI/AN youth nationwide.

(SEARCH for Diabetes in Youth is a national multi-center observational study focusing on physician-diagnosed diabetes in individuals < 20 years old. The study is funded by the CDC and the NIDDK [National Institute of Diabetes and Digestive and Kidney Diseases]).

• **Fig. 10.2** Rates of New Cases of Type 1 and Type 2 Diabetes Among People Less Than 20 Years Old, by Age and Race/Ethnicity, 2008–2009. (From National Diabetes Statistics Report 2014 – CDC. Accessed from <http://www.cdc.gov/diabetes/data/statistics/2014statisticsreport.html>.)

TABLE 10.1 Diagnosed and Undiagnosed Diabetes Among People Aged 20 Years or Older in the United States 2012

| | Number With Diabetes (million) | Percentage With Diabetes |
|-----------------|--------------------------------|--------------------------|
| Total | | |
| ≥20 years old | 28.9 | 12.3 |
| Age | | |
| 20–44 years old | 4.3 | 4.1 |
| 45–64 years old | 13.4 | 16.2 |
| Gender | | |
| Women | 13.4 | 11.2 |

Source: 2009–2012 National Health and Nutrition Examination Survey estimates applied to 2012 US Census Data. CDC National Diabetes Statistics Report, 2014.

of monogenic diabetes are heterogeneous, with phenotypes varying according to the specific mutation. Most forms share the following clinical features: affected individuals are typically diagnosed before the age of 25 years and are not insulin dependent. Women with monogenic diabetes may be first identified and diagnosed with diabetes during pregnancy and then are found to have continued hyperglycemia postpartum; genetic testing may be prompted by the recognition of a strong family history of diabetes (often two or more consecutive affected generations), as well as an absence of obesity or other clinical features suggestive of insulin resistance.

The most commonly affected genes identified in cases of monogenic diabetes are glucokinase (*GCK*) and hepatocyte nuclear factor (*HNF1A*, *HNF4A*). Glucokinase catalyzes the first and

rate-limiting step in glycogen storage and glycolysis; this enzyme effectively links insulin secretion to elevations in serum glucose. Mutations that inactivate *GCK* raise the glucose setpoint for insulin secretion. Women with *GCK* mutations usually have a stable, mild hyperglycemia with elevated fasting glucose levels most prominent. *HNF* genes encode transcription factors that play an important role in pancreatic beta-cell differentiation, development, and function; mutations in these genes typically produce a progressive defect in insulin secretion that results from progressive beta-cell failure. *HNF* mutations represent the most common cause of monogenic diabetes in most reported series to date: in a series reporting on monogenic diabetes in the United Kingdom, mutations in *HNF1A* and *HNF4A* accounted for approximately 50% and 10% of monogenic diabetes, respectively (Naylor and Philipson, 2011). *HNF1A* mutations in particular appear to have high penetrance, with 63% of affected individuals developing diabetes by age 25 years and 79% developing diabetes by age 35 years (Murphy et al., 2008; Naylor and Philipson, 2011).

Monogenic diabetes has several clinically relevant implications in pregnancy. The specific form of monogenic diabetes may affect responsiveness to certain therapies. Women with diabetes due to *HNF1A* or *HNF4A* mutations appear to be significantly more responsive to sulfonylureas (e.g., glyburide) than to biguanides (metformin). Increased sensitivity to sulfonylureas in *HNF1A* mutation carriers may be explained by sulfonylurea action on beta-cell potassium channels that are located downstream from the defect produced by the mutation; therefore if oral hypoglycemic therapy is preferred for treatment during pregnancy, glyburide is expected to be a more effective choice than metformin. Given the pathophysiology of diabetes resulting from *GCK* mutations, hyperglycemia in pregnancy may be more difficult to treat; this has been observed in clinical series (Bacon et al., 2015).

The genetic mutations leading to monogenic diabetes are inherited in an autosomal dominant fashion, so there is a 50%

risk of inheritance in the offspring. Whether or not the fetus is affected by a particular gene mutation may impact intrauterine growth. Affected fetuses with *HNF4A* mutations may have a greater likelihood of macrosomia and neonatal hypoglycemia. In an analysis of siblings discordant for the *HNF4A* mutation, affected infants had birth weights that were on average 790 g higher than unaffected infants; 56% of affected infants were macrosomic (birth weight >4000 g), as compared with 13% of those who were unaffected. Fifteen percent of affected infants demonstrated evidence of neonatal hypoglycemia (Pearson et al., 2007). In contrast, fetuses who have inherited the *GCK* mutation do not appear to demonstrate the excessive fetal growth that typically results from maternal hyperglycemia; studies have reported that affected fetuses may have a birth weight that may be 500–600 g lower than unaffected fetuses of mothers with monogenic diabetes due to *GCK* mutations (Spyer et al., 2001; Spyer et al., 2009; Bacon et al., 2015). Fetuses inheriting the *GCK* mutation may not respond to maternal (and therefore fetal) hyperglycemia by significantly increasing insulin secretion; therefore they may not experience the same pathologic growth patterns in response to maternal diabetes. Unaffected fetuses have a normally functioning glucokinase enzyme and are therefore at risk for hyperinsulinemia in response to maternal hyperglycemia, resulting in a greater risk of macrosomia. Knowledge of the fetal genotype during pregnancy might theoretically allow providers and patients to tailor diabetes management (e.g., recommend tight glycemic control when fetuses are unaffected but consider more relaxed glycemic targets when fetuses are affected). Despite the possible appeal of such a management strategy, the invasive testing (chorionic villous sampling or amniocentesis) necessary to determine the fetal genotype is unlikely to be accepted by patients given the potential for procedure-associated complications (although low risk). Some experts have suggested considering use of serial ultrasound assessment of fetal abdominal circumference and/or growth to help tailor diabetes management. In a general GDM population, maternal diabetes therapy guided by capillary blood glucose measurements versus a strategy of determining glycemic targets based on fetal abdominal circumference (high-risk abdominal circumference defined as ≥ 70 –75th percentiles for gestational age [GA]) appeared to perform comparably in terms of rates of macrosomia and neonatal morbidity (Kjos et al., 2001, 2007).

The management of diabetes during pregnancy may also have long-term effects on fetuses who inherit diabetes-causing mutations. Studies have demonstrated that individuals who inherit *HNF1A* mutations from their mothers often have earlier age at diabetes diagnosis and at initiation of insulin therapy than those who inherited their mutation from their fathers (Colom and Corcoy, 2010); this observation may suggest that the intrauterine environment can impact the phenotype produced by these mutations and further highlights the importance of good maternal metabolic control during pregnancy in optimizing long-term outcomes in her offspring.

Gestational Diabetes

Historically, the reported incidence of diabetes in pregnancy ranges from 6%–7%; 80%–90% of these cases are due to GDM, which is defined by the presence of elevated blood sugars, resulting from insulin resistance arising for the first time during pregnancy. It is likely that physiologic changes of pregnancy produce these elevated blood sugars in women with an underlying predisposition. In pregnancy, insulin resistance increases with advancing gestation in response to increasing levels of human placental lactogen, progesterone, cortisol, and prolactin. Human placental lactogen and

• BOX 10.1 Criteria for the Diagnosis of Diabetes (American Diabetes Association)

Clinical signs and symptoms of hyperglycemia – e.g., polyuria, polydipsia
Hemoglobin A_{1c} $\geq 6.5\%$
Fasting plasma glucose ≥ 126 mg/dL
Random plasma glucose ≥ 200 mg/dL
75-g, 2-hour oral glucose tolerance test with 2-hour value ≥ 200 mg/dL

prolactin antagonize the effects of insulin at the cellular level. Progesterone decreases gastrointestinal motility, which may enhance carbohydrate absorption. To a certain extent, insulin resistance in pregnancy is likely a physiologic adaptation as it helps to maintain a steady supply of nutrients to the growing fetus.

Given the underlying pathophysiology of insulin resistance, there is likely to be some overlap between women with type 2 diabetes and women diagnosed with GDM. Because of the likelihood that underlying type 2 diabetes may be first diagnosed in pregnancy (and therefore mistaken for GDM), it is recommended that women with risk factors for type 2 diabetes be screened early in pregnancy (Box 10.1). Risk factors that merit early diabetes testing include the following: a previous history of GDM; history of impaired glucose tolerance; and obesity (body mass index [BMI] ≥ 30 kg/m²). The American Diabetes Association also recommends consideration of testing for diabetes in women who are overweight (BMI 25–29 kg/m²) and who have one of the following additional risk factors: physical inactivity, a first-degree relative with diabetes, history of delivery of a macrosomic infant (defined as birth weight greater than 9 lbs), having an ethnic background associated with elevated risk of diabetes (African-American, Latina, Native American, Asian-American, or Pacific Islander), or having a history of polycystic ovarian syndrome or chronic hypertension. Women who have negative early screens for diabetes should be retested for GDM later in pregnancy. Universal testing for GDM at 24–28 weeks' gestation is recommended.

Currently in most of the United States, two-step testing is recommended with a 50-g glucose challenge test as the initial screen, followed by definitive diagnostic testing with a 100-g 3-hour oral glucose tolerance test; this testing approach is endorsed by the American College of Obstetricians and Gynecologists (ACOG). The International Association of Diabetes and Pregnancy Study Groups (IADPSG) recommends a one-step testing strategy utilizing a 75-g 2-hour oral glucose tolerance test. The IADPSG selected diagnostic thresholds for this test based on blood glucose levels that were associated with an odds ratio of 1.75 for various adverse outcomes evaluated in the Hyperglycemia and Adverse Pregnancy Outcome study (Metzger et al., 2010). It has been estimated that use of the IADPSG testing strategy would significantly increase the diagnosis of GDM (as many as 18% of pregnant American women may be diagnosed with GDM). The clinical benefits and cost-effectiveness of using the IADPSG testing recommendations require continued study (NICHD Consensus Development Conference panel, 2013).

Diabetic Embryopathy

In women with pregestational diabetes, poor periconceptional glycemic control has been associated with a significantly increased risk of embryopathy resulting in congenital malformations. The background rate of congenital malformations in the general population is around 2%–3%; in women with pregestational diabetes

the reported incidence of major congenital anomalies is approximately threefold higher at 6%–9%. The risk of congenital malformations increases with increasing maternal periconception hemoglobin A_{1c} (HbA_{1c}); women with a first trimester HbA_{1c} greater than or equal to 7.5% had a ninefold increased risk of congenital malformation than those women with good glycemic control (Miller et al., 1981; Temple et al., 2002; Nielsen et al., 2006). Extremely elevated maternal HbA_{1c} levels (i.e., ≥10%) in early pregnancy are associated with fetal malformation rates of 20%–25% (Greene, et al., 1989; Kitzmiller et al., 1996). Notably, the increased risk of fetal malformations in offspring of diabetic women reflects an increased risk of both major and minor congenital malformations. In studies evaluating the risk of diabetic fetopathy, major congenital malformations were often defined as any malformation anticipated to be lethal, likely to cause serious future handicap, or require surgical repair.

In a Finnish prospective cohort study of 709 offspring born to 488 women with type 1 diabetes, poor glycemic control in the first trimester (HbA_{1c} ≥9.4% at GAs <14 weeks) was associated with a sixfold increased relative risk (RR) of major fetal malformation (8.2% vs 1.4% in offspring of nondiabetic control patients) (Suhonen et al., 2000). In a Danish prospective cohort study of 933 singleton pregnancies in women with type 1 diabetes, 10.9% of the offspring to women with periconceptional HbA_{1c} greater than or equal to 10.4% had a major congenital malformation as compared with only 2.8% of the background population (Jensen et al., 2009).

Preconception care emphasizing the importance of good glycemic control before and during early pregnancy has been demonstrated to be associated with lower rates of congenital anomalies (Ray et al., 2001); achievement and maintenance of HbA_{1c} levels in the normal range (e.g., ≤6%) periconceptionally appears to confer a risk of congenital malformations that does not differ from the background population risk.

There is no anomaly that is pathognomonic for maternal diabetes. A wide range of congenital malformations affecting multiple organ systems have been reported in pregnancies complicated by pregestational diabetes. Pregestational diabetes confers a 26-fold increase in odds of caudal regression syndrome, but a large retrospective cohort study demonstrated that only 17% of all caudal regression cases occurred in women with diabetes (Garne et al., 2012). The most frequently encountered anomalies in the setting of maternal diabetes affect the cardiovascular system (3.5 times increase in odds in women with diabetes as compared to the background population) as well as the CNS (Table 10.2). Multiple congenital anomalies have been identified in 8%–13% of infants of diabetic mothers (IDMs) (Eidem et al., 2010; Garne et al., 2012).

High levels of glucose and ketone bodies (e.g., β-hydroxybutyrate) have been shown to be teratogenic in animal models. Hyperglycemia leads to the increased production of reactive oxygen species, which can alter cell membranes, cause mitochondrial dysfunction, or promote pathologic apoptosis; all of these alterations can contribute to embryopathy (Ornoy et al., 2015). Animal studies have demonstrated that hyperglycemia-induced excessive apoptosis affects neural progenitor cells, contributing to nervous system malformations (Yang et al., 2015). Hyperglycemia-related oxidative stress has also been shown to have a direct effect on the proliferation and migration of neural crest cells, which play a critical role in the development of the fetal heart; in animal models, these changes resulted in cardiac outflow tract defects (Morgan et al., 2008).

Congenital malformation risk in pregestational diabetics may be further increased by exposure to medications that may be teratogenic. Women with diabetes have a greater risk of comorbid medical

TABLE 10.2 Congenital Anomalies Reported in Fetuses of Diabetic Mothers

| Organ System | Anomalies |
|-------------------------------|--|
| Central nervous system | Spina bifida Anencephaly Hydrocephalus |
| Cardiovascular system | Ventricular septal defect Tetralogy of Fallot Transposition of the great arteries Hypoplastic left heart syndrome Coarctation of the aorta Atrial septal defect Pulmonic stenosis Double-outlet right ventricle Truncus arteriosus |
| Genitourinary tract | Hydronephrosis Renal agenesis Ureteral duplication Hypospadias |
| Gastrointestinal tract | Intestinal atresias Anal atresia |

conditions such as chronic hypertension or complications of long-standing diabetes (e.g., nephropathy). In this setting, angiotensin-converting enzyme (ACE) inhibitors may be prescribed outside of pregnancy; these medications are known to be teratogenic: in a nondiabetic population, first trimester exposure has been associated with a 2.7-fold increased risk of major congenital malformations, including a fourfold increase in the risk of CNS anomalies and a 3.7-fold increase in the risk of cardiovascular anomalies (Cooper et al., 2006). Exposure to ACE inhibitors beyond the first trimester of pregnancy also has adverse effects, including fetal renal failure, oligohydramnios with its associated consequences (e.g., pulmonary hypoplasia if early oligohydramnios is severe and persistent), fetal growth restriction, and increased risk of stillbirth.

Antenatal detection of congenital malformations usually requires targeted second trimester ultrasound evaluation. Systematic reviews evaluating the accuracy of ultrasound diagnosis at GAs less than 24 weeks report detection rates of 16%–44% for all anomalies (Grandjean et al., 1999; Whitworth et al., 2010); the overall detection rate of major lethal anomalies is higher, at 84% (Reddy et al., 2014). Detection rates differ based on the type of malformation – detection rates are higher for major malformations such as anencephaly and spina bifida than for more subtle malformations such as unilateral renal agenesis or small ventricular septal defects. Rates of detection also likely vary based on GA at the time of ultrasound exam, expertise of the sonographer, and patient factors (e.g., maternal obesity).

Cardiac anomalies comprise up to 40%–50% of congenital malformations encountered in IDMs; it is estimated that there is a fivefold increase in risk of congenital cardiac anomalies in IDMs as compared with infants of women without diabetes (Rowland et al., 1973; Wren et al., 2003). In a case-control study of around 8000 live and stillborn infants weighing greater than or equal to 500 g and greater than or equal to 20 weeks' gestation, the absolute risk of a major cardiovascular system defect was 8.5 per 100 live births in offspring of insulin-dependent diabetic mothers; in that population, the absolute risk of a cardiovascular malformation in

infants born to nondiabetic mothers was 0.8 per 100 live births (Becerra et al., 1990).

Given the increased risk of morbidity and mortality with some cardiac malformations, antenatal diagnosis is vital for optimization of obstetric and neonatal care. Some studies have suggested that antenatal diagnosis of complex cardiac malformations such as hypoplastic left heart syndrome or transposition of the great arteries may be associated with an improvement in neonatal outcomes as compared with infants in whom these malformations were first diagnosed postnatally. Routine second trimester fetal ultrasound evaluation of the basic cardiac views (four-chamber and outflow tract views) has a sensitivity of 60%–85%. Fetal echocardiography has been shown to have an increased sensitivity of 85%–95%, with positive predictive value of 90% and negative predictive value of 93% for the diagnosis of many types of cardiac malformations (Bakiler et al., 2007). Given the increased risk of cardiac malformations in fetuses of diabetic women, many American institutions recommend screening fetal echocardiography in addition to a detailed second trimester fetal anatomic survey.

Fetal Growth and Macrosomia

Maternal diabetes is associated with an increased risk for abnormal fetal growth. Pregestational diabetics with microvascular complications (especially diabetic nephropathy) or concomitant chronic hypertension may have an increased risk of intrauterine growth restriction, which may be related to abnormalities in placental structure or perfusion.

Excessive fetal growth is the aberrant pattern more commonly encountered in diabetic pregnancies. Large for gestational age (LGA) is commonly defined as birth weight greater than the 90th percentile for GA. Macrosomia is more variably defined: birth weight greater than 4000 g or greater than 4500 g without adjustment for GA. Both pregestational and gestational diabetic pregnancies have a greater prevalence of LGA and macrosomic infants: LGA has been reported to occur in 25%–42% pregestational diabetics (Jovanovic-Peterson et al., 1991). Birth weights greater than or equal to the 90th percentile have been reported to occur in 42%–62% of type 1 diabetic pregnancies, 30%–56% of type 2 diabetic pregnancies, and in 10%–20% of pregnancies complicated by gestational diabetes. For infants born to nondiabetic mothers, the rate of macrosomia (when defined as birth weight ≥ 4000 g) has been reported to be 7%–8% (Langer et al., 1991), and the rates of LGA have been reported to be 8%–14% (Jovanovic-Peterson et al., 1991).

Excessive fetal growth is probably stimulated by maternal hyperglycemia as explained in the classic Pedersen hypothesis. Maternal glucose readily crosses the placenta by facilitated diffusion; fetal glucose levels are maintained at a level that is approximately 70%–80% of the maternal concentration of glucose. Maternal insulin does not cross the placenta. Fetal hyperglycemia stimulates fetal pancreatic beta-cell hypertrophy and increased insulin production. Insulin acts as a potent growth factor in utero – fetal hyperinsulinemia and concomitant increases in insulin-like growth factors (IGFs) probably results in increased protein, lipid, and glycogen synthesis that contributes to increased fetal growth (Langer, 2000). The excess fetal growth seen in IDMs appears to be characterized by increased fat deposition as well as observation of visceral enlargement. Fetal insulin activity in response to fetal glucose levels becomes evident at around 20 weeks' gestation. Insulin receptor levels in target organs such as the fetal liver appear to become maximal in the mid-trimester, with insulin receptor binding affinities increasing as pregnancy progresses.

Maternal glycemic control has been identified as a predictor of abnormal fetal growth. Studies have demonstrated a positive linear association between maternal glucose levels and risk of LGA birth weights (HAPO Study Cooperative Research Group, 2008). Elevated maternal HbA_{1c} levels and nonfasting as well as mean glucose levels have been associated with risk of abnormal fetal growth (Jovanovic-Peterson et al., 1991; Gandhi et al., 2008). Clinical studies have also demonstrated a positive correlation between fetal insulin and IGF levels and birth weight. Amniotic fluid insulin levels have been shown to correlate with risk of macrosomia as well as childhood obesity (Metzger et al., 1990; Carpenter et al., 2001). Demonstrated a significant increase in fetal cord blood IGF-I levels in the late third trimester (34 weeks through term); these fetal IGF-I levels correlated positively with newborn weights in their cohort of nondiabetic women.

The clinical pattern of abnormal fetal growth in the context of maternal diabetes appears to be characterized by accelerated disproportionate growth in the second half of pregnancy. Studies evaluating fetal growth utilizing ultrasound assessment demonstrated that LGA IDMs had evidence of accelerated growth as early as 17–24 weeks' gestation, with maximal growth occurring late in the third trimester. Observations of accelerated fetal abdominal circumference growth have been made in diabetic pregnancies and associated with LGA birth weights (Greco et al., 2003; Mulder et al., 2010; Hammoud et al., 2013). LGA IDMs have evidence of increased adiposity when compared with LGA infants born to nondiabetic women. In an evaluation of LGA neonates, IDMs had significantly increased fat mass and increased percentage body fat as compared with infants whose mothers had normal gestational glucose tolerance (Durnwald et al., 2004). In general, fetal fat deposition seems to occur almost entirely within the third trimester and appears to be very sensitive to the effects of fetal insulin.

Antenatal diagnosis of macrosomia has been made difficult by limitations in the accuracy of fetal sonographic estimations of fetal weight. Measurements of the fetal head (head circumference and biparietal diameter), abdominal circumference, and femur length are incorporated into a formula (usually derived from nonlinear regression analysis) to estimate fetal weight; fetal weight percentiles are then determined from one of several fetal growth curves that, historically, have been derived from various geographic locations using representative subpopulations. Most experts consider a mean percent error in estimated fetal weight of $\pm 10\%$ to be clinically relevant for accuracy. Studies evaluating the accuracy of third-trimester ultrasound estimates of fetal weight show that sonographic estimates fall within 10% of birth weight in 50%–75% of cases; the reported mean percentage differences between ultrasound-predicted and actual birth weight have ranged from 6%–15% (Schwartz et al., 2016).

Sensitivities for the antenatal diagnosis or prediction of birth weight greater than or equal to 4000 g has been estimated to be as low as 53%–59% (Porter et al., 2015). In a cohort of women with gestational diabetes, third-trimester ultrasound estimation of fetal weight significantly overestimated the prevalence of LGA infants; of those women identified as having an estimated fetal weight greater than or equal to the 90th percentile, only 23% actually delivered an LGA neonate (Scifres et al., 2015). Some data suggest that prediction of clinically relevant LGA (e.g., LGA infants who will have the greatest risk of shoulder dystocia) may be improved by the use of customized fetal growth curves, which take into account clinical factors such as fetal gender, maternal height, weight, parity, and ethnicity that are physiologic determinants of fetal growth (Larkin et al., 2011).

Macrosomic IDMs have a greater risk of shoulder dystocia, birth trauma (including perinatal acidosis), cesarean delivery, and of neonatal complications such as severe hypoglycemia and hyperbilirubinemia (Ballard et al., 1993). Shoulder dystocia is defined as difficulty and delay in delivery of the fetal shoulders/body following delivery of the fetal head; this often results from impaction of the anterior fetal shoulder behind the maternal pubic bone. Shoulder dystocia requires additional obstetric maneuvers to effect delivery; typical management includes McRoberts maneuver, which produces hyperflexion of the maternal hips to widen the pelvic outlet, suprapubic pressure to attempt to dislodge the impacted anterior fetal shoulder, and/or delivery of the fetal posterior arm.

Shoulder dystocia represents an obstetric emergency as delayed delivery is associated with an increased risk of perinatal asphyxia (potentially related to impaired oxygenation from occlusion of the umbilical cord during delayed delivery). Both the condition of shoulder dystocia and the obstetric maneuvers required to effect delivery may lead to an increased risk of birth trauma, including brachial plexus injuries and clavicular or humeral fractures. In a general obstetric population, reported rates of shoulder dystocia range from 0.2%–3%. Macrosomia and maternal diabetes have been identified as independent risk factors for shoulder dystocia. In a nondiabetic population, macrosomia, when defined as birth weight greater than 4500 g, was associated with a 13-fold increase in the odds of shoulder dystocia as compared with non-macrosomic infants; the rate of shoulder dystocia in a nondiabetic population with macrosomic infants was 23% (Froehlich et al., 2016). When controlling for birth weight and other confounders, diabetes (pregestational and gestational) is an independent risk factor for shoulder dystocia, with a threefold increase in odds (Nesbitt et al., 1998). Women with the highest risk for shoulder dystocia are diabetics with a macrosomic infant; shoulder dystocia rates as high as 35% have been reported in diabetic women who deliver an infant with a birth weight between 4750 and 5000 g (Young and Ecker, 2013). In deliveries complicated by shoulder dystocia, IDMs have a greater rate of birth trauma than infants of nondiabetic mothers; in a review of shoulder dystocia cases in the state of California, 24% of IDMs and 7.3% of infants of nondiabetic mothers had evidence of birth trauma (Nesbitt et al., 1998).

Birth trauma appears to occur at an increased rate in macrosomic infants even in the absence of shoulder dystocia. In a cohort of infants with a birth weight greater than or equal to 4000 g, 7% had evidence of a traumatic birth injury – most commonly Erb's palsy and clavicular or humeral fractures (Cordero et al., 1998). Macrosomic infants also have an increased risk of requiring cesarean delivery; cesarean delivery rates as high as 50%–60% for infants with birth weights greater than or equal to 4000 g have been reported (Cordero et al., 1998).

Stillbirth and Perinatal Mortality

Pregnancies complicated by diabetes have been noted to have an increased risk of stillbirth (usually defined as fetal demise diagnosed at GAs at or beyond 20 weeks) as well as perinatal mortality. Perinatal mortality has varying definitions; the most inclusive definition is a fetal death at 20 weeks or more or an infant death before 28 days of life (Mathieson et al., 2011). The most recent US National Vital Statistics Report from 2013 demonstrated a fetal death (stillbirth) rate of 5.96 per 1000 live births. The perinatal mortality rate was 9.98 per 1000 live births plus fetal deaths for the time period of evaluation (MacDorman and Gregory, 2015).

This report did not specify fetal death rates or perinatal mortality rates for pregnancies complicated by specific medical or obstetric comorbidities. Studies evaluating the risk of perinatal mortality in pregnancies complicated by diabetes may have been limited by variable definitions of perinatal mortality as well as a lack of uniform agreement regarding how to determine if maternal diabetes was a direct contributor to the demise.

The stillbirth risk among women with pregestational diabetes has been reported to be as high as five times the risk of stillbirth noted in the general population (Casson et al., 1997; Wood et al., 2003). In the United States, a multicenter population-based case-control study identified maternal diabetes as an independent risk factor for stillbirth, accounting for 5.6% of all stillbirths in the cohort (for stillbirths occurring at GAs of ≥ 24 weeks, the adjusted odds ratio of stillbirth in women with diabetes was 3.47, 95% confidence interval [CI] 1.86–6.49) (Stillbirth Collaborative Research Network Writing Group, 2011).

The reported risk of stillbirth may differ in women with type 1 diabetes as compared with those with type 2 diabetes. In large Danish cohort studies, incidences of stillbirth among women with type 1 diabetes ranged from 18–28 per 1000 births as compared with a rate of 4.5 per 1000 births in the general population (Lauenborg et al., 2003; Jensen et al., 2004). The risk of stillbirth is similarly elevated in women with type 2 diabetes, although the magnitude of risk that has been reported has varied, with some studies suggesting worse outcomes in type 2 diabetes while others suggest comparable outcomes in both type 1 and type 2 diabetes. Large retrospective cohort studies conducted in Denmark and in New Zealand demonstrated perinatal mortality rates two to three times higher in women with type 2 diabetes as compared with those who have type 1 diabetes (Cundy et al., 2000; Clausen et al., 2005). It is possible that the risk of perinatal mortality may be increased in pregnancies complicated by type 2 diabetes because these women have an increased risk of obesity. Obesity has been shown to be an independent risk factor for adverse pregnancy outcomes, including stillbirth and gestational hypertensive disorders, which can also impact fetal growth and risks of prematurity (O'Brien et al., 2003; Athukorala et al., 2010; Aune et al., 2014; Yao et al., 2014). A systematic review evaluating population-based studies reporting on risk factors for stillbirth in high-income countries (such as the United States, Canada, the United Kingdom, Australia, and the Netherlands) demonstrated that diabetes is a maternal medical comorbidity with one of the strongest associations with stillbirth. In this systematic review, women with preexisting diabetes had an adjusted odds of stillbirth 2.9 times that of women without diabetes; however, the calculated population-attributable risk of diabetes to stillbirths is 3%–5%. In comparison to diabetes, maternal overweight and obesity (defined as maternal BMI >25 kg/m²) had a higher population-attributable risk for stillbirth (8%–18%). Maternal overweight and obesity were identified as the highest ranking modifiable risk factor for stillbirth in high-income countries (Flenady et al., 2011).

The risk of perinatal mortality in pregnancies complicated by GDM is not clear: several studies have suggested an increased risk of perinatal mortality with GDM, while other studies have not demonstrated a significant association. Some women with GDM are likely to be type 2 diabetics who just happen to be identified during pregnancy; therefore the possibility of misclassification might explain the discrepancy in reported results. A large retrospective cohort study evaluating outcomes of singletons delivered in California at 36–42 weeks' gestation demonstrated that there was an increased risk of stillbirth in women with GDM as compared with

women without diabetes, though the absolute risks are low: women with GDM experienced 17.1 stillbirths per 10,000 deliveries, whereas nondiabetic women had 12.7 stillbirths per 10,000 deliveries (RR 1.34, 95% CI 1.2–1.5). This same study also evaluated the risk of expectant management in pregnancies complicated by GDM and concluded that at 39 weeks' gestation, the risk of expectant management (stillbirth and infant mortality risks) appears to exceed the risks of delivery (Rosenstein et al., 2012). Results from similar studies inform recommendations for consideration of delivery at 39–40 weeks' gestation in pregnancies complicated by GDM requiring medical therapy. Women with diet-controlled GDM have not been shown to have greater risk of stillbirth (Karmon et al., 2009), and their delivery planning is managed accordingly.

Several studies evaluating risks of adverse outcomes in the setting of maternal diabetes have identified an association between poor glycemic control and increased risk of perinatal mortality. The etiologies of perinatal mortality that may be relevant in pregnancies complicated by diabetes include severe congenital malformations, growth abnormalities (e.g., fetal growth restriction in women with microvascular complications of diabetes), prematurity, and intrauterine asphyxia (Dudley, 2007). Each of these etiologies is anticipated to occur with increased frequency in the setting of poor maternal glycemic control and severity of diabetes. Poorly controlled diabetes early in pregnancy may contribute to abnormalities in placental development: high glucose levels have been shown to have an inhibitory effect on human trophoblast invasion (Belkacemi et al., 2005). Placental pathology of this nature might be hypothesized to impact placental function and therefore impact risk of intrauterine demise.

Intrauterine asphyxia may result from fetal hyperinsulinemia that is thought to increase fetal oxygen consumption and therefore decrease fetal oxygen tension; this proposed pathophysiology seems supported by the observation that some fetuses of women with type 1 diabetes have higher plasma lactate levels and lower blood pH levels than normal when evaluated by third-trimester cordocentesis (Bradley et al., 1991). This lactic acidemia observed in some of the fetuses of diabetic women may contribute to the risk of perinatal mortality.

One etiology of fetal death that is unique to diabetes is maternal DKA. Pregnancy represents a time of increased risk of DKA as several of the physiologic changes of pregnancy predispose to DKA. Pregnancy results in increased insulin resistance, enhanced lipolysis, and ketogenesis. Pregnant women are also in a state of compensated respiratory alkalosis; therefore there is decreased buffering capacity (lower plasma bicarbonate levels) in the setting of acidosis. Complications unique to pregnancy such as nausea and vomiting further increase risk of DKA.

The perinatal mortality rate in the setting of maternal DKA has been reported to be 9%–35%. The exact mechanism of fetal death in the setting of maternal DKA is not completely understood. It is known that maternal ketone bodies freely cross the placenta and contribute to fetal acidosis. Maternal DKA is also characterized by significant maternal hypovolemia (resulting from osmotic diuresis), which likely results in decreased uteroplacental perfusion. Maternal acidemia also shifts the maternal oxyhemoglobin dissociation curve leftward, thus further compromising oxygen delivery to the fetus. Decreased fetal oxygenation and metabolic acidemia are likely significant contributors to fetal mortality in this setting. Women who develop DKA during pregnancy are often critically ill; as expected, fetal status often reflects maternal illness. Fetuses often demonstrate evidence of intrauterine distress during fetal heart

rate monitoring. In this setting, obstetric management prioritizes maternal stabilization; emergent cesarean delivery for fetal distress resulting from DKA before adequate maternal treatment and stabilization is associated with high risk of maternal morbidity and mortality. Fortunately, early recognition of maternal DKA and aggressive, efficient initiation of treatment can lead to maternal improvement and effective intrauterine resuscitation of the fetus.

Prevention of perinatal mortality in pregnancies complicated by diabetes primarily relies on ensuring good maternal glycemic control throughout gestation. Increased antenatal surveillance to try to identify at-risk fetuses is also an accepted part of the obstetric management of pregestational diabetic women. Given the conflicting data regarding risks of perinatal mortality in women with GDM, current expert guidelines recommend antenatal testing for women with GDM who require medication therapy for hyperglycemia at the same time suggesting that testing may be deferred in women with GDM who are well controlled on dietary therapy alone and who are otherwise at low risk for perinatal complications.

Maternal Preeclampsia

Preeclampsia is a gestational hypertensive disorder; the diagnosis of preeclampsia is made with the onset after 20 weeks' gestation of persistent elevations in systolic blood pressure (≥ 140 mmHg) or diastolic blood pressure (≥ 90 mmHg) in women with previously normal blood pressure as well as the new onset of proteinuria. Proteinuria is defined as greater than or equal to 300 mg of protein per 24-hour urine collection or a protein/creatinine ratio greater than or equal to 0.3. The diagnosis of preeclampsia may also be made in the absence of proteinuria if there are other complications such as thrombocytopenia, renal insufficiency, impaired liver function, pulmonary edema, or neurologic symptoms (such as cerebral or visual symptoms) or HELLP syndrome (ACOG Task Force on Hypertension in Pregnancy, 2013). In addition to the aforementioned maternal complications, preeclampsia is also associated with an increased risk of fetal complications such as intrauterine growth restriction and oligohydramnios that are likely to reflect altered placental perfusion in the setting of preeclampsia. Women with severe forms of preeclampsia are also at increased risk of placental abruption. Delivery is the only definitive treatment for preeclampsia. In the setting of maternal or fetal complications resulting from preeclampsia, delivery is medically indicated as the risk of perinatal and maternal morbidity and mortality is elevated (Clark et al., 2008; Kuklina et al., 2009).

Women with pregestational diabetes are at increased risk for developing preeclampsia. The prevalence of preeclampsia in the general population has been estimated at 4.6% (Abalos et al., 2013), and the frequency of preeclampsia in women with diabetes has been reported to be 2–4 times higher than the general population. Among women with diabetes, the risk of preeclampsia appears to increase with increasing duration of diabetes as well as with the presence of microvascular complications. In a US prospective observational cohort study, 11% of women with a duration of diabetes of less than 10 years developed preeclampsia as compared with 22% of women with diabetes of duration 10–19 years. Thirty-six percent of women with proliferative diabetic retinopathy and/or diabetic nephropathy developed preeclampsia in this cohort. The women with diabetic nephropathy with a baseline level of proteinuria of greater than or equal to 300 mg in a 24-hour urine collection had an increased risk of small for GA infants and of preterm delivery (Sibai et al., 2000).

Obstetric Management of Diabetes in Pregnancy

Preconception Care

Care of the pregestational diabetic woman should ideally begin before pregnancy. As discussed previously, preconception care of a woman with diabetes can improve outcomes; if good glycemic control is achieved and maintained throughout conception and early pregnancy, rates of miscarriage and congenital malformations are often indistinguishable from the general population in women with uncomplicated diabetes (*The Diabetes Control and Complications Trial Research Group, 1996*). Preconception care also allows for identification of teratogenic medications and hopefully substitution with safer alternatives (e.g., discontinuation of ACE inhibitors and transition to other antihypertensives that are compatible with pregnancy). Given the elevated risk of neural tube defects in diabetic pregnancies, folic acid supplementation should be initiated before conception. Identification of microvascular complications of diabetes such as retinopathy and nephropathy before pregnancy allows opportunities for treatment of these conditions (e.g., laser therapy for treatment of cases of proliferative retinopathy); optimization of these conditions will hopefully prevent progression of disease during pregnancy. Women with microvascular complications of diabetes also appear to have an additional increased risk of adverse pregnancy outcomes; for example, women with diabetic nephropathy have an increased risk of having a fetus who develops intrauterine growth restriction and also have a risk of gestational hypertensive disorders such as preeclampsia that exceeds the risk conferred by diabetes alone. Low-dose aspirin has been evaluated as a possible intervention to reduce the risk of preeclampsia in women who have an a priori elevated risk of developing this obstetric complication, and its use has been found to confer a modest reduction in the risk of developing preeclampsia. Low-dose aspirin in pregnancy appears to have a good safety profile. The US Preventive Services Task Force has recommended the use of low-dose aspirin in women at elevated risk of developing preeclampsia including women with pregestational diabetes (type 1 or type 2) (*Henderson et al., 2014; LeFevre, 2014*).

Identification of women who have additional risk factors for adverse pregnancy outcomes may allow for pregestational optimization of medical comorbidities as well as closer maternal and fetal monitoring during pregnancy.

Medical Therapy for Diabetes in Pregnancy

In general, maintaining good glycemic control is a primary goal of the management of diabetes in pregnancy. The ideal target range for maternal blood glucose in pregnancy is not clear, though it is well established that average maternal blood glucose must be maintained at a much lower level than what is considered acceptable in the nonpregnant setting because fetal and neonatal complications occur with increased frequency even when maternal glucose levels are modestly elevated. Currently, expert consensus recommendations indicate the following targets for glycemic control in women with gestational diabetes: fasting capillary blood glucose (CBG) of less than 95 mg/dL, 1-hour postprandial CBG levels below 140 mg/dL, or 2-hour postprandial levels less than 120 mg/dL; pharmacologic therapy is strongly recommended for women whose CBGs exceed these thresholds (*ACOG, 2013*). In women with pregestational diabetes, expert consensus recommends the following goals: fasting CBG less than or equal to 90 mg/dL, 1-hour postprandial

CBG less than or equal to 130–140, or 2-hour postprandial levels less than or equal to 120 mg/dL (*ADA 2016 Standard of Medical Care in Diabetes*).

Results from the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study raise the question of whether stricter glucose levels should be targeted. The HAPO study was a prospective multinational study that evaluated the relationship between maternal hyperglycemia and adverse pregnancy outcomes that has been attributed to maternal diabetes. The relationship between results from a 2-hour, 75-g oral glucose tolerance test and the following primary outcomes were evaluated: birth weight greater than the 90th percentile for GA; cord blood C-peptide levels greater than the 90th percentile; primary cesarean delivery; and clinical neonatal hypoglycemia. Maternal glucose values at each time point (fasting, 1 hour, and 2 hours after glucose challenge) were positively associated with the primary outcomes in a linear fashion. There was no apparent threshold glucose level above which risk for an adverse outcome was clearly elevated; frequencies of adverse outcomes rose steadily with worsening maternal hyperglycemia (*HAPO Study Cooperative Research Group, 2008*).

As many adverse fetal/neonatal outcomes such as macrosomia and neonatal hypoglycemia are thought to result from fetal hyperinsulinemia in response to maternal hyperglycemia, it may be relevant to consider the maternal blood glucose levels in women without diabetes. A review of 12 studies evaluating the glycemic profile of nondiabetic women demonstrated fasting glucose levels of 71 ± 8 mg/dL (mean \pm 1 standard deviation); 1-hour postprandial levels of 109 ± 122 mg/dL; and 2-hour postprandial levels of 99 ± 109 mg/dL. These results suggest that further clinical study of maternal goals for glycemic control is warranted.

Maintaining maternal glycemic control is made more difficult by physiologic changes of pregnancy that lead to worsening insulin resistance with advancing GA. Increasing levels of human placental lactogen and changes in levels of cortisol and prolactin are likely to exacerbate insulin resistance. Insulin sensitivity has been demonstrated to decrease by as much as 56% by 36 weeks' gestation. For women with pregestational diabetes, third-trimester insulin requirements may be two to three times greater than prepregnancy doses. Obese women with type 2 diabetes may demonstrate extreme levels of insulin resistance.

All women with diabetes require dietary therapy during pregnancy. Consultation with a registered dietician is an important part of care to ensure that the nutritional requirements of pregnancy are met and the distribution of calories from carbohydrates, proteins, and fats contributes to optimal glycemic control. In obese women, in particular, attention to dietary therapy is also critical to the goal of preventing excessive weight gain. Excessive weight gain appears to be an independent contributor to some adverse outcomes such as fetal macrosomia.

Medical management of maternal hyperglycemia in women with pregestational diabetes commonly relies on subcutaneous insulin. Insulin does not cross the placenta so does not have a risk of adverse impact on fetal development. Insulin has been well-studied in pregnancy and has been shown to be effective in achieving good maternal glycemic control and in reducing the risk of adverse obstetric outcomes.

Women with GDM or mild type 2 diabetes may be treated with oral medications such as metformin or glyburide instead of insulin. The use of oral medications, especially for the treatment of GDM, has become increasingly popular following the publication of clinical trials that demonstrated the effectiveness of these medications. Metformin has long been used in type 2 diabetes; it acts primarily

by decreasing hepatic glucose production, decreasing intestinal absorption of glucose, and by improving insulin sensitivity in peripheral tissues. Metformin has a low risk of producing hypoglycemia. In a randomized clinical trial comparing metformin with insulin for the treatment of GDM, there was no significant difference in neonatal outcomes, including hypoglycemia, birth trauma, respiratory distress, or hyperbilirubinemia (Rowan et al., 2008). It was notable that 46% of women in the metformin group ultimately required insulin in order to maintain glycemic control; these women were more likely to be overweight and appeared to have had higher fasting glucose values at study entry. Metformin therapy had the additional benefit of being associated with significantly less maternal weight gain than insulin therapy alone. Metformin is known to cross the placenta, but neonatal outcomes in IDMs who were exposed to metformin in utero appear to be reassuring overall.

Glyburide has also become an increasingly popular treatment option for GDM. Glyburide acts on the pancreas to promote and increase insulin secretion. When compared with insulin therapy in GDM, glyburide has been reported to be equally effective in achieving glycemic goals (Langer, 2000). Glyburide is also known to cross the placenta; given the actions of this medication on the pancreas, concern has been raised about the potential metabolic implications of in utero exposure to glyburide. Metaanalyses evaluating studies of glyburide versus insulin have suggested an approximately twofold increase in risk of macrosomia and in neonatal hypoglycemia with glyburide therapy in GDM (Poolsup et al., 2014; Jiang et al., 2015; Balsells et al., 2015). Given the complex regulation of fetal growth, it is difficult to know the degree to which abnormal growth can be attributed to glyburide exposure. Further studies regarding the long-term consequences of in utero glyburide exposure are needed.

Antenatal Monitoring

As discussed previously, pregnancies complicated by diabetes are at increased risk for abnormal fetal growth as well as perinatal asphyxia and stillbirth. These complications warrant increased antenatal surveillance in the third trimester. Results of testing or changes in the pattern of testing may help to inform decisions regarding timing and mode of delivery.

Fetal acidemia may produce characteristic changes on biophysical tests. Tests currently utilized include maternal assessment of fetal movement, fetal heart rate testing (nonstress test or contraction stress test), and ultrasound biophysical profile testing.

Maternal assessment of fetal movement is a frequently recommended component of fetal monitoring programs. Studies have noted that decreased fetal movement may be perceived in the period preceding fetal death. There does not appear to be a superior method for evaluation of fetal movement: an example of a protocol that has been suggested includes counting distinct fetal movements and considering 10 movements within 2 hours reassuring (Moore et al., 1989).

Fetal heart rate testing relies on a mature and normally functioning fetal autonomic system; in this setting, baseline fetal heart rate should be between 120 and 160 beats per minute, with moderate variability, and accelerations that occur with fetal movement. The pattern described corresponds with fetal heart rate reactivity. Fetal heart rate testing as a method of screening for fetal compromise is probably of limited clinical utility at earlier GAs. In an uncompromised fetus, the likelihood of a nonreactive fetal heart rate test is greater at preterm GAs: between 24 and 28 weeks' gestation, 50% of fetal heart rate tests are nonreactive; between 28 and 32 weeks' gestation, 15% of tests are nonreactive. In order to avoid false-positive

results on fetal heart rate testing, most centers do not initiate these screening tests until 32–34 weeks unless there are indications for earlier testing (e.g., concern for fetal growth restriction, poorly controlled maternal diabetes, or hypertension etc.).

At any GA, fetal heart rate decelerations that are greater than 1 minute in duration or that are late in timing relative to a contraction are abnormal and are associated with an increased risk of perinatal complications including fetal death; therefore further evaluation and consideration of delivery may be indicated based on the clinical context of these findings.

Biophysical profile testing relies on sonographic evaluation of amniotic fluid volume (measurement of a single pocket of fluid of at least 2-cm depth), fetal movement, demonstration of fetal tone (by extension and flexion of a fetal limb or opening and closing of a hand), and fetal breathing movements. Each component of this examination is given a score of 0 if absent or 2 if present. If a fetal nonstress test is also performed, then a reactive test is given a score of 2 while a nonreactive test is given a score of 0. Biophysical profile scores of 8–10 are considered normal and reassuring. A score of 6 is considered equivocal and requires additional evaluation. A score of 4 or less is considered abnormal and requires intervention.

It is not clear that antenatal fetal testing as described above is useful in preventing stillbirth or perinatal asphyxia, but these tests may be clinically useful in providing reassurance that fetal status is stable so as to allow for continued expectant management until a favorable term GA is reached. If stillbirth within 1 week of testing is used as the primary outcome of interest for antenatal fetal testing, both fetal heart rate testing and biophysical profile testing appear to have high negative predictive values (>99%) for reactive nonstress tests and biophysical profile scores of 8–10. In diabetic pregnancies (especially if poorly controlled), more frequent testing may be warranted as the fetal testing strategies above may miss fetal compromise precipitated by acute maternal metabolic changes. The appropriate frequency of fetal testing in pregnancies complicated by diabetes has been difficult to establish. Twice-weekly testing is commonly recommended in most centers. This testing strategy has been evaluated in a large retrospective cohort that demonstrated an overall low stillbirth rate, with the observation that none of the stillbirths occurred within 4 days of the last antepartum test (Kjos et al., 1995).

Evaluation of fetal growth also contributes to the assessment of fetal status. As discussed previously, third-trimester evaluation of fetal growth is significantly limited by increasing inaccuracy of predicted fetal weights as gestation progresses. Despite these limitations, third-trimester ultrasound assessment of growth is commonly recommended, given the risk of pathologic growth in diabetic pregnancies. For women with microvascular complications of diabetes, concomitant chronic hypertension, and preeclampsia, fetal growth restriction may confer further elevated risk of perinatal morbidity and mortality, thus necessitating additional antenatal testing (e.g., fetal umbilical Doppler studies) to help inform obstetric management. While fetal macrosomia has been difficult to accurately diagnose antenatally, findings suggestive of predicted birth weight above 4500 g appear to be associated with an increased risk of shoulder dystocia. In this setting, it may be reasonable to consider cesarean delivery in an attempt to avoid shoulder dystocia and resultant birth trauma.

Delivery Planning

Women with diet-controlled gestational diabetes appear to have a low risk of perinatal complications; therefore early delivery is

not indicated, and expectant management is usually appropriate. For pregestational diabetics and gestational diabetics requiring medication management, timed early delivery has been considered in an attempt to reduce perinatal morbidity and mortality. Strategies of scheduled delivery at 38–39 weeks for women with GDM have been evaluated in clinical trials; results have suggested that while there may be some potential reduction in macrosomia or LGA with earlier delivery, there does not seem to be a significant reduction in shoulder dystocia, neonatal hypoglycemia, or perinatal death (Kjos et al., 1993; Witkop et al., 2009). Given recent evidence that suggests early term births (at 37–39 weeks) may be associated with differences in rates of neonatal complications (such as respiratory complications) as compared with deliveries at term (39–40 weeks), it is probably reasonable for most well-controlled pregestational and gestational diabetics to plan for a delivery at around 39 weeks' gestation. Delivery at GAs greater than 40 weeks' gestation may be associated with a greater risk of macrosomia and shoulder dystocia (Lurie et al., 1996) and does not appear to be associated with other improvement in neonatal or maternal outcome. Elective cesarean delivery in fetuses suspected of macrosomia has also been considered. As discussed previously, macrosomic infants have a greater risk of shoulder dystocia and IDMs have a greater risk of birth trauma resulting from shoulder dystocia. Based on evaluation of reported rates of shoulder dystocia and birth trauma at different birth weights, it has been proposed that it may be reasonable to consider cesarean delivery for fetuses with estimated fetal weights greater than 4500 g. The potential neonatal benefit of cesarean delivery in these cases must be balanced against potential maternal morbidity associated with cesarean delivery. Studies evaluating potential benefit of cesarean delivery for presumed macrosomia estimate that up to 588 elective cesarean deliveries for an estimated fetal weight of 4500 g may need to be performed to prevent one case of a permanent brachial plexus palsy (Garabedian et al., 2010).

Intrapartum Diabetes Management

The goals of intrapartum diabetes management are to maintain strict glycemic control and to avoid ketosis. Titration of an intravenous infusion of short-acting insulin is a common strategy for maintaining blood sugars at less than 140 mg/dL (some expert opinions recommend levels <120 mg/dL). Maintenance of blood sugars at this level may be helpful in reducing neonatal hypoglycemia. Maternal hyperglycemia intrapartum has been shown to be associated with higher rates of neonatal hypoglycemia, probably by worsening the fetal hyperinsulinemic response. After delivery, the IDM will no longer have a steady supply of maternal glucose as a fuel, but higher insulin levels persist.

Maternal Obesity

The worsening obesity epidemic is contributing to the global rise in type 2 diabetes and gestational diabetes, thus resulting in an increased risk of the perinatal complications attributable to diabetes. Importantly, maternal obesity even in the absence of diabetes has been found to be an independent risk factor for adverse obstetric, fetal, and neonatal outcomes. Specifically, maternal prepregnancy obesity has been associated with an increased risk of congenital anomalies, stillbirth, macrosomia, hypertensive disorders of pregnancy (e.g., preeclampsia), stillbirth, cesarean delivery, as well as pediatric obesity (ACOG, 2015).

Obese women have been noted to have offspring with a 2.2-fold increased odds of open neural tube defects as well as an increased

risk of hydrocephalus, cardiovascular anomalies, cleft lip and/or palate, as well as limb reduction anomalies (Stothard et al., 2009). This elevated risk of congenital malformation is likely to be compounded by the presence of pregestational diabetes if there is suboptimal glycemic control. Despite the recognition of elevated risk of congenital malformations in offspring of obese women with diabetes, ultrasound evaluation for fetal anomalies in obese women is more challenging; studies estimate a 20% lower detection rate for anomalies (Dashe et al., 2009; Aagaard-Tillery et al., 2010).

The risk of stillbirth is also elevated in overweight and obese women; in several studies, the risk of perinatal mortality was shown to increase with increasing severity of maternal obesity (Salihu et al., 2007; Aune et al., 2014). Maternal prepregnancy obesity, as defined by a BMI of greater than or equal to 30 kg/m², has been identified as an independent risk factor for macrosomia and LGA birth weight (Willman et al., 1986). In a cohort of singleton pregnancies, both maternal diabetes and maternal obesity were identified as independent risk factors for LGA birth weight; in the case of maternal obesity, the adjusted odds ratio for delivering an LGA infant is 1.6, while for pregestational diabetes, the adjusted odds ratio is 4.4 (Ehrenberg et al., 2004). Given the greater prevalence of obesity as compared with pregestational diabetes, it is likely that the proportion of LGA infants delivered by obese women will exceed that of diabetic women. Similarly to what is observed in macrosomic IDMs, LGA infants born to obese women appear to have increased adiposity as compared with infants of nonobese women; these children may go on to have an increased risk of childhood obesity and metabolic abnormalities (Boney et al., 2005; Catalano et al., 2009).

Neonatal Complications

Hypoglycemia

IDMs are at increased risk of neonatal hypoglycemia; this is one of the more common neonatal morbidities encountered in IDMs, with reports citing rates of neonatal hypoglycemia as high as 25%–50%; studies in which good maternal glycemic control were reportedly achieved suggest a lower incidence of neonatal hypoglycemia in the range of 5%–15% (Cordero et al., 1998). The reported rates of neonatal hypoglycemia have varied in the medical literature; this is probably due to the continued debate about clinically meaningful definitions of hypoglycemia. Severe or persistent hypoglycemia is known to cause adverse neurologic and endocrine sequelae; however, it is not clear what levels of neonatal glucose are optimal to minimize risk of these complications in IDMs. Defining these optimal newborn glucose levels is likely further confounded by the observation that preterm and low birth weight neonates are more likely to have lower blood sugars (Cornblath et al., 2000; Garcia-Patterson et al., 2012).

Most studies have defined neonatal hypoglycemia as blood glucose levels below 35–45 mg/dL, but clearly not all infants who have hypoglycemia as defined by these levels demonstrate evidence of adverse effects (Garcia-Patterson et al., 2012). In a large prospective cohort study evaluating neonates born at GAs of 35 weeks or older, hypoglycemia (defined as a blood glucose concentration of <47 mg/dL) when treated did not appear to be associated with an increased risk of neurosensory impairment when assessed at 2 years of age. It was also notable that the lowest blood glucose concentration observed in these neonates as well as the number of hypoglycemic episodes did not predict the child's

neurodevelopmental outcome at age 2 years (McKinlay et al., 2015). Many infants with hypoglycemia (if defined as blood glucose <40 mg/dL) may initially be asymptomatic. Symptoms attributable to hypoglycemia may include tremor, jitteriness, irritability, lethargy, and hypotonia; severe cases of hypoglycemia may also lead to convulsions, apnea, or cyanosis.

Neonatal hypoglycemia in IDMs is most likely due to fetal hyperinsulinism: i.e., a response to fetal hyperglycemia resulting from maternal hyperglycemia. After birth, the infant no longer receives a steady supply of maternal glucose; if there is hyperinsulinemia originating in utero, then the neonatal period is characterized by a relative imbalance of insulin as compared with neonatal glucose supply. This imbalance leads to a risk of hypoglycemia evolving within the first few hours of life with a nadir in blood glucose levels reported at 1–3 hours of life in most infants.

IDMs with neonatal hypoglycemia have been observed to have elevated cord blood C-peptide and serum insulin levels at birth (Andersen et al., 1985). Based on the pathophysiology of hypoglycemia in IDMs, it is not surprising that poor maternal glycemic control in the third trimester and in the intrapartum period is a predictor of neonatal hypoglycemia (Andersen et al., 1985; Kline and Edwards, 2007). Maternal plasma glucose levels at delivery have been shown to correlate strongly and negatively with neonatal glucose concentrations at 2 hours of life; in observational studies, rates of neonatal hypoglycemia were significantly lower if maternal blood glucose levels were less than 130 mg/dL (Andersen et al., 1985; Flores-le-Roux et al., 2012). Intrapartum maternal hyperglycemia and resulting fetal hyperglycemia probably stimulate the fetal pancreas to release more insulin, with this effect persisting through delivery and for the first few hours thereafter. Macrosomia has also been shown to be a risk factor for neonatal hypoglycemia, with some studies suggesting a prevalence of 30%–50% in LGA IDMs; this observation is not surprising, as excessive fetal growth has also been attributed to the effects of fetal hyperinsulinemia (Maayan-Metzger et al., 2009; Flores-le-Roux et al., 2012).

In addition to optimizing maternal glycemic control throughout gestation, strict maternal glycemic control in labor or in the hours immediately preceding delivery (e.g., if scheduled cesarean section) will help to prevent or reduce rates of neonatal hypoglycemia. Current expert recommendations for maternal intrapartum glycemic control vary; ACOG recommends maintaining maternal blood glucose levels between 70 and 110 mg/dL for pregestational diabetics (ACOG Practice Bulletin, 2005). Postpartum management that emphasizes the importance of early (within the first hour of life) and liberal breastfeeding may also help to prevent neonatal hypoglycemia (Sarkar et al., 2003; Chertok et al., 2009; Maayan-Metzger et al., 2014).

Respiratory Distress

IDMs may also be at greater risk of respiratory complications and respiratory distress syndrome (RDS) as compared with same GA infants born to nondiabetic mothers. This increased risk of respiratory compromise appears to affect even late-preterm and early-term IDMs, though studies evaluating risk of respiratory distress in IDMs at GAs greater than 38 weeks vary in their results. In a large retrospective cohort evaluating infants born at GAs of 34 weeks or greater, gestational diabetes was identified as an independent risk factor for severe neonatal respiratory failure (requiring admission to the neonatal intensive care unit or the need for ventilator support at 24 hours of age) (Vignoles et al.,

2011). The pathophysiology of respiratory distress in IDMs is thought to result from a delay in pulmonary maturation and surfactant production, leading to a relative surfactant deficiency (Singh and Feigelson, 1983). Insulin appears to have a role in regulating fetal lung maturation; in a cell culture model of type II alveolar cells, exposure to elevated levels of insulin reduced choline incorporation into surfactant phosphatidylcholine (Engle et al., 1983). Fetal hyperinsulinemia may have an inhibitory effect on glycogenolysis, which contributes to a decrease in the synthesis of phosphatidylglycerol (PG).

Given these concerns, many experts have recommended that fetal lung maturity be evaluated prior to considering delivery in women with diabetes at GAs less than 38–39 weeks when there is not an absolute indication for delivery (e.g., preeclampsia or non-reassuring fetal testing). Delivery prior to 39 weeks may be considered clinically when there is persistent suboptimal or worsening maternal glycemic control that is not responsive to usual medical therapy or when there is concern for significant risk of adverse fetal outcomes (e.g., in the setting of macrosomia and polyhydramnios). Currently available antenatal tests for evaluating fetal lung maturity require amniocentesis to facilitate the identification or quantification of pulmonary phospholipids or the quantification of lamellar bodies, which are the storage form of surfactant released by type 2 pneumocytes. Amniocentesis performed in the third trimester is generally considered a safe procedure, with low rates of complications that require same-day delivery (Stark et al., 2000). The lecithin/sphingomyelin (L/S) ratio is one of the quantitative tests used to evaluate likelihood of pulmonary maturity. The amniotic fluid concentration of lecithin usually increases in the third trimester, whereas sphingomyelin levels tend to remain relatively stable. L/S ratio values above 2 in the late third trimester have been associated with a high likelihood of pulmonary maturity. L/S ratios are altered by the presence of blood or meconium in the amniotic fluid. PG is one of the last pulmonary phospholipids to be identified in the amniotic fluid; its presence in the amniotic fluid has been associated with pulmonary maturation. Unlike the L/S ratio, the test for identification of PG is not impacted by the presence of blood or meconium in the amniotic fluid. Both the L/S ratio and the PG assay require a significant amount of technical expertise and cost to perform; therefore the lamellar body count (LBC) test, which is automated and more widely accessible, has become increasingly popular as a test of fetal lung maturity. Lamellar bodies are the storage form of surfactant released by type 2 pneumocytes; lamellar bodies are similar in size to platelets so the quantity of lamellar bodies can be estimated using the same automated counter used to obtain platelet counts. LBCs greater than 50,000/μL are positively correlated with pulmonary maturation. If there is blood in the amniotic fluid specimen, the results of the LBC may be affected.

Studies evaluating the performance of the L/S ratio as a predictor of fetal pulmonary maturation in diabetic pregnancies have been mixed, with some studies concluding that using a higher threshold with L/S greater than 3.0 as indicative of lung maturity in diabetic pregnancies performs better (Kitzmillier et al., 1978). Conversely, other studies have demonstrated the appropriateness of utilizing the general threshold of L/S greater than 2.0 as indicative of fetal pulmonary maturation (Kjos et al., 1990; Tabsh et al., 1982). The values of the PG and LBC tests that have been considered indicative of fetal pulmonary maturation in the general population appear to be valid in pregnancies complicated by diabetes. In general, tests of fetal lung maturity have a much better negative predictive value for RDS than positive predictive value.

Antenatal Corticosteroids for Reduction in Risk of Respiratory Distress Syndrome

In clinical settings in which early preterm birth (at GAs <34 weeks) is anticipated, antenatal corticosteroids may be considered to reduce the risk of RDS and other neonatal complications of prematurity. In the setting of maternal diabetes, the decision to administer antenatal corticosteroids must also take into consideration the potential maternal impact of these medications. Corticosteroid administration can lead to maternal hyperglycemia by promoting hepatic gluconeogenesis and impairing insulin sensitivity (Tamez-Perez et al., 2015). Significant maternal hyperglycemia resulting from the administration of antenatal corticosteroids must be anticipated, because if left untreated there may be an increased risk of maternal complications such as DKA in women with type 1 diabetes (Bedalov et al., 1997; Fisher et al., 1997). An evaluation of the impact of antenatal corticosteroid use in pregnant diabetic women demonstrated a significant increase in insulin requirements for the 5 days following medication administration. Peak insulin requirements were noted on days 2–3 after administration, with an approximately 40% increase in insulin dosage needed to achieve glycemic control (Mathiesen et al., 2002).

Because of the potentially adverse maternal effects resulting from corticosteroid administration, women with diabetes were excluded from the recent National Institute of Child Health and Human Development multicenter trial evaluating the role of corticosteroid therapy in pregnancies at risk of late preterm birth between 34 and 36 weeks' gestation. This trial demonstrated a significant decrease in the need for neonatal respiratory support and a decrease in the rate of severe respiratory complications in those infants whose mothers received corticosteroids prior to delivery; however, neonatal hypoglycemia was significantly more common in the group of infants who had been exposed to betamethasone in utero (24% in the betamethasone group vs 15% in the placebo group, RR 1.60, 95% CI 1.37–1.87) (Gyamfi-Bannerman et al., 2016). These results have led ACOG to advise the consideration of betamethasone administration in singleton pregnancies at risk for late preterm birth, but ACOG acknowledges that women with pregestational diabetes were not included in the Antenatal Late Preterm Steroids trial so the effect of late preterm corticosteroids in this population is unknown (ACOG Committee Opinion, 2016). In this setting, the potential adverse effects of late preterm corticosteroids is likely difficult to justify in women with pregestational diabetes.

Hypertrophic Cardiomyopathy

IDMs have an increased risk of developing hypertrophic cardiomyopathy. The most common echocardiographic findings include asymmetric interventricular septal enlargement, with hypertrophy of the ventricular free walls noted to a lesser extent. On average, most series reporting on the prevalence of hypertrophic cardiomyopathy note that 30%–40% of IDMs have evidence of these cardiac changes on imaging, but only approximately 5% of infants will manifest symptoms suggestive of congestive heart failure. Heart failure may be caused by interventricular septal hypertrophy, leading to obstruction of the left ventricular outflow tract. Most symptomatic infants require only supportive care. In almost all cases, the myocardial hypertrophy is found to resolve spontaneously within 6–12 months of life.

The underlying etiology of hypertrophic cardiomyopathy seen in IDMs is still incompletely understood; it is hypothesized to be

related to fetal hyperinsulinemia (Hayati et al., 2004). As noted in other organs, increased insulin and related factors such as IGFs have been found to stimulate hyperplasia and hypertrophy. In myocardial cells, insulin inhibits the enzyme glycogen synthase kinase-3, which is a negative regulator of myocardial growth; its inhibition might contribute to abnormal myocardial hypertrophy. Further evidence in support of fetal hyperinsulinemia in the pathogenesis of hypertrophic cardiomyopathy comes from the observation that infants with congenital hyperinsulinism (in the absence of maternal diabetes) also appear to have an increased risk of hypertrophic cardiomyopathy. In a series of 25 infants with congenital hyperinsulinism who required neonatal echocardiogram evaluation for symptoms (e.g., arrhythmias, auscultated murmurs, etc.), 40% were found to have evidence of hypertrophic cardiomyopathy with features similar to that found in IDMs (Huang et al., 2013). Findings from serial fetal echocardiographic evaluations have also raised the hypothesis that structural cardiac changes may represent an adaptive response to altered fetal cardiac function early in gestation. Fetuses of diabetic mothers demonstrated impaired diastolic function (prolonged isovolumetric relaxation time and lower early/atrial [E/A] ratio) and poorer global cardiac function (increased myocardial performance index or Tei index) as compared with fetuses of nondiabetic mothers. The fetuses of diabetic mothers went on to develop evidence of interventricular septal thickening in the third trimester but had no persistent evidence of cardiac dysfunction later in gestation. (Russell et al., 2008). Maternal metabolic control during organogenesis is known to have a significant impact on fetal cardiac development, as discussed earlier in this chapter.

Predictors of symptomatic cardiomyopathy in IDMs are limited at this time. Despite evidence that supports a role for fetal hyperinsulinemia in the development of hypertrophic cardiomyopathy, the association between maternal glycemic control and this fetal/neonatal complication is not clear. Clinical studies evaluating the correlation between fetal/neonatal hypertrophic cardiomyopathy and maternal HbA_{1c} values have been mixed in their conclusions. Investigators have reported evidence of septal hypertrophy even in fetuses of women with well-controlled diabetes. Third-trimester fetal echocardiography has been used to measure the myocardial performance index (as an indicator of global cardiac function) and the E wave/A wave peak velocity (E/A) ratio at the mitral valve, as an indicator of ventricular diastolic function, in an attempt to identify fetuses of diabetic mothers who are at increased risk of adverse perinatal outcome (including symptomatic cardiomyopathy). In a small series (Bhorat et al., 2014), these fetal echocardiographic measurements appeared to have reasonable sensitivity and specificity for the prediction of a composite of adverse perinatal outcomes, but given the low prevalence of the most serious of these outcomes (symptomatic cardiomyopathy, perinatal death), the predictive value and the cost-effectiveness of these measures are probably limited at this time.

Hypocalcemia and Hypomagnesemia

Calcium and magnesium are actively transported across the placenta to the fetus throughout gestation. At delivery, the transfer of these minerals is terminated and neonatal levels of these minerals are expected to decrease in most infants, but IDMs appear to have an increased risk of more significant declines in levels of calcium and magnesium.

Neonatal hypocalcemia is often defined as a serum calcium level less than 7 mg/dL or an ionized calcium level less than 4.4 mg/

dL (1.1 mmol/L). Studies have historically reported that up to 50% of IDMs may have hypocalcemia in the neonatal period, though more recent reports suggest a lower prevalence of 4%–5% (Cordero et al., 1998). Most affected infants will be asymptomatic. This condition is often found to resolve spontaneously. It is suspected that neonatal hypocalcemia may result from a delayed transition from fetal to neonatal parathyroid action in calcium metabolism as fetal parathyroid glands are relatively inactive until after delivery. The risk of neonatal hypocalcemia is further increased in the setting of prematurity as well as perinatal asphyxia.

Neonatal hypomagnesemia is defined as a serum magnesium level below 1.5 mg/dL. Hypomagnesemia has been reported to affect as many as 40% of IDMs, although most usually remain asymptomatic. Neonatal hypomagnesemia appears to be most clinically relevant in the context of significant hypocalcemia, as concurrent hypomagnesemia makes the treatment of hypocalcemia more difficult. Magnesium deficiency probably has adverse effects on parathyroid function, thus exacerbating hypocalcemia. The pathophysiology underlying neonatal hypomagnesemia is not clear. Some experts have hypothesized that fetal hypomagnesemia may result from lower maternal magnesium levels in the setting of diabetes from increased maternal urinary losses or renal dysfunction.

Clinical signs and symptoms of hypocalcemia and hypomagnesemia are similar to those of hypoglycemia and include jitteriness, irritability, tachypnea, and possibly seizures in severe cases. Unlike hypoglycemia, which often presents within the first few hours of life, symptoms of hypocalcemia and hypomagnesemia typically present later at 24–72 hours after birth (Barnes-Powell, 2007).

Polycythemia

Polycythemia, defined as a venous hematocrit above 65% or hemoglobin greater than 20 g/dL, is also observed more often in IDMs. Polycythemia has been observed in approximately 3% of all infants born at sea level, with a slightly higher prevalence of 5% in infants born at higher altitudes. Among IDMs, the reported prevalence has ranged from 5%–40% (Tyrala, 1996). Fetal hyperglycemia leads to increased catabolism and increased oxygen consumption resulting in decreased oxygen tension. If chronic, this relative fetal hypoxemia is thought to stimulate erythropoiesis, resulting in polycythemia. Some small series have reported an association between maternal diabetic control (as represented by HbA_{1c} levels) and neonatal hematocrit. Higher erythropoietin levels have been noted in the amniotic fluid of women with diabetes. IDMs have also been shown to have increased erythropoietin levels at birth; erythropoietin levels in IDMs have also been shown to correlate with amniotic fluid glucose and insulin levels as well as cord blood insulin levels.

Infants with polycythemia may be at greater risk of complications resulting from hyperviscosity; reported complications include ischemia and infarction such as within the kidneys or within the CNS. These complications attributable to hyperviscosity may explain the increased incidence of renal vein thrombosis and stroke that have previously been reported in IDMs. Polycythemia in utero may also lead to deficient iron stores in the liver, brain, and heart; iron deficiency in these organs may then predispose to myopathies and altered neurodevelopment.

Infants with clinically significant polycythemia are likely to appear plethoric and may demonstrate sluggishness or lethargy. Treatment is currently initiated on the basis of symptoms and clinical status; treatment is not recommended on the basis of hematocrit value alone as the hematocrit level does not appear to

correlate well with clinical hyperviscosity. Reported therapies include intravenous fluid administration and possible consideration of partial volume exchange transfusion in some severe cases.

Hyperbilirubinemia

IDMs also appear to be at greater risk for hyperbilirubinemia than infants born to nondiabetic mothers. In studies that defined hyperbilirubinemia as a serum bilirubin level greater than 12 mg/dL or any bilirubin level requiring phototherapy, the prevalence of hyperbilirubinemia in IDMs was 25% (Cordero et al., 1998); other series report a hyperbilirubinemia prevalence of 10%–13% in IDMs. This increased risk might be attributable to polycythemia (larger source of bilirubin to be conjugated by the liver prior to excretion), ineffective erythropoiesis with an increased red blood cell turnover, as well as to immaturity of hepatic bilirubin conjugation and excretion. Macrosomic IDMs appear to have the greatest risk of hyperbilirubinemia; this probably reflects the role of poor maternal glycemic control during pregnancy as IDMs of these women are most likely to be macrosomic and polycythemic.

Breastfeeding

Breastfeeding is important to the health and well-being of all children; the benefits of breastfeeding may be even greater in IDMs. As mentioned previously, early and liberal breastfeeding in the immediate neonatal period may help to prevent hypoglycemia or effectively treat mild cases of hypoglycemia.

The positive effects of breastfeeding for IDMs are likely to extend significantly beyond the neonatal period. Intrauterine exposure to maternal diabetes and/or obesity appears to be associated with an increased risk of childhood obesity; successful breastfeeding of durations greater than or equal to 6 months may mitigate some of this risk. There has been increasing evidence of the long-term impact of the intrauterine environment on the development of adulthood diseases. Several cohort studies have suggested an increased risk of diabetes and obesity in childhood or adolescence in the offspring of diabetic mothers (Dabelea et al., 2000; Damm, 2009; Vohr and Boney, 2008). Studies conducted in the Pima Indian population demonstrated that offspring of diabetic mothers had a significantly increased risk of type 2 diabetes (12-fold increased odds) when compared to offspring of nondiabetic mothers (Pettitt et al., 1991). Offspring of diabetic mothers also were noted to be heavier than offspring of nondiabetic mothers when evaluated at ages 5–9 years and at 10–14 years; the greater risk of obesity in offspring of diabetic women persisted even among those who had a normal birth weight (Pettitt et al., 1987).

A longer duration of breastfeeding has been associated with a decreased risk of offspring childhood obesity. A metaanalysis of studies conducted in general populations demonstrated that each month of breastfeeding was associated with a 4% decrease in risk of being overweight in childhood (Harder et al., 2005). In offspring of diabetic mothers, breastfeeding duration of greater than or equal to 6 months has also been associated with decreased measures of adiposity such as waist circumference and measures of visceral and subcutaneous abdominal fat (Crume et al., 2011, 2012). The obstetric community has increasingly recognized the importance of recommending and advocating for breastfeeding among diabetic mothers; these women are anticipated to benefit from additional medical support, given the effects of breastfeeding on diabetes. During lactation, women with diabetes have a significant decrease in insulin or oral hypoglycemic medication requirements; in fact,

women (especially type 1 diabetic women) are at an increased risk of hypoglycemia during breastfeeding. Predelivery counseling by their obstetric and/or endocrinologic providers can help to facilitate an appropriate medical management and safety plan during lactation; close follow-up during the postpartum period with attention to issues unique to lactation in the context of diabetes can help to optimize breastfeeding success.

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11

Maternal Medical Disorders of Fetal Significance

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KEY POINTS

- Diagnostic imaging carries a risk of fetal radiation exposure. However, in most cases the actual fetal dose in properly performed procedures is low. While x-ray and computed tomography imaging should be avoided during pregnancy if possible, these studies are not contraindicated if needed.
- Delay in diagnosis of medical and surgical conditions in pregnancy is more common, in part because certain signs and symptoms may be similar to those related to pregnancy.
- The previously utilized pregnancy drug classification is being abandoned because most drugs have no definitive human studies documenting their safety or harm.
- Adverse pregnancy outcomes in patients with lupus are more frequently seen in those with renal disease, antiphospholipid antibody syndrome, thrombocytopenia, and active disease.
- Immune thrombocytopenia is associated with a potential risk of neonatal thrombocytopenia, which is not predicted by maternal disease status. However, gestational thrombocytopenia does not incur the same risk.
- Cardiovascular disease in pregnancy is increasing in frequency and has become a major contributor to maternal mortality.
- The evaluation and management of most cancers should not be delayed for the sake of pregnancy and for fear of harm to the fetus. Surgery and most chemotherapeutic agents have been successfully used during the second and third trimesters.
- Epilepsy is associated with an increased rate of poor reproductive outcomes regardless of medication use. Medications used to control seizures do incur an increased rate of fetal anomalies, but the benefits to mother and fetus usually outweigh their risk.
- Selective serotonin reuptake inhibitors are considered first-line medication therapy for depression in pregnancy.

Pregnancies complicated by an underlying maternal medical condition increase the potential of a suboptimal perinatal outcome. The trend of delaying conception has undoubtedly increased the rate of medical comorbidities. Therefore the obstetrician is increasingly faced with patients affected by a medical problem that is unrelated to pregnancy. While diagnostic and therapeutic options for a nonpregnant adult in most cases can be effectively applied during pregnancy, there are concerns about whether these will adversely affect the fetus or newborn.

Furthermore, the alterations of maternal anatomy and physiology during pregnancy may require adjustments of therapeutic options.

Two simple (but potentially difficult to answer) questions should be addressed when a specialist is caring for a pregnant patient with a known medical problem. First, is the condition itself affected by the anatomic or physiologic alterations of pregnancy? Secondly, how does the medical problem affect the women, her fetus, or the ultimate perinatal outcome? A further concern is how to evaluate a newly suspected medical problem during pregnancy. Pregnancy itself can be a confounder as many symptoms experienced are similar to those of certain medical or surgical conditions, leading to either a lack or delay of diagnosis. Providers may be reticent to perform needed imaging studies, to proceed with indicated surgical procedures, or to prescribe medications over concerns regarding risk to the fetus. Delays of diagnoses of de novo medical problems due to pregnancy have been reported. Unfortunately, in some cases, lack of timely recognition and initiation of appropriate therapy may result in poor maternal or fetal outcomes.

General Principles in the Diagnosis and Management of Medical Complications During Pregnancy

Diagnostic Radiation

Diagnostic radiography may be associated with fetal radiation exposure even when studies target maternal anatomy remote from the pregnant uterus (Table 11.1). The fetal consequences of radiation exposure are both dose and time dependent (Table 11.2). However, for most diagnostic procedures, actual fetal exposure is relatively low. The practitioner should limit the amount of radiographic testing if at all possible, but indicated studies should never be withheld because of pregnancy. Lead shielding of the abdomen and pelvis and careful selection of the type of study should be undertaken to minimize the fetal dose. If the amount of fetal exposure is less than 5 rad, there appears to be no significant increased risk of malformations. There is, however, a slightly increased risk of childhood cancer if the fetus is exposed to doses of greater than 10 mGy (Doll and Wakeford, 1997). To simplify the various measures of exposure, 1 radiation absorbed dose (rad), 1 Roentgen equivalents man (rem), 10 milliGray (mGy), and 10

TABLE 11.1 Estimated Conceptus Doses From Selected Imaging Studies

| Radiographic and Fluoroscopic Examinations | Typical Conceptus Dose (mGy) |
|--|------------------------------|
| Cervical spine (anteroposterior, lateral) | <0.001 |
| Extremities | <0.001 |
| Chest (posteroanterior, lateral) | 0.002 |
| Thoracic spine (anteroposterior, lateral) | 0.003 |
| Abdomen (anteroposterior) | |
| 21-cm patient thickness | 1 |
| 33-cm patient thickness | 3 |
| Lumbar spine (anteroposterior, lateral) | 1 |
| Limited IV pyelogram | 6 |
| Small-bowel study | 7 |
| Double contrast barium enema | 7 |
| CT Scan | |
| Head CT | 0 |
| Chest CT | |
| Routine | 0.2 |
| Pulmonary embolus | 0.2 |
| CT angiography of coronary arteries | 0.1 |
| Abdominal | |
| Abdominal, routine | 4 |
| Abdominal/pelvis | 25 |

CT, Computed tomography; IV, intravenous; mGy, milligray.

Adapted from McCollough CH, Schueler BA, Atwell TD, et al. Radiation exposure and pregnancy: when should we be concerned? *Radiographics*. 2007;27:909–917.

milliSievert (mSv) can be considered equivalent (Wang et al., 2012). Iodinated contrast agents have not been shown to be teratogenic. There is a theoretical concern of fetal hypothyroidism, but there have been no reported cases resulting from the use of these agents (Tirada et al., 2015). Use of these agents may also be justifiable with the aim of reducing further radiation exposure from repeated studies.

Magnetic resonance imaging (MRI) has not been shown to have adverse effects on the fetus. However, its safety has yet to be definitively determined. It is generally recommended that MRI be avoided in the first trimester, unless the benefit outweighs the risk (Duchene et al., 1991; Kanal et al., 2007). Gadolinium contrast should be used during pregnancy with extreme caution, given the lack of human safety data and unknown half-life in the fetal compartment (Kanal et al., 2007).

Surgery During Pregnancy

Approximately 2% of pregnant women require surgery for a non-obstetrical condition. The most common indications are appendicitis and cholecystitis (Juhász-Böss et al., 2014). Indicated surgery during pregnancy is relatively safe. The potential for adverse perinatal

consequences is increased when compared with patients not requiring surgery. However, delay of needed procedures in some cases clearly increases the risk of poor maternal or fetal outcome. A recent review of 54 papers regarding surgery during pregnancy (Cohen-Kerem et al., 2005) concluded the following:

1. The risk of maternal death is low.
2. Surgery and general anesthesia do not appear to be major risk factors for spontaneous abortion.
3. Elective termination rates are similar to the general population.
4. The rate of congenital anomalies does not appear to be increased.
5. Acute appendicitis, particularly with peritonitis, appears to be a risk of surgery-induced labor or fetal loss.
6. Urgent surgical procedures should be performed when needed.

Since appendectomy is the most common nonobstetric surgery performed during pregnancy (1 in 766 births), the data regarding outcomes are relatively robust. The rates of fetal loss and early delivery are higher for complex appendicitis (6% and 11%, respectively) versus simple appendicitis (2% and 4%, respectively). Pregnancy confounds the diagnosis and, potentially, the therapy with a reluctance to operate. The negative appendectomy rate in pregnancy is higher when compared with nonpregnant cases (23% vs 18%). Unfortunately, surgery for appendicitis and the finding of a normal appendix is still associated with an increased risk of loss (3%–4%) (McCory et al., 2007; Ito et al., 2012). While laparoscopy is being utilized increasingly during pregnancy, there may be an associated increased risk of loss when compared with laparotomy for suspected appendicitis (Wilasrusmee et al., 2012). However, the type of surgical procedure, whether laparoscopic versus open, should be determined by the surgeon, preferably in consultation with the obstetrics team.

Guidelines to optimize outcome for pregnant patients undergoing surgery are relatively simple. Anesthetic options are unchanged when compared with nonpregnant patients, as both regional and general anesthesia can be safely administered. However, if general anesthesia is being used, the maternal airway must be protected to prevent aspiration. Gastrointestinal motility is reduced in pregnancy and the stomach may contain significant residual contents hours after eating. A lateral decubitus position is preferred to optimize maternal venous return and uteroplacental perfusion. Preoperative counseling with an obstetrician and neonatologist is recommended, particularly if the gestational age is greater than or equal to 22 weeks to give the surgical team direction regarding interventions should fetal distress be encountered. Fetal heart rate monitoring, if feasible, can also facilitate assessment of fetal status.

Medication Usage

Maternal medication use during pregnancy requires the provider to understand two basic principles:

- Alterations of maternal anatomy and physiology may alter the effective dose.
- Maternal medications may enter the fetal circulation with resulting fetal exposure.

There are significant physiologic alterations in pregnancy which may alter the bioavailability, distribution, clearance, and half-life of a medication. Absorption is altered due to nausea and vomiting, gastric volume and pH changes, increased gastrointestinal transit time, and differences in activity of drug-metabolizing enzymes in the gut. Increased body weight and plasma volume as well as reduced albumin alter the volume of distribution. Hepatic and placental enzymatic activity and increased glomerular filtration

TABLE 11.2 American College of Radiology Summary of the International Commission on Radiological Protection Suspected in Utero-Induced Deterministic Radiation Effects

| Menstrual or Gestational Age | Conception Age | RADIATION DOSE | | |
|-------------------------------|-------------------------------|------------------|--|--|
| | | <50 mGy (<5 rad) | 50–100 mGy (5–10 rad) | >100 mGy (>10 rad) |
| 0–2 weeks (0–14 days) | Before conception | None | None | None |
| 3rd and 4th week (15–28 days) | 1st–2nd week (1–14 days) | None | Probably none | Possible spontaneous abortion |
| 5th–10th week (29–70 days) | 3rd–8th week (15–56 days) | None | Potential effects are scientifically uncertain and probably too subtle to be clinically detectable | Possible malformations increasing in likelihood as dose increases |
| 11th–17th week (71–119 days) | 9th–15th week (57–105 days) | None | Potential effects are scientifically uncertain and probably too subtle to be clinically detectable | Increased risk of deficits in intelligence quotient or mental retardation that increase in frequency and severity with increasing dose |
| 18th–27th week (120–189 days) | 16th–25th week (106–175 days) | None | None | Intelligence quotient deficits not detectable at diagnostic doses |
| >27 weeks (>189 days) | >25 weeks (>175 days) | None | None | None applicable to diagnostic medicine |

mGy, MilliGray; RAD, radiation absorbed dose.

Data from Tirada N, Dreizin D, Khatri NF, Akin EA, Zeman RK. Imaging pregnant and lactating patients. *Radiographics*. 2015;35:1751–1765.

rates will influence drug clearance (Zhao et al., 2014). Examples include levothyroxine and phenytoin, both medications in which the dose often must be increased through gestation.

The awareness that certain maternal drugs may induce congenital malformations, notably thalidomide in the early 1960s, led the Food and Drug Administration (FDA) to develop a maternal drug classification system (categories A–D + X). The premise for such a classification was to provide prescribing providers with a brief summary of potential fetal risk. Most drugs (65%–70%) were category C, which meant that while animal studies had shown adverse effects, there were no adequate human studies (Greene, 2015). Half of all pregnant women receive drugs in categories C and D (Andrade et al., 2004). Since so many drugs are category C, providers may assume that they are safe. The FDA has recently published the “Pregnancy and Lactation Labeling Rule,” effective 2015, eliminating the ABCDX classification for any new drug applications. The rule will expand for prescription drugs approved after 2001 (US Food and Drug Administration, 2006). The new subheadings under the pregnancy subsection will include exposure registry, risk summary, clinical considerations, and data. It is a reasonable assumption that, with the exception of a few medications, the most commonly prescribed drugs have some theoretical or proven fetal risk. Their choice and usage during pregnancy should be dictated by the premise that the maternal benefits clearly outweigh the fetal risk.

Autoimmune Disorders

Systemic rheumatoid illnesses are seen more commonly in women than men and with presentations commonly in young adulthood and middle age. Therefore pregnancy complicated by an autoimmune condition is common. There are an estimated 4500 pregnancies complicated by systemic lupus erythematosus (SLE) in the United States annually (Ramires de Jesus et al., 2015). Other autoimmune conditions seen during pregnancy include rheumatoid arthritis and scleroderma. While perinatal implications may differ, depending on the diagnosis, there are enough similarities among most rheumatologic disorders that there are common themes in maternal

management, including work-up, medication usage, and fetal screening. Furthermore, some patients may have findings consistent with an autoimmune condition but lack enough criteria to warrant a specific diagnosis. Finally, there are individuals lacking clinical rheumatologic manifestations that exhibit autoantibodies that have maternal, fetal, or neonatal implications. The nuances of all autoimmune disorders are too broad to allow discussion in this chapter. Therefore the focus will be on SLE and common antibody disorders with specific perinatal implications.

Systemic Lupus Erythematosus

SLE or lupus is a disease that can affect multiple organs. Early studies reported increased risks of prematurity, preeclampsia, and lupus flare, and therefore pregnancy was ill-advised. However, while recent literature has suggested more reasonable outcomes, there is an increased risk of prematurity (47%), fetal loss (23%), miscarriage (16%), growth restriction (14%), and early (13%) and late (12%) preeclampsia (Clark et al., 2005; Alijotas-Reig et al., 2015). In the absence of antiphospholipid antibody syndrome (APS) or significant renal insufficiency, fertility does not seem impacted (Stanhope et al., 2012). As with most autoimmune conditions, there is not uniform agreement on whether pregnancy alters the disease course. However, risk factors associated with a lupus flare include active disease within 6 months before conception, history of multiple flares, and discontinuation of hydroxychloroquine.

Risk factors for poor pregnancy outcomes in patients with lupus include proteinuria, renal insufficiency, APS, thrombocytopenia, or active maternal disease at the time of conception (Strojan and Baer, 2012). Those with nephritis, APS, or who have anti-Sjögren syndrome related-antigen A (anti-SSA or anti-Ro antibodies) may have increased risks of altered perinatal outcomes. Adverse outcomes include spontaneous abortion and intrauterine growth restriction (IUGR), and the risk of superimposed preeclampsia and stillbirth are potentially increased. Therefore assessment of the patient's baseline disease activity, as well as exploration for evidence of multiorgan system dysfunction, is vital to aid in counseling and

TABLE 11.3 Laboratory Testing of Patients With Systemic Lupus Erythematosus During Pregnancy

| Timing | Purpose | Test | Comment |
|---|---|---|---|
| Preconception or initial prenatal visit | Screen for organ system dysfunction and baseline for comparison should a lupus flare or preeclampsia be suspected | Urinalysis | Assessment of renal disease activity |
| | | 24-hour urine for total protein or urine protein:creatinine ratio | 24-hour urine collection for proteinuria recommended if abnormal protein:creatinine ratio |
| | | Complete blood count | Assess for thrombocytopenia |
| | | Chemistry panel | |
| | Screen for risk of neonatal lupus syndrome | Anti-SSA (Ro) and Anti-SSB (La) antibodies | If present, screen for fetal congenital heart block |
| | Screen for disease activity and baseline for comparison should a lupus flare or preeclampsia be suspected | Anti-double-stranded DNA antibody Complement levels | Complement levels may increase in a normal pregnancy |
| Monthly | | Urinalysis | Urine protein:creatinine ratio if urinalysis suspects proteinuria |
| | | Creatinine | If prior history of lupus nephritis or prior renal dysfunction is noted |
| Every trimester | | Anti-double-stranded DNA antibody Complement levels | |
| | | Complete blood count | |
| | | Chemistry panel | |

management. Comanagement with rheumatologists and/or nephrologists is vital to improve maternal and fetal outcomes.

Initial prenatal evaluation of a patient with lupus includes taking a history, such as disease activity, organ system involvement, and current medical therapy. The latter is important in determining whether there is an increased fetal risk, as some medications used are generally contraindicated during pregnancy (methotrexate, mycophenolate mofetil). Fortunately, most patients planning pregnancy are not using these agents. Prednisone, azathioprine, and hydroxychloroquine are more commonly used medications with reasonable safety profiles during pregnancy.

Chemical screening for disease activity should be performed. This includes anti-double-stranded DNA and complement levels. Antiphospholipid antibodies, anti-Ro, and anti-La (otherwise known as anti-Sjögren syndrome related-antigen B or anti-SSB) antibodies should be measured. The patient should also have baseline assessments for proteinuria, renal function, hepatic enzymes, and platelet count not only to assess her status but also to formulate a baseline profile (Table 11.3). Since a lupus flare may mimic preeclampsia, comparison of her repeated studies against those obtained earlier may be helpful, as the management of these two conditions differs.

Medication management, including type and amount, is dictated by disease activity and the presence of APS. Most patients are managed with steroids, hydroxychloroquine, or azathioprine, and alterations of these should probably not be dictated by pregnancy. While there may be theoretical concerns with their usage, these drugs have to be balanced against the risk of uncontrolled lupus (Table 11.4).

Assessment of disease activity should include the presence of maternal symptoms as well as regular complement and anti-double-stranded DNA levels. Regular assessment of fetal growth by ultrasound and antepartum fetal testing should be initiated in the latter third trimester. A sudden increase in the patient's blood

pressure or level of proteinuria must be regarded as a potential sign of either a lupus flare or preeclampsia. At times, differentiation between the two is challenging, but every effort should be made to do so, as therapies for both are different.

Antiphospholipid Antibody Syndrome

APS was originally described as an autoimmune disease characterized by circulating antiphospholipid antibodies (aPL) and venous thrombosis, recurrent pregnancy loss, and occasionally thrombocytopenia (Hughes, 1983). This disorder may be isolated or associated with other autoimmune diseases. The presence of aPL is found in about 1%–5% of healthy women. However, these antibodies are found in 15% of women who have recurrent abortions (Ruiz-Irastorza et al., 2010) and in 30%–40% in those with SLE (Ünlü et al., 2015). The presence of aPL in the latter is a risk factor for a poor pregnancy prognosis.

Few women with aPL will develop disease, and the prevalence of APS is estimated to be only 50/100,000 (Gomez-Puerta et al., 2014). The diagnosis of APS must include clinical criteria (vascular thrombosis, unexplained death of a morphologically normal fetus >10 weeks' gestation, premature birth <34 weeks due to preeclampsia or placental insufficiency, or ≥three otherwise unexplained abortions <10 weeks) as well as the presence of aPL (lupus anticoagulant, anticardiolipin antibody, or anti-β₂-glycoprotein antibody) on greater than two occasions, at least 12 weeks apart (Miyakis et al., 2006). For those with a prior history of venous or arterial thrombosis, prophylactic anticoagulation with heparin throughout pregnancy and continuing through 6 weeks' postpartum is usually recommended. In patients with recurrent pregnancy loss, low-dose aspirin and heparin may reduce pregnancy loss by 50% (Empson et al., 2002). Therefore even in the absence of a thrombotic history, in those with a history of stillbirth or recurrent pregnancy loss,

TABLE 11.4 Medications Used for Rheumatic Disorders

| Commonly Used During Pregnancy | Maternal and Fetal Outcomes | Recommendations |
|--|--|--|
| Prednisone, prednisolone | Possible risk for orofacial clefts | Treatment of flares or maintenance therapy |
| Fluorinated corticosteroids (betamethasone, dexamethasone) | Cross the placenta. May be associated with poor fetal growth or adverse neurologic outcomes if used in multiple doses | Use only if fetal treatment is being considered (neonatal lupus syndrome) |
| Hydroxychloroquine | Considered safe in doses commonly used in SLE and RA | Safe to continue in pregnancy |
| Azathioprine | May be associated with IUGR, transient immune alterations | Long track record of use in pregnancies complicated by SLE. Safe to continue if necessary |
| Nonsteroidal anti-inflammatory drugs | May be associated with fetal ductus arteriosus constriction and impaired renal function with third trimester use | Avoid in third trimester. Limit use to very short duration (48–72 hours) |
| Sulfasalazine | No increased risk in doses commonly used in RA | Use folic acid supplementation preconception and during pregnancy |
| Heparin | Does not cross placenta | If using low-molecular-weight heparin, may require changing to unfractionated heparin after 36 weeks for maternal anesthesia |
| IVIg | No identifiable fetal risk | May be used in refractory cases of immune thrombocytopenia |
| Contraindicated | | |
| Methotrexate | Increased risk for congenital malformations | Conception should be delayed for at least 3 months after last dose |
| Leflunomide | Embryotoxicity | Discontinue 2 years prior to conception |
| Mycophenolate mofetil | Increased risk for congenital malformations | |
| May Be Used | | |
| Cyclosporine | Often used in patients with organ transplants. No increased risk of congenital malformations | May be used in patients with SLE |
| Cyclophosphamide | Use in first trimester associated with fetal anomalies or loss. Second- and third-trimester use may be associated with fetal growth issues and impaired neurologic development | Use only if mother's life is at risk and other options have been exhausted |
| Rituximab | Associated with transient B-cell depletion in the second and third trimesters | Inadequate safety data to support routine use in pregnancy Neonatal live virus vaccination should be delayed by 6 months |

IUGR, Intrauterine growth restriction; IVIg, intravenous immunoglobulin; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

Adapted from Baer AN, Witter FR, Petri M. Lupus and pregnancy. *Obstet Gynecol Surv.* 2011;66: 639–653. Makol A, Wright K, Amin S. Rheumatoid arthritis and pregnancy. *Drugs.* 2011;71:1973–1987. Hyrich KL, Verstappen SMM. Biologic therapies in pregnancy: the story so far. *Rheumatology.* 2014;53:1377–1385.

prophylactic heparin and low-dose aspirin should be offered during pregnancy for up to 6 weeks' postpartum ([American College of Obstetrics and Gynecology, 2012](#)).

Neonatal Lupus

Anti-Ro and anti-La antibodies can be present in asymptomatic women. The former are more often seen in women with Sjögren syndrome but can be also found in women with other autoimmune disorders ([Mecacci et al., 2007](#)). These antibodies cross the placenta

and have been associated with congenital heart block. In the absence of a prior infant with congenital heart block, the prospective fetal risk is low, at 2%. However, in patients with a prior history of an affected fetus or neonate, the recurrence risk is 15%–20% ([Izmirly et al., 2010](#)). The morbidity and mortality of these infants are significant, the latter ranging between 10% and 29% ([Saxena et al., 2014](#)). Other consequences include potential need for a pacemaker or cardiac transplantation.

The injury to the atrioventricular node most often occurs between 16 and 24 weeks, and there is the potential for fibrosis that can

extend to the endocardium and myocardium. No prenatal interventions have been shown to reverse complete heart block (Eliasson et al., 2011). However, there may be some improvement in outcome with the use of fluorinated steroids if second-degree heart block is detected. There are potential fetal consequences of maternal steroids, including adrenal insufficiency, as well as neurodevelopmental and growth abnormalities (Saxena et al., 2014). It is suggested that women exhibiting these antibodies undergo fetal echocardiography beginning in the second trimester (16–18 weeks) and repeated every 1–2 weeks until 28 weeks (Donofrio et al., 2014). In those with a prior child affected with congenital heart block, use of hydroxychloroquine may be associated with a reduced risk of recurrence (Izmirly et al., 2012).

Immune Thrombocytopenia

Approximately 7%–10% of pregnancies are complicated by thrombocytopenia (Meyers, 2012). The most common etiology is gestational thrombocytopenia (75%), followed by preeclampsia (15–20%) and immune thrombocytopenia (ITP) (3%). By definition, ITP is immune mediated, and the responsible antibody may cross the placenta. The incidence is approximately 1/1000 pregnancies (Gill and Kelton, 2000). It is associated with an increased risk of neonatal thrombocytopenia, with 15%–50% of newborns having platelets less than $100 \times 10^9/L$, 8%–30% less than $50 \times 10^9/L$, and 1%–5% less than $20 \times 10^9/L$ (Meyers, 2012). Unfortunately, there is no reliable variable that accurately predicts the potential for neonatal thrombocytopenia; hence a number of interventions had been proposed in the past to reduce the risk of spontaneous fetal or neonatal bleeding. Strategies such as performing routine cesarean section, fetal scalp platelet assessment in labor, or percutaneous fetal scalp platelet counts were not found to be helpful and were potentially associated with increased maternal or fetal morbidity. Therefore it is recommended that delivery mode should not be altered for the diagnosis of ITP (Payne et al., 1997).

Maternal management for this condition in the first and second trimesters of pregnancy is essentially unchanged when compared with a nonpregnant patient (Piatek et al., 2015). If the maternal platelet count is greater than $30,000/\mu L$, no treatment is necessary. Below this threshold, or if there is evidence of spontaneous bleeding, corticosteroids and intravenous immunoglobulin are usually first-line therapies. Both are relatively safe in pregnancy and usually associated with a good maternal response. Refractory cases are much more difficult to manage. Options of large-dose anti-D antibodies have been tried. Splenectomy is usually a last resort but can be performed in the second trimester or at the time of cesarean section (Piatek et al., 2015).

Gestational thrombocytopenia (GTP) is generally a benign condition that sometimes can be difficult to delineate from ITP. However, the salient features include a lack of prior maternal history of severe thrombocytopenia and a maternal platelet count that is usually above $80,000/\mu L$. Other potential causes for a low platelet count should be explored, including severe preeclampsia, autoimmune disease, medication usage, and viral illness. True GTP has no associated risk of neonatal thrombocytopenia. The one potential consequence of GTP may be the reluctance of some anesthesiologists to place a regional anesthetic if the platelet count is too low.

Heart Disease

Heart disease has now become a leading cause of maternal mortality, with cardiomyopathy and acquired cardiovascular diseases

contributing to 26% of maternal deaths in the United States (McGregor et al., 2015). This increase may be due to the more rigorous evaluations of maternal mortality cases than were previously performed, as well as the older, aging pregnant population. In 1970, approximately 1% of first-time mothers were greater than 35 years old. Two decades later, this incidence had increased to 8%. Advances in age are often accompanied by comorbidities such as diabetes, hypertension, and obesity.

Hemodynamic alterations of pregnancy begin early in gestation. Maternal blood volume increases by 50%, with an accompanied decrease in systemic vascular resistance. The cardiac output increases by 30%–50% and heart rate by 15%. The labor process further increases cardiac output (Ouzounian and Elkayam, 2012; Lewey and Haythe, 2014). Furthermore, there are intra- and extravascular fluid shifts that occur after delivery. A previously compromised heart may not tolerate these changes. Attempts to stratify maternal risk have resulted in various scoring systems. The World Health Organization (WHO) classification defines risk based on cardiac condition. However, it is recognized that pregnancy-related risks may be additive: thus a “lower risk” WHO classification of 1 or 2 may be upgraded in the presence of cyanosis, low ejection fraction, or prior cardiovascular event. The highest risk (WHO 4) includes individuals with pulmonary hypertension, severe left ventricular dysfunction, previous peripartum cardiomyopathy with residual impairment of left ventricular dysfunction, left heart obstruction, or Marfan syndrome with a dilated aorta (Thorne et al., 2006). In these patients, pregnancy is not advisable given the high risk of maternal mortality.

Cardiomyopathy

Cardiomyopathy obviously can predate pregnancy, but a form of this condition is unique to gestation. Peripartum cardiomyopathy requires the onset to occur within 1 month prior to delivery or within 5 months after. It is estimated to occur in 1/3000 live births (Mielniczuk et al., 2006). The incidence is higher in African-American women, and other risk factors include hypertension, parity, and multiple gestations (Lewey and Haythe, 2014). The etiology is unknown but potentially mediated by inflammation, autoimmune processes, endothelial dysfunction, and oxidative stress (McGregor et al., 2015). Presentation may be indolent, as symptoms may mimic those commonly encountered in pregnancy. Furthermore, patients with preeclampsia can have significant left-ventricular dysfunction and pulmonary edema, which may confuse the picture, albeit more transiently.

The work-up includes an electrocardiogram. There is a high prevalence of abnormalities in peripartum cardiomyopathy. Echocardiography may reveal depressed left-ventricular function and dilation. Usually the ejection fraction is less than 45% (Lewey and Haythe, 2014). B-type natriuretic peptide levels are normally increased in pregnancy, but there can be a further increase as a consequence of left-ventricular end-diastolic pressure (McGregor et al., 2015). Treatment is supportive and similar to that of preexisting dilated cardiomyopathy. Complications include cardiogenic shock, arrhythmias, thromboembolism, and death. While 25%–50% of patients with peripartum cardiomyopathy may recover by 6 months, one quarter do not improve. The mortality may approach 15% (McGregor et al., 2015). There is an increased risk in subsequent pregnancies, particularly in those who have not had full recovery of reduced ejection fraction and heart failure (Elkayam et al., 2001).

Congenital Heart Disease

Congenital heart disease (CHD) affects approximately 1% of the population (Hoffman and Kaplan, 2002). In the last decade, there has been a significant increase in the prevalence of CHD complicating deliveries from 6.4–9 per 10,000 hospitalizations (Thompson et al., 2015). This is not surprising given the advances in surgical intervention that have improved long-term outcomes and numbers of patients reaching childbearing age. With the exception of hypertensive disorders and thromboembolic events, obstetric complications do not seem to be increased in these patients. However, cardiac complications are found in about 11% of patients and include arrhythmias, heart failure, and cardiovascular events (Drenthen et al., 2007). Myocardial infarction, cerebrovascular accidents, and mortality were noted in about 2% of patients. In those patients with more complex CHD, including Ebstein's anomaly, transposition of the great vessels, pulmonary atresia with ventricular septal defects, and Eisenmenger syndrome, the preterm delivery rate ranged between 22% and 65%. The offspring mortality is higher, probably caused by prematurity. The infants have a higher incidence of CHD, between 0.6% and 8%, depending on the maternal lesion (Drenthen et al., 2007).

Women with CHD should be evaluated before conception to advise them of the maternal and fetal risks of pregnancy. Many patients have limited or inaccurate knowledge of their disease. The history of the actual defect and subsequent surgical and other interventions should be obtained. Electrocardiography and echocardiography should be performed. The latter should focus on ventricular and valve functions, pulmonary artery pressure, and the presence of any prosthetic material. Since about 18% of patients have a defined chromosomal or Mendelian abnormality, formal genetic screening should be performed (Brickner, 2014).

Pregnancy management requires a multidisciplinary team, including cardiology, high-risk obstetrics, and anesthesiology. If necessary, cardiac catheterization or percutaneous interventions can be considered. Cardiopulmonary bypass can be performed during pregnancy, but there is an increased risk of prematurity and fetal death, particularly with urgent, high-risk surgery and maternal comorbidity (John et al., 2011). Medical control of arrhythmias, if necessary, is usually safe. However, use of β -blocker or calcium channel blocker may be associated with suboptimal fetal growth. In most cases, vaginal delivery is preferred. Cesarean section may be considered for women with severe aortic stenosis or pulmonary hypertension.

Coronary Artery Disease

Coronary artery disease is a significant cause of maternal mortality. In a recent survey, it may account for up to 20% of maternal cardiac deaths (CMAC, 2011). Acute myocardial infarction may complicate up to 6/100,000 pregnancies (James et al., 2006). In the UK data, all of the women who died had at least one risk factor for coronary artery disease. Unfortunately, in half of all women who died of ischemic heart disease, their care was deemed substandard (CMAC, 2011), possibly because of symptoms otherwise attributed to pregnancy or a reluctance to pursue a work-up. It appears that spontaneous coronary artery dissection is more common during pregnancy. Patients with ST segment elevation myocardial infarction should be treated similarly to those who are not pregnant. This includes percutaneous coronary intervention (Emmanuel and Thorne, 2015). If stenting is required, bare metal stents should preferably be used to minimize the need for long-term

dual antiplatelet therapy (Regitz-Zagrosek et al., 2011). Aspirin and clopidogrel appear to be safe in pregnancy. Longer-duration antiplatelet therapy is generally recommended if a drug-eluting stent is placed. Once clopidogrel is no longer deemed necessary, single-agent antiplatelet therapy with aspirin is appropriate. β -Blockers are relatively safe to use in pregnancy but carry a theoretical risk of suboptimal placental perfusion.

Renal Disease

Chronic kidney disease (CKD) is increasing in prevalence, affecting up to 3% women of childbearing age (Piccoli et al., 2010). It is estimated that 1/750 pregnancies is complicated by stages 3–5 CKD (Williams and Davison, 2008) (Table 11.5). Since there is no accurate ability to measure glomerular filtration rate in pregnancy, women with CKD have been arbitrarily classified into three categories based on the serum creatinine level: mild, less than 1.5 mg/dL; moderate, 1.5–2.5 mg/dL; and severe, greater than 2.5 mg/dL (Vellanki, 2013). Whether the newer staging of CKD is better than the latter classification in predicting pregnancy outcomes is unknown.

Pregnancy may influence the rate of decline of renal function. While it appears unlikely when the baseline creatinine is less than 1.5 mg/dL, patients with hypertension are more likely to develop progressive disease (Jungers et al., 1995). However, the same cannot be said for those with moderate impairment (creatinine >1.5 mg/dL). A significant risk of deterioration of maternal renal function can be seen in up to 40% of patients with progression to end-stage disease in 10% (Jones and Hayslett, 1996). The risk of irreversible deterioration of renal function is significant in those with marked impairment of renal function irrespective of the type of disease when accompanied by uncontrolled hypertension (Jungers et al., 1991).

When compared with women without CKD, those with kidney disease have a 52% increased odds of preterm delivery, 33% increased odds of cesarean section, 71% increased odds of neonatal intensive care unit (NICU) admission, and a twofold increased odds of low birth weight (Kendrick et al., 2015). The effect of mild-to-moderate CKD on pregnancy is dependent on the degree of renal impairment and the presence of underlying hypertension. In patients with mild renal dysfunction, successful outcomes are usually above 80% but with a complication rate of 26% (Lightstone,

TABLE
11.5

Stages of Chronic Kidney Disease

| Stage | Description | Glomerular Filtration Rate (mL/min ²) |
|-------|---|---|
| 1 | Kidney damage with normal or raised GFR | >90 |
| 2 | Kidney damage with mildly lower GFR | 60–89 |
| 3 | Moderately lower GFR | 30–59 |
| 4 | Severely low GFR | 15–29 |
| 5 | Kidney failure | <15 or dialysis |

GFR, Glomerular filtration rate.

From Vellanki K. Pregnancy in chronic kidney disease. *Adv Chronic Kidney Dis.* 2013;20: 223–229.

2011). However, there are increased rates of IUGR, preterm birth, and preeclampsia. In those with severe renal impairment, there are increased rates of infertility, miscarriage, and poor perinatal outcome. These pregnancies are complicated by growth restriction (65%), preterm birth (90%), preeclampsia (60%), and perinatal death (10%) (Williams and Davison, 2008).

Ideally, patients with CKD should be seen prior to pregnancy. Preconception counseling regarding maternal and perinatal outcomes is dependent on the patient's baseline renal function, degree of hypertension, and various comorbid conditions. For example, those with severe renal impairment may be advised to consult with a nephrologist to determine whether medication therapy or dialysis should be considered. Implementing therapies that may optimize her medical status in the non-gravid state is recommended to improve outcome. Treatment with antihypertensive medications should be adjusted if necessary to reduce fetal consequences, particularly with the use of angiotensin-converting enzyme inhibitors or receptor blockers that are known teratogens. If the degree of renal impairment is unknown, a 24-hour urine for creatinine clearance and total proteinuria should be performed. Furthermore, screening for active renal processes such as glomerulonephritis, lupus nephritis, reflux, and infection is advisable, as treatment may improve the degree of renal dysfunction. Low-dose aspirin should be considered in early pregnancy to potentially reduce the rate of preeclampsia.

Management of pregnancy includes initial office visits every other week and then weekly after 32 weeks, as well as serial ultrasounds to evaluate fetal growth. The urine should be evaluated every 4–6 weeks for infection, proteinuria, and hematuria. The blood pressure targets should ideally be between 120/70 mmHg and 140/90 mmHg, making adjustments of antihypertensive medications as necessary. Maternal creatinine and hemoglobin levels are also regularly assessed, the frequency dependent on the patient's baseline renal dysfunction (Williams and Davison, 2008).

Cancer

Principles

Cancer complicating pregnancy is rare, with an estimated frequency of 1 case per 1000 live births. The trend of delaying childbearing to later maternal age and the increased rates of cancer in general have increased the incidence over the past four decades (Salani et al., 2014). The sites or types of cancer in pregnancy, in descending order of frequency, are cervical, breast, ovarian, lymphoma, melanoma, brain, and leukemia (Table 11.6) (Haas, 1984; Jacob and Stringer, 1990). Pregnancy probably complicates the diagnosis, given the overlap of symptoms, compromised physical examination, hesitation to obtain testing, and restrictions of imaging and biochemical markers. All of these factors may contribute to a delay in diagnosis.

Finding a malignancy during gestation poses a unique set of issues that must be addressed with care. Will the pregnancy accelerate the malignant process? Are the accepted therapies appropriate for the mother, and are they safe for the unborn fetus? Will delay of therapy adversely affect the mother? Should the pregnancy be terminated, or should the child be delivered prematurely to maximize treatment of the mother with no resultant risk to the child?

Few conditions in pregnancy require as meticulous and multidisciplinary approach as cancer. Providers unaccustomed to managing pregnant women may be reticent recommending definitive cancer therapy, for fear of the surgical or teratogenic risks to the

TABLE 11.6

Cancers That Can Complicate Pregnancy

| Site/Type | Incidence (Per Number of Gestations) |
|------------|--------------------------------------|
| Cervix | 1:2000–1:10,000 |
| Breast | 1:3000–1:10,000 |
| Melanoma | 1:1000–1:10,000 |
| Ovary | 1:10,000–1:100,000 |
| Colorectal | 1:13,000 |
| Leukemia | 1:75,000–1:100,000 |
| Lymphoma | 1:1000–1:6000 |

Data from Pentheroudakis G, Pavlidis N, Castiglione M, ESMO Guidelines Working Group. Cancer, fertility and pregnancy: ESMO clinical recommendations for diagnosis, treatment and follow-up. *Ann Oncol* 2009;20(Suppl 4):S178–S181.

fetus. Therefore input regarding diagnosis and treatment must be acquired not only from an oncologist but also from the obstetrician, perinatologist, pediatrician, neonatologist, and dysmorphologist. The patient and her family must be involved in decision making. Information should include the risks of the disease and its potential therapies, as well as the limitations of current knowledge about cancer in pregnancy and any uncertainties of outcomes. Furthermore, depending on the gestational age at diagnosis, discussions regarding termination and potential iatrogenic prematurity are important.

In most cases, there should not be any delays in diagnostic procedures. As discussed previously, most imaging studies are relatively safe in pregnancy, but the exact modality can be modified to lessen the risk of fetal exposure, providing there is no reduction in an ability to make a diagnosis. Biopsies and indicated surgery should not be delayed for the sake of the pregnancy.

Chemotherapy

Administering agents that impair cell division in pregnancy is a concern for both the mother and the care team. Often the patient is more concerned about this issue than about the underlying cancer. Cytotoxic chemotherapy should be avoided in the first trimester because of the high incidence of spontaneous abortion and the potential teratogenic effects on the fetus. For a few agents with confirmed teratogenic effects (e.g., methotrexate, amethopterin, and chlorambucil), chemotherapy must be avoided during organogenesis. The use of folic acid antagonists in first-trimester cancer treatment raises the specific problem of possible induction of neural tube defects, because these lesions are known to be folate sensitive. For a list of the more common chemotherapeutic agents that have been used in pregnancy, refer to Table 11.7.

The literature regarding most chemotherapeutic agents is limited, consisting of a few collected series; therefore these agents should be used cautiously, with their potential harm to the fetus balanced against their benefit to the maternal condition. Little is also known regarding the long-term outcomes of fetuses exposed to chemotherapeutic agents in utero. The National Cancer Institute in Bethesda, Maryland, USA, maintains a registry in the hopes of determining the delayed effects. A small series of fetuses exposed to chemotherapeutic agents for acute leukemia revealed normal mental development with follow-up between 4 and 22 years (Aviles

TABLE 11.7 Common Chemotherapeutic Agents and Uses

| Class | Drug | Common Uses |
|--|----------------------------------|---|
| Alkylating Agents | | |
| Bind covalently to DNA, causing cross-links and strand breaks | Busulfan | Leukemias |
| | Chlorambucil | Lymphomas, leukemias |
| | Cyclophosphamide | Breast, ovary, lymphomas, leukemias |
| | Melphalan | Ovary, leukemia, myeloma |
| | Procarbazine | Lymphomas |
| Antimetabolites | | |
| Act as a false substrate during DNA and RNA synthesis, resulting in truncated proteins | 5-Fluorouracil | Breast, gastrointestinal |
| | 6-Mercaptopurine | Leukemias |
| | Methotrexate | Trophoblastic disease, lymphomas, leukemias, breast |
| | 6-Thioguanine | Leukemias |
| Antibiotics | | |
| Bind with DNA, prevent RNA synthesis, generate highly reactive free radicals | Bleomycin | Cervix, lymphomas |
| | Daunorubicin | Leukemias |
| | Doxorubicin | Leukemias, lymphomas, breast |
| Antimitotic Agents | | |
| Stop mitosis in M phase of cell cycle | Vincristine | Leukemias, lymphomas |
| | Vinblastine | Breast, lymphomas, choriocarcinomas |
| Taxanes | | |
| Inhibit disassembly of microtubules | Tamoxifen | Breast, lymphomas, choriocarcinoma |
| | Paclitaxel | Breast, ovarian |
| Platinum Compounds | | |
| Bind to DNA, causing cross-links and apoptosis | Cisplatin | Ovary, cervix, sarcoma |
| Other | | |
| | L-Asparaginase | Leukemias |
| | Hydroxyurea | Leukemias |
| | All- <i>trans</i> -retinoic acid | Leukemias |

Data from Neoplastic diseases. In Cunningham FG, MacDonald PC, Gant NF, et al., eds. *Williams Obstetrics*, ed. 20, Stamford, CT: Appleton and Lange; 1997; Ngu S, Ngan HY. Chemotherapy in pregnancy, *Best Pract Res Clin Obstet Gynaecol*. 2016;33:86–101.

and Niz, 1988). A recent case-control study involving 129 children, 96 of whom were exposed to chemotherapy in utero, suggested that maternal cancer with or without treatment did not impair cognitive, cardiac, or general development (Amant et al., 2015).

The risk of teratogenicity does not appear to be higher with combination chemotherapy than with single-agent therapy (Selig et al., 2012). Studies performed thus far involve small numbers of patients, with power insufficient to show a statistic difference, but there seems not to be a trend. There is an increased rate of small for gestational age babies and premature labor in mothers receiving cytotoxic chemotherapy (Van Calsteren et al., 2010). Theoretical consequences, such as bone marrow suppression, immune suppression, and anemia, could occur in the fetus. As a result, the timing of chemotherapy should account for the anticipated date of delivery. Data regarding safety for breastfeeding the neonate of

a mother receiving cancer chemotherapy are limited. For this reason, the majority of agents are contraindicated in nursing mothers.

Radiation Therapy

The deleterious effects of irradiation on the fetus have been discussed previously. Adjuvant radiation during pregnancy should be avoided if possible. However, there are small case series of radiotherapy in pregnancy, primarily for lymphoma (Eyre et al., 2015). There are several considerations for a pregnant woman undergoing radiation therapy. First, the dose used in estimating risk should be the amount that the fetus actually receives. For example, axillary or neck irradiation for lymphoma involves a lower direct fetal exposure than direct pelvic irradiation for cervical cancer. Secondly, the magnitude of radiation scatter to the pelvis must be considered. External

shielding does not prevent internal reflection of the ion beam. Thirdly, the advancing size of the uterus actually increases the amount of radiation exposure of the fetus, because of the closer proximity of the nonpelvic irradiation. Therefore an 8-week-old fetus may actually receive a smaller radiation dose from supraclavicular irradiation than a 30-week-old fetus. Fourthly, will the fetus concentrate the radiation and therefore increase its delivered dose? This is exemplified by the use of radioactive iodine (^{131}I) for maternal thyroid conditions. The actual rad dose is markedly higher in the fetus, because the fetal thyroid concentrates the iodine.

Cervical Cancer

Cervical cancer is one of the more common gynecologic malignancies in pregnancy. The incidence of cervical intraepithelial neoplasia is approximately 130 per 100,000 gestations and of invasive disease, 3.3 per 100,000 gestations (Al-Halal et al., 2013). A Papanicolaou test smear should be performed for all patients at their first prenatal visit and subsequent evaluation for an abnormal result should not be altered because of pregnancy. Colposcopy with cervical biopsy remains the mainstay of diagnosis. The greater vascularity of the cervix during pregnancy predisposes bleeding. An experienced colposcopist may be able to defer actual biopsy in cases of possible visual findings of a noninvasive process. However, if cancerous invasion is suspected, or if the physician is uncertain of the visual findings, biopsy is necessary. If microinvasive disease is confirmed by biopsy, cone biopsy is required to rule out frankly invasive disease. This procedure is undertaken with caution during pregnancy, because of the associated high rate of bleeding complications and miscarriage. Cervical conization may raise the risk of incompetence or preterm labor. The assistance of a gynecologic oncologist is preferred, given these unique sets of potential consequences. A shallow cone biopsy will reduce the risk of subsequent cervical weakness.

The therapy for invasive cervical cancer is based on the disease stage and gestational age as well as the patient's decision regarding pregnancy continuation and future fertility. Therapy can involve external beam radiation, internal radiotherapy (brachytherapy), or surgery. In most cases, delay of definitive therapy by 4–14 weeks may be acceptable. Pregnancy does not seem to accelerate the growth of the tumor. However, patient counseling is important. In the extremely previsible gestation, the likelihood of achieving a safe gestational age for the fetus without worsening the stage or spread of the cancer in the mother must be balanced against parental desires based on ethical or religious beliefs. Conversely, it might be reasonable to delay definitive therapy until a time when delivery would not likely result in a long-term disability because of extreme prematurity.

Breast Cancer

Breast cancer is the most common malignancy of women, with approximately 1 in 8 women affected in their lifetimes (Goldman and O'Hair, 2009): 0.2%–3.8% of cases are diagnosed during pregnancy (Vinatier et al., 2009). The incidence of breast cancer during pregnancy or within a year of delivery is 1.3 in 10,000 live births or 10–30 per 100,000 pregnancies (Isaacs, 1995; Smith et al., 2001a). Pregnancy does not seem to influence the actual course of the disease; however, there appears to be a higher risk of delay in diagnosis and a trend toward more advanced stages at diagnosis in pregnant, compared with nonpregnant, women.

The diagnostic procedures for breast cancer should not be altered during pregnancy. Any suspicious mass should undergo biopsy.

Mammography, although discouraged for routine screening in pregnancy, can be used safely if indicated. The fetal radiation exposure is negligible – approximately 0.001–0.01 mGy (Langer et al., 2015). Mammography may be more difficult to interpret due to the physiologic changes of pregnancy, and ultrasound examination may be a useful alternative. Metastatic evaluation may also be limited because of a reluctance to utilize bone and liver scans during pregnancy. MRI can be used safely in the second and third trimesters.

Surgical therapy for breast cancer should not be delayed because of pregnancy. The risks of mastectomy and axillary node dissection appear to be low (Isaacs, 1995). Radiation therapy is usually not recommended during pregnancy because of the risk of beam scatter to the pregnant uterus. If the pregnancy is to continue and the patient has evidence of tumor invasion in the lymph nodes, adjuvant chemotherapy is often given. The timing of delivery should account for the following factors:

- When would the fetus have a reasonable chance for survival with a low risk of severe permanent morbidity?
- Can the number of cycles of chemotherapy be minimized with an earlier delivery? In addition, avoiding delivery just before or just after administration of chemotherapy is important to reduce the risk of immunosuppression and infection.
- How long can radiotherapy be delayed without increasing the risk of metastatic spread of the tumor?

Approximately 10% of women treated for breast cancer become pregnant, the majority within 5 years of diagnosis. Data from small series suggest that pregnancy does not influence the rate of recurrences or of distal metastasis (Dow et al., 1994). While ideal timing of pregnancy after treatment for breast cancer is controversial, it seems reasonable to delay childbearing for at least 1–2 years, which is the time of the highest rate of recurrence (Landa et al., 2015). Breastfeeding may be possible in women who have undergone conservative breast cancer surgery.

Ovarian Cancer

Most ovarian cancer occurs in women older than 35 years. Delayed childbearing has been more widely accepted, exemplified by British birth rates doubling in women older than 30 years and tripling in women older than 40 years since 1975 (Palmer et al., 2009), in addition to a twofold increased birth rate among US women older than 40 years since 1981 (Martin et al., 2009). It would not be surprising for the rate of ovarian and other cancers during pregnancy to increase. However, the current estimate of actual ovarian malignancies in pregnancy is low and estimated to range from 1 in 10,000 to 1 in 50,000 deliveries (Palmer et al., 2009). Whereas most ovarian cancers are epithelial in origin, borderline epithelial and germ cell tumors (dysgerminomas and malignant teratomas) are more common in pregnancy.

The widespread use of ultrasonography, particularly in the first two trimesters, has been helpful in identifying adnexal masses. Fortunately, most are functional cysts (13%–17%) or benign teratomas, serous or mucinous cystadenomas, endometriomas, or paraovarian cysts (Hoover and Jenkins, 2011). Actual malignancy is rare and is estimated at 5% of the ovarian masses found. The risk is higher in nonpregnant females, approaching 15%–20%. Surgery for a suspected ovarian mass occurs in approximately 1 per 1000 pregnancies. Most procedures are performed not for suspected malignancy but because of concern about torsion and rupture. The incidence of adnexal torsion ranges from 1%–50%,

but more recent data suggest rates between 7% and 22% (Goh et al., 2014), and there appears to be a trend with increasing rates in masses greater than 6 cm (Yen et al., 2009). The maximal times of risk of these events are at the end of the first trimester, when the uterus elevates beyond the true pelvis, and at the time of delivery.

The characterization of an ovarian process can be aided by ultrasonography or MRI, but these modalities are not definitive. Ultrasound scoring systems that use size and character poorly predict malignancy but have a better negative predictive value (Lerner et al., 1994). Although an ovarian cyst, particularly if it is simple in nature, is probably not malignant, the patient must be cautioned that histologic diagnosis is more definitive. Indications for surgical exploration include a complex mass, a persistent simple cyst greater than 8 cm, or one that is symptomatic (Leiserowitz, 2006). The optimal time for laparotomy is during the second trimester. At that time, there is minimal interference from the gravid uterus and less risk of fetal loss, and the theoretical concerns of teratogenic exposure to anesthetic agents are avoided. Some patients opt for more conservative management; they should be counseled that they have a potential risk of requiring emergent surgery for an acute event such as torsion or rupture.

If a malignancy is confirmed at the time of laparotomy, treatment and staging are no different than for a nonpregnant woman. Frozen-section diagnosis, peritoneal washings, omentectomy, and sub-diaphragmatic biopsy are performed. Depending on the cell type and the stage, treatment can range from removal of the affected adnexa to complete hysterectomy and bilateral oophorectomy. Chemotherapy may be given during pregnancy if necessary. Fortunately, most epithelial ovarian cancers found in pregnant women are usually of a lower stage, with 59% of reported cases being stage I (Palmer et al., 2009).

Survivors of Childhood Cancer

Given the improvements of therapy for childhood cancer, a large number of these individuals have survived into adulthood. Some are unable to conceive because of high-dose radiation or cytotoxic chemotherapy. The risk of decreased fertility for patients exposed to pelvic radiation therapy may as high as 32% (Georgesescu et al., 2008). Those who remain fertile may have concerns regarding whether their treatment increases the risk of adverse pregnancy outcomes. Although data are limited, female cancer survivors treated with radiation therapy appear to have increased risks of premature delivery, low birth weight, and miscarriage. There is no evidence that female partners of male cancer survivors treated with radiation have these excess risks (Reulen et al., 2009).

Maternal Seizure Disorders

Epilepsy is the most common major neurologic disorder in pregnancy. Approximately 18 million women are affected worldwide, and 40% of those are of childbearing age. The estimated prevalence in pregnancy is 0.2%–0.7% (Chen et al., 2009). The pattern of maternal seizures ranges from complex partial to generalized tonic-clonic (grand mal) and generalized absence (petit mal) seizures. Physiologically, seizures arise from paroxysmal episodes of abnormal brain electrical discharges; when associated with motor activity, they are termed convulsive.

The effect of pregnancy on the frequency and severity of the seizure disorder has been difficult to ascertain because of limited prospective data. EURAP, an international registry of Antiepileptic Drugs and Pregnancy, recently reported on more than 1800 patients

whose seizure frequency and treatment were recorded. Fifty-eight percent of patients had no seizures during their pregnancy. When using first-trimester seizure activity as a reference, 64% had no change in frequency in the second and third trimester, 6% improved, and 12% deteriorated (EURAP Study Group, 2006). The only exception was that tonic-clonic seizures occurred more frequently in women using oxcarbazepine monotherapy; this has been confirmed by an Australian registry in which seizures occurred in 50% of pregnant women with epilepsy who were receiving therapy. However, in a subset that had no seizures for 12 months before pregnancy, there was a 50%–70% reduced frequency during gestation (Vajda et al., 2008). In patients in whom higher numbers of seizures occur during gestation, decreased plasma concentrations of antiepileptic medications have been hypothesized as causative. The fall in plasma drug levels during pregnancy may be due in part to increased protein binding, reduced absorption, and increased drug clearance. The adequacy of prepregnancy seizure control can influence a patient's course during gestation. Patients whose seizures were poorly controlled tended to have more frequent seizures during pregnancy, whereas patients who had no seizures for 2 years before pregnancy had only a 10% chance of experiencing seizures during gestation. These latter patients may be candidates for stopping therapy or considering monotherapy if they have previously required multiple antiepileptic drugs (Schmidt et al., 1983; Walker et al., 2009).

Perinatal Risk

For reasons that are not clear, women with seizures have more obstetric complications and a higher rate of poor perinatal outcomes. A recent systematic review comparing women with epilepsy to those without revealed increased odds of spontaneous miscarriage, antepartum and postpartum hemorrhage, hypertensive disorders, labor induction, cesarean section, preterm birth, and fetal growth restriction (Viale et al., 2015).

Earlier publications suggested an increased risk of congenital malformations in children of mothers with epilepsy even without prenatal use of antiepileptic drugs. More recent data appear to refute this. A study comparing patients with epilepsy to matched controls revealed that women receiving no medication had no increased rate of congenital malformations; however, monotherapy was associated with an increased risk of embryopathy (odds ratio [OR] 2.8). Furthermore, the frequency was even higher with use of two or more drugs (OR 4.2) (Holmes et al., 2001). A metaanalysis revealed the OR of malformations in those with untreated epilepsy was similar to nonepileptic controls (OR 1.92, 95% confidence interval [CI] 0.92–4) but a higher prevalence of a major congenital malformation in those exposed to antiepileptic medications (OR 3.26, 95% CI 2.15–4.93) (Fried et al., 2004). The malformation risk correlates with the number of medications used and is dose dependent in those receiving monotherapy (Tomson et al., 2011). Recent updates from five international registries have reported malformation rates ranging from 3.7%–8.0% (with monotherapy) and 6%–9.8% (with polytherapy) (Meador et al., 2008). Specific malformations associated with antiepileptic medications include a fivefold rise in the rate of orofacial clefts (Friis et al., 1986), an increase in the rate of congenital heart disease, particularly with trimethadione (Friis and Hauge, 1985), and a 3.8% incidence of neural tube defects in fetuses exposed to valproic acid (Samrén et al., 1997). Facial abnormalities (e.g., midface hypoplasia) are not specific to any particular antiepileptic drug; they have been seen with phenytoin, carbamazepine, and trimethadione. Some antiepileptic medications can adversely affect postnatal cognitive

TABLE 11.8 Clinical Features of the Fetal Hydantoin Syndrome

| | |
|----------------------------|----------------------------------|
| Craniofacial abnormalities | Broad nasal ridge |
| | Wide fontanel |
| | Low-set hairline |
| | Broad alveolar ridge |
| | Metopic ridging |
| | Short neck |
| | Ocular hypertelorism |
| | Microcephaly |
| | Cleft lip with or without palate |
| | Abnormal or low-set ears |
| | Epicanthal folds |
| | Ptosis of eyelids |
| | Coloboma |
| | Coarse scalp hair |
| Limb abnormalities | Smallness or absence of nails |
| | Hypoplasia of distal phalanges |
| | Altered palmar crease |
| | Digital thumb |
| | Dislocated hip |

Data from Briggs GC, Freeman RK, Yaffe SJ. *Drugs in Pregnancy and Lactation*, ed.7, Baltimore: Lippincott Williams & Wilkins; 2005.

development. Although conclusive data are lacking, there may be an increased adverse effect, particularly with valproate (Meador et al., 2008; Tomson and Battino, 2009).

Fetal Hydantoin Syndrome

The classic features of the fetal hydantoin syndrome are facial clefting, a broad nasal ridge, hypertelorism, epicanthal folds, distal phalangeal hypoplasia, and growth and mental deficiencies; however, these effects also result from the use of other antiseizure medications (Table 11.8). The postulated cause of this syndrome is the teratogenic action of a common epoxide intermediate of these medications. The hydantoin syndrome was found to develop in fetuses with inadequate epoxide hydrolase activity (Buehler et al., 1990). This enzymatic deficiency appears to be recessively inherited. It appears that preconception folic acid supplementation can reduce the risk of major congenital malformations in women taking antiepileptic medication (Harden et al., 2009).

Management

Management of the pregnant patient with epilepsy is based on keeping her free of seizures. Theoretically, this goal reduces maternal physical risk and lowers the incidence of fetal complications. Preconception counseling is preferable and should entail (1) adjusting medication doses into the therapeutic range, (2) attempting to limit the patient to one drug if possible, and (3) choosing an agent with the least risk of teratogenesis. Frank discussion of the various risks of each agent should be conducted, particularly the risks associated with valproic acid and trimethadione. Usually if the patient's disease is adequately controlled with one agent, it rarely needs to be changed, because the risks of increasing seizure activity are believed to outweigh the potential for reducing congenital malformations.

Patients taking antiepileptic medications should also take folic acid supplements (800–1000 µg) before conception, because

inhibition of folate absorption has been proposed as a teratogenic mechanism, particularly with phenytoin. However, there is some controversy about the use of higher doses of folic acid, as there is some evidence of potential drug–drug interactions and animal evidence that high levels may have adverse effects on fetal brain development (Asadi-Pooya, 2015). Maternal serum screening for neural tube defects, fetal ultrasound, and echocardiography should be performed in the mid-trimester. During gestation, the anticonvulsant levels should be checked monthly, and the dose should be adjusted accordingly, particularly with the use of lamotrigine, carbamazepine, and phenytoin (Harden et al., 2009). Although the evidence is less clear with other agents such as phenobarbital, valproate, primidone, and ethosuximide, serial level assessment should not be discouraged. Medications should not be changed unless they prove ineffective at the optimal serum level. If a patient reports greater seizure activity, the serum drug level should be checked immediately. A common reason for increased seizures is that the patient is not taking her medication, usually because she fears its teratogenicity.

Mothers taking phenytoin, phenobarbital, or primidone may have a higher incidence of neonatal coagulopathy as a result of vitamin K-dependent clotting factor deficiency. Although maternal vitamin K supplementation in the third trimester may be reasonable, there is insufficient evidence to determine whether it will reduce neonatal hemorrhagic complications (Harden et al., 2009).

Mental Health Disorders

It is estimated that, annually, over 500,000 pregnancies are complicated by maternal mental health disorders (ACOG, 2008). If inadequately recognized or treated, patients may be at increased risk of poor nutrition; maladaptive behaviors such as smoking, drinking, and illicit drug use; poor compliance with medical care; and deficits of mother–child bonding. Furthermore, there appears to be an increased perinatal risk with mental disorders and pregnancy (Table 11.9). Women with preexisting mental illness have a higher recurrence risk in the puerperium. Patients with suspected mental illness should also be assessed for substance abuse and thyroid dysfunction. A multidisciplinary approach is advantageous. In rare cases, the patient's mental competency is an issue, which may require legal assistance to determine who may make medical decisions for the patient.

Depression

Depression ranks as the fourth leading cause of disability worldwide, and recognized prevalence appears to be increasing (Dossett, 2008). Rates of depression during pregnancy are 7%–11% in the first trimester, 9%–13% in the second trimester, and 9%–12% in the third trimester (Bennett et al., 2004; Gavin et al., 2005). The prevalence over the entire pregnancy course is 18.4% (Gavin et al., 2005). The obstetrician must be aware that life events such as miscarriage, infertility, and complicated pregnancy in patients with risk factors are likely to precipitate depression; therefore there should be a low threshold for diagnosis and treatment of mood alterations in such patients. Alternatively, perinatal loss experienced by a woman without predisposing risk factors will probably lead to a grief reaction or adjustment disorder, which may be misdiagnosed as depression.

Chronic medical conditions that are associated with a high prevalence of depression and may occur in women of childbearing age include renal failure, cancer, AIDS, and chronic fatigue or pain. Antihypertensives, hormones, anticonvulsants, steroids,

TABLE 11.9 Impact of Psychiatric Illness on Pregnancy Outcome

| Illness | Teratogenic Effects | IMPACT ON OUTCOME | | Treatment Options |
|-------------------|---|---|---|---|
| | | Obstetric | Neonatal | |
| Anxiety disorders | N/A | Increased incidence of forceps deliveries, prolonged labor, precipitate labor, fetal distress, preterm delivery, and spontaneous abortion | Decreased developmental scores and inadaptability; slowed mental development at 2 years of age | Benzodiazepines Antidepressants Psychotherapy |
| Major depression | N/A | Increased incidence of low birth weight, decreased fetal growth, and postnatal complication | Increased newborn cortisol and catecholamine levels, infant crying, rates of admission to NICUs | Antidepressants Psychotherapy ECT |
| Bipolar disorder | N/A | Increased incidence of low birth weight, decreased fetal growth, and postnatal complication | Increased newborn cortisol and catecholamine levels, infant crying, rates of admission to NICUs | Lithium Anticonvulsants Antipsychotics ECT |
| Schizophrenia | Congenital malformations, especially cardiovascular | Increased incidence of preterm delivery, low birth weight, small for gestational age, placental abnormalities, and placental hemorrhage | Increased rates of postnatal death | Antipsychotics |

ECT, Electroconvulsive therapy; N/A, not applicable; NICUs, neonatal intensive care units.

From ACOG Committee on Practice Bulletins—Obstetrics. ACOG Practice Bulletin. Clinical management guidelines for obstetrician-gynecologists number 92, April 2008 (replaces practice bulletin number 87, November 2007). Use of psychiatric medications during pregnancy and lactation, *Obstet Gynecol.* 2008;111:1001–1020.

chemotherapeutics, and antibiotics can cause depression. Alcoholism and substance abuse may manifest as depression. Underlying personality disorders complicate the diagnosis of depression by confusing the clinical presentation, in addition to contributing to the secondary effect of many physicians' avoidance of patients suffering from such disorders.

Supportive therapy alone is rarely effective for major depression. Therapeutic interventions include psychotherapy and medication. Electroconvulsive therapy (ECT) has been used in pregnancy, usually for refractory severe cases of psychotic depression, schizophrenia, and bipolar disease. There are some potential complications with ECT, including fetal bradycardia (2.7%), preterm contractions, or labor (3.5%), and potential complications of anesthesia including aspiration (Anderson et al., 2009).

Medication is by far the most common treatment modality. However, it is associated with concerns about fetal exposure, leading patients or providers to limit or discontinue medication use. Unfortunately those who discontinue antidepressants before pregnancy have a 68% risk of relapse as opposed to a 26% risk in those whose medication was maintained. In those who discontinued medication and suffered a relapse, 50% did so in the first trimester and 90% by the second trimester (Cohen et al., 2006). Another study found that among those who discontinued antidepressants, over half had to restart them in pregnancy (Roca et al., 2013).

The maternal risk of depression can be significant, including an increased risk of suicidal ideation (Einarson et al., 2001). Therefore careful assessment regarding the need for medication, counseling regarding the risks of medication discontinuation versus the potential concerns regarding the fetal impact of their use, and ongoing evaluation of patients during pregnancy are paramount.

In general, treatment of depression is effective in approximately 70% of cases. Most antidepressant medications currently prescribed during pregnancy are selective serotonin reuptake inhibitors (SSRIs), which have an advantage over the tricyclic antidepressants by not

causing orthostatic hypotension. While they are commonly used in pregnancy, complications of fetal exposure have not been fully established. SSRIs as a group have not been consistently associated with specific birth defects (Byatt et al., 2013). However, paroxetine has been associated with a small but increased risk of congenital heart defects (Källén and Otterblad Olausson, 2007; Grigoriadis et al., 2013). Although data remain inconsistent, current recommendations are to avoid paroxetine as a first-line antidepressant.

One concern regarding the use of SSRIs is the association with persistent pulmonary hypertension of the newborn (PPHN); however, the incidence remains low, at approximately 10 in 1000 fetuses exposed after 20 weeks' gestation (Chambers et al., 2006). This study was one of the first to link SSRIs to potential newborn harm. In five subsequent studies, two studies have found a small but statistically increased risk, but three studies did not (Robinson, 2015). In reviewing the potential relationship between SSRI and PPHN, there are a number of risk factors for this disorder that are more commonly seen in depressed women, including obesity, smoking, cesarean section, and early delivery (Occhiogrosso et al., 2012). Other controversies regarding SSRIs and their link to prematurity and low birth weight when systematically evaluated suggest that the medication is not the most likely cause but, rather, the underlying depression.

Approximately 30% of newborns exposed to SSRIs prior to birth develop poor neonatal adaptation syndrome (PNAS) (Weiskopf et al., 2015). This is usually transient and most often does not require specific medical care. PNAS affects the central nervous system, motor function, and the gastrointestinal and respiratory systems. NICU admissions are more common (Lund et al., 2009). Reduction of medication dose in the latter third trimester in the hope of reducing adverse neonatal outcomes does not appear to be beneficial (Warburton et al., 2010). Long-term outcome data suggest that maternal antidepressants do not cause autism or affect the neurodevelopment of offspring (Robinson, 2015). Therefore given the lack of consistent data suggesting significant fetal harm,

SSRIs are currently considered the first-line antidepressant for use in pregnancy (Weisskopf et al., 2015). Furthermore, reducing the dose or discontinuing SSRIs in the latter part of pregnancy does not appear to improve neonatal outcome.

There have been concerns regarding the use of SSRIs during breastfeeding. Fluoxetine has an active metabolite with a long half-life and is found in higher concentrations in infants (Eberhard-Gran et al., 2006). Short-term neonatal effects have been reported, including increased crying, decreased sleep, and irritability, particularly with fluoxetine and citalopram. However, the relative infant dose ingested by the child is less than 10% of the maternal dose, and this level is lower than the fetal exposure (Weisskopf et al., 2015). In some cases such as prematurity, where there is immaturity of drug elimination pathways, there may be potential for “toxicity” (irritability, agitation, sleep disturbances, etc.). However, this is usually reversible with breastfeeding discontinuation. There are clear benefits of breastfeeding that must be accounted for in decisions regarding those needing SSRIs, particularly at the highest time of maternal risk. The current available data do not support a contraindication for lactation (Weisskopf et al., 2015).

Postpartum Psychosis

A severe disorder, postpartum psychosis, is fortunately rare, occurring in 1–4 per 1000 births (Weissman and Olfson, 1995). This condition is more worrisome than postpartum depression, because of the patient’s inability to discern reality from the periods of delirium. Patients at risk for postpartum psychosis may have underlying depression, mania, or schizophrenia. Other risks are younger age and family history. The recurrence rate is approximately 25%. The peak onset of symptoms is between 10 and 14 days after delivery. Recognition of this disorder is extremely important to the protection of the patient and her family.

Schizophrenia

The prevalence of schizophrenia is approximately 1% in the general population (Myers et al., 1984); it is associated with delusions, hallucinations, and incoherence. Morbidity due to this mental illness is higher than that due to any other. There appears to be a genetic component to the etiology; schizophrenia develops in approximately 10% of offspring of an affected person. Concordance of schizophrenia in identical twins reaches 65%. There is some speculation and controversy as to whether low birth weight (Smith et al., 2001b) and obstetric complications (Kendell et al., 2000) are associated with a higher rate of schizophrenia.

Because the peak age of incidence is approximately 20 years and women are affected more often than men, it is unrealistic to assume that obstetricians will never encounter patients with schizophrenia. There appear to be higher rates of cesarean section and surgical vaginal delivery in affected patients (Bennedsen et al., 2001a). Offspring of women with schizophrenia may have a higher rate of sudden infant death syndrome and congenital malformations (Bennedsen et al., 2001b). However, it is difficult to ascertain whether these risks are independent of other factors such as smoking, poor socioeconomic status, and use of certain medications.

Treatment is achieved primarily through the use of psychotropic medication. The potential for teratogenesis appears low with the older-generation medications in the phenothiazine class, but most data for this issue were derived from the use of lower doses given to patients with hyperemesis gravidarum. Antipsychotic medication does cross the placenta. Current recommendations include avoiding use in the first trimester if possible, the use of lower doses or higher-potency alternatives, and cessation of medication 5–10 days before delivery (Herz et al., 2000). The use of most antipsychotics in breastfeeding is associated with an unknown risk (Briggs et al., 2005) (Table 11.10).

TABLE 11.10 Summary of Current Knowledge of Drug Excretion Into Breast Milk, Drug Concentrations in Infant Serum, Adverse Effects in the Child, and Breastfeeding Recommendations for Different Psychotropic Drugs

| Class or Drug | Drug Transfer Into Breast Milk | Infant Plasma Concentrations | Adverse Effects in the Child | Breastfeeding Recommendations |
|---------------------------------|--------------------------------|---|---|--|
| SSRIs | Low transfer | Low plasma concentrations | Case reports of adverse effects in infants exposed to fluoxetine and citalopram | Compatible with breastfeeding; however, fluoxetine and citalopram may not be drugs of first choice |
| TCAs | Low transfer | Low plasma concentrations (except doxepin) | No suspected immediate adverse effects observed (except doxepin) | Compatible with breastfeeding; however, doxepin should be avoided |
| Other antidepressants | Limited data | Limited data | Limited data | Insufficient data |
| Benzodiazepines | Low transfer | High plasma concentrations with longer-acting drugs with active metabolites | Case reports of CNS depression reported for diazepam | Sporadic use of short-acting benzodiazepines unlikely to cause adverse effects |
| Lithium | Low transfer | Dose received by the infant is high | Limited data; some reports of toxicity in the infant | Limited data; however, breastfeeding should be avoided |
| Carbamazepine, sodium valproate | Low transfer | Low plasma concentrations | Some case reports of various adverse effects in the infant | Generally more compatible with breastfeeding than lithium |
| Lamotrigine | High transfer | High plasma concentrations | Limited data | None able to be made |
| Novel antipsychotics | Moderate transfer | Variable plasma concentrations | Limited data | None able to be made |

CNS, Central nervous system; SSRIs, selective serotonin reuptake inhibitors; TCAs, tricyclic antidepressants.

Modified from Eberhard-Gran M, Eskild A, Opjordsmoen S. Use of psychotropic medications in treating mood disorders during lactation: practical recommendations. *CNS Drugs*. 2006;20:187–198.

Lithium, used primarily in mania, is associated with a higher rate of Ebstein anomaly. Although the incidence of this consequence is low, either discontinuing the medication in the first trimester or continuing its use with careful counseling is a viable alternative. Fetal echocardiography should be performed in women who have used lithium in early pregnancy.

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12

Hypertensive Complications of Pregnancy

THOMAS R. MOORE

KEY POINTS

- Hypertensive disorders of pregnancy are classified as chronic hypertension, gestational hypertension, preeclampsia (PE) with and without severe features, and chronic hypertension with superimposed PE.
- Pharmacologic management of chronic hypertension should be reserved for women with sustained elevations in blood pressure at or above 160 mmHg systolic or 110 mmHg diastolic.
- Magnesium sulfate administration to prevent eclampsia is used for those PE cases with severe features.
- Delivery in cases of PE without severe features should be accomplished at or beyond 37 completed gestational weeks. When severe features are present, delivery should be accomplished expeditiously (regardless of gestational age) once maternal stabilization has been achieved.

Hypertension is the most common medical problem in pregnancy, affecting 10%–15% of all pregnant women. As the third most common cause of maternal mortality after thromboembolic disease (15%) and hemorrhage (11%), hypertensive disorders account for almost 10% of maternal deaths in the United States (Creanga et al., 2015). Complications arising from hypertensive disorders have profound effects on the fetus and neonate and thus are a major source of perinatal mortality and morbidity. Preeclampsia (PE) is a leading cause of maternal and perinatal morbidity and mortality, with an estimated 50,000–60,000 PE-related deaths per year worldwide.

Unfortunately the incidence of PE has increased by 25% in the United States during the past two decades (Shih et al., 2016). Furthermore, since PE has been shown to be a risk factor for future cardiovascular and metabolic disease in women, the importance of proper perinatal management and, ideally, prevention has never been more important (Behrens et al., 2016).

Classification of Hypertensive Disorders of Pregnancy

The diagnostic criteria for the hypertensive complications of pregnancy have undergone significant revision in the past several

years (ACOG, 2002; Lindheimer et al., 2010; WHO, 2011; Magee et al., 2014). In 2013, the American College of Obstetricians and Gynecologists (ACOG) convened a Task Force to summarize the current state of knowledge about PE and other hypertensive disorders in pregnancy and to translate this information into practice guidelines (ACOG, 2013). Hypertension during pregnancy was defined by four categories (Table 12.1):

1. Chronic hypertension predating pregnancy
2. Gestational hypertension (GH) occurring after 12 weeks' pregnancy
3. Preeclampsia–eclampsia
4. Chronic hypertension with superimposed PE

Importantly, proteinuria is no longer required for the diagnosis of PE if other abnormal findings are present. PE is now defined as hypertension plus proteinuria *or* hypertension plus other systemic abnormalities:

Hypertension in pregnancy is defined when blood pressure is:

- Greater than 140 mmHg systolic or greater than or equal to 90 mmHg diastolic on two occasions at least 4 hours apart after 20 weeks' gestation in a woman with a previously normal blood pressure. The requirement for documenting hypertension twice over an interval of at least 4 hours is shortened to minutes if systolic pressure is greater than or equal to 160 mmHg or diastolic pressure is greater than equal to 110 mmHg.

Proteinuria is defined when urine studies show:

- Greater than or equal to 300 mg per 24-hour urine collection *or*
- Protein/creatinine ratio greater than or equal to 0.3 *or*
- Dipstick reading of 1+ in a voided urine specimen if more precise laboratory-based results are not readily available

In the presence of hypertension but absent proteinuria, PE is diagnosed when any of the following are present:

- Platelet count less than 100,000/ μ L.
- Elevated liver transaminase levels to twice normal values
- Newly elevated serum creatinine greater than 1.1 mg/dL or a doubling in the absence of other renal disease
- Pulmonary edema *or*
- New-onset cerebral or visual disturbances

Since fetal growth restriction is managed similarly in pregnant women with and without PE, it has been deleted as a criterion for severe PE.

TABLE 12.1 Classification of Hypertensive Disorders of Pregnancy

| Category | Definition |
|---|---|
| Chronic hypertension | Hypertension present before pregnancy or diagnosed before 20 weeks' gestation. Hypertension is defined as either systolic BP of ≥ 140 mmHg or diastolic BP of ≥ 90 mmHg or both. Diagnosis optimally should be based on two observations at least 4 hours apart, although with severe BP elevations shorter intervals may suffice. |
| Gestational hypertension | New-onset hypertension after 20 weeks' gestation in the absence of proteinuria that normalizes postpartum Change diagnosis to chronic hypertension if blood pressure elevation does not resolve after delivery. |
| Preeclampsia–eclampsia | New-onset hypertension (usually after 20 weeks' gestation) with new-onset proteinuria (300 mg/24 hours or a protein/creatinine ratio of at least 0.3) or one or more of the following: <ul style="list-style-type: none"> – thrombocytopenia ($<100,000/\mu\text{L}$) – elevated liver transaminases (twice normal) – renal insufficiency (serum creatinine >1.1 mg/dL or doubling creatinine level without preexisting renal disease) – pulmonary edema – new-onset cerebral or visual disturbances |
| Chronic hypertension with superimposed preeclampsia | Preeclampsia occurring in a woman with hypertension predating pregnancy |

BP, Blood pressure.

Data from American College of Obstetricians and Gynecologists. Hypertension in Pregnancy. Washington, DC: American College of Obstetricians and Gynecologists; 2013.

Chronic Hypertension

Up to 5% of pregnant women have chronic hypertension, which is diagnosed when hypertension is present before pregnancy or recorded before 20 weeks' gestation. However, when hypertension is first noted in a patient after 20 weeks' gestation, it may be difficult to distinguish chronic hypertension from pregnancy-induced hypertension or PE. In such cases, the precise diagnosis might not be made until after delivery. Hypertension that is first diagnosed during the second half of pregnancy and persists postpartum is diagnosed as chronic hypertension.

Chronic hypertension has an adverse effect on pregnancy outcome. A recent summary of 55 studies of women with chronic hypertension, encompassing 795,221 pregnancies, indicated that women with chronic hypertension had pooled incidences of superimposed PE (25.9%), cesarean section (41.4%), preterm delivery at less than 37 weeks' gestation (28.1%), birth weight less than 2500 g (16.9%), neonatal unit admission (20.5%), and perinatal death (4.0%) (Bramham et al., 2014). The adverse effects on fetal

and maternal perinatal outcomes are related to the severity of the preexisting hypertension. When chronic hypertension is secondary to maternal chronic renal disease, a large (506,000 pregnancies) systematic review found significantly elevated odds of PE (odds ratio [OR] 10.36), premature delivery (OR 5.72), small for gestational age (OR 4.85), and pregnancy failure (OR 1.80) (Zhang et al., 2015). Women with untreated severe chronic hypertension are also at increased risk for cardiovascular complications during pregnancy, including stroke (Brown and Whitworth, 1999).

The majority of cases of chronic hypertension seen in pregnancy are idiopathic (essential hypertension), but causes of secondary hypertension should always be sought because pregnancy outcome is worse in women with secondary hypertension. Renal disease (e.g., chronic renal failure, glomerulonephritis, renal artery stenosis), cardiovascular causes (coarctation of the aorta, Takayasu arteritis), and, rarely, Cushing disease, Conn syndrome, and pheochromocytoma should be excluded through physical examination, history, and more detailed testing if needed.

All patients with chronic hypertension should be evaluated early in pregnancy with serum urea, creatinine, and electrolyte measurements, urinalysis, and 24-hour urine collection for protein and creatinine clearance determinations. Reassessment should be performed in each trimester and more frequently if the patient's condition deteriorates.

Antihypertensive Treatment of Chronic Hypertension in Pregnancy

Randomized trials have shown that antihypertensive treatment of chronic hypertension in pregnancy does not improve fetal outcome (ACOG, 2013), with rates of preterm delivery, abruption, intra-uterine growth restriction (IUGR), and perinatal death similar in treated and untreated women. Therefore treatment is usually reserved for patients whose hypertension places them at a significant risk of maternal stroke (systolic blood pressure of ≥ 160 mmHg or diastolic pressure of ≥ 110 mmHg). In patients with less severe hypertension who were taking medications before conception, discontinuing therapy with close surveillance is recommended. The risk of superimposed PE is not changed by antihypertensive therapy, so its development should be tracked carefully.

The choice of antihypertensive agent for use in pregnancy is governed by avoiding adverse effects on the fetus. Because excessive lowering of maternal blood pressure to less than 140 mmHg systolic or less than or equal to 90 mmHg diastolic (140/90 mmHg) can compromise uterine perfusion, with consequent slowing of fetal growth with ultimate reduction in fetal oxygenation, maternal pressures should be maintained in the 140–155 mmHg systolic and 90–105 mmHg diastolic range. The drugs most commonly used in pregnancy are listed in Table 12.2.

Labetalol is a mixed α_1 -adrenergic, β_1 -adrenergic, and β_2 -adrenergic blocker, and it is the most frequently used medication. Some pure β -blockers have been associated with a significant increase in the risk of IUGR (e.g., atenolol), and the mixed adrenergic blockade produced by labetalol is thought to mitigate this unwanted effect (Pickles et al., 1989). Labetalol is also used intravenously to manage severe hypertension accompanying PE.

Methyldopa, a centrally acting antihypertensive agent, does not impair uteroplacental perfusion and has a wide therapeutic margin. However, methyldopa has a rather slow onset of action, with prolonged time to therapeutic effect (days), and compliance with methyldopa therapy may be impeded by side effects such as sedation in some patients.

TABLE 12.2 Drugs Commonly Used to Treat Chronic Hypertension in Pregnancy and Their Modes of Action

| Drug | Mode of Action | Typical Dose |
|------------|---|--------------------------------------|
| Methyldopa | Centrally acting antihypertensive | 0.5–3 g/day in 2–3 divided doses |
| Labetalol | Mixed α -adrenergic and β -adrenergic blockers | 200–2400 mg/day in 2–3 divided doses |
| Nifedipine | Calcium channel blocker | 30–120 mg/day in slow-release form |

Adapted from American College of Obstetricians and Gynecologists; Task Force on Hypertension in Pregnancy. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. *Obstet Gynecol.* 2013;122:1122–1131.

Calcium channel blockers (e.g., nifedipine) have been used mainly as second-line drugs, usually in long-acting, extended-release forms. Calcium channel blockers appear to be as effective as methyldopa and labetalol with minimal fetal side effects (Levin et al., 1994).

Hydralazine, a potent peripheral vasodilator, is frequently used intravenously to treat acute hypertensive emergencies in pregnancy (blood pressure >160/110 mmHg). Its role as an oral agent in the management of chronic hypertension is limited to a second or third-line choice. Long-term use of hydralazine may be associated with a lupus-like syndrome in some patients.

Although diuretics are used extensively in nonpregnant adults with hypertension, there is little role for their use in women with chronic hypertension in pregnancy. Diuretics can potentially reduce or prevent the plasma volume expansion seen in normal pregnancy (Sibai et al., 1984), an effect that might impede fetal growth, although the evidence for this is mixed. Most authorities restrict the use of diuretics in pregnant patients to those with cardiac dysfunction or pulmonary edema.

Angiotensin-converting enzyme inhibitors and angiotensin receptor blockers should not be used during pregnancy. In the second and third trimesters, these agents are associated with malformation of the fetal calvarium, fetal renal failure, oligohydramnios, pulmonary hypoplasia, and fetal and neonatal death (Buttar, 1997). Angiotensin-converting enzyme inhibitors appear to be safe when taken in the first trimester (Steffensen et al., 1998), but a patient who conceives while taking an angiotensin receptor blocker or angiotensin-converting enzyme inhibitor should be switched to a safer alternative as soon as possible. Similar precautions apply to the use of angiotensin receptor blockers in pregnancy.

Antenatal Fetal Surveillance in Chronic Hypertension

As women with chronic hypertension are at increased risk of slowing of fetal growth and of superimposed PE, antenatal surveillance in women with chronic hypertension should include careful screening for signs of superimposed PE and serial ultrasonographic evaluations every 3–6 weeks. All patients should perform fetal movement counts from 28 weeks' gestation onward, and cases with slowing of fetal growth should be followed with twice-weekly nonstress tests with amniotic fluid index or a weekly ultrasound biophysical profile.

• BOX 12.1 Management of Pregnant Women With Chronic Hypertension

Monitoring

- Daily home BP monitoring
- Fetal growth sonography every 4 weeks
- Fetal biophysical testing at least weekly from 32–34 weeks

Avoid

- Low sodium diets
- Weight loss prescriptions
- Limitations of moderate exercise

Prophylaxis

If prior pregnancy had PE with severe features and delivery was <34 0/7 weeks, begin daily low-dose aspirin (81 mg) by 15 weeks' gestation.

Antihypertensive Medications

- Maintain systolic BP 120–160 mmHg; diastolic BP 80–105 mmHg
- Utilize labetalol, nifedipine, or methyldopa if antihypertensives needed
- Avoid ACE inhibitors, ARBs, renin inhibitors, and mineralocorticoid receptor antagonists.

Delivery

- At 37 0/7 weeks unless PE with severe features
- If <37 0/7 weeks, administer antenatal corticosteroids
- If PE with severe features
 - Soon after maternal stabilization if uncontrollable BP, eclampsia, pulmonary edema, abruptio placentae, nonreassuring fetal status
 - Administer antepartum and postpartum magnesium sulfate.

ACE, Angiotensin-converting enzyme; ARBs, angiotensin receptor blockers; BP, blood pressure; PE, preeclampsia.

If the sonographic estimated fetal weight tapers below the 10th percentile or abdominal circumference is a much smaller percentile than the head, more intensive fetal surveillance is indicated and IUGR is diagnosed. In such cases, sonography should be performed at 10-day to 21-day intervals with attention paid to amniotic fluid volume and trending of each biometric parameter. Serial monitoring of umbilical Doppler waveforms in IUGR has been shown to reduce perinatal mortality while not increasing labor induction or cesarean delivery (ACOG, 2013). Indications for delivery in the hypertensive patient with fetal IUGR include absence of growth of the head and abdomen over a 10-day interval, oligohydramnios, biophysical score of less than 6, or reversal of end-diastolic velocity on the umbilical Doppler waveform.

Women with renal impairment and chronic hypertension have a markedly higher risk of poor perinatal outcome than women without renal impairment. The incidence of impaired fetal growth is directly related to the degree of renal impairment, and women undergoing dialysis are at particular risk for fetal growth failure, preterm delivery, and fetal death, even with optimal management. Those who start dialysis during pregnancy are at the greatest risk, with only a 50% chance of a surviving infant (Hou, 1999).

Gestational Hypertension

Management of women with GH and PE is summarized in Box 12.1. GH is defined as new-onset hypertension occurring after 20 weeks' pregnancy in the absence of signs of PE. Because a woman with apparent GH at 36 weeks' gestation can rapidly evolve into

PE at 39 weeks' gestation, the diagnosis of GH should always evoke caution and vigilance. Only if the patient's blood pressure returns to normal postpartum, without development of signs of PE during the pregnancy, should the final diagnosis of GH be applied. During pregnancy, GH is indistinguishable from preeclampsia-in-evolution. Therefore all patients with GH should be regarded as being at risk for progression to PE.

The earlier that GH is evident, the greater the risk of PE. When the diagnosis is made before 30 weeks' gestation, more than one-third of patients will develop PE, whereas the risk is less than 10% when the diagnosis is made after 38 weeks' gestation. Use of antihypertensive agents to treat patients with GH should be avoided, given the risk of concurrent PE and the lack of evidence supporting improved fetal outcome. GH tends to recur in subsequent pregnancies and predisposes women to hypertension in the future (Marin et al., 2000).

Preeclampsia–Eclampsia

Apparently unique to humans, the underlying causes of PE remain poorly elucidated. It is evident that the clinical manifestations of PE arise from vascular endothelial dysfunction that ultimately involves the central nervous, renal, hepatic, and cardiovascular systems. In its full-blown form, PE can produce a profound coagulopathy and liver, respiratory, or cardiac failure and maternal death.

PE is categorized as having severe features when systemic complications (e.g., renal, hepatic, pulmonary, or hematologic) are present (Box 12.2). This distinction is important, because when the presence of severe features is documented at any gestational age, the only appropriate treatment option is delivery, whereas expectant management may be acceptable in a woman who has mild disease and is remote from term.

Etiology

Although the precise etiology of PE remains uncertain, numerous factors are associated with elevated risk (Table 12.3). Up to 10% of primigravid patients have mild PE, and approximately 1% have severe disease. The most widely accepted theory for the pathophysiology of PE is based on pathologic observations in the placenta indicating hypoperfusion in the maternal spiral arteries with resultant hypoxia. The placenta then releases substances into the maternal circulation that adversely affect maternal endothelial function, leading to the clinical syndrome of widespread vascular dysfunction, which is recognized as the syndrome of PE (Myers and Baker, 2002). Individual responses to the process of progressive vascular dysfunction vary in severity and timing in a manner that seems to have genetic, familial, and immunologic components. For example, PE occurring in a first-degree relative confers a fourfold increase in risk of the disease in siblings and children (Chesley and Cooper, 1986). Population studies have suggested that women exposed to the antigenic effects of sperm before conception have a lower rate of PE than do women who conceive with lesser degrees of exposure, although the evidence is inconclusive (Koelman et al., 2000).

The endothelial dysfunction that characterizes PE (Roberts, 1999) manifests as greater reactivity to circulating vasoconstrictors such as angiotensin, reduced production of endogenous vasodilators such as prostacyclin and nitric oxide (Ashworth et al., 1997), increased vascular permeability, and an increased tendency toward platelet consumption and coagulopathy. The end result is

• BOX 12.2 Management of Pregnant Women With Gestational Hypertension or Preeclampsia

Women With Gestational Hypertension or Preeclampsia Without Severe Features

Monitoring

- Fetal movement daily
- BP checks twice weekly
- Platelet counts and liver enzymes weekly
- Fetal growth sonography every 2–4 weeks
- If fetal growth restriction is detected, assess umbilical artery Doppler.

Avoid

- Antihypertensive medications unless >160/110 mmHg
- Strict bed rest prescription

Delivery

- At or beyond 37 0/7 weeks unless PE with severe features
- Magnesium sulfate need not be administered for the prevention of eclampsia.
- Regional neuraxial anesthesia (spinal or epidural) may be performed.
- Monitoring of BP should be performed for the first 72 h and again at 7–10 days.

Women With PE and Severe Features

- Administer parenteral magnesium sulfate and continue without interruption until at least 24–48 h postpartum.
- If 34 0/7 weeks or more, deliver after maternal stabilization
- If less than 34 0/7 weeks
 - Delivery may be delayed in facilities with adequate maternal and neonatal intensive care while antenatal corticosteroids for fetal lung maturity are administered.
 - Delivery should not be delayed if there is coexisting uncontrollable hypertension, eclampsia, pulmonary edema, abruption placentae, or nonreassuring fetal status.
- If previable gestational age, deliver soon after maternal stabilization
- If sustained systolic BP >160 mmHg and/or diastolic BP >110 mmHg, administer antihypertensive medication.

Prevention of Recurrent Preeclampsia

If prior PE and delivery occurred prior to 34 0/7 weeks, begin daily low-dose aspirin (81 mg) prior to 15 weeks.

BP, Blood pressure; PE, preeclampsia.

Data from American College of Obstetricians and Gynecologists. Hypertension in Pregnancy. Washington, DC: American College of Obstetricians and Gynecologists; 2013.

TABLE 12.3 Risk Factors for Development of Preeclampsia

| Factor | Relative Risk |
|-------------------------------------|---------------|
| Primigravida | 3 |
| Age >40 years | 3 |
| African-American race | 1.5 |
| Family history | 5 |
| Chronic hypertension | 10 |
| Chronic renal disease | 20 |
| Antiphospholipid syndrome | 10 |
| Insulin-dependent diabetes mellitus | 2 |
| Multiple gestation | 4 |

hypertension, proteinuria secondary to glomerular injury, edema, and a tendency toward extravascular fluid overload with intravascular hemoconcentration.

Prediction

Perhaps one of the most important contributions that prenatal care makes to maternal and fetal outcomes is the detection of PE and the prevention of eclampsia (Karbhari et al., 1972; Backe and Nakling, 1993). A wide variety of biochemical and physical tests have been proposed as screening tools for early detection of PE (Dekker and Sibai, 1991), but even the most widely used biochemical tests have poor predictive values. Uric acid levels are elevated in many cases of PE, but the sensitivity of the measurement is low (Lim et al., 1998). Clinicians should be aware of the limitations of routine urine testing for detection of proteinuria, with standard dipstick testing being notoriously inaccurate (Bell et al., 1999).

Doppler ultrasonographic assessment of the vascular dynamics in the uterine arteries during the second trimester has been proposed as a screening tool in populations in which obstetric ultrasonography is routine (Cnossen et al., 2008). Up to 40% of women who develop PE have abnormal waveforms, and this finding was reported to be associated with a sixfold rise in the risk of PE (Papageorgiou et al., 2002). As no randomized trials to date have demonstrated convincingly the ability of uterine artery Doppler studies to predict PE, its use is currently not recommended (ACOG, 2013).

The role of angiogenic factors in the pathophysiology of PE has become increasingly clear. Vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), which bind to fms-like tyrosine kinase-1 (Flt-1) and soluble fms-like tyrosine kinase-1 (sFlt-1) receptors, have a critical role in angiogenesis and placental development. Flt-1, VEGF, and PlGF factor promote angiogenesis and placental vasculogenesis, whereas sFlt-1, VEGF, and PlGF inactivate those proteins, resulting in disordered angiogenesis and endothelial dysfunction. Levels of sFlt-1 are elevated in women with PE, and these elevated levels of sFlt-1 precede the features of clinical PE.

Zeisler et al. (2016) recently reported a multicenter, prospective study of the ratio of sFlt-1 to PlGF in women between 24 weeks' and 37 weeks' gestation who presented with a clinical suspicion of PE or the HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count). An sFlt-1:PlGF ratio of 38 was the optimal cutoff in distinguishing between women in whom PE would develop and those in whom it would not develop in the next week. In a validation cohort of 550 women, a ratio of 0.38 or lower had a negative predictive value of 99.3% (95% confidence interval [CI], 97.9–99.9).

Despite the clear negative predictive value of an sFlt-1:PlGF ratio of less than 38 for PE diagnosis in the subsequent week, the clinical utility of this fact in managing pregnant women remains unclear. Thus at present, serum screening for PE risk is not recommended.

Prevention

If an accurate predictor of PE could be identified, the next logical step would be the application of a preventive or ameliorative treatment. Unfortunately, attempts to identify an effective treatment have proved equally difficult. Given the recognized association between vascular endothelial dysfunction and PE, prostaglandin inhibitors have been proposed as a candidate for prophylaxis or treatment. Numerous trials (Duley et al., 2001) have been conducted

with low-dose aspirin, based on the idea that the ability of aspirin to irreversibly inhibit production of the vasoconstrictive prostaglandin thromboxane would promote greater activity of prostacyclin, a vasodilatory prostaglandin. This ability of aspirin would help to maintain patency in the maternal placental vascular bed and limit or prevent the evolution of PE.

In a recent systematic review of 34 randomized controlled trials (Bujold et al., 2010) of women at risk for recurrent PE, low-dose aspirin started at 16 weeks or earlier was associated with a significant reduction in PE (9.3% treated vs 21.3% control) and IUGR (7% treated vs 16.3% control), whereas aspirin started after 16 weeks was not. Low-dose aspirin started at 16 weeks or earlier also was associated with a reduction in severe PE (0.7% treated vs 15.0% control) and preterm birth (3.5% treated vs 16.9% control). Of note, all studies for which aspirin had been started at 16 weeks or earlier included women identified to be at moderate or high risk for PE.

Calcium supplementation has been proposed as a preventive treatment on the basis of the known vasodilatory effect of calcium and impressive results in earlier, small studies (Atallah et al., 2000). Similarly, it has been suggested that antioxidants may have a role in PE prevention, but the only available trial to date showed mixed results, with improvements in biochemical indices in women receiving vitamins C and E, although perinatal outcomes were not different in treated and untreated groups (Chappell et al., 1999). Of concern was the finding that women in whom PE developed despite vitamin therapy had markedly worsened PE than controls in whom the disease developed.

Two Cochrane systematic reviews (Hofmeyr et al., 2010, 2014) found that daily calcium supplementation significantly reduced the risk of PE and hypertension with and without proteinuria. However, women who received calcium supplements had a significantly higher risk of developing HELLP syndrome. Calcium supplementation had no effects on the risk of developing eclampsia, maternal death, or maternal admission to the intensive care unit. There was no effect of calcium supplementation on preterm birth, although a subgroup analysis suggested that there were fewer preterm births among women who received between 1.5 g and 2 g of elemental calcium per day. There was no effect on low birth weight, admission to a neonatal intensive care unit, stillbirth, and neonatal death. Thus in populations with low calcium intake, calcium supplementation is a reasonable intervention of uncertain net benefit.

Antepartum Management

Given the current inability to predict or prevent PE in the majority of cases, clinicians should actively manage established disease and thus minimize maternal and fetal morbidity. The recognition that PE has a form with severe features is of great value in escalating management and minimizing morbidity (Box 12.3). Mild disease is generally managed expectantly with frequent fetal and maternal biophysical assessments until 37 weeks or there is evidence of severe features. The appearance of severe features mandates prompt delivery in all but highly selected cases regardless of gestational age.

Patients with a diagnosis of PE should be regularly evaluated for severe features. This includes a 24-hour urine collection; complete blood count with platelet measurements; determination of serum uric acid, blood urea nitrogen, and creatinine levels; and evaluation of liver transaminases. Fetal size should be estimated with ultrasonography; the presence of IUGR (estimated fetal weight less

• BOX 12.3 Preeclampsia and Severe Features

Preeclampsia Diagnosis

- Systolic blood pressure ≥ 140 mmHg or diastolic pressure of ≥ 90 mmHg twice at least 4 h apart
- Proteinuria ≥ 300 mg/24 h (not required for diagnosis)

Severe Features

- Systolic blood pressure ≥ 160 mmHg or diastolic pressure of 110 mmHg on two occasions at least 4 hours apart while the patient is on bed rest
- Pulmonary edema
- HELLP syndrome: thrombocytopenia (platelet count $<100,000/\mu\text{L}$), elevated liver enzymes to twice normal concentration, severe persistent right upper quadrant or epigastric pain
- Renal dysfunction with serum creatinine concentration >1.1 mg/dL or a doubling of the serum creatinine concentration in the absence of other renal disease
- Symptoms suggestive of end-organ involvement: headache, visual disturbance, epigastric, or right upper quadrant pain
- Eclampsia

From American College of Obstetricians and Gynecologists; Task Force on Hypertension in Pregnancy. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. *Obstet Gynecol.* 2013;122:1122–1131.

than the 10th percentile), while no longer a criterion for severe PE, is a sign of jeopardy for the fetus. Patients with mild disease at 37 weeks' gestation or more should be delivered, because prolonging pregnancy further increases the risks of maternal and fetal morbidity. Patients at earlier gestational ages should be closely monitored with sequential clinical and laboratory evaluations. Such monitoring often begins in the hospital and may be continued in an outpatient setting with appropriate supervision. Fetal well-being should be evaluated until delivery by means of kick counts and regular nonstress tests or modified biophysical profiles. There is no evidence that antihypertensive therapy influences progression of nonsevere PE, and it may actually be dangerous by masking worsening hypertension; therefore oral antihypertensive therapy should be avoided during expectant management.

Conversely, severe hypertension requires prompt treatment with rapid-acting antihypertensive agents if stroke and placental abruption are to be avoided. Intravenous hydralazine is well established as a first-line drug for this purpose, although there is a growing experience with other agents, including intravenous labetalol and oral nifedipine (Duley and Henderson-Smart, 2000a) (Table 12.4). The aim of treatment is to lower blood pressure into the mild PE range ($<160/100$ mmHg) to reduce the risk of stroke and other maternal cardiovascular complications. Parenteral magnesium sulfate to prevent eclampsia should be administered in all cases of severe disease.

Severe features of PE can also present with atypical central nervous system abnormalities with associated headache, visual deficits, confusion, seizures, and, in the most severe cases, intracranial hemorrhage. This constellation of findings, when demonstrated on brain magnetic resonance imaging, has been termed the *posterior reversible encephalopathy syndrome* (PRES). Neuroimaging typically demonstrates cerebral edema in the parietal and occipital lobes. In addition to PE/eclampsia, PRES (Postma et al., 2014) has been associated with various nonobstetric conditions such as post transplantation or with autoimmune disease, in combination with immunosuppressive therapy or high-dose chemotherapy for various

TABLE 12.4 Drugs for Acute Treatment of Hypertension in Severe Preeclampsia

| Drug | Dosage |
|-------------|---|
| Hydralazine | 5 mg IV or IM, then 5–10 mg every 20–40 min as required, to a total of 30 mg or Constant intravenous infusion 0.5–10 mg/h |
| Labetalol | 10–20 mg IV, then 20–80 mg every 20–30 min to a maximum of 300 mg or Constant intravenous infusion 1–2 mg/min |
| Nifedipine | 10–20 mg PO, repeat in 30 min, then 10–20 mg every 2–6 h |

IM, Intramuscular; IV, intravenous; PO, per oral.

malignant conditions. Long-term sequelae of PRES after PE/eclampsia and other PRES-related conditions are poorly described.

More commonly, patients show evidence of intravascular microangiopathy leading to the hemolysis, elevated liver enzymes, and low platelets of HELLP syndrome. The full-blown clinical syndrome of HELLP carries a significant maternal risk related to coagulopathy and hepatic dysfunction, even hepatic rupture (Weinstein, 1982). Most patients with HELLP never experience catastrophic features of the syndrome because delivery is initiated before their condition deteriorates to a critical level.

Preeclampsia and Fetal Risk

While the practice of prompt delivery in cases of severe PE optimizes both maternal and newborn outcome, this practice increases the incidence of prematurity and its attendant complications. IUGR is not uncommon in severe PE, and there may be evidence of progressive deterioration in fetal well-being, with progressive placental dysfunction. Infants delivered before 34 weeks' gestation will benefit from antenatal steroid therapy – even as little as 8 hours of therapy before delivery may have benefit. Even those at gestations between 34 0/7 and 36 and 6/7 weeks of gestation will derive measurable benefit from a course of maternal corticosteroids (Gyamfi-Bannerman et al., 2016). The incidence of respiratory distress syndrome is lower in infants of mothers with PE who are delivered preterm than in those of age-matched controls without antenatal steroid exposure (Yoon et al., 1980). Nonetheless, the morbidity of such infants is greater because of hypoxemic insults received in utero. Infants born to mothers with PE may also have thrombocytopenia or neutropenia, which further complicates their newborn course (Fraser and Tudehope, 1996).

Intrapartum Management

Patients with PE and severe features should be delivered expeditiously once the maternal condition is stabilized. In most cases induction of labor can be undertaken safely, reserving cesarean section for obstetric indications such as breech presentation, placenta previa, and concerning fetal status. The only exception to prompt delivery may be a case of severe PE limited to proteinuria and intermittently severe hypertension but remote from term (<28 weeks' gestation). Such patients may be managed conservatively within a high-risk

• BOX 12.4 Magnesium Sulfate Therapy for Prevention of Eclampsia

- Bolus 4–6 g IV over 20 min
- Continuous infusion 1–2 g/h
- Follow up levels every 6–8 h to target 4–6 mEq/L
- Continue infusion 24 h after delivery or 24 h after seizure if seizure occurs despite magnesium therapy

IV, Intravenous.

center while antenatal corticosteroids are administered (Sibai et al., 1990). Patients with severe PE at less than 24 weeks' gestation should be offered termination of the pregnancy.

All women with PE and severe features should receive magnesium sulfate as seizure prophylaxis (Box 12.4). Current guidelines do not require magnesium sulfate for those with gestational hypertension/PE lacking severe findings. The safety and therapeutic superiority of magnesium sulfate over other agents (e.g., diazepam) have been validated in multiple randomized trials (Duley et al., 2003).

Intrapartum blood pressure should be maintained in the mild PE range (<160/105 mmHg) using intravenous antihypertensive agents (labetalol, hydralazine, or nifedipine). Epidural anesthesia is indicated for pain control and may aid in blood pressure management. Careful attention to fluid balance, especially avoiding overload, should be maintained. After delivery, the preeclamptic process typically begins to resolve rapidly.

Eclampsia

Eclampsia, by definition a “severe feature,” is the occurrence of generalized tonic-clonic seizures in association with PE. It affects approximately 1 in 2500 deliveries in the United States and may be much more common in developing countries, affecting as many as 1% of parturients. Up to 10% of maternal deaths are due to eclampsia in the United States (Creanga et al., 2015).

Most cases of eclampsia occur immediately prior to or within 24 hours of delivery. Almost half of seizures occur before the patient's admission to the labor and delivery department, approximately 30% are intrapartum, and the remainder are postpartum. There is a considerable drop in the risk of eclampsia by 48 hours postpartum, with seizures occurring in less than 3% of women beyond that time. Most patients have antecedent symptoms that

are suggestive of PE, although in some cases eclampsia may occur without warning. If eclampsia is left untreated, repetitive seizures become more frequent and of longer duration, and ultimately status eclampticus may develop. Maternal and fetal mortality are as high as 50% in severe cases, especially if the seizures occur while the patient is remote from medical care.

Randomized controlled trials have demonstrated the clear superiority of magnesium sulfate for the treatment of eclampsia over all other anticonvulsants (Duley and Gulmezoglu, 2002; Duley and Henderson-Smart, 2002b, 2002c). Intravenous magnesium sulfate is given as a 4 g bolus over 5 minutes followed by a maintenance infusion of 1–2 g/h for 24–48 hours after delivery. Subsequent seizures can be treated with further bolus injections. In refractory cases, second-line treatment with other anticonvulsants may be required. Rarely, the patient may require pharmacologic paralysis and mechanical ventilation.

Delivery after an eclamptic seizure should take place expeditiously but in a controlled, careful manner. There is little value in performing an emergency cesarean section (Coppage and Polzin, 2002) in response to a seizure. Stabilization and optimization of both maternal and fetal status are important prerequisites for delivery procedures. Once magnesium sulfate has been administered and maternal vital signs controlled, vaginal delivery is preferable in most cases. Potential indications for cesarean include a significantly unfavorable cervix, evidence of ongoing fetal compromise, or inability to achieve acceptable blood pressure control. Infants born to mothers after eclampsia require careful observation after birth.

Suggested Readings

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13

Prenatal Drug Exposure

LINDA D. WALLEN AND CHRISTINE A. GLEASON

KEY POINTS

- National and global substance use have reached epidemic proportions.
- The legalization of marijuana has resulted in new concerns for fetal and infant exposure, with only a minor increase in our knowledge of the short- and long-term effects of marijuana in this population.
- The recent focus of perinatal substance use has grown to include widely prescribed substances, including opiates and antidepressants.
- The importance of establishing and maintaining consistent protocols in managing neonatal abstinence syndrome (NAS) has been clearly demonstrated.
- For women with substance use or misuse, whether licit or illicit, the choice to breastfeed is often challenging, and healthcare professionals are equally challenged in making recommendations. The known benefits of breastfeeding and human milk must be weighed against the potential risks to the infant, most of which are not well understood.

Substance use and misuse during pregnancy has been recognized as a problem for more than a century, but in the past decade the problem has reached near epidemic proportions. This has been due in part to increasingly widespread use of prescription painkillers, sedatives, and antidepressants in the United States. Prenatal exposure to psychotropic substances, both legal (such as alcohol, nicotine, prescription painkillers, and, recently, cannabinoids) and illegal (such as heroin, cocaine, and methamphetamines) can cause maternal, fetal, and neonatal complications. These include poor fetal growth, preterm birth, placental abruption, fetal distress, spontaneous abortion, stillbirth, fetal malformations, neonatal neurobehavioral dysfunction, and increased risk for sudden infant death syndrome (SIDS). Although substance abuse occurs in all socioeconomic classes, illegal drug abuse is more frequently associated with unhealthy lifestyles, poor access to prenatal care, untreated health problems, poverty, stress, and psychological disorders. Because of these socioeconomic confounders as well as the confounders of polysubstance exposure and the influence of various postnatal environmental factors, it is often difficult to determine the effects of maternal use of one specific drug on the fetus and newborn. This chapter addresses the epidemiology of perinatal substance use and abuse; the effects of specific drugs on the fetus and newborn; maternal issues and their effects on the newborn; identification of pregnancies and babies at risk; management of drug-exposed neonates; and the long-term effects of perinatal substance exposures. The discussion will focus

on substances that are known, or believed, to be associated with significant perinatal and neonatal morbidity: alcohol, tobacco, nicotine, opioids, cocaine, marijuana, and methamphetamine.

Epidemiology of Perinatal Substance Exposure

Prevalence

According to a 2014 national survey, approximately 10% of Americans aged 12 years or older used an illicit drug in the past 30 days. This prevalence rate is higher than in any year from 2002 through 2013 and has been driven primarily by increasing marijuana use and the nonmedical use of prescription pain relievers (SAMHSA, 2014; Center for Behavioral Health Statistics and Quality, 2015).

Determining prevalence rates for perinatal substance use, whether in general or for a specific substance, is quite challenging. Under-reporting of substance use by pregnant women, unreliable drug use survey and detection methods, frequent polydrug/substance use, and differing societal attitudes toward use of illicit and licit substances by pregnant women are just a few of those challenges. There are, nevertheless, several sources for data regarding perinatal substance exposure. Long-term follow-up studies of perinatal substance exposure, such as the Maternal Lifestyle Study developed in the early 1990s to follow substance-using pregnant women and their offspring, have published data regarding prevalence of specific drug exposures at the initiation of those studies (Shankaran et al., 2007). But that epidemiologic data, now decades old and with new ascertainment methodologies and social changes, have limited applicability to the present.

Another data source is the Substance Abuse and Mental Health Services Administration (SAMHSA), an agency of the US Department of Health and Human Services, which conducts an annual US National Survey on Drug Use and Health (NSDUH), periodically updating and revising their methods. Data from the 2013 survey revealed that 5.4% of pregnant women 15–44 years old reported current illicit drug use, higher than the 2005–2006 survey results (4.0%) and lower than the rate among women in this age group who were not pregnant (11.4%). The rates of illicit drug use by pregnant women also varied considerably by age group – 14.6% among pregnant women 15–17 years old, 8.6% among women 18–25 years old, and 3.2% among women 26–44 years old. Regarding alcohol, 9.4% of pregnant women 15–44 years old reported current alcohol use, lower than the 12% reported use in the 2005–2006 survey and significantly lower than the rates for

nonpregnant women (55.4%). Regarding cigarette smoking, 15.4% of pregnant women reported cigarette use, not significantly different from 2005–2006 (16.5%) but significantly lower than the rate for nonpregnant women (24.0%) (SAMHSA, 2014).

PRAMS, the Pregnancy Risk Assessment Monitoring System, provides another source for information on substance exposure before, during, and after pregnancy. Developed in 1987, PRAMS is a cooperative surveillance project of the US Centers for Disease Control and Prevention (CDC) and individual state health departments. It is designed to monitor maternal behaviors and experiences among women who deliver liveborn infants and currently includes 40 states and New York City, covering about 78% of all US births. PRAMS surveillance data results have historically demonstrated wide geographic, age-related, and racial/ethnic variation in tobacco, alcohol, and illicit drug use. For example, in a 2009 survey, an average of 54.2% of women 18–44 years old reported alcohol use during the 3 months prior to becoming pregnant. The highest rate was reported among whites (65.7%) and those 35–44 years old (56.5%). There was remarkable variation between states, ranging from 24% in Utah to 71.6% in Vermont (Centers for Disease Control and Prevention, 2014a). Pregnant women are more likely to decrease both recreational substance and “hard drug” use once they know they are pregnant. However, there is wide variation among substances studied, depending on the success of targeted public policy campaigns, licit versus illicit substances, and availability of treatment programs for substance-abusing or dependent women. For example, the 2013 NSDUH data regarding alcohol use in pregnancy showed that current alcohol use among pregnant women during the second and third trimesters was lower than during the first trimester (5.0 and 4.4% vs 19.0%). Disappointingly, there was less of a reduction in current cigarette use, with 19.9% in the first trimester, 13.4% in the second trimester, and 12.8% in the third trimester (SAMHSA, 2014).

Health Policy

In the 1980s, with rising cocaine use and the emergence of crack cocaine, national attention was turned to drug use during pregnancy, with a general public outcry being the result. Children born to crack addicts were widely believed to be irrevocably damaged, and public opinion was that mothers should be punished. As the foster care system became overwhelmed, and as evidence to the contrary emerged, public opinion regarding prenatal drug use began to shift, turning more toward maternal treatment and prevention rather than punishment. As Lester et al. (2004) stated, the initial overreaction of the public “in which drug-exposed children were characterized as irrevocably and irreversibly damaged” shifted “to a perhaps equally premature excessive ‘sigh of relief’ that drugs such as cocaine do not have lasting effects, especially if children are raised in appropriate environments.”

Unfortunately, this change in the public discourse regarding health policy interventions for substance use and abuse during pregnancy has not yet led to significant changes in the availability of women-centered drug treatment services, although the need for, and effectiveness of, such treatment is high (Terplan et al., 2015a). The focus of current policy is (or should be) to provide appropriate medical care for substance-using pregnant women, including medical management of their chemical dependency and programs to decrease substance use during pregnancy. However, there is still significant stigmatization of individuals with substance abuse disorders and pregnant women suffer additional stigma due to the potential for fetal harm, thus calling into question maternal

“fitness,” which may lead to a number of punitive measures. As Terplan et al. (2015b) recently wrote, “pregnant women who use substances deserve compassion and care, not pariah-status and punishment.” Further, there remains the important step of recognizing that “the idea that illegal drugs are more harmful to the unborn fetus than legal drugs is incorrect” (Thompson et al., 2009). Future research and intervention need to include programs to educate women of childbearing age of the significant effects of both legal and illegal substance use on the early-gestation fetus, starting even before pregnancy may be recognized.

A promising step in the right direction was recently taken by the US Congress, which turned its attention to increasingly widespread prescription opioid use/misuse and the resulting neonatal abstinence syndrome (NAS) epidemic. Lawmakers passed the “Protecting Our Infants” Act of 2015, which aims to reduce the number of newborn infants exposed to opioids and who may thus develop NAS. This act of Congress calls for the development of recommendations, programs, strategies, and research relevant to prenatal opioid use by the Agency for Healthcare Research and Quality and the Department of Health and Human Services as well as the provision of assistance to states by the CDC in collecting relevant public health data.

Perinatal Exposure to Specific Substances (Tables 13.1–13.2)

Alcohol

History/Epidemiology

Before 1970, the detrimental effects of alcohol abuse during pregnancy were believed to be related only to drunkenness, such as an increased risk for accidents. There was a widely held belief that the placenta formed a protective barrier between alcohol and the fetus. This belief was finally repudiated by studies in the United States (Jones and Smith, 1973) and France (Lemoine et al., 1967) describing fetal alcohol syndrome (FAS). These studies led to the 1989 US federal law requiring that warning labels be placed on all alcoholic beverage containers regarding alcohol-related birth defects. In 2005, the Surgeon General reissued an advisory for women who are, or might become pregnant – urging abstinence from alcohol consumption to eliminate the risk for fetal alcohol spectrum disorders. Despite this extensive public health campaign, a CDC analysis of 2011–2013 Behavioral Risk Factor Surveillance System data found that among pregnant women, the prevalence of any alcohol use was 10.2% and of binge drinking was 3.1%. This was significantly lower than the prevalence among nonpregnant women 15–44 years old (53.6% and 18.2%, respectively) but disappointingly similar to prevalence estimates reported for 2006–2010. A comprehensive approach is needed to achieve the *Healthy People 2020* objectives, which are to increase the percentage of pregnant women reporting abstinence from any alcohol use to 98% and to increase the percentage reporting abstinence from binge drinking to 100% (Centers for Disease Control and Prevention, 2015).

Pharmacology and Biologic Actions

Alcohol is a mood-altering substance that enhances the effects of the inhibitory neurotransmitter γ -aminobutyric acid and lessens the effect of the excitatory neurotransmitter glutamate, thus acting as a central nervous system (CNS) depressant or sedative. Alcoholic beverages come in many forms, and for centuries they have been

TABLE 13.1 Enhanced Risk for Various Events or Processes After Substance Use During Pregnancy^a

| Event or Process | Ethanol | Cigarettes | Marijuana | Opiates | Cocaine | Amphetamines |
|---------------------------------|---------|------------|-----------|---------|---------|--------------|
| Malformations | + | — | — | — | — | — |
| Abortion/stillbirth | + | + | ? | + | + | + |
| Intrauterine growth restriction | + | + | — | + | + | + |
| Prematurity | — | + | ? | + | + | + |
| Withdrawal | ? | + | +/- | + | — | — |
| Central nervous system sequelae | + | ? | + | ? | + | ? |
| Sudden infant death syndrome | + | + | ? | + | ? | ? |
| Foster care | + | — | — | + | + | + |

^aAlthough risk is increased, the risk ratio ranges for many from 1–2 for these associations.

? = Possible association that has not been confirmed.

TABLE 13.2 Summary of Effects of Prenatal Drug Exposure

| | Nicotine | Alcohol | Marijuana | Opiates | Cocaine | Methamphetamine |
|---|------------------------|---------------|-----------|------------------------|------------------------|-----------------|
| Short-Term Effects/Birth Outcome | | | | | | |
| Fetal growth | Effect | Strong effect | No effect | Effect | Effect | Effect |
| Anomalies | No consensus on effect | Strong effect | No effect | No effect | No effect | No effect |
| Withdrawal | No effect | No effect | No effect | Strong effect | No effect | ^a |
| Neurobehavior | Effect | Effect | Effect | Effect | Effect | Effect |
| Long-Term Effects | | | | | | |
| Growth | No consensus on effect | Strong effect | No effect | No effect | No consensus on effect | ^a |
| Behavior | Effect | Strong effect | Effect | Effect | Effect | ^a |
| Cognition | Effect | Strong effect | Effect | No consensus on effect | Effect | ^a |
| Language | Effect | Effect | No effect | ^a | Effect | ^a |
| Achievement | Effect | Strong effect | Effect | ^a | No consensus on effect | ^a |

^aLimited or no data available.

From Behnke M, Smith VC, Committee on Substance Abuse, Committee on Fetus and Newborn. Prenatal substance abuse: short- and long-term effects on the exposed fetus. *Pediatrics*. 2013;131:e1009–e1024.

consumed for diverse reasons: celebrations, relaxation, religious ceremonies, and medicinal purposes (alcohol is a sedative and tocolytic agent). The alcohol contained in alcoholic beverages is ethanol, which is absorbed in the digestive tract and into the body fat and bloodstream. Ethanol is metabolized to acetaldehyde by alcohol dehydrogenase (ADH), primarily in the liver. ADH is then metabolized to acetate by aldehyde dehydrogenase and eventually eliminated as water and CO₂. Although acetaldehyde is short-lived, it can cause significant tissue damage, which is particularly evident in the liver, where most alcohol metabolism takes place. Pregnant women have slower rates of alcohol clearance, probably related to hormonal alterations in the activity of the alcohol-metabolizing enzymes; this leads to slower clearance of alcohol compared with nonpregnant women consuming the same amount of alcohol (Shankaran et al., 2007).

Complications of Pregnancy

Heavy drinking carries a higher risk of cardiovascular and hepatic complications in women compared with men, and the alcohol-associated mortality rate is also considerably higher (Smith and Weisner, 2000). These factors alone can clearly complicate a woman's pregnancy. In addition, nutritional deficiencies and poor diet can affect general health and dentition, which can negatively affect a pregnancy. Alcoholism is a chronic disease that is often progressive and can be fatal. Pregnant alcoholics often have related medical disorders such as cirrhosis, pancreatitis, and alcohol-related neurologic problems. These disorders can affect the health and well-being of their fetus.

Alcohol affects prostaglandin levels, increasing levels of its precursors in human placental tissue and thus affecting fetal development and parturition. In fact, researchers have used this

knowledge to test the effect of aspirin, which inhibits alcohol-induced increases in prostaglandin levels, on reducing alcohol-induced fetal malformations in a mouse model (Randall, 2001). Specific obstetric complications of heavy drinking may relate to alterations in prostaglandin levels, including an increased risk for spontaneous abortion, placental abruption, and alcohol-related birth defects such as FAS.

Fetal Growth

Intrauterine growth restriction (IUGR) is one of the most consistent findings of prenatal exposure to alcohol (Hannigan and Armant, 2000). Growth deficit begins in utero and continues throughout childhood (Williams et al., 2015). The facial features and the growth restriction become less noticeable during adolescence and puberty (Streissguth et al., 1991; Williams et al., 2015).

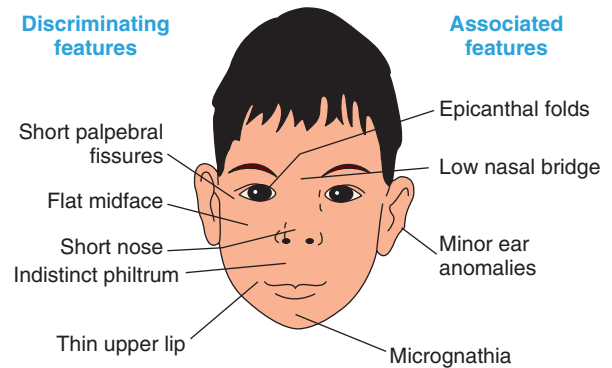
Fetal Alcohol Spectrum Disorders

FAS was first described by Lemoine et al. (1967), a Belgian pediatrician who observed a common pattern of birth anomalies in children born to alcoholic mothers in France. This description was followed by a landmark article by Jones and Smith (1973) that reported similar features in several children born to alcoholic mothers in the United States.

It is unclear how much alcohol exposure is necessary to cause fetal teratogenicity, and even high consumption levels do not always result in the birth of a child with FAS (Abel and Hannigan, 1995). However, a woman with a previously affected child is at increased risk for having a child with FAS if she consumes alcohol during a subsequent pregnancy. The adverse effects of alcohol on the fetus are related to gestational age (GA) at exposure, the amount of alcohol consumed, and the pattern of consumption (e.g., binge drinking), maternal peak blood alcohol concentrations, maternal alcohol metabolism, and the individual susceptibility of the fetus. Studies show that maternal peak blood alcohol levels are affected by maternal nutrition, age, body size, and genetic disposition (Maier and West, 2001). In addition, various risk factors increase susceptibility to FAS, including advanced maternal age and confounding factors such as nonwhite race, poverty, and socioeconomic status (Abel, 1995; May and Gossage, 2001). In the United States, the incidence of FAS is tenfold higher for African Americans living in poverty than for white middle-class women (Abel, 1995). Despite the differences in incidence of FAS worldwide, reports consistently indicate poverty or socioeconomic status as major determinants of FAS (Abel, 1995; May et al., 2000).

Features of FAS include characteristic facial dysmorphism (short palpebral fissures, midface hypoplasia, broad flat nasal bridge, flat philtrum, and thin upper lip [Fig. 13.1]), prenatal and postnatal growth deficiency, and variable CNS abnormalities. Skeletal anomalies, abnormal hand creases, and ophthalmologic, renal, and cardiac anomalies have been described in children with FAS but less frequently than the facial dysmorphism and CNS abnormalities that include structural brain defects (e.g., dysgenesis of the corpus callosum and cerebellar hypoplasia), cognitive abnormalities, delayed brain development, and signs of neurologic impairment, including lifelong behavioral and psychosocial dysfunction. In 1996, the Institute of Medicine further defined the criteria for the diagnosis of FAS and proposed a new term – alcohol-related neurodevelopmental disorder (ARND). This term includes structural CNS and cognitive abnormalities in children with confirmed fetal exposure to alcohol. Unlike FAS, a diagnosis of ARND does not require the presence of facial or other physical abnormalities. In 2000, the American Academy of Pediatrics (AAP) Committee on

FACIES IN FETAL ALCOHOL SYNDROME



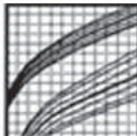





• **Fig. 13.1** Facies in Fetal Alcohol Syndrome. (From Streissguth AP, Little RE. Alcohol, pregnancy, and the fetal alcohol syndrome, ed. 2, unit 5 of *Alcohol use and its medical consequences: a comprehensive slide teaching program for biomedical education*. Developed by Project Cash of the Dartmouth Medical School. Reproduced with permission from Milner-Fenwick, Inc., Timonium, Michigan, 1994.)

Substance Abuse published these new definitions with an explanatory drawing (Fig. 13.2), and their recommendations were updated in 2015 (Williams et al., 2015).

The incidence of FAS in the United States has been estimated to vary from 0.5–9 cases per 1000 live births (Bertrand et al., 2005; Williams et al., 2015). FAS is recognized more frequently in the United States than in other countries and is most common (4.3%) among women who report heavy drinking (Abel, 1995). Accurate incidence and prevalence rates of FAS are difficult to obtain because of wide variations in methodologies used for estimation of rates and because the clinical diagnosis is often missed in the neonatal period.

FAS is diagnosed from the history and physical findings. No laboratory tests are available for clinical use to quantify the extent of alcohol exposure during fetal life. There are also no clinical methods for validating maternal self-reporting of alcohol use, quantifying the level of fetal exposure, or predicting future disability after fetal exposure. Koren et al. (2002) have proposed meconium fatty acid ethyl ester levels as a potential biologic marker for fetal alcohol exposure, but relevance of these levels to childhood outcomes remains to be studied. Investigators have shown that pediatricians fail to recognize FAS in the newborn and do not always inquire about alcohol exposure during pregnancy (Stoler and Holmes, 1999). One promising screening tool is the use of averaged cranial ultrasound images to examine the size and shape of the corpus callosum, which is typically dysgenic in FAS (Bookstein et al., 2005). Guidelines to aid in the earlier recognition and referral of infants and children with FAS and fetal alcohol spectrum disorder have been published recently (Bertrand et al., 2005; Hoyme et al., 2005). FAS is not a problem just for neonatologists. Adolescents who were exposed prenatally to alcohol have a different approach to alcohol than their nonexposed peers, with an increased risk for earlier use and subsequent alcohol abuse (Baer et al., 2003). In an Australian study of education records at age 8–9 years old, children with heavy prenatal alcohol exposure during the first trimester or binge drinking later in pregnancy were twice as likely not to achieve the benchmark for reading and writing (O’Leary et al., 2013b). Children with FAS continue to have serious disabilities into adulthood (Williams et al., 2015). Although the facial features and

| | | | | | | |
|--|---|---|---|--|---|---|
| FAS with confirmed maternal exposure | | | | | | |
| | | | | | | |
| FAS without confirmed maternal exposure | | | | | | |
| | | | | | | |
| Partial FAS with confirmed exposure | | | OR | OR | OR | |
| | | | | | | |
| Alcohol-related birth defects (ARBD) | | | | | | |
| | | | | | | |
| Alcohol-related neurodevelopmental disorder (ARND) | | | | | | |
| | | | | | | |
| | A | B | C | D | E | F |
| | Confirmed Exposure to Alcohol | Facial Anomalies | Growth Retardation | CNS Abnormalities | Cognitive Abnormalities | Birth Defects |
| |  |  |  |  |  |  |

Adapted from *Fetal Alcohol Syndrome: Diagnosis, Epidemiology, Prevention, and Treatment*. 1996;4–5. Letter designations in the figure indicate the following:

- A. Confirmed maternal alcohol exposure indicates a pattern of excessive intake characterized by substantial, regular intake or heavy episodic drinking. Evidence of this pattern may include frequent episodes of intoxication, development of tolerance or withdrawal, social problems related to drinking, legal problems related to drinking, engaging in physically hazardous behavior while drinking, or alcohol-related medical problems such as hepatic disease.
- B. Evidence of a characteristic pattern of facial anomalies that includes features such as short palpebral fissures and abnormalities in the premaxillary zone (e.g., flat upper lip, flattened philtrum, and flat midface)
- C. Evidence of growth retardation, including at least one of the following:
- Low birth weight for gestational age
 - Decelerating weight over time not caused by nutrition
 - Disproportional low weight to height
- D. Evidence of CNS neurodevelopmental abnormalities, including at least one of the following:
- Decreased cranial size at birth
 - Structural brain abnormalities (e.g., microcephaly, partial or complete agenesis of the corpus callosum, cerebellar hypoplasia)
 - Neurologic hard or soft signs (as age appropriate), such as impaired fine motor skills, neurosensory hearing loss, poor tandem gait, poor eye-hand coordination
- E. Evidence of a complex pattern of behavior or cognitive abnormalities that are inconsistent with developmental level and cannot be explained by familial background or environment alone, such as learning difficulties; deficits in school performance; poor impulse control; problems in social perception; deficits in higher level receptive and expressive language; poor capacity for abstraction or metacognition; specific deficits in mathematical skills; or problems in memory, attention, or judgment

F. Birth defects associated with alcohol exposure include:

| | | |
|----------|--|---|
| Cardiac | Atrial septal defects Ventricular septal defects | Aberrant great vessels Tetralogy of Fallot |
| Skeletal | Hypoplastic nails Shortened fifth digits Radioulnar synostosis Flexion contractures Camptodactyly | Clinodactyly Pectus excavatum and carinatum Klippel-Feil syndrome Hemivertebrae Scoliosis |
| Renal | Aplastic, dysplastic, hypoplastic kidneys Horseshoe kidneys | Ureteral duplications Hydronephrosis |
| Ocular | Strabismus Retinal vascular anomalies | Refractive problems Secondary to small globes |
| Auditory | Conductive hearing loss | Neurosensory hearing loss |
| Other | Virtually every malformation has been described in some patient with FAS. The etiologic specificity of most of these anomalies to alcohol teratogenesis remains uncertain. | |

Alcohol-related effects indicate clinical conditions in which there is a history of maternal alcohol exposure and where clinical or animal research has linked maternal alcohol ingestion to an observed outcome. There are two categories, alcohol-related neurodevelopmental disorder and alcohol-related birth defects, which may cooccur. If both diagnoses are present, then both diagnoses should be rendered.

• **Fig. 13.2** Diagnostic Classification of Fetal Alcohol Syndrome and Alcohol-Related Effects. CNS, Central nervous system; FAS, fetal alcohol syndrome. (From the American Academy of Pediatrics. Committee on Substance Abuse and Committee on Children with Disabilities. Fetal alcohol syndrome and alcohol-related neurodevelopmental disorders. *Pediatrics*. 2000;106:358–361. Reproduced with permission from the American Academy of Pediatrics, 2000.)

growth restriction are no longer as distinctive as during childhood, mental retardation continues to have a significant effect. Adults with FAS have behavior, socialization, and communication dysfunction, and on average they function at the second-grade or third-grade level. A significant number of FAS patients do not achieve fully independent living. Earlier recognition and intervention for children with FAS and its variants may help to minimize eventual adulthood disabilities and help to prepare adolescents and young adults with the disorder for independent living (Bertrand et al., 2005; Williams et al., 2015).

Cigarette Smoking and Nicotine

History/Epidemiology

Nicotine – in the form of cigarettes (including electronic cigarettes), smokeless tobacco, and nicotine replacement patches – remains the substance used most often during pregnancy and the most influential factor adversely affecting perinatal outcomes, particularly preterm birth. Cigarette smoking in the United States has decreased significantly over the past 25 years, thanks to a number of effective public health strategies, including funding tobacco control programs, increasing the price of tobacco products, enacting comprehensive smoke-free policies, and reducing tobacco advertising and promotion. Unfortunately, these efforts have not been as effective for pregnant women. Data from the 2012–2013 NSDUH survey revealed that 24% of reproductive-aged women and 15% of pregnant women smoked cigarettes (SAMHSA, 2014). This is a reduction in prevalence compared to 2002–2003 (30% and 18%, respectively) but far lower than was hoped and considerably lower than the targets set by the *Healthy People 2020* initiative to increase abstinence from cigarette smoking to 98.6% of pregnant women.

Women who smoke during pregnancy are more likely to use opioids, alcohol, cocaine, amphetamines, and marijuana than women who do not smoke (SAMHSA, 2014). Cigarette smoking is especially prevalent among pregnant women with opioid use disorders, with smoking estimates as high as 95% (Ram et al., 2015). Treatment programs for substance use disorders, particularly those targeted at opioid use, are in an ideal position to develop concomitant smoking cessation programs.

Pharmacology and Biologic Actions

Cigarette smoke contains a complex mixture of approximately 4000 compounds, including nicotine and carbon monoxide, which can adversely affect the fetus (Lester et al., 2004). In rodents, nicotine releases chemicals in the reward center of the brain, which likely triggers the euphoria that smokers experience. Nicotine activates nicotinic acetylcholine receptors, and these receptors remain depressed for a longer time after their activation stops, which probably accounts for compulsive smoking (Morrell et al., 2008). Nicotine crosses the placenta and concentrates in fetal blood and amniotic fluid, where its levels significantly exceed maternal blood concentrations (Haustein, 1999). The serum concentration of cotinine, the primary metabolite of nicotine, is used to quantitate the level of smoking and fetal exposure. Cotinine has a half-life of 15–20 hours, and because its serum levels are tenfold higher than those of nicotine, this substance may represent a better marker for intrauterine exposure (Lambers and Clark, 1996).

Complications of Pregnancy

Although the exact mechanism of the adverse effects of smoking on pregnancy is unknown, cigarettes contain numerous potentially

toxic compounds that affect fetal health in a number of ways. Nicotine and its metabolites can act as vasoconstrictors, and a study in pregnant rhesus monkeys demonstrated a nicotine-associated decrease in uterine blood flow (Jauniaux and Burton, 2007; Salihu and Wilson, 2007), which might provide a partial explanation for the association between maternal cigarette smoking and low birth weight. Theories regarding mechanisms for the adverse effects of smoking on fetal health include direct vasoconstrictive effects of nicotine on uteroplacental blood flow, the induction of fetal hypoxia from carbon monoxide production, direct toxic effects and indirect effects of altered maternal nutritional intake, and altered maternal and placental metabolism (Jauniaux and Burton, 2007). When pregnant women smoke cigarettes, the resulting increased levels of carbon monoxide cross the placenta and form carboxyhemoglobin in the fetus, with resulting hypoxemia (Jauniaux and Burton, 2007). Supporting this theory, serum erythropoietin levels are higher in tobacco smoke-exposed infants at delivery, a finding that is presumed to reflect fetal hypoxia (Jazayeri et al., 1998). In addition to the fetal hypoxia theory, there have recently been studies demonstrating that nicotine may act as a developmental neurotoxin targeting nicotinic acetylcholine receptors (Lester et al., 2004; Jauniaux and Burton, 2007) and may disturb protein metabolism during gestation, leading to decreased serum amino acids in umbilical cord blood (Jauniaux et al., 2001).

Maternal smoking has also been shown to affect the length of gestation in a dose-dependent manner, with a higher risk of preterm delivery (Jaakkola et al., 2001) and a twofold increase in the incidence of placental abruption (Jauniaux and Burton, 2007). Perinatal mortality is increased in pregnant smokers, probably reflecting the increases in rates of prematurity, placental abruption, and placenta previa in women who smoke. Mothers who smoke during pregnancy commonly continue to smoke during their infants' childhood. Asthma and other chronic conditions are more common in infants who are exposed to passive smoking (Burke et al., 2012).

Fetal Growth

The effect of smoking on fetal growth is significant and dose dependent (Jauniaux and Burton, 2007). Studies have shown lower birthweights associated with levels of nicotine exposure, with a 1 g reduction in birthweight observed for every microgram per milliliter increase in maternal serum cotinine level (Eskenazi et al., 1995). Investigators have shown a dose-dependent relationship between the amount of smoking and the extent of fetal growth restriction and birthweight reduction (Jaakkola et al., 2001; Jauniaux and Burton, 2007).

Both reducing and ceasing cigarette smoking during pregnancy have been shown to be beneficial and to lead to improved fetal growth. Lieberman et al. (1994) have shown that if pregnant women stop smoking during the third trimester, their infants' weights are indistinguishable from those of a nonsmoking population. Other investigators report that even a modest reduction in smoking is associated with improved fetal growth (Walsh et al., 2001).

Marijuana

History/Epidemiology

Marijuana, or cannabis, has been used for medicinal purposes for over 5000 years, but it was not classified as a legitimate medical compound in the *United States Pharmacopeia* until 1851. In 1937, marijuana was criminalized in the United States (against the advice of the American Medical Association) and was given schedule I status, although patients could still obtain it at cannabis dispensaries

and a growing number of statewide programs. Several cannabis-derived medicines have been developed and are available in the United States and Canada, including dronabinol (schedule III) and nabilone (schedule II), both of which were US Food and Drug Administration approved in 1985 for treatment of nausea and vomiting associated with cancer chemotherapy (Borgelt et al., 2013). Recreational use of marijuana increased in the 1960s, especially among young people, and today it is the most commonly used illicit drug in the United States (SAMHSA, 2014; Center for Behavioral Health Statistics and Quality, 2015).

In the United States, a major shift in societal attitudes toward marijuana has occurred in the past decade, leading to increasing legalization of its use as well as increased availability and potency. This societal shift has largely been due to the widespread belief that marijuana is less “risky” compared to most other substances (both licit and illicit) as well as to growing belief in, and some evidence for, its potential medicinal properties. Although marijuana use remains illegal (in 2016) under federal law (Title 21 US State Code Controlled Substances Act), more than half of US states and the District of Columbia have legalized “medical” marijuana use, and several have also legalized recreational use for those 21 years of age and older. In 2013, 7.5% of the US population reported use of marijuana within the last month, up from 5.8% in 2007 (NIDA Nationwide Trends, 2016).

Cannabis use by pregnant women is similarly on the rise. Using US data from the 2007–2012 NSDUH, Ko et al. (2015) reported that 10.9% of pregnant women and 14% of nonpregnant women of childbearing age used marijuana in the past year and that 3.9% of pregnant and 7.6% of nonpregnant women used it in the past month. Among past-year marijuana users, near daily use was reported by 16.2% of pregnant women, and 18.1% met criteria for abuse and/or dependence. A 2010 study in the UK found that a majority of women who used cannabis prior to pregnancy continued both their use and their levels of usage while pregnant (Moore et al., 2010). An emerging concern is the use of marijuana to treat hyperemesis gravidarum or the more common “morning sickness,” with conflicting reports on its effectiveness (Westfall et al., 2006; Roberson et al., 2014).

Pharmacology and Biologic Actions

Marijuana refers to the dried leaves, flowers, stems, and seeds from the hemp plant *Cannabis sativa*, while hashish refers to the resin that the plant produces. The main psychoactive ingredient is δ -9-tetrahydrocannabinol (THC), a small, highly lipophilic molecule that binds to the endogenous cannabinoid receptors (CB1 and CB2) and modifies the release of several neurotransmitters. Its primary biologic effects include pleasant euphoria and a sense of relaxation; heightened sensory perception; increased appetite; laughter; altered perception of time; and impaired coordination, decision making, short-term memory, concentration, and learning. Less common effects include anxiety, fear, distrust, panic, and acute psychosis. Marijuana can be smoked in hand-rolled cigarettes (joints), pipes (bongs), or blunts (emptied cigars refilled with marijuana). To avoid inhaling smoke, vaporizers can be used to pull the active ingredients from the marijuana (vaping). Users can also mix marijuana in edibles such as brownies, cookies, or candy. Smoking THC-rich resins extracted from the marijuana plant, a practice called “dabbing,” is on the rise. These extracts can deliver very large amounts of THC to users.

Although controversial, there is emerging evidence that chronic marijuana users can develop dependence or addiction, as well as signs of withdrawal following abrupt discontinuation (Hasin et al.,

2015). Whether or not neonates who have had chronic perinatal marijuana exposure can experience withdrawal is unknown. THC can be stored in body tissues for as long as 30 days in chronic users. THC readily crosses the placenta and collects in the amniotic fluid, while its major metabolite does not. In an animal model, fetal plasma levels were approximately 10% of maternal levels after an acute exposure and higher after repetitive exposures (Bailey et al., 1987).

Complications of Pregnancy

There is limited evidence to suggest that marijuana use during pregnancy has significant perinatal effects. There is scant data regarding its potential effects on stillbirth, primarily because prior studies of marijuana use in pregnancy excluded women with stillbirth. Varner et al. (2014) recently reported an increased risk of stillbirth among women who used marijuana during pregnancy, as demonstrated by THC in umbilical cord homogenates, but there was partial confounding by maternal tobacco use. Studies regarding marijuana and preterm birth have yielded mixed results. Some studies suggest increased risk, while others demonstrate no association. This is probably due to poor quantification of marijuana exposure as well as lack of documentation of the indication(s) for preterm birth in many of these studies.

Regarding birth defects, the evidence is relatively clear: both prospective and retrospective studies have not demonstrated an association between marijuana use and major congenital anomalies. Unfortunately, most of these studies are based on birth defects registries with incomplete ascertainment of confounding factors and the potential for recall bias (Metz and Stickrath, 2015).

Fetal Growth

There have been numerous long-term outcome studies regarding the effect of marijuana use during pregnancy on fetal growth, and most have shown marginal to no effect (Metz and Stickrath, 2015). The Generation R Study, for example, used serial ultrasound (not birthweight) to assess fetal growth. Early gestation–exposed fetuses grew 11 g per week less than nonexposed fetuses; the effect was more pronounced if there was continued use throughout pregnancy. The clinical significance of this finding is questionable (El Marroun et al., 2009). More recently, Warshak et al. (2015) examined associations between marijuana exposure and adverse outcome, excluding women with polysubstance abuse and stratifying for concurrent tobacco use. They reported no increased risk of adverse obstetric outcomes or fetal anomalies but did note an increase in small for gestational age (SGA) infants and neonatal intensive care unit (NICU) admission.

Long-Term Effects of Perinatal Cannabis Exposure

The potential for marijuana use during pregnancy to cause adverse fetal effects is clear. Endocannabinoid receptors are found in the human fetal brain by the 14th week of gestation and while the role of these receptors is not well understood, they modulate neural proliferation, migration, and differentiation of lineage-committed cells. Both supraphysiological stimulation of the endogenous cannabinoid system and disruption of developing neurotransmitter systems by exogenous cannabinoids have the potential to adversely affect fetal brain development (Alpar et al., 2016).

Animal studies of maternal marijuana exposure have shown alterations in neurotransmitter and neuroendocrine systems of the offspring, especially the dopaminergic pathways (Fernandez-Ruiz et al., 1999) and marked increase in hyperactivity, and persistent effects on learning and memory (Antonelli et al., 2005; Moreno

et al., 2005). Jutras-Aswad et al. (2009) studied postmortem human fetal brains from 17–22 week elective terminations with a self-reported history of marijuana use during pregnancy. Reduced dopamine receptors in the amygdala were noted, with the effect most prominent in male fetuses. The finding correlated with the amount of marijuana used during pregnancy.

There have been several longitudinal studies of the effects of perinatal marijuana use and infant outcomes, including the Ottawa Prenatal Prospective Study, the Generation R Study, and the Maternal Health Practices and Child Development (MHPCD) study. These studies are flawed by several factors, including polysubstance use, variability in dosing and frequency, and reliance on self-reported use. In addition, there are the universal confounders for perinatal drug use studies such as, socioeconomic, genetic, or environmental factors. However, although the data are not strong and the effects are uncertain, these studies suggest that prenatal marijuana exposure may cause problems with neurodevelopment. The MHPCD study began subject recruitment in 1982 and continues to follow the large cohort of the offspring of pregnant women who used THC during the first trimester. At age 3 years, altered sleep patterns but no cognitive effects were reported; at age 6 years, decreased verbal reasoning if there was greater exposure; at age 10 years, decreased attention, more impulsivity, and worse academic performance in reading and spelling; and at age 14 years, lower scores in reading, math, and spelling and earlier onset of substance use. Most recently, it was reported that prenatal marijuana exposure was a significant predictor of offspring marijuana use at age 22 years (Sonon et al., 2015).

Prenatal marijuana exposure is reported to be associated with deficiencies in executive function in 9–12 year olds (Fried et al., 1998; Fried and Smith, 2001), and recent reports have found that prenatal marijuana exposure has a significant effect on school-age intellectual performance (Richardson et al., 2002; Goldschmidt et al., 2004, 2008).

Although current evidence suggests that the adverse effects of prenatal marijuana exposure are uncertain (Jaques et al., 2014; ACOG [American College of Obstetricians and Gynecologists], 2015b), this evidence is based upon studies that were often begun decades ago and thus may not be relevant to today's pregnant users. Today's marijuana is 6–7 times more potent than it was in the 1970s, and there are many more ways to consume cannabis now, some of which result in significantly higher levels, and longer duration, of the active ingredient. As Warner et al. (2014) so eloquently titled a 2014 review, "It's not your mother's marijuana."

Opioids (Including Prescription Painkillers)

History/Epidemiology

Opioid is a generic term applied to alkaloids derived from the opium poppy, to their synthetic analogues, and to compounds synthesized in the body. Opium derivatives have been used as analgesics for centuries and remain the most effective analgesics available. Opioids of clinical interest are morphine, heroin, methadone, meperidine, oxycodone, codeine, and fentanyl (Rudd et al., 2015). Perinatal problems associated with maternal opium use were first reported in the late 1800s. Since the 1950s, heroin use, particularly among women, has been endemic in most major US cities although heroin addiction during pregnancy was still relatively rare (Shankaran et al., 2007).

However, in recent years, there has been a significant increase in prescription opioid use and misuse in the United States, and this has resulted in an increase in opioid dependence and addiction.

According to the United Nations Office on Drugs and Crime, an emerging phenomenon among opioid-dependent drug users in the United States has been the replacement of synthetic opioids by heroin, due to both increased heroin availability and its lower cost. In addition, the reformulation of one of the main prescription opioids of abuse, OxyContin, now makes it more difficult to snort or inject (UN Office on Drugs and Crime, World Drug Report 2014).

Women of reproductive age have not been spared from this surge in opioid use and misuse. In a recent CDC report using commercial insurance claims and Medicaid data from 2008–2012, approximately 28% of privately insured and 39% of Medicaid-enrolled women 15–44 years old filled an opioid medication each year for five consecutive years. The most frequently prescribed opioids were hydrocodone, codeine, and oxycodone. There were significant regional and racial/ethnic differences; among privately insured women, opiate prescriptions were highest among those residing in the South and among Medicaid-enrolled women, prescriptions were highest among non-Hispanic whites. Women 15–19 years old, regardless of their healthcare coverage, were least likely to fill a prescription for an opioid (Ailes et al., 2015). Opioid prescriptions for pregnant women have similarly been increasing. Patrick et al. (2015) used prescription data for enrollees in the Tennessee Medicaid program between 2009 and 2011 and found that 28% of pregnant women filled at least one opioid prescription. Women who were prescribed opioids were more likely to have depression or anxiety disorder and to smoke tobacco, increasing the risk of their offspring developing NAS (Patrick et al., 2015). Bateman et al. (2014) studied opioid utilization in pregnancy in a large cohort of private insurance beneficiaries from across the United States from 2005–2011 and reported that opioids were dispensed to 14% of pregnant women, with approximately 6% receiving opioids during all three trimesters. The prevalence of exposure varied significantly by region, being lowest in the Northeast and highest in the South.

Pharmacology and Biologic Actions

Opioids are either drugs derived from the opium poppy or synthesized compounds that have similar biologic actions. The prototype opioid is morphine, and all opioids relate to it. For example, heroin (diacetylmorphine) exerts its effects by being metabolized to morphine, as does codeine, which is methylated morphine. Other opioids, such as methadone and oxycodone, are structurally unlike morphine but share its pharmacologic properties, because they stimulate similar opioid receptors. Specific opioid receptors (μ , δ , κ) have been identified in the nervous system and bowel that are activated by endogenous opioids, such as the naturally occurring endorphins and enkephalins (Vaccaro and Kastin, 2000). As modulators of the sympathoadrenal system, endogenous opioids are important during periods of diverse forms of stress. Activation of these receptors by the endogenous opioids has physiologic effects, including analgesia, drowsiness, respiratory depression, decreased gastrointestinal motility, nausea, vomiting, and alterations in the endocrine and autonomic nervous systems. Activation of these same endogenous opioid receptors by exogenous opioid drugs has similar clinical effects, producing euphoria, sleepiness, and decreased sensitivity to pain, as well as adverse effects such as constipation and nephrotic syndrome (ACOG, 2012).

The use of opioid drugs can result in the development of tolerance, physiologic dependence, and addiction. Tolerance leads to a shortened duration of the action of opioids and a decrease in the intensity of the drug's action, followed by the need for higher

doses to obtain the same clinical effect. Tolerance is believed to result from continued occupancy of the opioid receptor. Continuous administration of opioids therefore leads to the more rapid onset of tolerance (Suresh and Anand, 2001). With physiologic dependence, there is a need for further drug administration to prevent withdrawal symptoms (agitation, dysphoria, temperature instability). Addiction is a more severe form of dependence that involves a complex pattern of drug-seeking behavior (Christensen, 2008).

Complications of Pregnancy

Obstetric complications associated with maternal use of opioids include a higher incidence of spontaneous abortion, premature delivery, preterm labor, placental abruption, chorioamnionitis, impaired fetal growth, and fetal distress. In women who use opioids during pregnancy, the incidence of preterm labor and premature delivery ranges from 25%–35% (Fajemirokun-Odudeyi et al., 2006; Pinto et al., 2010). Maternal opioid use is also associated with higher rates of meconium-stained amniotic fluid, lower Apgar scores, and an increased incidence of syphilis, hepatitis C (HCV), and HIV infection at birth (Fajemirokun-Odudeyi et al., 2006). The rates of maternal complications of pregnancy are further increased when drug use is added to infection with HIV.

The etiology of these opioid-associated pregnancy complications is multifactorial. Maternal lifestyle, malnutrition, infections, and polydrug effects are likely to result in poor perinatal outcomes, including poor intrauterine growth and prematurity. Because the drug supply is often episodic, the pregnant addict is subject to episodes of withdrawal and overdose, thereby subjecting the fetus to intermittent episodes of hypoxia in utero, hindering growth and raising the risk of spontaneous abortion, stillbirth, fetal distress, and prematurity. Infants born to mothers who are addicted to opioids are more likely to be of low birth weight, to be premature, and to suffer from infection and perinatal asphyxia (Christensen, 2008; ACOG, 2012).

Fetal Growth

Initial reports from studies addressing the effects of maternal opioid use on the fetus suggested that infants exposed to opioids in utero have a higher incidence of IUGR and smaller head circumference (Fajemirokun-Odudeyi et al., 2006; Pinto et al., 2010). Pinto et al. (2010) reported an association with heroin abuse and methadone use and increased prematurity, low birth weight, and reduced fetal growth. However, other studies which controlled for confounding factors have not demonstrated a significant relationship between opioid use and prematurity, low birth weight, or IUGR (Sharpe and Kuschel, 2004; Minozzi et al., 2008).

Long-Term Effects of Perinatal Opioid Exposure

A number of studies suggest that exposure to exogenous opioids during fetal development may produce lifelong alterations in brain development. Significant developmental and learning deficits have been described in both methadone-exposed and heroin-exposed children. For example, Bunikowski et al. (1998) have reported a higher incidence of abnormalities in intellectual performance, developmental retardation, and neurologic abnormalities in a group of opioid-exposed infants compared with a control group. However, the exposed infants had a higher incidence of prematurity (38%) than the control infants (0) and had an unusually high incidence of seizures (15%). These important considerations temper the findings of that study.

A more recent report following a cohort of opioid-exposed infants documented lower Bayley Mental Development Index scores

at 18 months old, lower Stanford Binet Intelligence Scale scores at 3 years, and decreased scores on the Vineland Social Maturity Scale and Reynell Language Scale at 3 years (Hunt et al., 2008). However, a similar study showed no mental, motor, or behavioral deficits at 3 years after controlling for birthweight and environmental risk factors (Messinger et al., 2004). Other investigators have shown normal development for opioid-exposed infants during the first 2 years of life after data were controlled for socioeconomic status and birthweight (Behnke et al., 2013). Using regression analysis, researchers have shown that the amount of prenatal care obtained by the mother and the postnatal home environment were more predictive of the infant's future intellectual performance. Conversely, the amount of maternal opioid use during pregnancy was not found to be predictive. Ornoy et al. (2001) have shown that children exposed to heroin but adopted at an early age performed better on intelligence testing than did opioid-exposed infants who were raised in the homes of their biologic parents. The same investigators found a higher rate of attention deficit disorders in children exposed to opioids regardless of home environment, with the highest incidence in children who were raised in the homes of their biologic parents. Numerous studies point also to the importance of the home environment in optimizing child development. Variable outcomes of these studies may depend on the amount and type of drug use or on other covariates such as nutritional status, poverty, psychosocial problems, and parental educational level.

Cocaine

History/Epidemiology

The euphoria-producing effect of cocaine was exploited extensively in the United States in the late 19th and early 20th centuries, when the agent was an active ingredient in a number of widely used over-the-counter elixirs and tonics. Cocaine use markedly decreased after the Harrison Narcotic Act of 1914 and the supervening Comprehensive Drug Abuse Prevention and Control Act of 1970, which classified cocaine as a schedule II drug (i.e., one of "high abuse potential with restricted medical use," similar to opioids, barbiturates, and amphetamines). Cocaine's reputation as a glamour drug, the widely held misconception that cocaine is not addictive, and the development and marketing of "crack," a cheap version of cocaine, were major factors in the resurgence in the use of the drug. Growing concern regarding the effects of maternal cocaine use on pregnancy outcomes was one of the reasons that the US Congress passed the 1986 Narcotics Penalties and Enforcement Act, which imposed severe penalties on any person convicted of either possessing or distributing the drug; however, this law did not appreciably alter the fact that cocaine and other stimulants had become the drugs of choice for women in the United States. By the 1990s, studies based on urine toxicology screening reported a prevalence of cocaine use among pregnant women of 5% in New York City, 1.1% in a geographic sample in California, and less than 0.5% in private hospitals in Denver, Colorado (Burke and Roth, 1993). Cocaine use has been declining in North America since 2006, according to the 2014 United Nations World Drug Report, partly due to a sustained shortage, although a slight increase in prevalence has been observed in the United States, along with an increase in maritime cocaine seizures (United Nations World Drug Report, 2014).

Pharmacology and Biologic Actions

Cocaine is a highly psychoactive stimulant with a long history of abuse. A naturally occurring anesthetic of the tropane family of

alkaloids, cocaine is obtained from the *Erythroxylon coca* plant, which is indigenous to the mountain slopes of Central and South America. The coca leaf has been chewed or made into a stimulant tea by the natives of these areas to decrease fatigue and hunger. The pharmacologic actions of cocaine include inhibition of postsynaptic reuptake of norepinephrine, dopamine, and serotonin neurotransmitters by sympathetic nerve terminals, thus allowing higher concentrations of these neurotransmitters. In adults, cocaine binds strongly to neuronal dopamine reuptake transporters, thereby increasing postsynaptic dopamine at the mesolimbic and mesocortical levels and producing the addictive cycle of euphoria and dysphoria (Malanga and Kosofsky, 1999). Tryptophan uptake is similarly inhibited, altering serotonin pathways with resultant effects on sleep.

Cocaine use leads to a sense of well-being, increased energy, increased sexual achievement, and an intense euphoria or “high.” The sympathomimetic action can have potentially devastating physiologic effects on the cardiovascular system. In adults, cocaine has been associated with cerebral hemorrhage, cardiac arrest, cardiac arrhythmias, myocardial infarction, intestinal ischemia, and seizures. Chronic use is associated with anorexia, nutritional problems, and paranoid psychosis and can ultimately result in neurotransmitter depletion and a “crash,” characterized by lethargy, depression, anxiety, severe insomnia, hyperphagia, and cocaine craving.

Two forms of cocaine are commonly used – cocaine hydrochloride and cocaine base – which are either extracted by organic solvents or precipitated as crack through the use of ammonia and baking soda. Cocaine hydrochloride is a water-soluble white powder that is used orally, intranasally (“snorting”), or intravenously (“running”). Intravenous users are more likely to have a history of heroin abuse and often use the drug in combination with heroin (known as *speedballing*). Cocaine hydrochloride decomposes on heating and is, therefore, cocaine converted to the free base for inhalation. “Freebasing” involves extracting cocaine from aqueous solution into an organic solvent such as ether. Crack, the most widely available form of freebase, is almost pure cocaine; when smoked, it readily enters the bloodstream to produce levels similar to those occurring with intravenous use. Crack cocaine is popular in urban minority communities, where it may be smoked in combination with phencyclidine or PCP (known as *spacebasing*). Crack smoking appears to be particularly reinforcing and is associated with compulsive use, binges, and acceleration of the addictive process.

Cocaine and some of its metabolites readily cross the placenta and achieve pharmacologic levels in the fetus (Schenker et al., 1993). Amniotic fluid may serve as a reservoir for cocaine and its metabolites and prolong exposure to vasoactive compounds. The extent to which cocaine or its metabolites are responsible for aberrant fetal growth, neurodevelopmental sequelae in exposed infants, and the range of congenital malformations reported in the literature may be less than suggested by uncontrolled case reports early in the cocaine-epidemic era. The confounding effects of increased use of multiple drugs, tobacco, alcohol, nutritional deficits, and decreased use of prenatal care among cocaine users make interpretation of the causal relationships between gestational cocaine exposure and intrauterine growth and subsequent neurobehavioral development difficult (Pinto et al., 2010). These identified confounders might serve to explain the reported effects attributed to cocaine in clinical series, although significant effects on the fetus and newborn have been reported in more recent studies that controlled for many confounders (Bada et al., 2002; Shankaran et al., 2007).

Complications of Pregnancy

Adverse perinatal outcomes associated with cocaine use are believed to be largely due to the vasoconstrictive effects of cocaine on uterine blood supply (Woods et al., 1987). An increase in maternal mean arterial blood pressure, a decrease in uterine blood flow, and a transient rise in fetal systemic blood pressure after an intravenous cocaine infusion have been described in fetal sheep along with significant fetal hypoxemia associated with changes in uterine blood flow (Woods et al., 1987). Maternal hypertension and intermittent fetal hypoxia contribute to the higher risks for placental abruption and IUGR seen in cocaine-exposed infants (Pinto et al., 2010; Behnke et al., 2013).

To date, no well-defined cocaine-associated syndrome has been identified, and the teratogenic potential of cocaine remains controversial. Earlier reports had suggested that cocaine-exposed infants had a higher rate of limb reduction anomalies, heart defects, ocular anomalies, intestinal atresia or infarction, and other vascular disruption sequences. However, the preponderance of more recent data from multiple studies has failed to demonstrate higher rates of other congenital anomalies among cocaine-exposed infants (Behnke et al., 2013).

Women who use cocaine during pregnancy are at higher risk for stillbirths, spontaneous abortions, placental abruption, IUGR, anemia and malnutrition, and maternal death from intracerebral hemorrhage. Cocaine directly stimulates uterine contractions because of its α -adrenergic, prostaglandin, or dopaminergic effects, with resulting greater risk for fetal distress and premature deliveries. Placental abruption appears to be related to cocaine use only when the drug is used shortly before delivery (Ostrea et al., 1992). Cocaine use significantly increases the odds ratio (OR) for prematurity, low birth weight, premature rupture of membranes, and IUGR (Bada et al., 2002, 2005; Shankaran et al., 2007; Pinto et al., 2010).

Overall, because of the higher risks of premature delivery, the frequency of respiratory distress syndrome is greater in cocaine-exposed infants. Cocaine-exposed infants less frequently require surfactant administration and intubation for respiratory distress syndrome; however, the risks of bronchopulmonary dysplasia are similar in infants who have and those who have not been exposed to cocaine during gestation (Hand et al., 2001).

Fetal Growth

Infants exposed to cocaine in utero have lower birthweight, smaller birth length, and smaller head circumference (Bada et al., 2002, 2005; Pinto et al., 2010; Behnke et al., 2013). Cocaine is hypothesized to reduce fetal growth via vasoconstriction of uteroplacental vessels with consequent decreased fetal substrate and oxygen delivery (Schempf, 2007). Several studies have shown a dose-response effect of cocaine exposure on fetal growth. In the Maternal Lifestyle Study, cocaine-exposed infants were 1 week younger in GA, and after controlling for confounders, cocaine exposure was associated with decrements in birthweight (151 g), length (0.71 cm), and head circumference (0.43 cm) at 40 weeks' gestation (Shankaran et al., 2007). After adjusting for the effects of birthweight, GA, sex, maternal height, maternal weight gain, and other drug use, newborns with a high exposure to cocaine, as measured by radioimmunoassay of cocaine metabolites in maternal hair, had a disproportionately smaller head circumference even for their birthweight, resulting in “head wasting” (Bateman and Chiriboga, 2000).

Long-Term Effects of Perinatal Cocaine Exposure

No major neurologic deficits in motor development have been reported after in utero exposure to cocaine. Although infants exposed

to cocaine were reported to have lower motor skills at 1-month testing, they displayed significant improvements over time. Both higher and lower levels of tobacco use were related to poor motor performance (Shankaran et al., 2007).

Subtle neurobehavioral abnormalities have been reported in older studies and more recently using the NICU Network Neurobehavioral Scale (NNNS) to evaluate infants in the month after birth. Cocaine-exposed infants manifest a range of neurobehavioral abnormalities that were initially described as drug withdrawal but are more likely caused by acute intoxication. Signs are present at birth and wane as cocaine and the metabolite benzoylecgonine are cleared from plasma. The infants are hypertonic, irritable, and tremulous (Hudak et al., 2012), and they may have abnormal crying, sleep, and feeding patterns. Tachycardia, tachypnea, and apnea have been noted in two blinded, controlled studies, with significant elevations in cardiac output, stroke volume, mean arterial blood pressure, and cerebral artery flow velocity resolving by day 2, which is consistent with an intoxicant effect of cocaine (van de Bor et al., 1990a, 1990b). Cocaine-exposed infants may have abnormal electroencephalograms or clinical seizures, perhaps the result of toxicity from the metabolite benzoylecgonine; however, neonatal seizures attributable directly to maternal use of cocaine are rare (Legido et al., 1992).

Persistent behavioral, neurologic, and rearing problems have been reported in children exposed to both cocaine and opioids (Hunt et al., 2008). No significant differences in mean developmental scores were noted in a group of children exposed to cocaine plus polydrugs compared with a group without drug exposure. Other investigators reported no differences between infants who have and those who have not been exposed to cocaine in mean cognitive, psychomotor, or language quotients at age 36 months (Kilbride et al., 2000).

The neurodevelopmental problems among children exposed to cocaine may occur either from a direct teratogenic drug effect during gestation or from the effects of the social environment in which the developing infant is reared (Behnke et al., 2013). Singer et al. (2002) reported that cocaine-exposed infants are twice as likely to have significant cognitive but not motor delays at 2 years, and they demonstrated a downward trend in mean developmental scores by 2 years, which is consistent with a deleterious effect of the environment, parental stimulation, socioeconomic status, or possibly other, indirect effects of drugs on the developing CNS. Other studies have not consistently demonstrated this association (Frank et al., 2001). In contrast, the Maternal Lifestyle Study, after controlling for covariates, found no differences in Bayley scores between cocaine-exposed, opioid-exposed, and control infants for the first 3 years of life (Shankaran et al., 2007), although subtle effects on cognitive subscales were reported at 3 years (Messinger et al., 2004).

A more recent report of volumetric magnetic resonance imaging in thirty-five 12-year-old children exposed to cocaine in utero found smaller total parenchymal volumes, lower cortical gray matter volumes, and smaller head circumferences with prenatal substance exposure. The decreases were statistically significant only for prenatal cigarette exposure and for infants exposed to all substances studied (cocaine, tobacco, marijuana, and alcohol) (Rivkin et al., 2008). As in other studies, exposure to multiple substances clearly has more potential to have detrimental effects on the developing brain.

Amphetamines

History/Epidemiology

Amphetamines have surpassed cocaine as the primary illicit drugs used by pregnant women in many states. Methamphetamine (or

“crystal”) has been the primary form abused, because it can be produced locally and fairly cheaply. Greater restrictions on the importing of cocaine have also contributed to resurgence in amphetamine use. Amphetamines have always been popular among adolescents, especially females, and, accordingly, women of child-bearing age are at high risk for perinatal abuse. The Infant Development, Environment, and Lifestyle (IDEAL) study chose four major US cities with known methamphetamine-abusing populations and reported methamphetamine use in 5.2% of pregnant women. As in previous reports, these women frequently used other substances: 25% used tobacco, 22.8% used alcohol, 6% used marijuana, and 1.3% used barbiturates (Arria et al., 2006).

Pharmacology and Biologic Actions

Amphetamine (α -methylphenethylamine) was synthesized in 1887 and introduced in the United States in 1931. The *N*-methylated form, methamphetamine (or “crystal”), is increasingly abused because it readily dissolves in water for injection and it sublimates (converts directly from a solid to gas) when smoked (known as *ice*). The amphetamine isomers have similar clinical effects and can be distinguished only in the laboratory. Amphetamines were initially marketed for the treatment of obesity and narcolepsy and continue to be used for the treatment of attention deficit disorders in children. Amphetamines are classified as schedule II drugs, like cocaine and narcotics. Amphetamines are taken orally, inhaled, or injected. The clinical effects and toxicity of these agents are often indistinguishable from those of cocaine. The primary difference is in the duration of action. The psychotropic effects of cocaine are of a short duration, 5–45 minutes. The effects of amphetamines may last from 2–12 hours. Methamphetamine exposure has direct and indirect effects on the fetus, with increases in maternal blood pressure and restrictions in delivering nutrients and oxygen to the fetus (Smith et al., 2003). The clinical effects of amphetamines resemble those of cocaine. Like cocaine, amphetamines are sympathomimetics, and they potentiate the actions of norepinephrine, dopamine, and serotonin. In contrast to cocaine, amphetamines appear to exert their CNS effects primarily by enhancing the release of neurotransmitters from presynaptic neurons. Amphetamines can block reuptake of released neurotransmitters; they can also exert a weaker direct stimulatory action on postsynaptic catecholamine receptors.

Complications of Pregnancy

The medical and obstetric complications of amphetamine use are similar to those described for cocaine use. Amphetamine toxicity has been described as more intense and prolonged than cocaine toxicity. Visual, auditory, and tactile hallucinations are common, and microvascular damage has been seen in the brains of chronic users. Amphetamine withdrawal is characterized by prolonged periods of hypersomnia, depression, and intense, often violent paranoid psychosis. Obstetric complications include a higher incidence of stillbirth. Methamphetamine use is also associated with an increased incidence of premature delivery and placental abruption. Methamphetamine users who stop using earlier in gestation have rebound weight gain, suggesting that the anorexic effects are limited to continuous use (Smith et al., 2003). Like the pregnancies of cocaine users, the pregnancies of amphetamine users are characterized by poor prenatal care, sexually transmitted diseases, and cardiovascular problems including placental abruption and postpartum hemorrhage. The risk of cerebrovascular accidents is lower in pregnant amphetamine users than in pregnant cocaine users, but the mechanism for this difference is not understood.

Perinatal problems associated with maternal amphetamine use include prematurity and IUGR (Smith et al., 2006; Behnke et al., 2013). Fetal growth restriction, leading to smaller head circumference and lower birthweight, can result from the vasoconstrictive effects of norepinephrine or other vasoactive amines or from diminished maternal nutrient delivery as a consequence of the anorectic effect of amphetamine. Systemic effects from altered norepinephrine metabolism explain the transient bradycardia and tachycardia reported in exposed infants. Studies have failed to show consistent patterns of malformations in amphetamine-exposed infants, although several studies report cleft lip and cleft palate in association with amphetamine and methamphetamine exposure during early gestation (Behnke et al., 2013).

Fetal Growth

The IDEAL study followed 84 methamphetamine-exposed and 1534 unexposed infants (confirmed by screening for meconium). Both groups included alcohol, tobacco, and marijuana use but excluded opioids, PCP, and lysergic acid diethylamide (LSD) (Smith et al., 2003, 2006; Arria et al., 2006). Cocaine and methamphetamine use occurred together in 13% of the methamphetamine users, and tobacco, alcohol, and marijuana use were also more frequent in the methamphetamine users. Methamphetamine-exposed infants were 3.5-fold more likely to be SGA (less than the 10th percentile; 18% incidence). Methamphetamine contributed significantly to low birth weight, even after correcting for confounders such as low socioeconomic status, GA, and tobacco exposure.

Long-Term Effects of Perinatal Amphetamine Exposure

Early neurobehavioral changes have been associated with maternal methamphetamine use; heavy use was associated with lower arousal, increased lethargy, and increased CNS stress (Smith et al., 2008). However, a detailed analysis in a subgroup of the same population found that these same abnormalities on the NNNS were associated with maternal depression and that prenatal methamphetamine exposure was not associated with additional neurodevelopmental differences (Paz et al., 2009).

In methamphetamine-exposed infants, neurodevelopmental abnormalities have been described to persist as late as 14 years (Cernerud et al., 1996). Intellectual capacity does not appear to be diminished among exposed infants. These children are described as exhibiting disturbed behavior, including hyperactivity, aggressiveness, and sleep disturbances. Eriksson et al. (2000) reported that neurobehavioral abnormalities appear to be associated with the extent and duration of fetal exposure and with the severity of head growth restriction. In this study, children with the most severe problems were those born to mothers who abused amphetamines throughout pregnancy and were reared in homes with an addicted parent. Alterations in growth have been reported after prenatal exposure, with striking gender differences (Cernerud et al., 1996). Drug-exposed boys in Sweden were taller and heavier, and girls were smaller and lighter, than national standards. This finding suggests that fetal amphetamine exposure affects the onset of puberty, and amphetamines may interfere with neurodevelopment of the adenohypophysis. Children of amphetamine abusers appear to be at high risk for social problems, including abandonment, abuse, and neglect.

Selective Serotonin Reuptake Inhibitors

Prescribed medications can also have significant fetal and neonatal effects. The prevalence of depression during pregnancy is estimated

to be 14%–23%, and in the United States up to 13% of pregnant women use antidepressants during pregnancy (Yonkers et al., 2009). Selective serotonin reuptake inhibitors (SSRIs) are the most commonly prescribed antidepressants. They selectively inhibit reuptake of serotonin in presynaptic nerve terminals and increase the synaptic concentration of serotonin. They are better tolerated and have fewer side effects than tricyclic antidepressants because they do not affect other neuroreceptors (Lattimore et al., 2005). Animal studies have shown that serotonin appears as a neurotransmitter in the early fetal brain, but its role in development is not clearly delineated. Fluoxetine and its active metabolite cross the placenta and enter the fetal brain (Lattimore et al., 2005), although the significance of this finding is not known.

Studies of SSRI use in pregnant women have not shown any consistent pattern of malformations with first trimester use (Lattimore et al., 2005; Yonkers et al., 2009). There are no adverse pregnancy outcomes associated with SSRI use, including no evidence of growth restriction or increased fetal loss. Longer in utero exposure to antidepressants is more likely to result in earlier delivery, and low birth weight is increased (Lattimore et al., 2005; Yonkers et al., 2009).

The effects of maternal SSRI use, if any, are typically seen in the early neonatal period, with exposed infants being more likely to be admitted to an intensive care nursery. Poor neonatal adaptation is increased and includes tachypnea, irritability, jitteriness, hyper-tonia, sleep disturbance, temperature instability, poor feeding, hypoglycemia, and seizures. These neonatal symptoms are consistent with a gradual resolution of a hyper serotonergic state rather than a withdrawal syndrome and usually resolve spontaneously within the first 2 weeks (Lattimore et al., 2005; Moses-Kolko et al., 2005; Hudak et al., 2012). A small increase in risk for persistent pulmonary hypertension of the newborn has been associated with maternal SSRI use late in pregnancy (Huybrechts et al., 2015). A small study of neurodevelopment found possible motor effects in the first 18 months, but larger studies are needed (Lattimore et al., 2005; Yonkers et al., 2009).

Identifying Pregnancies and Babies at Risk

Pregnancies

Identification of perinatal substance abuse, in order to intervene and protect the health and well-being of both mother and child, has been a goal of practitioners for decades, ever since the scope of the problem became known – particularly for alcohol. Urine toxicology testing was initially believed to be the best approach, and the universal approach was used in many busy labor and delivery units, particularly in urban centers. In 1994, an ACOG Technical Bulletin concluded that urine toxicology testing had limited ability to detect substance abuse and therefore recommended against universal toxicology screening and for alternative screening methods. In 2003, the US Congress passed the Keeping Children and Family Safe Act. This law requires each state (as a condition of receiving federal funds under the Child Abuse Prevention and Treatment Act) to develop policies and procedures designed “to address the needs of infants born and identified as being affected by illegal substance abuse or withdrawal symptoms resulting from prenatal drug exposure.” This law included a requirement that healthcare providers notify child protective services regarding prenatal substance exposure but differed from the providers’ legal responsibility to report suspected child abuse or neglect because the former “shall not be construed to be child abuse” and “pregnant

TABLE 13.3 Risk Indicators for Gestational Substance Exposure

| | |
|----------|---|
| Maternal | No or late prenatal care Precipitous labor Placental abruption Repeated spontaneous abortions Hypertensive episodes Severe mood swings Previous unexplained fetal demise Myocardial infarction or stroke |
| Newborn | Jittery, with normal blood glucose level Marked irritability with excessive crying NEC in an otherwise healthy term infant Neurobehavioral abnormalities Significant hypotonia or hypertonia Unexplained IUGR Unexplained poor suck and feeding Unexplained seizures or apneic spells Clinical signs of narcotic withdrawal |

IUGR, Intrauterine growth restriction; NEC, necrotizing enterocolitis.

women should not be punished for adverse perinatal outcomes” (Washington State Department of Health, 2016). Each state was expected to develop its own guidelines for identification of at-risk pregnancies. A 2015 ACOG Committee Opinion (2015a) stated that best practices included universal screening questions followed by brief interventions or referrals. A number of screening tools have been developed including T-ACE (tolerance, annoyed, cut down, eye-opener), TWEAK (tolerance, worried, eye-opener, amnesia, K/cut down), and 4Ps Plus (parents, partner, past, pregnancy + assess for substance abuse, domestic violence) (Chang, 2001; Chasnoff et al., 2005). Identification of at-risk pregnancies concentrated on the maxim “if you don’t ask, they won’t tell.” The universal use of standard screening questionnaires is strongly recommended for obstetricians (ACOG, 2011, 2012, 2015a). Therefore a history of drug and alcohol use should be routinely included in the initial contact with every pregnant patient. To be effective, the history taking must be nonjudgmental and must occur in the context of other lifestyle questions. When a positive history of use is obtained, intervention should begin immediately. The person taking the history should be prepared to offer preliminary counseling on risk reduction and concrete referrals for treatment programs, although access to drug programs is often restricted, inadequate, or delayed.

Although the screening questionnaire approach works well for women who seek prenatal care, it is not useful for the significantly higher percentage of substance-using pregnant women who do not seek or actively avoid prenatal care. For these women, it is more helpful to identify maternal risk indicators for perinatal substance abuse at the time of delivery. Use of one of the screening questionnaires, in addition to drug testing, increases the likelihood of identifying at-risk pregnancies and neonates, allowing for earlier referral for treatment or specialized interventions. Several of these maternal risk indicators for perinatal substance abuse were identified in the 2004 ACOG statement (Table 13.3) (ACOG, 2015a).

Babies

It is important for practitioners to know the difference between a substance-exposed newborn and a substance-affected newborn.

The Washington State Department of Health (2016) defines them as follows:

- Substance exposed:
 - tests positive for substances at birth *or*
 - mother tests positive for substances at time of delivery *or*
 - is identified by medical practitioner as having been prenatally exposed to substances
- Substance affected:
 - has withdrawal symptoms resulting from prenatal substance exposure *or*
 - demonstrates physical and behavioral signs that can be attributed to prenatal exposure to substances and is identified by a medical practitioner as affected

For the identification of either of these groups of infants, assessment of both maternal and newborn risk indicators (see Table 13.3) is essential. If risk indicators suggest perinatal substance abuse, then consideration should be given to newborn drug testing. For urine testing, there is a poor correlation between maternal and newborn tests. The earliest newborn urine will contain the highest concentration of substances, but the first urination may be missed and urine output in the first day is often scant. However, some drug metabolites such as cocaine are present for 4–5 days, and marijuana metabolites may persist for weeks. The disadvantages of newborn urine drug testing are that it primarily reflects substance exposure during the preceding 1–3 days, and alcohol is nearly impossible to detect. Meconium drug testing (at term) reflects substance exposure during the second half of gestation, has a high sensitivity for opioids and cocaine, and can assess for more drugs than urine testing. The cost is similar to newborn urine testing, but it generally takes longer to obtain results. Other newborn drug tests include hair – which is costly and has a high sensitivity for cocaine, amphetamines, and opioids but not for marijuana – and umbilical cord segments, which is an evolving technology that is becoming widely available and appears to reflect earlier exposure and gives results similar to meconium testing (Farst et al., 2011).

Pregnancy Management

Ideally, perinatal substance use or abuse should be identified by universal screening procedures during prenatal visits; this gives the practitioner an opportunity to intervene, with the goal being prevention of significant obstetric and neonatal complications related to substance abuse. Screening procedures and counseling should include the following topics:

Antepartum

- Initial screening for hepatitis, HIV, and tuberculosis (if not part of routine prenatal care) and ongoing screening for sexually transmitted infections
 - Referral to drug dependency/addiction treatment program, if appropriate
 - Discussion of possible drug effects on the fetus and newborn
 - Discussion of contraception and prevention of sexually transmitted disease
 - Discussion of breastfeeding issues related to alcohol and drug use
- ### Intrapartum
- Effects of recent drug use on labor and fetal well-being
 - Pain management (women in methadone or alternative opioid treatment programs will be less responsive to opioid pain medications)
 - Intrapartum prophylaxis for HIV and herpes simplex virus infections
 - Need for social services involvement

Postpartum

- Breastfeeding issues (related to both drug use and infection)
- Contraception and pregnancy prevention including tubal ligation
- Support for continuation in, or initiation of, a drug treatment program
- Child Protective Services notification

Maternal Treatment for Opioid Addiction

Methadone Maintenance

Methadone maintenance continues to be the primary treatment for opioid dependence. Potential benefits of maternal methadone maintenance are numerous and include the prevention of opioid withdrawal symptoms in the mother, better medical and prenatal care, improved health and growth of the fetus, and a decrease in both the use of illicit drugs and the potential for perinatal infections (ACOG, 2012). Methadone maintenance programs associated with comprehensive medical and psychosocial services for the pregnant woman are even more beneficial.

Detoxification of a pregnant heroin user is infrequently attempted because maternal drug withdrawal is believed to be associated with subsequent fetal withdrawal, fetal asphyxia, and spontaneous abortions (ACOG, 2012). In the United States, most pregnant, narcotic-addicted women are treated with daily methadone rather than a program of detoxification. Some authorities, however, have urged the reappraisal and reevaluation of the benefits of methadone maintenance in pregnancy.

In the Netherlands, women enrolled in a methadone program had higher rates of prenatal care, which were associated with higher birthweights and reduced prematurity in the offspring (Soepatmi, 1994). Others have shown that higher methadone doses are associated with improved head circumference and increased GA at delivery (Hagopian et al., 1996). Improved birthweights are believed to be to the result of a stable intrauterine environment uncomplicated by periods of intoxication and withdrawal, as well as less stress and better nutrition in the mother (ACOG, 2012).

Several investigators have found that neonatal withdrawal symptoms, birthweight, length of pregnancy, and the number of days infants require treatment for abstinence do not correlate with maternal methadone dosage (Cleary et al., 2010). Studying maternal and neonatal serum levels of methadone does not help clarify this dilemma. Investigators have found no correlation between neonatal serum levels of methadone and the maternal methadone dose at delivery, the maternal serum levels, or the severity of withdrawal symptoms in the neonates (Hudak et al., 2012). Other researchers have reported that neonatal signs of withdrawal correlate with the rate of decline of the neonatal plasma level during the first few days of life (Doberczak et al., 1993).

There are no definitive guidelines for methadone doses during pregnancy, and there is continuing controversy over the most appropriate dose of methadone maintenance during pregnancy. Current recommendations are to adjust the methadone dose to avoid withdrawal symptoms and decrease the likelihood of maternal use of illicit drugs (ACOG, 2012). High-dose methadone maintenance ranges between 60 and 150 mg/day. Medically supervised withdrawal from opioids in the pregnant women is not recommended due to high relapse rates (Jones et al., 2008b).

There is a growing body of literature promoting breastfeeding for infants of mothers in methadone maintenance programs as both beneficial and safe. Studies have shown that breastfed infants tend to have less need for pharmacotherapy for NAS, despite low

and unpredictable levels of methadone in breast milk and in infant serum. Maternal serum levels, breast milk levels, and infant serum levels do not correlate with maternal methadone dose (Jansson et al., 2008; Hudak et al., 2012).

Buprenorphine (Suboxone, Subutex)

Buprenorphine is an alternative opioid substitute that was first introduced in France in 1996. It is increasingly used instead of methadone for the treatment of opioid addiction because it has fewer autonomic side effects than methadone, lower risk of overdose, fewer drug interactions, and improved compliance and treatment efficacy (ACOG, 2012). Recent studies comparing methadone with buprenorphine in pregnancy found that both agents improve pregnancy outcome with decreased risk of growth restriction, abortion, preterm labor, and fetal death (Jones et al., 2010; Noormohammadi et al., 2016). Small randomized trials have compared methadone with buprenorphine treatment during pregnancy and found no difference in the neonatal outcomes (Jones et al., 2008a; Minozzi et al., 2008). The larger Maternal Opioid Treatment: Human Experimental Research (MOTHER) trial (Jones et al., 2010) found that although the infants exposed to buprenorphine or methadone were treated for abstinence syndrome at the same rate, infants exposed to buprenorphine received significantly lower total amounts of morphine and had a shorter hospital length of stay. The difference in length of stay was not significant after post hoc corrections for level of opioid dependence (Noormohammadi et al., 2016). A systematic review in 2014 concluded that risk of infant treatment for withdrawal, total morphine dose, and length of hospital stay were lower in infants of buprenorphine-treated women, but confounding variables contributed to these findings (Brogly et al., 2014). Disadvantages of buprenorphine use are the lack of long-term data on infant and child outcomes and increased patient dropout rate because of dissatisfaction with the drug effect. If a woman is well controlled on methadone before pregnancy, continued methadone maintenance is recommended (ACOG, 2012).

Human Immunodeficiency Virus and Other Viral Infections

Nationwide, intravenous drug abusers are the second largest risk group for HIV infection and the largest risk group for HCV infection. In the United States, other risk factors for HIV are multiple sexual partners. Since the mid-1990s, the rate of mother-to-child transmission has decreased significantly due to implementation of antenatal HIV testing and treatment during pregnancy, labor, and in the neonatal period. However, most mother-to-child transmission occurs during the intrapartum period, and there is a significant risk of transmission through breastfeeding (American Academy of Pediatrics Committee on Infectious Diseases, 2015b). Every infant born to a substance abuser should be evaluated for HIV infection, and universal precautions should be observed. The AAP Committee on Infectious Diseases (2015b) recommends rapid HIV testing of any mother whose HIV status is not known, with appropriate consent as required by local law. Many states have now adopted a policy promoting testing with an option to opt out, rather than requiring consent for testing (Branson et al., 2015).

Intravenous drug use places the woman at risk for multiple infectious complications, including cellulitis, thrombophlebitis, hepatitis, endocarditis, syphilis, gonorrhea, and AIDS. In a prospective study undertaken in Canada, a 5-year incidence of HIV seroconversion was 13.4%; the rate of conversion associated with injection of heroin or cocaine was 40% higher in women than in

men (Spittal et al., 2002). Opioid abusers are also less likely to receive prenatal care or to obtain late prenatal care (ACOG, 2012). Heroin-addicted mothers are often poorly nourished, have iron-deficiency anemia, and are hepatitis positive when compared with nonusers.

HCV is another chronic infectious condition that is spread by parenteral exposure to infected blood and can be acquired by the newborn in the perinatal period. The most common route for HCV infection of children is maternal-to-child transmission. The most common risk factors for adults acquiring infection are injection drug use or multiple sexual exposures in individuals with concomitant HIV infection. The 2015 AAP Red Book (American Academy of Pediatrics Committee on Infectious Diseases, 2015a) states that “seroprevalence [of HCV] among pregnant women in the United States has been estimated at 1% to 2%,” and the risk of perinatal transmission averages 5%–6% from women who are HCV-RNA positive at the time of delivery. Maternal coinfection with HIV has been associated with increased risk of perinatal transmission of HCV. Antibodies to HCV and HCV RNA have been detected in colostrum, but the risk of HCV transmission is similar in breastfed and bottlefed infants, so breastfeeding is currently allowed.

Neonatal Management After Gestational Substance Exposure

General

Although at higher risk for medical complications, the majority of infants of drug-using women do not require intensive neonatal care; however, symptomatic infants often need more nursing care. Physical examination on admission should document a GA assessment, birthweight, head circumference, and length. Infants should be examined carefully for evidence of malformations, dysmorphic facial features, or both. Studies such as electroencephalography and brain imaging may add diagnostic or prognostic information when physical or neurologic abnormalities are not consistent with drug exposure, but these procedures are not indicated for most drug-exposed infants. If indicated by neonatal or maternal risk indicators, toxicology testing should be performed on neonatal urine, meconium, and/or umbilical cord segments as soon as possible after birth. Infants whose mothers were not screened for HIV should undergo screening for perinatal HIV exposure and other infections such as hepatitis B, HCV, and syphilis, as clinically indicated. In some states, rapid testing of the newborn for HIV is “required by law if the mother refuses to be tested” so that appropriate treatment of the infant can be started before 12 hours of age. Rapid screening detects only HIV-1, the most common serotype of HIV in the United States, but it can miss HIV-2 (American Academy of Pediatrics Committee on Infectious Diseases, 2015a).

Cocaine-exposed infants weighing more than 1500 g have longer hospital stays and increased need for therapies, procedures, intravenous fluid, and formula feeding. These infants also undergo more investigations for sepsis, more NICU admissions, and more social and family problems delaying discharge (Bada et al., 2002). In the Maternal Lifestyle Study, cocaine-exposed infants had a higher frequency of infection (odds ratio [OR] 3.1, 99% confidence interval [CI] 1.8–5.4) and neurologic signs and symptoms (adjusted OR 1.7, 99% CI 1.0–2.1). Neurologic signs were highest in the infants exposed to opioids and cocaine but remained significantly increased in infants exposed to cocaine alone. Smoking also increased

the risk for neurologic signs and symptoms (Shankaran et al., 2007). The association between cocaine exposure and fetal hypoxic-ischemic episodes creates special concerns. Maternal cocaine use exposes infants to a higher than expected risk of problems with postasphyxial syndrome, and organ dysfunction from hypoxic-ischemic injury should be investigated and treated. Feedings in premature infants with cocaine exposure should be started cautiously, because premature infants exposed to cocaine may be at increased risk for necrotizing enterocolitis. In addition, after controlling for gender, GA, birthweight, maternal parity, ethnicity, and polydrug use, heavy cocaine use during pregnancy was associated with a slightly higher risk of subependymal hemorrhage (Shankaran et al., 2007).

Using the NNNS, subtle differences in behavior have been detected in drug-exposed newborn infants (Lester et al., 2002; Smith et al., 2008). Cocaine-exposed infants showed lower arousal, and with heavy cocaine use they showed lower regulation and higher excitability than did unexposed infants (Lester et al., 2002). There were no stress or abstinence signs associated with cocaine exposure. Marijuana use was associated with more stress and abstinence signs and higher excitability scores (Lester et al., 2002). Low birth weight was also significantly correlated with poorer regulation and higher excitability. Methamphetamine exposure was also associated with increased stress signs, especially in first-trimester use. Heavy methamphetamine use was related to lethargy, lower arousal, and increased physiologic stress (Smith et al., 2008). Neonatal neurologic abnormalities similar to a mild withdrawal syndrome, consisting of hypertonicity, irritability, and jitteriness, have been reported after in utero marijuana exposure but without documented evidence of long-term sequelae (Hudak et al., 2012). Finally, gestational nicotine exposure and SSRI exposure clearly alter the newborn neurobehavioral scores, and both have also been reported to elevate neonatal abstinence scores (Law et al., 2003; Godding et al., 2004; Moses-Kolko et al., 2005).

In the Maternal Lifestyle Study, only 100 women were identified as isolated users of opioids, and a similar number used cocaine and opioids. Transient but dramatic neurobehavioral signs are present in the first week of life as symptoms of opioid withdrawal (increased irritability, jitteriness, poor feeding, sweating, sneezing; Shankaran et al., 2007). See the discussion under Neonatal Abstinence Syndrome.

Breastfeeding and Drug Exposure

The AAP reaffirmed the many benefits of breastfeeding and the consumption of human milk in their 2012 policy statement on infant nutrition (American Academy of Pediatrics, 2012), and the World Health Organization (WHO) and CDC have set goals for increasing breastfeeding throughout the world. In the United States, the rates of breastfeeding have continued to rise. In the CDC’s recent breastfeeding report card, 79.2% of infants born in 2014 were breastfed; 49.4% were still breastfeeding by 6 months of age, with 18.8% still exclusively breastfeeding by 6 months (Centers for Disease Control, 2014b).

For women with substance use or misuse, whether licit or illicit, the choice to breastfeed is often challenging, and healthcare professionals are equally challenged in making recommendations. The known benefits of breastfeeding and human milk must be weighed against the potential risks to the infant, most of which are not well understood. There are many issues to consider, including lactation pharmacology issues, maternal behaviors that may be

dangerous during breastfeeding, coexisting risk factors or conditions, polydrug use/abuse, and infection risks. In addition, there are numerous infant factors to consider, including age and underlying medical conditions (including prematurity) that may affect drug clearance, accumulation, and/or toxicity.

The most current and comprehensive information regarding drug transfer to human milk, bioavailability, and potential toxicity are available on the Internet, at LactMed (<http://toxnet.nlm.nih.gov/newtoxnet/lactmed.htm>). For many drugs, this information is limited to data from animal studies, with uncertain relevance to human infants, or from anecdotal case reports. In addition, there are virtually no data regarding the many confounding factors (noted above) that probably increase the potential for adverse effects on the breastfeeding infant.

For infants whose mothers engage in active, ongoing use of “street” drugs (heroin, cocaine, methamphetamines, marijuana), the risks of breastfeeding outweigh any potential benefits. Use of street drugs is often associated with risky behaviors and poor maternal health and nutrition. In addition, illicit drugs are often mixed with dangerous adulterants and contain variable drug doses with a higher risk for drug overdose.

According to a recent survey, approximately half of breastfeeding women in Western countries consume alcohol, at least occasionally (Hastrup et al., 2014). Human milk alcohol levels generally parallel maternal blood alcohol levels. Heavy alcohol use during lactation has been associated with decreased milk production via interference with the milk ejection reflex. Studies evaluating infant effects of maternal alcohol consumption during breastfeeding have yielded mixed results, including mild effects on infant sleep. Women are generally advised to wait 90–120 minutes after consuming alcohol before breastfeeding (Reece-Stremtan and Marinelli, 2015).

Maternal smoking is not an absolute contraindication to breastfeeding, although there are measureable levels of nicotine and cotinine in breast milk. However, lactating women are encouraged to stop if they can, both for their own health and to minimize the detrimental effects of secondhand smoke on their infant – including the association with SIDS (American Academy of Pediatrics, 2013).

Smoking marijuana may similarly increase the risk of SIDS, and there is the added potential for infant harm resulting from nursing while “high.” THC, the psychoactive compound in marijuana, is also contained in cannabis edibles and medicinal compounds. It is lipophilic and excreted and concentrated in breast milk, absorbed by infants, and can be stored in fat tissues for weeks. As with most drugs, there are very limited data regarding the effects of THC exposure, via breast milk alone, on infant development. The data that do exist are conflicting and based upon studies done decades ago, when the potency of marijuana was substantially less than it is today (Reece-Stremtan and Marinelli, 2015).

Breastfeeding is now generally supported for women who are engaged in treatment for substance use disorders and who have had a confirmed period of abstinence (from street drugs) prior to delivery. Similarly, women who are dependent upon opioids for chronic pain or other conditions are encouraged to breastfeed if they are medically able and if their drug dosing is stable. Concentrations of methadone and buprenorphine in human milk are low, and there are other significant advantages for the mother and infant. This includes increasing evidence supporting a reduction in the severity and duration of NAS when mothers on methadone maintenance breastfeed and emerging evidence that the same is true for buprenorphine (Jansson et al., 2008; O’Connor et al., 2013; Welle-Strand et al., 2013).

Neonatal Abstinence Syndrome

The incidence of neonatal narcotic withdrawal, commonly termed NAS, has been steadily increasing in the United States and Canada, with prevalence rates of 3.6 to 5.1 per 1000 live births (2009–2011) and NICU admission rates up to 27 cases per 1000 admissions (Tolia et al., 2015; Davies et al., 2016).

Clinical Findings

Classic NAS occurs secondary to maternal use of morphine, heroin, methadone, buprenorphine, and other prescription opioid analgesics (e.g., oxycodone) and may be combined with withdrawal or effects from antidepressants (particularly SSRIs), anxiolytics, nicotine, and other substances (Hudak et al., 2012; Kocherlakota, 2014). Clinical signs consist of a wide variety of neurologic problems (tremors, hyperirritability, excessive crying, hypertonia, and, infrequently, seizures), gastrointestinal and feeding problems (diarrhea, hyperphagia, or poor feeding), autonomic signs of dysfunction (fever, sweating, and sneezing), and respiratory problems (Tables 13.4–13.5). These signs are most often related to gestational opioid exposure but are relatively nonspecific, with the differential diagnosis including infection, meningitis, hypoglycemia, hypocalcemia, hyponatremia, intracranial hemorrhage, seizures, and stroke (Hudak et al., 2012). The signs of neonatal serotonin syndrome (or SSRI withdrawal) may also mimic those of NAS (Moses-Kolko et al., 2005; Boucher et al., 2008).

The timing of withdrawal signs from specific drug exposures can often be anticipated; for example, heroin withdrawal usually occurs within 24 hours of birth, whereas methadone withdrawal typically begins later, at approximately 48–72 hours after birth. The incidence of NAS in infants born to women using heroin or methadone is high, with wide ranges reported between 16% and 90% (Jones et al., 2010), and between 30% and 91% of infants with signs of NAS receive pharmacologic treatment for NAS with inpatient stays averaging 3–5 weeks (Kuschel, 2007; Dryden et al., 2009). Premature infants generally have milder signs of withdrawal and often show alternating periods of hyperactivity and lethargy, with tremors seen less commonly.

TABLE 13.4 Clinical Signs of Neonatal Abstinence Syndrome

| | |
|---|--|
| Central nervous system dysfunction | Excoriation (from frantic movement) Hyperactive reflexes Increased muscle tone Irritability, excessive crying, high-pitched cry Jitteriness, tremulousness Myoclonic jerks Seizures Sleep disturbance |
| Autonomic dysfunction | Excessive sweating Frequent yawning Hyperthermia |
| Respiratory problems | Nasal stuffiness, sneezing Tachypnea |
| Gastrointestinal and feeding disturbances | Inadequate oral intake Diarrhea (loose, watery, frequent stools) Excessive sucking Hyperphagia Regurgitation |

TABLE 13.5 Neonatal Neurobehavioral Signs After Fetal Drug Exposure

| Drug | Onset (days) | Peak (days) | Duration | Relative Severity | Likely NICU Admission | Symptoms |
|--------------|--------------|-------------|----------------------------|-------------------|-----------------------|-----------------|
| Alcohol | 0–1 | 1–2 | 1–2 days (up to 18 months) | Mild | No | ? |
| Amphetamine | 0–3 | — | 2–8 weeks | Mild | No | Neuro |
| Barbiturates | 0–1 | 1–2 | 1–14 days | Mild to moderate | No | Neuro |
| Caffeine | 0–1 | 1 | 1–7 days | Mild | No | Neuro, Resp |
| Cocaine | 0–3 | 1–4 | ? months | Mild | No | Neuro |
| Heroin | 0–3 | 3–7 | 2–4 weeks | Mild to severe | Yes | Neuro, Resp, GI |
| Methadone | 3–7 | 10–21 | 2–8 weeks | Mild to severe | Yes | Neuro, Resp, GI |
| SSRI | 0–3 | 1–3 | 1–4 weeks | Mild to moderate | No | Neuro, Resp, GI |
| Tobacco | 0–1 | 1–2 | 2–3 days | Mild | No | Neuro |

GI, Gastrointestinal; *Neuro*, neurologic; *NICU*, neonatal intensive care unit; *Resp*, respiratory; *SSRI*, selective serotonin reuptake inhibitor; *?*, not known.

A number of evaluation tools are used to assess the severity of opioid withdrawal after birth. Neonatal abstinence scores are scale-based using nursing observations of the severity of signs of withdrawal, and the modified Finnegan score is the most widely used scale (Finnegan, 1980; Hudak et al., 2012; Grim et al., 2013). Finnegan scoring requires rater training or else it is subject to wide inter-rater variability. Normal values for Finnegan scores in infants who have not been substance exposed have been reported; the 95%ile Finnegan score increases from 5.5 on day 1 to 7 on day 2, before decreasing to less than 6 from 48 to 72 hours (Zimmermann-Baer et al., 2010). The Lipsitz score was developed at the same time and is simpler to use, with a score greater than 4 indicating withdrawal. The Withdrawal Assessment Tool-1 was developed to monitor withdrawal from opioid infusions in pediatric intensive care units but is also used in some NICUs (Grim et al., 2013). The use of these scoring systems allows more objective quantification of the severity of the infant's withdrawal and the response to treatment. These scoring systems have shown good inter-observer reliability and can improve clinicians' ability to treat the withdrawing infant appropriately (Hudak et al., 2012; Grim et al., 2013).

The goal of medical management of opioid withdrawal is to avoid serious symptoms of NAS, such as seizures, and to maintain the infant's comfort while enabling the infant to feed, sleep, and gain weight in an appropriate manner. There are a number of reported threshold scores for initiating pharmacologic treatment (Finnegan NAS scores between 8 and 12) (Kuschel, 2007; Hudak et al., 2012; Kocherlakota, 2014), but none of these choices have been rigorously examined. The decision to begin treatment or to wean treatment should be influenced by the absolute score and other factors, such as the infant's age, comorbidities, other conditions leading to abnormal behavior, and a daily evaluation of the abnormal clinical elements observed in the scoring system (Kuschel, 2007). Standard medical practice is to combine both developmental and behavioral methods with pharmacologic interventions as necessary to control symptoms and signs of narcotic abstinence. Recent work has confirmed that use of a standard protocol for management of NAS decreases length of hospital stay and use of multiple drug therapy (Kocherlakota, 2014; Hall et al., 2015).

Treatment

Treatment for opioid withdrawal should always begin with supportive, nonpharmacologic measures such as swaddling, rocking, minimizing environmental stimulation, and avoiding unnecessary handling and irritation and should only progress to pharmacologic management when medically necessary. Breastfeeding is associated with less severe NAS, and mothers in a supervised drug treatment program should be encouraged to breastfeed and provide human milk (Hudak et al., 2012). Provision of adequate caloric intake for growth is essential as infants with NAS may require up to 150 kcal/kg per day to gain weight appropriately. Between 27% and 91% of infants exhibiting signs of NAS will receive pharmacologic treatment (Kocherlakota, 2014). The mainstay of treatment for opioid withdrawal is the use of opioids, either alone or in combination with other medications (Hudak et al., 2012; Grim et al., 2013; Kocherlakota, 2014). Medication is titrated for each infant according to the severity of the signs of withdrawal and abstinence scoring.

A 2006 survey in the United States showed that opioids, morphine or methadone, are the most common medication used for narcotic withdrawal, and phenobarbital is frequently used or added for polydrug exposure (Sarkar and Donn, 2006). A meta-analysis of seven studies found that opioid treatment reduced the time to regain birthweight and the duration of supportive care compared with supportive care alone; however, the length of hospital stay was increased (Osborn et al., 2005). Phenobarbital was also shown to be superior to diazepam for treating NAS, but small, randomized, controlled trials comparing morphine to phenobarbital for treatment of NAS suggested that opioids are superior at decreasing treatment duration and lowering NAS scores (Osborn et al., 2002; Jackson et al., 2004; Ebner et al., 2007).

A standard starting dose of morphine is 0.03–0.05 mg/kg given orally every 3–4 hours. This dose can be increased in increments of 0.03 to 0.1 mg until the symptoms are controlled. Daily doses of morphine for infants experiencing withdrawal at birth range from 0.024–1.3 mg/kg per day (Hudak et al., 2012). When the dose exceeds the 1.3 mg/kg per day, addition of a second drug is recommended (Kocherlakota, 2014).

Although preparations of oral morphine are most commonly used in recent studies, methadone is also reported as a treatment option (Kuschel, 2007; Kocherlakota, 2014). Methadone has a long duration of action and can be administered by either the oral or parenteral route. The initial recommended methadone dose is 0.05–0.1 mg/kg, followed by 0.025–0.05 mg/kg every 8–12 hours until symptoms are controlled. The long half-life of methadone makes it more difficult to titrate the dose, so once the symptoms are controlled, the total daily dose given is then divided into two doses and given every 12 hours (Kocherlakota, 2014).

In addition, sublingual buprenorphine has been reported as a treatment for NAS, but no large-scale studies are available (Kraft et al., 2011).

Phenobarbital has been used for signs of acute opioid withdrawal and is a frequently used second-line therapy for NAS, especially when maternal multidrug use is suspected (Kocherlakota, 2014). Phenobarbital does not, however, reduce significant physiologic signs of opioid withdrawal, such as diarrhea and seizures. At higher doses, phenobarbital has also been shown to impair infant sucking and cause excessive sedation. The doses of phenobarbital used are 5–20 mg/kg in the first 24 hours, followed by 2–4 mg/kg every 12 hours; the therapeutic blood level of phenobarbital for control of opioid withdrawal signs is not known. Combining an oral morphine solution and phenobarbital treatment was found to shorten duration of hospitalization and lessen the severity of withdrawal symptoms, compared with morphine treatment alone (Coyle et al., 2002, 2005). Compared with those treated with morphine alone, infants treated with morphine and phenobarbital were more interactive, had smoother movements, were easier to handle, and were less stressed. Dual treatment resulted in improved neurobehavioral organization during the first 3 weeks of life, which may indicate a more rapid recovery from opioid withdrawal (Coyle et al., 2005). A recent randomized clinical trial compared the addition of phenobarbital versus clonidine to morphine as a second-line therapy and found that the phenobarbital-treated infants had fewer morphine treatment days, with no difference in the cumulative total morphine dose. However, those infants were discharged home on phenobarbital therapy, from which they were slowly weaned over 1–8 months (Surran et al., 2013).

Clonidine, an α_2 -adrenergic receptor antagonist, is used in treating opioid withdrawal symptoms in older children and adults. In 2009, a randomized, controlled trial showed that adding clonidine to opioid treatment for NAS significantly reduced the number of treatment failures, the median length of opioid therapy, and the number of infants requiring high-dose opioids. There were reportedly no significant adverse events (i.e., hypertension, hypotension, bradycardia, or desaturations) related to clonidine use (Agthe et al., 2009). Surran et al. (2013) compared clonidine and phenobarbital as a second-line therapy for NAS in a randomized, single-center trial and found that clonidine was safe and efficacious. Infants treated with clonidine had more morphine treatment days and longer length of inpatient stay, probably due to inpatient weaning off clonidine. A recent randomized, controlled pilot study compared clonidine to morphine as first-line, single-drug therapy for NAS and found that morphine resulted in a significantly longer length of treatment. In addition, measurement of NNNS at 1 week and 2–4 weeks of age improved significantly with clonidine but not with morphine. One year follow-up did not show differences in motor, cognitive, or language scores between the treatments (Bada et al., 2015). Before clonidine begins to be more widely used for NAS, a larger clinical trial is indicated to investigate appropriate dosing regimens and determine long-term safety.

Once medications have been titrated to a level that controls the severity of opioid withdrawal and lowers the withdrawal scores, then tapering of the dosage should be started. A common method is to decrease the opioid dose by 10%–20% of the highest dose, either daily or every other day, with continued surveillance of withdrawal scores to assure the infant tolerates the decrease. It is not unusual to note increased signs of opioid withdrawal during the medication tapering. The goal of weaning is to allow infants to acclimate to a new and lower dose of medication while ensuring that they are comfortable and consolable and are able to sleep, eat, and gain weight appropriately. Objective measurements using established withdrawal scoring systems should be used to determine the rate and efficacy of medication tapering (Kocherlakota, 2014). Whether narcotics can safely be weaned more rapidly in infants has not been studied. One small study reported good results using oral morphine on an as-needed basis for elevated individual withdrawal scores, rather than providing regular doses of morphine every 3–4 hours (Ebner et al., 2007). Clonidine must be carefully weaned in the hospital because of the risk of rebound autonomic activity with possible hypertension and tachycardia; recent studies have proposed stepwise clonidine weans (Surran et al., 2013; Bada et al., 2015).

The average length of hospital stay for infants with NAS who are treated with medications varies from 8–78 days, with a stay of 21–30 days being common (Kuschel, 2007; Ebner et al., 2007; Bada et al., 2015). Investigation of outpatient management of detoxification may result in a shorter hospital stay, but with a more prolonged duration of neonatal treatment (Kuschel, 2007).

Similar principles apply to management of acquired or iatrogenic opioid and benzodiazepine dependency due to more prolonged need for these medications in some chronically ill NICU patients. Use of a withdrawal score should be implemented as soon as opioid doses are decreased. If there are signs of significant withdrawal, medication can be weaned more gradually over days to weeks (Hudak et al., 2012).

Prevention and treatment of NAS are areas where additional research is urgently needed, to define optimal treatment protocols, to improve care of both mothers and their infants, and to improve infant outcomes (McLemore et al., 2013; Jones and Fielder, 2015). There are preliminary reports of decreased regional brain volumes in neonates with NAS (Yuan et al., 2014). Improved neurodevelopmental and psychobehavioral follow-up is needed to identify long-term problems and needed services (Kocherlakota, 2014). Infants treated for NAS have been reported to have increased rates of rehospitalization during childhood for maltreatment, trauma, and mental and behavioral disorders, emphasizing the need for continued support of this group (Uebel et al., 2015).

Sudden Infant Death Syndrome

The incidence of SIDS was previously thought to be higher in cocaine-exposed infants, with a metaanalysis of 10 studies demonstrating a 4.1 OR for SIDS among cocaine-exposed infants (Fares et al., 1997) compared with infants not exposed to perinatal drugs. However, after data were controlled for concurrent use of other drugs, the increased risk for SIDS could not be attributed to intrauterine cocaine exposure alone but was believed to be caused by exposure to other illicit drugs and smoking. A recent analysis of exposed infants showed no association of maternal recreational drug use with SIDS but found an increase in SIDS associated with paternal marijuana use around the time of conception and during pregnancy (Klonoff-Cohen and Lam-Kruglick,

2001). Maternal alcohol use has also been associated with an increased risk of SIDS (O'Leary et al., 2013a).

There is a significant relationship between exposure to tobacco smoke and SIDS. A higher risk for SIDS is reported in infants exposed to maternal smoking with either antenatal or postnatal exposure (Salihu and Wilson, 2007). Additional evidence supporting a causal role includes a dose-response relationship (Rogers,

2008). With recent substantial decreases in SIDS risk (after “back to sleep”), maternal smoking is calculated to account for an increasing proportion of SIDS risk. Mitchell and Millerad (2006) suggest that one-third of all SIDS deaths might be prevented by eliminating in utero exposure to maternal smoking. Despite the increased risks of SIDS among drug-exposed and tobacco-exposed infants, home apnea monitoring is not indicated in the absence of other risk factors.

Summary

The magnitude of observed perinatal outcomes after illicit maternal substance use pales in comparison to the established health and developmental risks associated with tobacco, alcohol, and prescribed opioid exposure (Schempf, 2007). The greatest impact of illicit substance use may be the increased postnatal risks of

neglect, maltreatment, and disruptions in the home environment. Health policy must be directed at reducing all these complex factors associated with perinatal substance abuse and providing support for families and children with in utero substance exposure.

Suggested Readings

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14

Antepartum Fetal Assessment

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KEY POINTS

- Fetal ultrasound assessment in the first, second, and third trimesters
- Antepartum fetal testing in high-risk pregnancies (nonstress test, amniotic fluid volume, biophysical profile, Doppler velocimetry)

A primary objective of obstetric care is the assessment and prevention of adverse fetal and neonatal outcomes. Maternal care is an integral step toward this goal. Optimization of the maternal state, through monitoring and treatment of chronic conditions such as diabetes or hypertension or acute states like preeclampsia or preterm labor, is one important facet of care to achieve desirable perinatal outcomes. Monitoring and management of the fetus, although a more obvious step toward this goal, is somewhat less straightforward. Fetal assessment demands a view into the somewhat inaccessible intrauterine environment. Our ability to gain access to this space to gauge the needs and health of the fetus has improved dramatically with developments in technology and increased understanding of fetal physiology over the past three decades. As a result, perinatal morbidity and mortality have decreased considerably over that time (Fig. 14.1).

In general, antepartum fetal assessment utilizes various techniques to assess fetal health and well-being in pregnancies that are at increased risk of fetal death due to preexisting maternal conditions (chronic hypertension) or pregnancy-related complications (fetal growth restriction [FGR]). Selecting appropriate patients at risk for adverse perinatal events can enhance the prediction of these events, although some tests may be appropriate even for a low-risk population. The assessment may allow for certain therapeutic options – often, timely delivery – to prevent fetal harm. The overall goal of these efforts is to reduce perinatal mortality, although the reduction of morbidities such as cerebral palsy or preventable birth injury is intertwined with this objective. In antenatal assessment in the third trimester, the prediction and detection of fetal acidemia and hypoxemia form a central principle underlying these efforts.

It is important to make the distinction between antepartum and intrapartum fetal assessment: the latter is specifically related to monitoring the fetus during labor. The nature of labor affords certain advantages (e.g., dilation allows blood samples from the fetus) and restrictions (the lack of fluid after rupture of membranes

creates difficulties for ultrasound examination) that do not occur in the antenatal period. As a result, this chapter focuses only on events and assessment preceding labor.

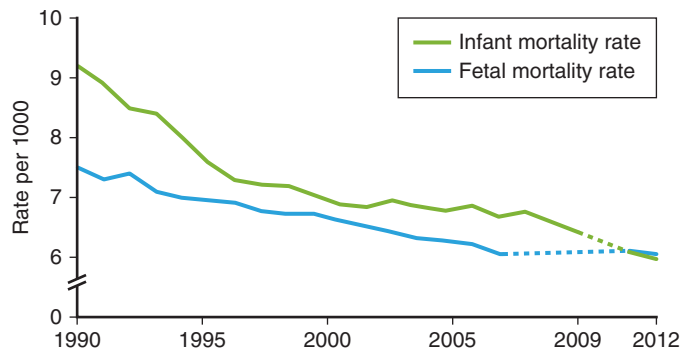
General Principles

Principles of Testing

Many of the tests used for antepartum fetal assessment are screening tests that will lead to further testing allowing for diagnosis and decision making; therefore it is important to note the principles guiding such tests. The outcome, principally perinatal morbidity and mortality, is a significant burden to both the individual and the overall healthcare system. The primary tools for assessment, ultrasound examination and fetal heart rate (FHR) monitoring, are generally easy, safe, and acceptable to patients. Screening has the potential to allow important and timely interventions, such as antenatal steroid administration or delivery. The predominant difficulty with fetal testing comes in the unproven utility of testing to improve outcomes. Furthermore, some tests, such as the nonstress test (NST), have high false-positive rates; therefore when used as a diagnostic test (e.g., to decide on delivery), they can lead to the overuse of interventions. The specificity and sensitivity of the tests vary, and the critical step to enhancing test performance is patient selection. The utility of fetal surveillance involves the judicious application of the tests in patients with specific risk profiles.

Fetal Physiology and Behavior

The first trimester (≤ 14 weeks' gestation) is mainly a time of system development and organogenesis. The hyperplastic enlargement during the first 11 weeks produces standard rates of growth, with deviation being rare. At the completion of the first trimester, the major organ systems have developed, allowing the opportunity during the second trimester to assess for anomalies in development. The second and third trimesters involve maturation of these systems. Fetal assessment, primarily performed in the third trimester, is concerned with the prediction or detection of fetal hypoxemia and acidemia. Integration of the fetal neurologic and cardiovascular systems as they relate to acid–base status is the cornerstone of this assessment. By the beginning of the third trimester, there is usually adequate maturation present in the neurologic and cardiovascular



• **Fig. 14.1** Fetal and Infant Mortality Rates in the United States, 1990–2012. Infant mortality rates are the number of infant deaths per 1000 live births. Fetal mortality rates are the number of fetal deaths of 20 weeks' gestation or later per 1000 live births and fetal deaths. (From CDC/NCHS, National Vital Statistics System.)

systems to enable meaningful fetal assessment. We are thus able to monitor the manifestations of hypoxemia and acidemia as shown by neurologic and cardiovascular changes. In pregnancies at extremely high risk for adverse perinatal outcome or stillbirth, fetal assessment may be performed in the second trimester, as early as 24 weeks' gestation.

Technology

The technologic underpinning of fetal assessment is ultrasound. FHR monitoring during the antepartum period depends on a Doppler cardiogram; movements of the fetal heart, in particular the sounds of the valves, are detected by this monitor. The time between the beats is translated into a heart rate, which is then graphically represented on a chart over time. This process produces the FHR monitoring strip that becomes the NST or contraction stress test (CST).

Contemporary ultrasound imaging technology involves a wide array of features, including B-mode (basic imaging), M-mode (mapping the movement of structures over time), pulsed Doppler (demonstrating flow velocity in a particular area, such as a vessel), color Doppler (showing intensity and direction of flow through shades of red and blue), and power Doppler (a more sensitive form of colorized Doppler). Magnetic resonance imaging (MRI) is often used to supplement ultrasound imaging, especially for imaging of the fetal brain.

Indications and Timing

Most fetal testing protocols involve a stepwise approach, and the first step is the selection of the appropriate patient. Suggested assessments for low-risk pregnancies include one ultrasound examination for dating and one for the basic anatomic survey. Prenatal risk assessments for chromosomal disorders, such as first-trimester risk assessment with maternal serum analysis and fetal nuchal translucency assessment, cell-free fetal DNA analysis, or the second-trimester maternal quadruple serum screen, are additional options (see Chapter 18 for additional discussion of these tests). Whereas up to 30% of perinatal morbidity may occur in low-risk patients, routine fetal testing beyond that described previously in a low-risk pregnancy is an ineffective use of resources.

High-risk pregnancies are those at greater peril for perinatal morbidity and mortality. These pregnancies often have more justification for targeted or detailed anatomic ultrasound examinations

• BOX 14.1 Common Indications for Antepartum Fetal Assessment: High-Risk Pregnancies

Fetal

- Abnormal fetal testing, fetal distress
- Decreased fetal movement
- Fetal growth restriction
- Monochorionic multiple gestation
- Oligohydramnios

Maternal–Fetal

- Placenta abruption (abruptio placenta)
- Alloimmunization
- Late term or postterm pregnancy
- Gestational hypertension or preeclampsia
- Gestational diabetes
- History of fetal death

Maternal

- Advanced maternal age (>40 years old)
- Cyanotic cardiac disease (severe)
- Hypertension, chronic
- Morbid obesity
- Pulmonary disease (severe)
- Chronic renal disease
- Systemic lupus erythematosus
- Hyperthyroidism

and for regular assessment of fetal growth or heart rate assessment. Common conditions requiring increased fetal surveillance are shown in Box 14.1.

Pregnancy dating should be confirmed at the earliest possible moment, and fetal anatomic screening is best accomplished in the second trimester, specifically at 18–20 weeks' gestation, when visualization of the anatomic features is adequate. However, standards for the timing of antepartum fetal assessment to survey for fetal compromise do not exist. Certainly, assessment with NST or biophysical profiles would have little utility before viability (approximately 24 weeks' gestation). Guidelines for initiating fetal testing for specific indications are largely based on the risk of fetal loss at a particular gestational age (GA). Most fetal testing algorithms begin antepartum fetal testing no earlier than 32 weeks' gestation. However, the most at-risk pregnancies may be monitored at earlier GAs when delivery would be considered for fetal benefit.

Fetal Assessment in Low-Risk Pregnancies

Ultrasound: Pregnancy Dating

The *estimated date of delivery* is defined at the beginning of pregnancy based on the best available information, including menstrual history, ultrasound data, and assisted reproduction technology. The median duration of a singleton pregnancy is 280 days (40 weeks) from the first day of the last menstrual period or 266 days (38 weeks) from the time of ovulation. *Term* is defined as 37 to 41 + 6 weeks' (259–294 days) gestation. This definition is further broken down in the subgroups: early term (37 weeks 0 days – 38 weeks 6 days), late term (41 weeks 0 days – 41 weeks 6 days). Preterm is defined as delivery prior to 37 weeks. This definition is further broken down into the subgroup late preterm (34 weeks 0 days – 36 weeks 6 days). Postterm pregnancies are defined as delivery at 42 weeks and beyond (Box 14.2). Given that the preterm and postterm

• BOX 14.2 Definition of Gestational Age Categories

Gestational Age Categories

| | |
|--------------------------|-----------------------|
| Term Pregnancy | 37 + 0 – 41 + 6 weeks |
| • Early term | 37 + 0 – 38 + 6 weeks |
| • Late term | 41 + 0 – 41 + 6 weeks |
| Preterm Pregnancy | <37 weeks |
| • Late preterm pregnancy | 34 + 0 – 36 + 6 weeks |

periods are associated with increased risks to the fetus and newborn, pregnancy dating provides an approximate expectation for the completion of the pregnancy and serves as a basis for the efficient and appropriate use of fetal surveillance, testing, and treatment. Accurate pregnancy dating by ultrasound has been associated with reduced diagnoses of growth restriction (Waldenström et al., 1992), reduced use of tocolysis for preterm labor (LeFevre et al., 1993), and a reduced need to intervene in postterm pregnancies (Neilson, 2000; Whitworth et al., 2015). Additionally, early ultrasound assessment is associated with increased diagnosis of multifetal pregnancies (Whitworth et al., 2015).

In a spontaneous pregnancy in a woman with regular cycles and normal menstrual periods, the last menstrual period is often an accurate way of dating a pregnancy. Menstrual dating is less accurate in women who are taking oral contraceptives, were recently pregnant, or have irregular periods or intermenstrual bleeding. In these cases, and others in whom there is uncertainty, ultrasound dating in the first trimester is accurate and effective. A fetal pole may be seen beginning at 6 weeks' gestation, and the fetal heartbeat should be visualized by 6 to 7 weeks' gestation (Doubilet et al., 2013). In the first trimester, measurement of the crown-rump length is accurate to within 5 to 7 days; therefore this measurement should take priority in dating a pregnancy when the timing of the last menstrual period suggests a GA outside this range of variation (Robinson and Fleming, 1975; Drumm et al., 1976; Savitz et al., 2002; American College of Obstetricians and Gynecologists [ACOG], 2014a). A first-trimester ultrasound examination is indicated to confirm an intrauterine pregnancy (i.e., exclude ectopic pregnancy), confirm fetal viability, document fetal number, and estimate GA. During this ultrasound, the maternal pelvis and ovaries may be assessed to look for the presence of abnormalities, including uterine fibroids and Müllerian anomalies and ovarian masses, such as a dermoid cyst. In the second trimester, ultrasound dating is less accurate (discrepancy from 7 to more than 14 days) but can nonetheless be helpful. Measurement of the biparietal diameter (BPD) of the fetal head, the most accurate parameter, can be accurate to within 7 to 10 days (Campbell et al., 1985; Waldenström et al., 1990). The BPD is also a parameter of choice because it is less affected by chromosomal anomalies, in particular Down syndrome (Cuckle and Wald, 1987). Usually in the second or third trimesters, several biometric measurements – such as BPD, head circumference, abdominal circumference (AC), and femur length – are recorded, and a computerized algorithm can generate an estimated GA. Additional fetal measurements, including other fetal long bones (i.e., humerus, ulna, and tibia) and transverse cerebellar diameter may also assist with estimating fetal GA (Goldstein et al., 1987; Melamed et al., 2009).

Ultrasound: Second and Third Trimesters

Perinatal ultrasound examination in the second and third trimesters can be classified broadly into three types: the basic or standard

• BOX 14.3 Images and Information Obtained During a Basic Fetal Anatomy Survey

| |
|--|
| Fetal number |
| Fetal viability |
| Fetal position |
| Fetal biometry |
| Placental location |
| Amniotic fluid volume |
| Assessment of maternal adnexa, uterus, and cervix |
| Fetal anatomy |
| Head, face, neck |
| Lateral cerebral ventricles |
| Choroid plexus |
| Midline falx |
| Cavum septi pellucidi |
| Cerebellum |
| Cistern magna |
| Upper lip |
| Chest |
| Heart |
| Four-chamber view |
| Left ventricular outflow tract |
| Right ventricular outflow tract |
| Abdomen |
| Stomach |
| Kidneys |
| Urinary bladder |
| Umbilical cord insertion site into the fetal abdomen |
| Umbilical cord vessel number |
| Spine |
| Cervical, thoracic, lumbar, and sacral spine |
| Extremities |
| Legs and arms |
| Sex |
| In multiple gestations and when medically indicated |

examination, the specialized (detailed) examination, and the limited examination. The standard or basic examination includes the determination of fetal number, fetal viability, fetal position, fetal biometry, placental location, amniotic fluid volume, the presence or absence of a maternal pelvic mass, and the presence of gross fetal malformations (ACOG, 2009) (Box 14.3). Most pregnancies can be evaluated adequately by this basic examination. If the patient's history, physical examination, or basic ultrasound examination suggests the presence of a fetal malformation, a specialized or detailed examination should be performed by a sonographer who is skilled in fetal evaluation. During a detailed ultrasound, which is best performed at 18 to 20 weeks' gestation, fetal structures are examined in detail to identify and characterize any fetal malformation. In addition to identifying structural abnormalities, a specialized ultrasound examination can identify sonographic markers of fetal aneuploidy. In some situations, a limited examination may be appropriate to answer a specific clinical question (such as fetal viability, amniotic fluid volume, fetal presentation, placental location, or cervical length) or to provide sonographic guidance for an invasive procedure (such as amniocentesis).

Current debate centers on who should undergo sonographic examination and what type of evaluation these patients should have. Advocates of routine sonography cite several advantages of universal ultrasound evaluation, including more accurate dating of pregnancy and earlier and more accurate diagnosis of multiple gestation, structural malformations, and fetal aneuploidy (discussed

later in [Ultrasound](#) section). Opponents of routine sonographic examination argue that it is an expensive screening test (\$100–\$250 for a standard examination) and that the cost is not justified by published research, which suggests that routine ultrasound examinations do not significantly change perinatal outcome ([Ewigman et al., 1993](#); [LeFevre et al., 1993](#); [Crane et al., 1994](#)). However, subsequent cost–benefit analyses regarding the routine use of ultrasound in low-risk pregnancies have concluded both cost savings ([Vintzileos et al., 2000](#)), if the ultrasound is performed in a tertiary care setting, and net loss, if the ultrasound is performed by less-experienced providers. Ultrasound performed in a country with socialized medicine proved cost saving ([Leivo et al., 1996](#)). Thus it may be the more-tempered approach to ultrasound screening with utilization of highly skilled sonographers and sonologists that allows the routine ultrasound screening of pregnancies be both effective and economical. Nonmedical use of obstetric ultrasound is not supported by either ACOG or the American Institute of Ultrasound in Medicine (AIUM) for many reasons, including the potential for false reassurance at the time of the ultrasound as well as no formal process for follow-up if an abnormality is identified ([ACOG, 2004](#)).

Second-trimester ultrasound examination may be indicated for various reasons, including uncertain dating, uterine size larger or smaller than expected for the estimated GA, vaginal bleeding, suspected multiple gestation, a medical disorder that can affect fetal growth and development (e.g., diabetes, hypertension, collagen vascular disorder), a family history of an inherited genetic abnormality, a suspected fetal malformation or growth disturbance, or suspected fetal death ([ACOG, 2009](#); [AIUM, 2013](#)). In the United States, most patients undergo a standard examination at 18 to 20 weeks' gestation to screen for structural defects. An understanding of normal fetal physiology is critical to the diagnosis of fetal structural anomalies. For example, extra-abdominal herniation of the midgut into the umbilical cord occurs normally in the fetus at 8 to 12 weeks' gestation and can be misdiagnosed as an abdominal wall defect. Placental location should be documented with the bladder empty, because overdistension of the maternal bladder or a lower uterine contraction can give a false impression of placenta previa. If placenta previa is identified at 18 to 22 weeks' gestation, serial ultrasound examinations should be performed to follow placental location. Presence of placenta previa at 15–17 weeks has about a 12% risk of being present at term, while presence later in the second trimester (24–27 weeks) confers a 49% risk of persistence at term ([Dashe et al., 2002](#)). The umbilical cord should also be imaged, including the number of vessels and its fetal and placental insertion.

The indications for third-trimester ultrasound examination are similar to that for second-trimester ultrasound. Fetal anatomy survey examinations and estimates of fetal weight become less accurate as GA increases, especially in obese women or in pregnancies complicated by oligohydramnios. However, fetal biometry and an anatomic survey should still be performed, because certain fetal anomalies, such as achondroplasia, may become evident for the first time later in gestation.

Fetal Movement Counting

Fetal movement (quickening) is typically perceived by the mother at 16 to 22 weeks' gestation. Fetal hypoxemia is typically associated with a reduction in fetal activity; the fetus is essentially conserving energy and oxygen for vital activities. A typical procedure for fetal movement counting consists of having the patient record the interval

taken to feel 10 fetal movements, usually after a meal when the fetus is more active. If 10 movements are not detected in 1 hour, further testing is often recommended. The data supporting fetal movement counting are mixed. A large international cluster randomized trial involving more than 68,000 patients demonstrated no benefit ([Grant et al., 1989](#)), and a Cochrane analysis found insufficient evidence to support this technique to prevent stillbirth ([Mangesi and Hofmeyr, 2007](#)). However, a more recent Norwegian study found a significant reduction in the number of stillbirths without a subsequent increase in the number of hospital visits for decreased fetal movement when a standardized approach to patient education regarding the recommended approach to decreased fetal movement ([Tveit et al., 2009](#)) was implemented. Fetal movement counting represents a low-technology screening test that can be applied easily to all pregnancies. Although its effectiveness in improving perinatal outcomes is debatable, it can be used as a cost-effective first-line strategy.

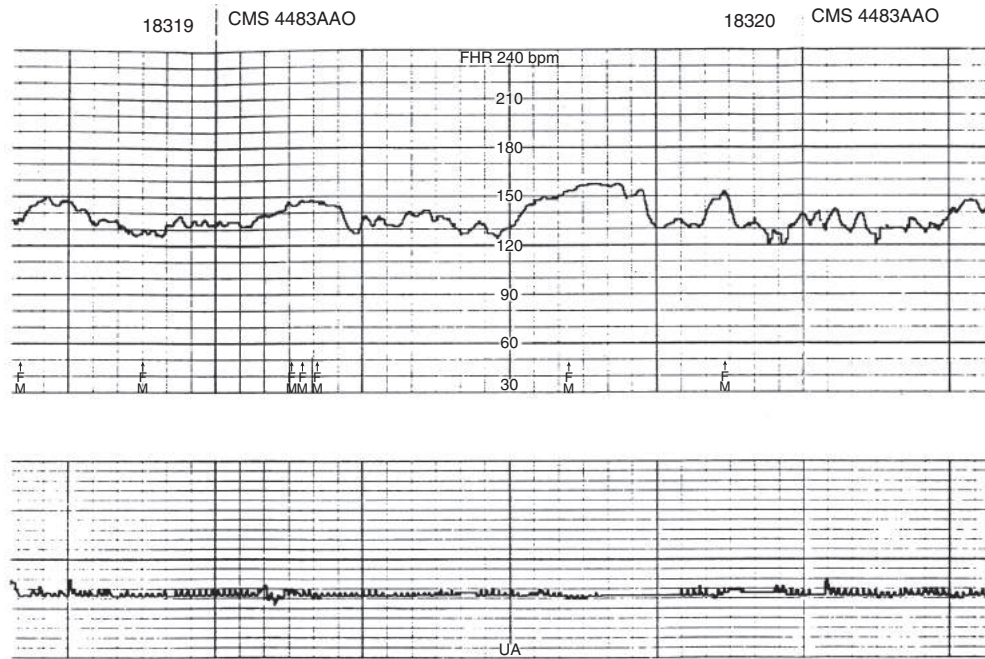
Fetal Assessment in High-Risk Pregnancies

Cardiotocography

Cardiotocography is the visual representation of FHR and uterine contractions. FHR has been recognized as an important indicator of fetal status since the 19th century, with Lejumeau Kergaradec of Switzerland being credited with the first accounts of direct fetal auscultation and the uterine soufflé in 1821. FHR monitoring is based on the principle that the fetal neurologic system, through its afferent and efferent networks, serves as a key mediator to demonstrate fetal well-being. Oxygenation, acidemia, and other vital functions are monitored by peripheral chemoreceptors and baroreceptors, which provide input on fetal status through afferent neurologic networks to the central nervous system (CNS). This information is processed by the CNS, and signals are conducted through efferent networks to produce peripheral changes, particularly to the heart via direct parasympathetic vagal neurons, direct sympathetic signals, or indirect sympathetic stimulation of catecholamine release. In this way, fetal cardiac activity can be seen as a surrogate for fetal oxygenation and acid–base status.

For many years, assessment of the FHR was limited to the fetoscope, a direct stethoscope attributed to Adolphe Pinard in 1876. In 1957, Orvan Hess and Ed Hon at Yale University (New Haven, CT) introduced electronic FHR monitoring as a window into the status of the fetus ([Hon and Hess, 1957](#)). This technology relied on direct monitoring through a scalp electrode; only years later would Doppler technology allow cardiac signals to be detected noninvasively. FHR monitoring became a tool for fetal assessment, as it was recognized that certain FHR patterns were associated with fetal compromise and poor fetal outcomes.

The basic elements of a FHR strip are baseline, variability, accelerations, and decelerations. A baseline of 110–160 beats per minute is normal. Variability is determined by the irregular fluctuations in amplitude and frequency in the baseline, and variability of fewer than 6 beats per minute is often abnormal. Accelerations are classified as visually apparent abrupt increases that peak at 15 beats per minute or more above the baseline and last 15 seconds or longer. Fetal movements often coincide with FHR accelerations. Finally, decelerations, often classified as early, variable, or late, are decreases in the FHR that have specific pathologic and physiologic associations. Although primarily focused on intrapartum monitoring, the 2008 National Institute of Child Health and Human Development workshop report on fetal monitoring provides an excellent



• **Fig. 14.2** A Normal Nonstress Test. Note two fetal heart rate accelerations exceeding 15 beats/min and lasting at least 15 seconds during the monitoring period. FHR, Fetal heart rate.

summary of the nomenclature and interpretation involved (Table 14.1) (Macones et al., 2008).

Nonstress Test

A normal result of an NST is defined as a 20-minute FHR tracing that contains two heart rate accelerations lasting 15 seconds or longer that peak 15 beats or more above the baseline: often this is called a *reactive NST* (Fig. 14.2). Modifications are made in reference to GA. NSTs for fetuses at less than 32 weeks' gestation are often considered reactive if the acceleration is 10 beats per minute or more above the baseline and lasts for at least 10 seconds (Glantz and Bertoia, 2011; Cousins et al., 2012). Furthermore, to account for the periodicity of 20–30-minute sleep cycles in the fetus, an NST that is not reactive over the first 20 minutes may be continued an additional 20–40 minutes. A nonreactive NST or an NST with specific abnormalities (e.g., high or low baseline, decelerations) should be followed by a biophysical profile (BPP). It is important to note that some abnormal states, such as a fetal CNS abnormality or maternal drug ingestion, may contribute to a nonreactive NST. In these cases, ultrasound examination may provide appropriate information to determine the diagnosis or required management.

Falsely normal NSTs occur at a rate of 3–5 per 1000 tests, although this does not account for a baseline rate of unpreventable fetal deaths (Freeman et al., 1982b). The difficulty with the NST really lies in its lack of specificity for fetal death or compromise; the false-positive rate may be as high as 50% (Freeman et al., 1982b).

The rather modest false-negative rate is likely because of the NST being a measurement of short-term hypoxemia. Indeed, longer-term fetal status can be measured through amniotic fluid assessment, because the amniotic fluid is correlated with fetal urinary output, which is a surrogate for renal perfusion. When combined with an assessment of amniotic fluid level, the false-negative rate

of the NST is reduced to 0.8 per 1000, although a 60% false-positive rate remains (Miller et al., 1996). Thus when the amniotic fluid level is combined with the NST – sometimes known as the *modified biophysical profile* – the risk of fetal death is reduced to negligible levels in high-risk populations (Clark et al., 1989). For these reasons, the modified biophysical profile is a modality of choice for monitoring the high-risk pregnancy.

Contraction Stress Test

The CST assesses the FHR response in the presence of contractions. This test improves on the specificity and sensitivity of the NST by assessing the fetal response to stress. In fact, the CST preceded the NST, although the NST became more favorable because of fewer contraindications, ease of administration, and reduced time and supervision necessary. Compared with the NST, there is a much lower incidence of falsely normal tests (0.4 per 1000), representing an eightfold reduction in the risk of fetal loss in one study (Freeman et al., 1982a).

Contractions are stimulated by the administration of intravenous oxytocin or through maternal nipple stimulation. Of course, the CST is contraindicated in patients in whom contractions should not be provoked, such as threatened preterm delivery or preterm premature rupture of membranes, prior classical cesarean delivery, or placenta previa. A minimum of three contractions over a 10-minute period of continuous FHR assessment are necessary for a satisfactory test interpretation. An unsatisfactory test should be followed by continued testing with a modification of the mode of contraction stimulation. A negative (i.e., normal) test result demonstrates no late decelerations, whereas a positive test result shows late decelerations after 50% or more of contractions (Fig. 14.3). A positive test result requires immediate further testing or evaluation, if not delivery. An equivocal test demonstrates late decelerations with less than 50% of contractions and requires further testing or monitoring. A test that encompasses a hyperstimulatory

TABLE 14.1 Interpretation of Antepartum Cardiotocography

| Term | Characteristic | Description |
|--------------|----------------|--|
| Baseline | Definition | Mean FHR, rounded to increments of 5 beats/min (e.g., 140, 145); need baseline duration of ≥ 2 min during a 10-min segment, between periodic or episodic changes, to determine baseline |
| | Bradycardia | <110 beats/min for >10 min |
| | Tachycardia | >160 beats/min for >10 min |
| Variability | Definition | Fluctuations of the baseline heart rate; measured from peak to trough |
| | Absent | Undetectable |
| | Minimal | Undetectable to ≤ 5 beats/min |
| | Moderate | 6–25 beats/min |
| | Marked | >25 beats/min |
| Acceleration | Definition | Abrupt increase ≥ 15 beats/min lasting ≥ 15 s |
| | Prolonged | ≥ 2 min and <10 min (≥ 10 min is a baseline change) |
| Deceleration | Definition | Decreases in the FHR |
| | Variable | Abrupt decrease onset to nadir <30 s; decrease ≥ 15 beats/min lasting ≥ 5 s to <2 min |
| | Early | Gradual decrease onset to nadir ≥ 30 s with contraction |
| | Late | Gradual decrease onset to nadir ≥ 30 s; nadir of deceleration occurring after peak of contraction |
| | Prolonged | Decrease ≥ 15 beats/min lasting ≥ 2 min, but <10 min (≥ 10 min is a baseline change) |
| | Recurrent | Occur with $\geq 50\%$ of uterine contractions in any 20-min window |
| | Intermittent | Occur with $<50\%$ of uterine contractions in any 20-min window |
| Contractions | Considerations | Frequency, duration, intensity, and relaxation |
| | Normal | ≤ 5 contractions per 10 min averaged over a 30-min window |
| | Tachysystole | >5 contractions per 10 min averaged over a 30-min window; should always be qualified as to the presence or absence of associated FHR decelerations |

FHR, Fetal heart rate.

contraction pattern (e.g., five contractions within 10 minutes or contractions lasting longer than 90 seconds) is also considered equivocal and requires further testing.

Ultrasound

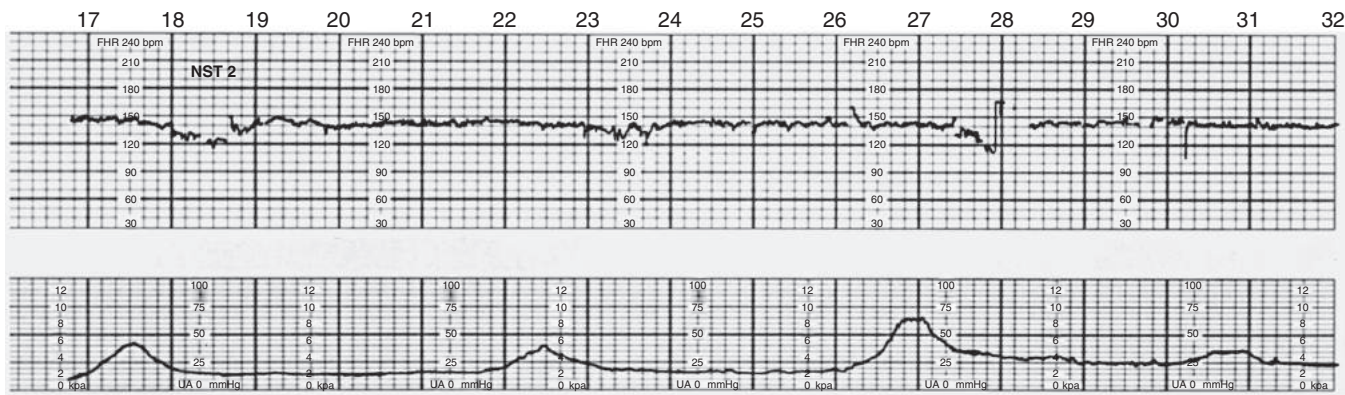
Although routine sonography for low-risk pregnant women is controversial, few would disagree that the benefits far outweigh the costs for high-risk patients. Given the higher risk for fetal complications such as anatomic anomalies or growth disturbances, a specialized examination is performed between 18 and 20 weeks' gestation in most high-risk pregnancies.

Additional ultrasound modalities are also available, including fetal echocardiography, three-dimensional (3D) sonography, and Doppler. Cardiac anomalies are the most common major congenital defects encountered in the antepartum period. A four-chamber view of the heart at the time of fetal anatomy survey at 18 to 20 weeks' gestation will detect only 30% of congenital cardiac anomalies, although the detection rate can be increased to approximately 65%–90% if the outflow tracts are adequately visualized (Kirk et al., 1994; Li et al., 2013), but this still leaves 10%–35% of all congenital cardiac anomalies undiagnosed. Factors that affect the sensitivity include type of practice (university-based vs community-based), operator training and experience, GA at the time of scan, maternal weight, fetal position, and type of defect present (Tegnander and Eik-Nes, 2006). For this reason, fetal echocardiography should be performed by a skilled and experienced sonologist at 20 to 22 weeks' gestation in all pregnancies at high-risk of a fetal cardiac anomaly; this includes pregnancies complicated by pregestational diabetes mellitus, a personal or family history of congenital cardiac disease (regardless of the nature of the lesion or whether it has been repaired), maternal drug exposure (e.g., lithium and valproic acid) (Bérard et al., 2007), and pregnancies conceived by in vitro fertilization, but not if the pregnancy was conceived using clomiphene citrate or ovarian stimulation or intrauterine insemination alone (Olson et al., 2005).

Compared with standard two-dimensional (2D) ultrasound, 3D ultrasound (or four-dimensional [4D] if fetal movements are included) allows for visualization of fetal structures in all three dimensions concurrently for the improved characterization of complex fetal structural anomalies and for storage of scanned images with 3D reconstruction at a later date or remote location (telemedicine). Unlike 2D ultrasound, 3D images are greatly influenced by fetal movements and are subject to more interference from structures such as fetal limbs, umbilical cord, and placental tissue. Because of movement interference, visualization of the fetal heart with 3D ultrasound is suboptimal.

In addition to rapid acquisition of images that can be later reconstructed and manipulated, 3D ultrasound has other potential advantages:

- Surface rendering mode can provide clearer images of many soft tissue structures. Such images can improve the diagnosis of certain fetal malformations, especially craniofacial anomalies (cleft lip and palate, micrognathia, ear anomaly, facial dysmorphism, club foot, finger and toe anomalies), intracranial lesions, spinal anomalies, ventral wall defects, and fetal tumors.
- 3D ultrasound may be useful in early pregnancy by providing more accurate measurements of the gestational sac, yolk sac, and crown-rump length. It may also allow for a more accurate midsagittal view of the fetus for measuring nuchal translucency.
- 3D ultrasound can also be used to measure tissue volume. Preliminary data suggest that the assessment of cervical volume



• **Fig. 14.3** A Contraction Stress Test. The fetal heart rate is plotted above the uterine contraction signal. Note the late deceleration after a contraction; this is a positive, or abnormal, test result. *FHR*, Fetal heart rate; *NST*, nonstress test.

may predict the risk of cervical insufficiency (Rovas et al., 2005), and measurement of placental volume in the first trimester may predict fetuses at risk of FGR (Schuchter et al., 2001).

Despite these advantages and the fact that 3D ultrasound has been available since the early 1990s, it has yet to live up to its promises. Although 3D ultrasound is unlikely to replace standard 2D imaging in the near future, it is a valuable complementary modality in obstetric imaging. As the technology improves, it is likely that perinatal ultrasound will evolve to resemble computed tomography (CT) and MRI.

Growth Assessment

Normal fetal growth is a critical component of a healthy pregnancy and the subsequent long-term health of the child. A systematic method of physical examination of the gravid abdomen was first described by Leopold and Spörlin (1894). Although this examination has several limitations, particularly in the setting of maternal obesity, multiple pregnancy, uterine fibroids, or polyhydramnios, it is safe, low cost, and well tolerated and may add valuable information to assist in antepartum management. Palpation is divided into four separate Leopold maneuvers. Each maneuver is designed to identify specific fetal landmarks or to reveal a specific relationship between the fetus and mother. For example, the first maneuver involves measuring fundal height. The uterus can be palpated above the pelvic brim at approximately 12 weeks' gestation. Thereafter, fundal height should increase by approximately 1 cm per week, reaching the level of the umbilicus at 20 to 22 weeks' gestation. Between 20 and 32 weeks' gestation, the fundal height (in centimeters, from the superior edge of the pubic symphysis) is approximately equal to the GA (in weeks) in healthy women of average weight with an appropriately grown fetus. However, there is a wide range of normal fundal height measurements. One study has shown a 6-cm difference between the 10th and 90th percentiles at each week of gestation after 20 weeks (Belizan et al., 1978). Moreover, fundal height is maximal at approximately 36 weeks' gestation, at which time the fetus drops into the pelvis in preparation for labor and the fundal height decreases. For these reasons, reliance on fundal height measurements alone will fail to identify more than 50% of fetuses with FGR (Gardosi and Francis, 1999). Serial fundal height measurements by an experienced obstetric care provider are more accurate than a single measurement and will lead to an improved diagnosis of FGR, with reported sensitivities as high as 86% (Belizan et al., 1978). However, a more recent

systematic review failed to conclude that serial fundal height measurements were superior to abdominal palpation alone due to a lack of randomized controlled trials (Robert et al., 2015).

If the clinical examination is not consistent with the stated GA, an ultrasound examination is indicated to confirm GA and to establish a more objective measure of fetal growth. Ultrasound examination may also identify an alternative explanation for the discrepancy, such as multiple pregnancy, polyhydramnios, oligohydramnios, fetal demise, or uterine fibroids.

For many years, obstetric sonography has used fetal biometry to define fetal size by weight estimation, although this approach has a number of key limitations. For example, regression equations used to create weight estimation formulas are derived primarily from cross-sectional data that rely on infants delivering within an arbitrary period of time after the ultrasound examination, and they assume that body proportions (i.e., fat, muscle, bone) are the same for all fetuses. Moreover, growth curves for healthy infants from 24 to 37 weeks' gestation rely on data collected from pregnancies delivered preterm, which should not be regarded as normal pregnancies and are likely to be complicated by some element of uteroplacental insufficiency, regardless of whether the delivery was spontaneous or iatrogenic. Despite these limitations, if the GA is well validated, the prevailing data suggest that prenatal ultrasound can be used to verify an alteration in fetal growth in 80% of cases and exclude abnormal growth in 90% of cases (Sabbagha, 1987).

Sonographic estimates of fetal weight are commonly derived from mathematical formulas that use a combination of fetal measurements, especially the BPD, AC, and femur length (Hadlock et al., 1984). Whereas the BPD may be the most accurate indicator of GA in the second or third trimesters, fetuses gain weight in their abdomen, making the AC the single most important measurement for fetal size. The AC is thus given more weight in these formulas. Unfortunately, the AC is also the most difficult measurement to acquire, and a small difference in the AC measurement will result in a large difference in the estimated fetal weight (EFW). The accuracy of the EFW depends on a number of variables, including GA (in absolute terms, EFW is more accurate in preterm or FGR fetuses than in term or macrosomic fetuses), operator experience, maternal body habitus, and amniotic fluid volume (measurements are more difficult to acquire if the amniotic fluid volume is low). Although objective, sonographic EFW estimations are not particularly accurate and have an error of 15%–20%, even in experienced hands (Anderson et al., 2007). Indeed, a sonographic EFW at term is no more accurate than a clinical

estimate of fetal weight by an experienced obstetric care provider or the mother's estimate of fetal weight if she has delivered before (Chauhan et al., 1992). Sonographic estimates of fetal weight must therefore be evaluated within the context of the clinical situation and balanced against the clinical estimate of fetal weight. Serial sonographic evaluations of fetal weight are more useful than a single measurement in diagnosing abnormal fetal growth. The ideal interval to evaluate fetal growth is every 3 to 4 weeks, with a minimum 10-day to 14-day interval necessary to see significant differences. Because of the inherent error in fetal biometric measurements, more frequent ultrasound determinations of EFW may be misleading. Similarly, the use of population-specific growth curves, if available, will improve the ability of the obstetric care provider to identify abnormal fetal growth. For example, growth curves derived from a population that lives at high altitude, where the fetus is exposed to lower oxygen tension, will be different from those derived from a population at sea level. Abnormal fetal growth can be classified as insufficient (i.e., FGR) or excessive (fetal macrosomia).

The definition of FGR has been a long-standing challenge for modern obstetrics. Distinguishing the healthy, constitutionally small fetus, defined as an EFW below the 10th percentile for a given week of gestation, from the nutritionally deprived, truly growth-restricted fetus has been particularly difficult. Fetuses with an EFW less than the 10th percentile are not necessarily pathologically growth restricted. Conversely, an EFW greater than the 10th percentile does not mean that an individual fetus has achieved its growth potential, and such fetuses may still be at risk of perinatal mortality and morbidity. As such, the most widely utilized definition of FGR is EFW less than the 10th percentile for GA in a well-dated pregnancy (ACOG, 2013; Copel and Bahtiyar, 2014). More restrictive definitions of FGR (EFW less than the 5th or 3rd percentiles) can identify fetuses at higher risk for pathologic growth restriction as opposed to constitutionally small fetuses. Additional findings suggestive of fetal compromise like oligohydramnios or abnormal umbilical artery Doppler velocimetry increase the likelihood of pathologic fetal growth restriction.

FGR can be classified into maternal, fetal, or placental etiologies (see Table 14.1). These distinct pathophysiologic mechanisms associated with FGR may occur in isolation or collectively but result in the common final pathway of suboptimal fetal growth and compromised fetal nutrition. Fetuses affected by poor antepartum growth may be further categorized as having symmetric or asymmetric FGR. In cases of symmetric FGR, both the fetal head size and body weight are reduced, indicating a global insult that probably occurred early in gestation. Symmetric FGR may reflect an inherent fetal abnormality (e.g., fetal chromosomal abnormality, inherited metabolic disorder, early congenital infection) or long-standing severe placental insufficiency caused by an underlying maternal disease (e.g., maternal hypertension, long-standing pregestational diabetes, or a significant collagen vascular disorder). Asymmetric FGR is characterized by suboptimal body growth with preserved head growth. It is more commonly observed in the third trimester and is thought to result from a later pathologic event, such as chronic placental abruption leading to uteroplacental insufficiency, in an otherwise uncomplicated pregnancy and healthy fetus.

Currently, patients with risk factors for FGR, those who develop obstetric complications, and those identified with lagging symphysial fundal height measurements are subsequently screened with ultrasound to assess fetal growth. This screening algorithm is in place in the United States, the UK, and various other countries; however, it is known to be imprecise and many fetuses at risk for

growth restriction as well as perinatal mortality are not identified with this system. For this reason, a prospective cohort study evaluated screening for FGR with a universal third-trimester ultrasound to assess fetal growth (Sovio et al., 2015). The authors found that standard screening for FGR identified 20% of small for gestational age (SGA) infants, and implementation of universal screening identified 57% of SGA infants. The increased sensitivity resulted in a decrease in specificity from 98% to 90%, resulting in an increased number of false-positive cases. Therefore implementation of a universal screening program to identify fetuses at risk for growth restriction and increased perinatal mortality would have to take into consideration the issue of over-identification of possible at-risk fetuses and subsequent overtreatment.

Early and accurate diagnosis of FGR coupled with appropriate intervention will lead to an improvement in perinatal outcome. If FGR is suggested clinically and by ultrasound examination, thorough evaluations of the mother and fetus are indicated. Referral to a maternal–fetal medicine specialist should be considered. Every effort should be made to identify the cause of FGR and to modify or eliminate contributing factors. Up to 20% of cases of severe FGR are associated with fetal chromosome abnormalities or congenital malformations, 25%–30% are related to maternal conditions characterized by vascular disease, and a smaller proportion are the result of abnormal placentation. However, in a substantial number of cases (50% or more in some studies), the cause of the FGR will remain uncertain even after a thorough investigation (Resnik, 2002).

Fetal macrosomia is defined as an EFW (not birthweight) of 4500 g or greater, measured either clinically or by ultrasound, and is independent of GA, diabetic status, or actual birthweight (ACOG, 2000). Fetal macrosomia refers to a single cutoff EFW; this should be distinguished from the large for GA fetus, which is one in whom the EFW is greater than the 90th percentile for GA. By definition, 10% of all fetuses are large for GA at any given GA. Fetal macrosomia is associated with an increased risk of cesarean delivery, operative vaginal delivery, and birth injury to both the mother (including vaginal, perineal, and rectal trauma) and the fetus (orthopedic and neurologic injury) (O'Sullivan et al., 1973; Widness et al., 1985; Magee et al., 1993; Kjos and Buchanan, 1999; ACOG, 2000). Shoulder dystocia with resultant brachial plexus injury (Erb palsy) is a serious consequence of fetal macrosomia and is further increased in the setting of diabetes because of the increased diameter of the upper thorax and neck of those fetuses.

Fetal macrosomia can be determined clinically, by abdominal palpation using Leopold maneuvers, or by ultrasound examination; these two techniques appear to be equally accurate (Watson et al., 1988). However, EFW measurements are less accurate in large (macrosomic) fetuses than in normally grown fetuses, and factors such as low amniotic fluid volume, advancing GA, maternal obesity, and the position of the fetus can compound these inaccuracies. Clinical examination has been shown to underestimate the birthweight by 0.5 kg or more in almost 80% of fetuses with macrosomia (Niswander et al., 1970). For these reasons, the prediction of fetal macrosomia is not particularly accurate, with a false-positive rate of 35% and a false-negative rate of 10% (Niswander et al., 1970; Watson et al., 1988). A number of alternative sonographic measurements have therefore been proposed in an attempt to better identify the macrosomic fetus, including fetal AC alone, umbilical cord circumference, cheek-to-cheek diameter, and upper arm circumference; however, these measurements remain investigational and should not be used clinically.

Despite the inaccuracy in the prediction of fetal macrosomia, an EFW should be documented either by clinical estimation or ultrasound examination in all women at high risk for fetal macrosomia at approximately 38 weeks' gestation. Suspected fetal macrosomia is not an indication for induction of labor, because induction does not improve maternal or fetal outcomes (ACOG, 2000). However, if the EFW is excessive, an elective cesarean delivery should be considered to prevent fetal and maternal birth trauma. Although controversy remains as to the precise EFW at which an elective cesarean delivery should be recommended, a suspected birthweight in excess of 4500 g in women with diabetes or 5000 g in women without diabetes is a reasonable threshold (ACOG, 2000, 2001, 2002).

Amniotic Fluid Assessment

Amniotic fluid plays a key role in the health and development of a growing fetus. Once considered an afterthought during the ultrasound examination of the fetus, evaluation of the amniotic fluid is now considered an integral part of ultrasound evaluation for fetal well-being. Amniotic fluid serves a number of important functions for the developing embryo and fetus. It provides cushioning against physical trauma; creates an environment free of restriction and or distortion, allowing for normal growth and development of the fetus; provides a thermally stable environment; allows the respiratory, gastrointestinal, and musculoskeletal tracts to develop normally; and helps to prevent infection (Hill et al., 1984).

The chorioamnion acts as a porous membrane early in pregnancy, allowing the passage of water and solutes across the membrane; there is little contribution from the small embryo. As the pregnancy progresses into the late first trimester, the diffusion of fluid across the fetal skin occurs, increasing the volume of amniotic fluid. In the second half of the pregnancy, the main sources of amniotic fluid come from fetal kidneys and lungs. The primary sources for removal of fluid are from fetal swallowing and absorption into fetal blood perfusing the surface of the placenta. As more fluid is produced than is resorbed by the fetal-placental unit, the volume of amniotic fluid increases throughout the first 32 weeks of pregnancy (Fig. 14.4). The volume peaks at approximately 32 to

33 weeks' gestation, and at this GA, equal amounts of fluid are produced and resorbed. After term, the amniotic fluid declines at a rate of 8% per week (Brace and Wolf, 1989).

Because amniotic fluid plays a critical role in the normal development of a fetus, the assessment of amniotic volume is an essential component of the ultrasound evaluation for fetal well-being. Subjective estimates of the amniotic fluid volume have been validated, but two ultrasound measurements – amniotic fluid index (AFI) and maximum vertical pocket (MVP) – have been developed to quickly and accurately assess the quantity of amniotic fluid surrounding the fetus.

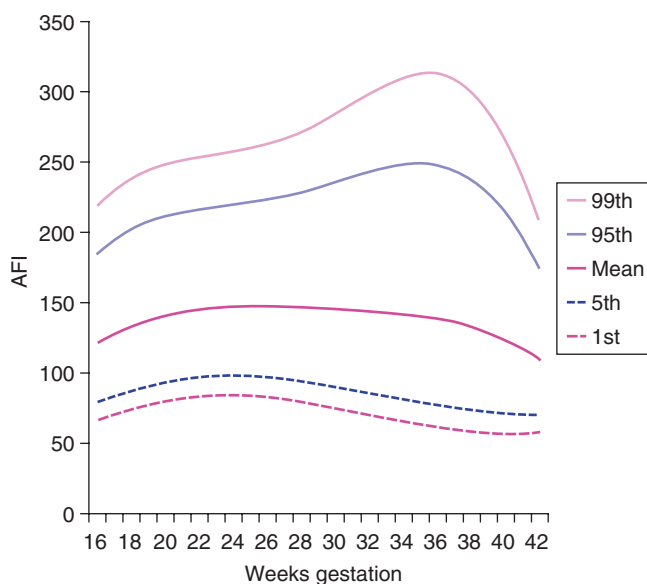
The AFI is a semiquantitative method for assessing the amniotic fluid volume with ultrasound. The gravid uterus is divided into four quadrants using the umbilicus, linea nigra, and external landmarks (Rutherford et al., 1987). The deepest amniotic fluid pocket is measured in each quadrant with the ultrasound transducer perpendicular to the floor. The four measurements are added together, and the sum is regarded as the AFI. Pockets filled with umbilical cord or fetal extremities should not be used for generating the AFI (Hill, 1997). Researchers and clinicians have used a variety of measurements to define abnormalities in amniotic fluid volume. However, the normal range of the AFI most commonly used in clinical practice is greater than 5 to less than 24 cm of fluid. Pregnancies with an AFI of greater than 5 are described as having oligohydramnios, and pregnancies with measurements greater than 24 cm are described as having polyhydramnios (Reddy et al., 2014).

The MVP is another semiquantitative method for assessing the fluid volume. The technique involves scanning the gravid uterus for the single deepest pocket of amniotic fluid that is free of umbilical cord and fetal parts and, with the transducer perpendicular to the floor, measuring the pocket of fluid (Manning et al., 1981b). Oligohydramnios is defined as a single measurement of less than 2 cm. Polyhydramnios is defined as a single measurement greater than 8 cm. In contrast to AFI, this method can be used for both singleton and multiple gestation pregnancies. Currently, MVP is preferred to AFI not only because of its ease to perform but also because the use of MVP compared with AFI results in fewer obstetric interventions without a significant difference in perinatal outcome (Nabhan and Abdelmoula, 2009).

Otherwise normal pregnancies affected by oligohydramnios are at increased risk for perinatal mortality as decreased amniotic fluid volume can be used as a proxy for declining uteroplacental perfusion and increasing placental dysfunction. Decisions to intervene in a pregnancy affected by oligohydramnios are based upon several factors such as GA, fetal condition, and maternal characteristics. First, rupture of membranes must be ruled out, as a low amniotic fluid volume in the setting of ruptured membranes is no longer predictive of poor placental perfusion. Expert opinion recommends delivery after 36 to 37 weeks' gestation in the setting of isolated and persistent oligohydramnios (MVP <2 cm) in an otherwise uncomplicated pregnancy (ACOG, 2014b).

Biophysical Profile

An NST alone might not be sufficient to confirm fetal well-being; in such cases, a BPP may be performed. The BPP refers to a sonographic scoring system performed over a 30-minute to 40-minute period designed to assess fetal well-being. The BPP was initially described for testing postterm fetuses but has since been validated for use in both term and preterm fetuses (Manning et al., 1981a, 1985, 1987; Vintzileos et al., 1983, 1987a, 1987b). Notably, BPP is not validated for use in active labor. The five variables



• **Fig. 14.4** Amniotic Fluid Index (in mm) Plotted Against Gestational Age. The curves represent percentiles. AFI, Amniotic fluid index.

described in the original BPP were gross fetal body movements, fetal tone (i.e., flexion and extension of limbs), amniotic fluid volume, fetal breathing movements, and NST (summarized in Table 14.2) (Manning, 1989). More recently, however, BPP is interpreted without the NST.

The individual variables of the BPP become apparent in healthy fetuses in a predictable sequence: fetal tone appears at 7.5 to 8.5 weeks, fetal movement at 9 weeks, fetal breathing at 20 to 22 weeks, and FHR reactivity at 24 to 28 weeks' gestation. Similarly, in the setting of antepartum hypoxia, these characteristics typically disappear in the reverse order in which they appeared (i.e., FHR reactivity is lost first, followed by fetal breathing, fetal movements, and finally fetal tone) (Vintzileos et al., 1987a). The amniotic fluid volume, which is composed almost entirely of fetal urine in the second and third trimesters, is not influenced by acute fetal hypoxia or acute fetal CNS dysfunction. Rather, oligohydramnios (decreased

amniotic fluid volume) in the latter half of pregnancy and in the absence of ruptured membranes is a reflection of chronic uteroplacental insufficiency, increased renal artery resistance leading to diminished urine output, or both (Oz et al., 2002); it predisposes to umbilical cord compression, thus leading to intermittent fetal hypoxemia, meconium passage, or meconium aspiration. Adverse pregnancy outcome (including abnormal FHR tracing, low Apgar score, and neonatal intensive care unit admission) is more common when oligohydramnios is present (Bochner et al., 1987; Tongsong and Srisomboon, 1993; Oz et al., 2002; Morris et al., 2003). Serial (weekly) screening of high-risk pregnancies for oligohydramnios is important, because amniotic fluid can become drastically reduced within 24 to 48 hours (Clement et al., 1987).

Although each of the five features of the BPP is scored equally (2 points if the variable is present or normal and 0 points if absent or abnormal, for a total of 10 points), they are not equally predictive of adverse pregnancy outcome. For example, amniotic fluid volume is the variable that correlates most strongly with adverse pregnancy events. The recommended management based on the BPP is summarized in Table 14.3 (Manning, 1989). A score of 8–10 out of 10 is regarded as reassuring; a score of 4–6 is suspicious and requires reevaluation, and a score of 0–2 suggests abnormal fetal testing – previously referred to as *fetal distress* (Manning et al., 1981a, 1981b). Evidence of abnormal fetal testing or oligohydramnios in the setting of otherwise normal fetal testing should prompt evaluation for immediate delivery (Vintzileos et al., 1987a, 1987b).

It is important to recognize that administration of maternal systemic steroids to promote fetal maturity may result in fetal behavioral changes. Subsequent to systemic steroid administration, fetal body movements may decrease, FHR variability may decrease, and fetal breathing episodes may be less frequent (Rotmensch et al., 2005; Verdurmen et al., 2013). These changes in fetal activity and FHR patterns are important to take into consideration when interpreting the various components of the fetal BPP.

TABLE 14.2 Fetal Biophysical Profile

| Element | Criterion (2 Points for Each Element Satisfied) |
|----------------|---|
| Breathing | ≥1 episode of breathing movements lasting 30 s |
| Movement | ≥3 discrete body or limb movements |
| Tone | ≥1 episode of active extension and flexion of limbs or trunk |
| Amniotic fluid | ≥1 pocket of amniotic fluid measuring ≥2 cm in two perpendicular planes |
| NST | ≥2 FHR accelerations lasting ≥15 s over 20 min |

FHR, Fetal heart rate; *NST*, nonstress test.

TABLE 14.3 Interpretation and Management of Biophysical Profile

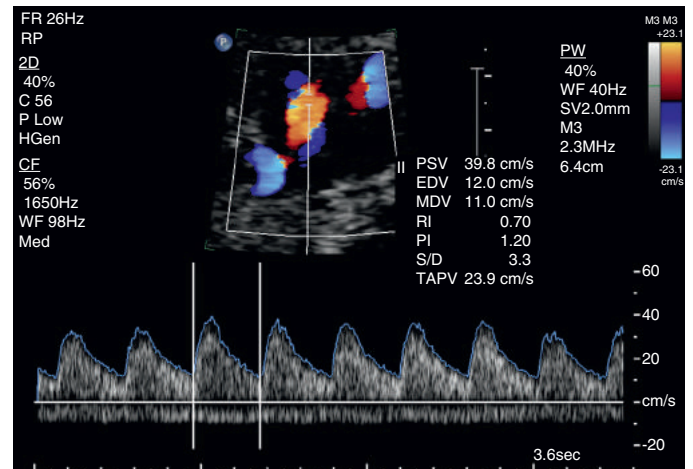
| Score | Comment | Perinatal Morbidity or Mortality Within 1 Week (No Intervention) | Management |
|--------------------------------|--|--|---|
| 10/10 | Normal | <1/1000 | No intervention |
| 8/8 | Normal | — | No intervention |
| 8/10 (abnormal NST) | Normal | — | No intervention |
| 8/10 (abnormal amniotic fluid) | Suspect chronic fetal compromise, renal anomaly, or rupture of membranes | 89/1000 | Rule out renal abnormality or rupture of membranes; consider delivery or prolonged observation if dictated by gestational age |
| 6/8 (other) | Equivocal, possible asphyxia | Variable | If fetus is mature, deliver; if immature, repeat test within 4–6 hours |
| 6/8 (abnormal amniotic fluid) | Suspect asphyxia | 89/1000 | Repeat 4–6 hours; consider delivery |
| 4/8 | Suspect asphyxia | 91/1000 | If ≥36 weeks' gestation or documented pulmonary maturity, deliver immediately; if not, repeat within 4–6 hours |
| 2/8 | High suspicion of asphyxia | 125/1000 | Immediate delivery |
| 0/8 | High suspicion of asphyxia | 600/1000 | Immediate delivery |

Adapted from Manning FA, Morrison I, Harman CR, Lange IR, Menticoglou S. Fetal assessment based on fetal biophysical profile scoring: experience in 19,221 referred high-risk pregnancies. *Am J Obstet Gynecol.* 1987;157:880–884.

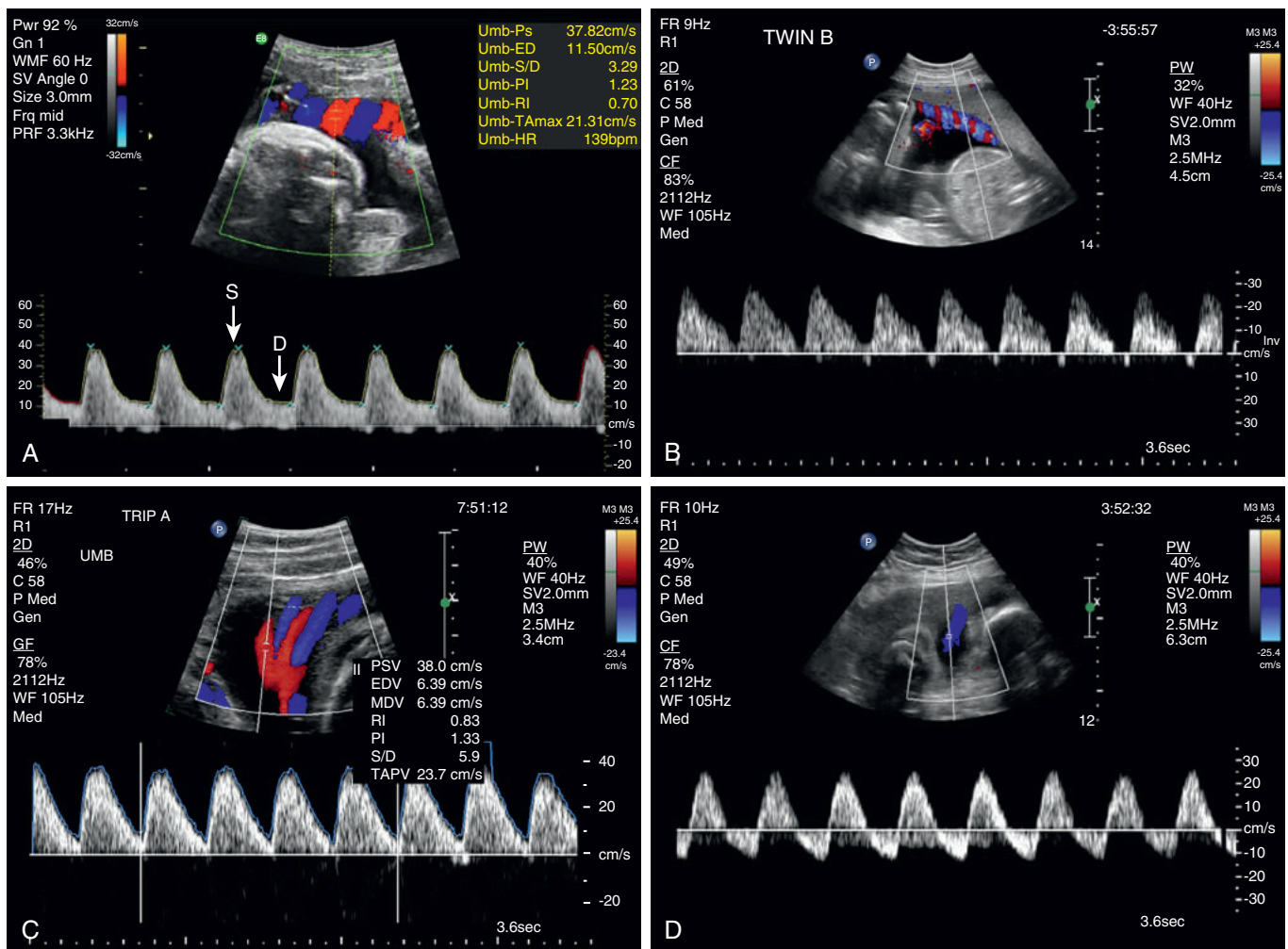
Doppler

Doppler velocimetry shows the direction and characteristics of blood flow, and it can be used to examine the maternal, utero-placental, or fetal circulations. Because of placental capacitance, the umbilical artery is one of the few arteries that normally has forward diastolic flow, and it is one of the most frequently targeted vessels during pregnancy (Fig. 14.5). Umbilical artery Doppler velocimetry measurements reflect resistance to blood flow from the fetus to the placenta. Factors that affect placental resistance include GA, placental location, pregnancy complications (placental abruption, preeclampsia), and underlying maternal disease (chronic hypertension).

Doppler velocimetry of umbilical artery blood flow provides an indirect measure of placental function and fetal status (Giles et al., 1985). Decreased diastolic flow with a resultant increase in systolic-to-diastolic ratio suggests increased placental vascular resistance and fetal compromise. Severely abnormal umbilical artery Doppler velocimetry (defined as absent or reversed diastolic flow) is an especially ominous observation and is associated with poor perinatal outcome, particularly in the setting of FGR (Fig. 14.6A–D)



• **Fig. 14.5** Normal Fetal Umbilical Artery Doppler Recording. EDV, End diastolic velocity; PSV, peak systolic velocity; S/D, systolic/diastolic ratio.

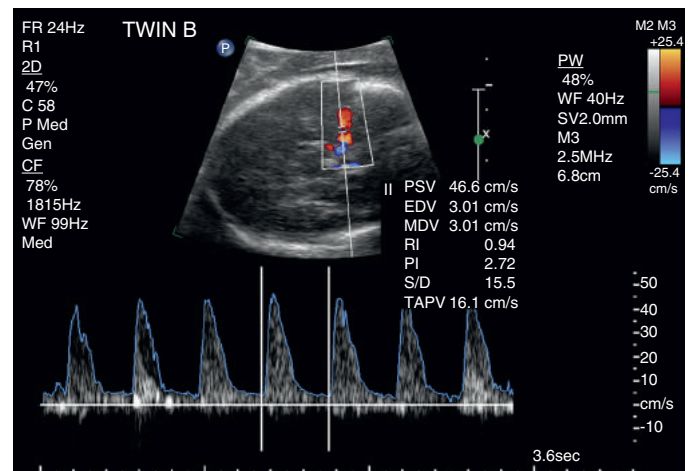


• **Fig. 14.6** Uterine Artery Doppler to Assess Growth Restriction. Increasing levels of placental resistance as seen in the umbilical artery at the time of diastole (D) as it relates to systole (S). Normal end-diastolic flow (A), decreased end diastolic flow (B), absent end-diastolic flow (C), and reversed end-diastolic flow (D). EDV, End diastolic velocity; PSV, peak systolic velocity; S/D, systolic/diastolic ratio.

(McCallum et al., 1978; Ducey et al., 1987; Rochelson et al., 1987; Trudinger et al., 1991; Wenstrom et al., 1991; Zelop et al., 1996). The overall mortality rate for fetuses with absent or reversed flow may be near 30% (Karsdorp et al., 1994). It should be noted that abnormal Doppler studies are often seen in cases of anatomic anomalies or chromosomal abnormalities, which should be noted when managing a case. A 2013 systematic review (Alfirevic et al., 2013) found evidence that use of umbilical artery Doppler ultrasound in high-risk pregnancies (FGR/suspected placental insufficiency) reduced the risk of perinatal deaths and resulted in less obstetric interventions.

The role of ductus venosus and middle cerebral artery (MCA) Doppler in the management of FGR pregnancies is not well defined. As such, urgent delivery should be considered in FGR pregnancies when the results of umbilical artery Doppler studies are severely abnormal, regardless of GA. However, it is unclear how to interpret these findings in the setting of a normally grown fetus. For these reasons, umbilical artery Doppler velocimetry should not be performed routinely on low-risk women. Appropriate indications include FGR, cord malformations, unexplained oligohydramnios, suspected or established preeclampsia, and possibly fetal cardiac anomalies. Umbilical artery Doppler velocimetry has not been shown to be useful in the evaluation of a variety of high-risk pregnancies, including diabetic and postterm pregnancies, primarily because of its high false-positive rate (Farmakides et al., 1988; Landon et al., 1989; Stokes et al., 1991; Baschat, 2004).

As such, in the absence of FGR, obstetric management decisions are not usually made on the basis of Doppler velocimetry studies alone. Nonetheless, new applications for Doppler technology are currently under investigation. An application that has proved extremely useful is the noninvasive evaluation of fetal anemia resulting from isoimmunization. When a fetus develops severe



• **Fig. 14.7** Middle Cerebral Artery Peak Systolic Velocity for Fetal Anemia Assessment. EDV, End diastolic velocity; PSV, peak systolic velocity; S/D, systolic/diastolic ratio.

anemia, cardiac output increases and there is a decline in blood viscosity, resulting in an increase in MCA blood flow, which can be demonstrated by measuring the peak velocity using MCA Doppler velocimetry (Fig. 14.7) (Mari et al., 1995). This demonstration can help the perinatologist to better counsel such patients about the need for cordocentesis and fetal blood transfusion. Doppler studies of other vessels – including the uterine artery, fetal aorta, ductus venosus, and fetal carotid arteries – have contributed considerably to our knowledge of maternal–fetal physiology but as yet have resulted in few clinical applications.

Summary

There are a variety of testing modalities available to the obstetrician for assessing fetal well-being in the antepartum period, each with specific applications, advantages, and disadvantages. As such, it is difficult to apply generalized protocols to the assessment of the fetus. A stepwise approach entails applying the appropriate tests for low-risk patients and identifying those patients, from the results of

those tests or from historical factors, for whom further testing is needed. Although many tests, including NST, fetal weight assessment, and uterine artery Doppler, may be somewhat nonspecific and may have misleading false-positive rates, combining those tests with others increases the specificity. Test results that raise concerns require further investigation or active management.

Suggested Readings

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- Complete references used in this text can be found online at www.expertconsult.com*

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15

Complicated Deliveries

KARA K. HOPPE AND THOMAS J. BENEDETTI

KEY POINTS

- Cesarean section occurs in approximately one-third of all births, with substantial variation among hospitals that cannot be entirely accounted for by preexisting maternal or fetal comorbidities.
- Multidisciplinary team training can reduce infant morbidity after shoulder dystocia.
- Operative vaginal birth with either forceps or vacuum has declined to a multi-decade low despite the low frequency of infant complications directly attributable to this method of delivery.
- Regardless of mode of delivery, both the obstetrician and pediatrician must be aware that the infant in breech presentation requires careful attention upon birth for the presence of hip dislocation and traumatic morbidity (soft tissue trauma, fracture, facial nerve paralysis, and brachial plexus palsy).
- Twin gestations have increased and now account for 3% of total births, 17% of preterm births, and approximately 25% of infants of low birth weight and very low birth weight.
- Vaginal birth after cesarean (VBAC) section should occur in delivery facilities capable of rapidly performing an emergent cesarean section, because this improves the likelihood of minimizing adverse neonatal sequelae.

Overview

Historically, childbirth was often regarded as a perilous undertaking. However, over the past century in the United States, perinatal and maternal mortality have dramatically fallen with advances in modern obstetric care, such as widespread use of antibiotics, easy access to expedient cesarean delivery, and better understanding of the proper use of instruments such as forceps and vacuum extraction (Ali and Norwitz, 2009). Indeed, adverse outcomes are generally uncommon in modern obstetrics and, unlike in the past, most labor and delivery concludes with a healthy mother and neonate. Nevertheless, complicated deliveries still exist, and knowledge of their conduct and sequelae is still required for the administration of proper maternal and infant care.

In this chapter we will first address the complicated vaginal delivery, with particular attention to neonatal outcomes. We will then discuss cesarean delivery and vaginal birth after cesarean (VBAC) delivery and what neonatal implications these may have. Before discussing complicated labor and its neonatal effects, it is

important to have a brief understanding of the conduct of normal labor and delivery. A comprehensive discussion of labor and delivery is beyond the scope of this chapter, and the interested reader is directed to *Williams Obstetrics*, 24th ed, Chapter 2 on normal labor (Cunningham et al., 2014).

Vaginal Delivery

The first stage of labor begins with the onset of regular uterine contractions with concomitant cervical dilation and effacement, and it ends with complete cervical dilation. The first stage is traditionally (Friedman, 1954) further subdivided into a latent phase, the length of which is variable and can last for several hours, and an active phase, which usually begins when the cervix is dilated 4 cm and is marked by further rapid, progressive cervical dilation and effacement. Often the diagnosis of the transition from latent to active phase labor is retrospective, because the time of onset of active labor is variable by patient. The second stage begins with complete cervical dilation and terminates with the expulsion of the fetus from the birth canal. The third stage of labor concludes with the delivery of the placenta.

Disorders of the conduct of labor are of either protraction, in which cervical dilation or fetal descent occurs but is at a rate much less than expected, or arrests. Both disorders are addressed by operative delivery if they are unresponsive to active medical management; this can be performed abdominally through cesarean section or vaginally by obstetric forceps or vacuum extraction if the cervix is fully dilated and specific criteria are fulfilled (see later discussion on [operative vaginal delivery](#)). All these modalities can have neonatal and maternal adverse effects, and the choice of instrument or mode of delivery must always be selected taking these potential morbidities into account.

In 2010, data from the Consortium on Safe Labor stimulated debate regarding whether the 60-year-old data of Friedman apply to currently laboring women (Zhang et al., 2010). In this retrospective study conducted at US hospitals, the duration of labor was analyzed in over 60,000 women, each of whom vaginally delivered a singleton vertex fetus and had a normal perinatal outcome. The 95th percentile rate of active phase dilation was found to be slower than the standard rate derived from Friedman's work. Two important differences were found.

First, from 4 to 6 cm, both nulliparous and multiparous women dilated more slowly than historically described. After 6 cm,

multiparous women dilated more rapidly than primiparous women. Secondly, the active phase often did not start until at least 6 cm. Although challenged by Friedman and Cohen, many obstetricians feel that the Consortium on Safe Labor data, rather than the standards proposed by Friedman, should inform evidence-based labor management (Cohen and Friedman, 2015). In 2012 a summary was published by a joint committee including the Eunice Kennedy Shriver National Institute of Child Health and Human Development, Society for Maternal-Fetal Medicine, and American College of Obstetricians and Gynecologists (ACOG) Workshop (Spong et al., 2012) regarding labor management guidelines largely based on the work of Zhang. Further studies will be necessary to determine whether the newer data from Zhang will supersede the work of Friedman.

Cesarean Section

A cause-and-effect relationship between cesarean delivery and improved neonatal outcomes in the United States has never been demonstrated. Currently, almost one mother in three is giving birth by cesarean section, a record level for the United States. The rate of cesarean delivery in the United States, which peaked in 2009 at 32.9%, had declined only slightly to 32.4% by 2014 (Hamilton et al., 2015). Recommendations from the US Department of Health and Human Services Healthy People 2010 recommend a cesarean section rate of less than 15% for a first pregnancy and 63% for previous cesarean sections. However, recent international data have called into question the safety of this low projection. In this extensive international study of World Health Organization (WHO) member countries, they found that a cesarean section rate up to 19% was associated with lower maternal and neonatal mortality than cesarean section rates lower than 19% (Molina et al., 2015).

There are, however, wide variations in cesarean delivery rates among individual states in the United States (Hamilton et al., 2015) and among individual hospitals within a given state (Main et al., 2012). This cesarean section variability was shown to be 10-fold (7%–70%) among birthing hospitals in the United States and varied from 17.5%–31.8% among the 50 states. This suggests that factors other than pregnancy risk indicators may heavily influence the current cesarean delivery rate. Liability fears have been suggested as a leading cause of the variability in cesarean section rates. One study has lent validity to this concern, showing that higher cesarean section rates were associated with reduced risk of litigation (Jena et al., 2015). This rise in cesarean delivery has been associated with a parallel drop in the vaginal operative delivery rate to less than 5%.

These two parallel trends have increased the cost of childbirth in the United States and are now presenting challenges to many state budgets, as nearly 50% of obstetric care cost is paid for with public funds (i.e., Medicaid). In response to a looming budget crisis, the state of Washington reduced hospital payment for uncomplicated cesarean delivery (DRG 371: Cesarean section) by reimbursing only the equivalent of a complicated vaginal birth (DRG 372: Normal delivery with problems), a projected savings of \$2 million per year. Many states are actively working to reduce the rate of cesarean delivery. Projects are under way to reduce elective delivery before 39 weeks' gestation, safely promote VBAC, and reduce the hospital and individual practitioner's variability in cesarean section rates. Whether this can be accomplished on a large scale without adversely affecting maternal and child health remains to be seen.

Cesarean section is usually performed through either a Pfannenstiel or vertical skin incision. The uterine incision is often made transversely in the lower uterine segment, because it minimizes intraoperative blood loss and future risk of rupture during subsequent labor, compared with a vertical or classical incision. The risk of rupture in future labor is thought to be 0.5%–1.0% for a low transverse incision (ACOG, 2010). However, risks of uterine rupture with previous classical incision have ranged from 1%–12% in women undergoing a trial of labor (Halperin et al., 1988; Rosen et al., 1991; Landon et al., 2004). The ACOG Practice Bulletin, Number 115 quotes a 4%–9% risk of uterine rupture with a vertical or T-shaped uterine incision (ACOG, 2010).

Cesarean delivery is also performed for disorders of protraction or arrest in the first stage of labor when conservative measures, such as oxytocin or amniotomy, fail to augment delivery or in the second stage when assisted or operative vaginal delivery is deemed unfeasible or unsafe.

A partial list of other accepted indications for cesarean delivery is as follows:

1. Fetal malpresentation (e.g., shoulder or breech)
2. Placenta previa
3. Prior classical uterine incision
4. Fetal status not reassuring, remote from vaginal delivery
5. Higher-order multiple gestation (triplet or greater)
6. Fetal contraindications to labor (alloimmune thrombocytopenia)
7. Maternal contraindication to labor (e.g., history of rectal or perineal fistulas from inflammatory bowel disease, large lower-uterine segment, or cervical leiomyoma preventing vaginal delivery)
8. Maternal choice after counseling regarding risks versus benefits

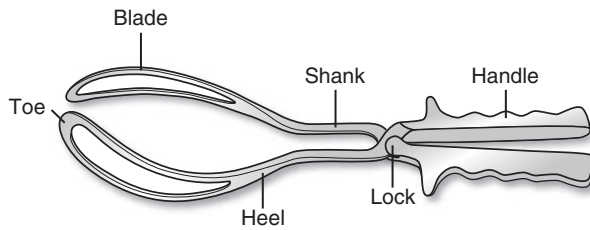
Operative Vaginal Delivery: Obstetric Forceps and Vacuum Extraction

Description of the Obstetric Forceps

Obstetric forceps have been used to facilitate vaginal deliveries since 1500 BC. Most commonly, the invention is credited to Peter Chamberlen and his brother, both obstetricians from England. Designed originally as a means of extracting fetuses from women who were at high risk of dying during childbirth, forceps now are an alternative to cesarean delivery in women with a protracted second stage of labor. Originally, many of these instruments were furnished with hooks and other accessories of destruction, and they were intended to save the mother but not the fetus. Over the last 500 years, the modern instruments in current use have been through hundreds of modifications, safer techniques have been established, and the overriding goal now includes delivering an intact, living baby and a healthy mother (Meniru, 1996; O'Grady et al., 2002).

Current obstetric forceps were first devised for practical use in the 16th and 17th centuries and were perfected over the past 300 years into the models in current use. Although there are many variations on the standard blueprint, depending on the indication for its use, all obstetric forceps have a similar design.

Forceps are made of stainless steel and consist of two blades (each approximately 37.5 cm long, crossing each other), a lock at the site of crossing, and a handle, whereby the instrument is grasped by the obstetrician. The part of the forceps that grasps the fetal head is the blade; this is further divided into the heel, which is the part closest to the lock, and the toe, which is the most distal



• **Fig. 15.1** Simpson forceps: a standard obstetric forceps with features common to all such instruments.

TABLE 15.1 Types of Obstetric Forceps in Most Common Use

| Type | Anatomic Modification | General Use |
|-------------------|---|--|
| Classic | | |
| Tucker–McClane | Solid blade | Nonmolded vertex |
| Simpson | Parallel shanks | Molded vertex or significant caput |
| Elliot | Convergent shanks | Nonmolded vertex |
| Laufe | Pseudofenestrated blade; divergent shanks | For preterm infants or EFW <2500 g |
| Rotational | | |
| Kielland | English lock; absent pelvic curve | For rotation of fetal vertex $\geq 45^\circ$ |
| Breech | | |
| Piper | Long handles with no pelvic curvature | For after-coming head in breech vaginal delivery |

EFW, Estimated fetal weight.

part of the blade. The blade can be either fenestrated – meaning the body of the blade is hollow – or solid to prevent fetal head compression. A further modification is pseudofenestration, in which a solid blade has a ridged edge, combining the advantages of easier applicability and less fetal trauma that a solid blade affords with the ease of traction of a fenestrated blade. Obstetric forceps also possess a rounded cephalic curve, which accommodates the fetal vertex, and a pelvic curve that mirrors the maternal pelvic curve (Fig. 15.1).

There are more than 60 different types of obstetric forceps described in the literature, but most of them are not used currently. The forceps used most commonly today are described in Table 15.1, along with their indications for use and the variations in anatomy, which distinguish one from the other.

Indications for Use of Obstetric Forceps

To an individual without obstetric training, the use of forceps can appear to be a dangerous and difficult undertaking, fraught with potential trauma for both the mother and fetus. It is true that the use of this instrument, if not performed carefully or appropriately, can have serious consequences. Nevertheless, with properly trained hands, and a proper appreciation of its use, forceps have traditionally been lifesaving for both mother and fetus.

The criteria for the safe application of obstetric forceps are as follows:

1. The cervix must be fully dilated.
2. The position of the fetal vertex must be known. Forceps should not be applied when the fetal presentation is in doubt.
3. The fetal vertex must be engaged within the maternal pelvis. Often in difficult or challenging labors, significant caput can lead to the false impression that fetal station is lower than in reality. For this reason, the obstetrician must be confident that the actual biparietal diameter has passed the pelvic inlet (engagement) as evidenced by the leading part of the fetal skull beyond the level of the ischial spines. In addition, when the presentation is occiput posterior, the leading point of the fetal skull may appear to be lower in the pelvis, although the biparietal diameter has not yet passed through the pelvic inlet, and can lead to an erroneous conclusion about fetal station.
4. When the forceps are properly applied, the sagittal suture must be exactly midway between the blades, and the lambdoidal sutures should be equidistant from the edge of the blade.
5. If these conditions are not met, the delivery with forceps should be reconsidered.

The ACOG has revised its 1988 classification of the type of forceps delivery, according to the station of the fetal vertex before forceps application (ACOG, 2015), which is as follows:

1. Outlet forceps – the fetal vertex is visible at the labia without manually separating them, and the fetal skull has reached the pelvic floor.
2. Low forceps – the leading point of the fetal skull is >2 cm beyond the ischial spines.
3. Mid forceps – the fetal head is engaged (0 to +1 station). The forceps should be applied only if cesarean delivery is not quickly or imminently possible with the fetus in distress, or there should be a high likelihood that the forceps operation will be successful.
4. High forceps – the vertex is not engaged (leading part not at the level of the ischial spines or beyond). Under these circumstances, the forceps must never be applied.

The usual indications for use of the obstetric forceps are:

1. Maternal exhaustion or inability to push (endotracheal intubation with sedation or paralysis; neuromuscular disease)
2. Fetal heart tracing not reassuring
3. Maternal contraindications to pushing (cardiopulmonary disease, cerebrovascular aneurysm)
4. After-coming head in a vaginal breech delivery

Forceps and Potential Neonatal Morbidity

As stated previously, forceps were used for hundreds of years without regard to fetal survival and were primarily needed to facilitate or terminate difficult labors for maternal benefit. Today, with the widespread availability of cesarean delivery, considerations turn to providing the best neonatal outcome possible; therefore the difficult forceps deliveries of the past have largely been abandoned. Nevertheless, forceps can still play a role in modern obstetrics if judiciously used and in some circumstances can provide a safer alternative to cesarean delivery for both mother and baby.

The difficulty in interpreting the obstetric literature, in regard to neonatal morbidity incurred by forceps, is that the classification for type of forceps was revised by the ACOG in 1988; therefore prior studies do not use the same clinical criteria used currently to select appropriate candidates for forceps use. Furthermore, residency training in operative vaginal delivery has dramatically decreased over the past 30 years, potentially increasing fetal risk. Consequently, for modern interpretation of adverse outcomes, one

must look to studies performed after the 1988 ACOG revision of the classifications.

The incidence of operative vaginal delivery in the United States has declined to 3.2% of births, ranging by hospital from 1% to 23% (Hamilton et al., 2015). Forceps deliveries have declined to 0.5%, while vacuum deliveries account for the majority of operative births. The reported failure rate for forceps delivery is approximately <5% (Murphy and Koh, 2007). In general the failure rate increases as the station from which delivery is attempted decreases. The indication for forceps use varies widely by clinical situation, and the neonatal morbidity that can result from a “difficult pull” in a patient with a transverse arrest with marked fetal asynclitism may be different from the quick delivery of a 2600 g fetus whose mother is unable to push, even if both deliveries are by low forceps. Asynclitism refers to the position of a baby in the uterus such that the head of the fetus is presenting first and is tilted to the shoulder, causing the fetal head to no longer be in line with the birth canal.

There are few randomized prospective trials specifically addressing the issue of neonatal morbidity arising from forceps operations, and many of those are over 20 years old. In addition, there appears to be a substantial difference regarding frequency of operative delivery and outcomes between the United States and other English-speaking countries (UK and Australia) (Murphy and Koh, 2007). Again, the liability atmosphere in the United States is most likely to account for this difference in operative vaginal birth frequency and experience.

The primary outcome that birth attendants wish to avoid when considering operative vaginal birth is birth trauma to the baby primarily and secondarily to the mother. A large population-based study in the United States describing over 11 million births showed that birth trauma (facial nerve injury, cephalhematoma, intracranial hemorrhage, need for mechanical ventilation) was more likely with operative vaginal birth than spontaneous birth and even higher if sequential (vacuum and forceps) operative attempts had been made (Demissie et al., 2004). Vacuum-assisted births were associated with lower rate of birth injury, seizures, and assisted ventilation than forceps-assisted births. A large population-based study in California, however, found no differences in outcome with forceps versus vacuum except for a higher incidence of facial nerve palsy with forceps delivery (Towner and Ciotti, 2007). The absolute frequencies of birth trauma in these two large series were very similar and, although the rate of birth trauma was higher than spontaneous vaginal birth, the overall frequencies were actually quite low. For example, when comparing forceps versus spontaneous vaginal delivery the rates of intracranial hemorrhage were 1.5/1000 versus 0.4/1000, mechanical ventilation was 4/1000 versus 2/1000, and facial nerve palsy was 4/1000 versus 0.2/1000. Table 15.2 shows the incidence of intracranial injury by delivery mode.

The hazard of making such comparisons is that in most instances the decision facing the birth attendant is not operative delivery versus spontaneous birth but operative birth versus cesarean delivery. In this regard the data from Towner provided some important information. When comparing fetal outcomes after successful operative vaginal birth, unsuccessful operative vaginal birth, and cesarean section done for labor disorder without attempt at operative vaginal birth, the latter two were similar. The primary finding of this study was that there appeared to be an irreducible number of fetal injuries associated with labor rather than the method of delivery.

The clinical dilemma that has probably changed the frequency of operative delivery in the United States also comes from data presented by Towner and Ciotti (2007) as well as others (Gardella

TABLE 15.2 Risk of Neonatal Intracranial Hemorrhage^a According to Type of Delivery

| Mode of Delivery | Incidence of Intracranial Injury |
|------------------------------|----------------------------------|
| Vacuum | 1 in 860 |
| Forceps | 1 in 664 |
| Combined vacuum and forceps | 1 in 256 |
| Cesarean, in labor | 1 in 907 |
| Cesarean, not in labor | 1 in 2750 |
| Spontaneous vaginal delivery | 1 in 1900 |

^aIntracranial hemorrhage was defined as subdural, cerebral, intraventricular, or subarachnoid.

Adapted from Towner D, Castro MA, Eby-Wilkens E, Gilbert WM. Effect of mode of delivery in nulliparous women on intracranial injury. *N Engl J Med*. 1999;341:1709–1714.

et al., 2001; Fong et al., 2014). The dilemma is what to do in the instances in which operative vaginal birth fails. Towner showed that fetal injury was higher in sequential vaginal birth, even if successful, than with successful single operative attempts. Gardella et al. (2001) found the same thing and expended the excess morbidity to the mother as well. However, Towner found that the excess morbidity to the baby after failed vaginal birth was not reduced by cesarean section. Another study looking at outcomes of failed operative delivery also found worse neonatal outcomes, but these were nearly all in infants who had fetal heart rate abnormalities listed as either the primary or secondary reason for operative delivery (Alexander et al., 2009). The knowledge of morbidity associated with failed vaginal births by most obstetricians in the United States has appeared to have changed behavior regarding forceps attempts. In the second stage of labor if efforts to achieve spontaneous vaginal birth are unsuccessful, cesarean delivery is increasingly offered rather than attempted vaginal birth with the risk of failure and increased fetal morbidity. In many hospitals in the United States, the percentage of labor-related cesarean sections done in the second stage of labor exceeds 40%.

Vacuum Delivery: Indications, Uses, and Comparison With Forceps Procedures

Operative vaginal delivery for the indications listed previously can also be performed by the vacuum extractor. Vacuum extraction was first described in 1705 by Dr. James Yonge, an English surgeon, several decades before the invention of the obstetric forceps. However, it did not gain widespread use until the 1950s, when it was popularized in a series of studies by the Swedish obstetrician Dr. Tage Malmström. By the 1970s, the vacuum extractor had almost completely replaced forceps for assisted vaginal deliveries in most northern European countries, but its popularity in many English-speaking countries, including the United States and the UK, was limited. By 1992, however, the number of vacuum-assisted deliveries surpassed the number of forceps-assisted deliveries in the United States, and by the year 2000 approximately 66% of operative vaginal deliveries were by vacuum (Hillier and Johanson, 1994; Ali and Norwitz, 2009). This trend has continued to 2014, with approximately 85% of operative vaginal attempts performed with vacuum extractors (Hamilton et al., 2015).



• **Fig. 15.2** A soft, bell-shaped vacuum extractor (*top*) and a rigid, mushroom-shaped vacuum extractor (*bottom*). (Courtesy Cooper Surgical, Trumbull, CT, USA.)

The situations that indicate the use of the vacuum and the requirements that must be fulfilled for its correct use are identical to those for the obstetric forceps. The original device consisted of a metal cup but, currently, Silastic cups (flexible or semirigid) have replaced the metal varieties. Care must be taken in its application to ensure that an adequate seal with the fetal head has been created and that no maternal soft tissue is trapped between the suction device and the fetus. Traction is then applied to the fetal head in the line of the birth canal in an effort to assist delivery. It is also cautioned that rocking movements or torque to the cup should not be used. The premature infant (<34 weeks) is a relative contraindication to vacuum application. It is generally advised that no more than three detachments occur before attempts at vacuum extraction are abandoned (Ali and Norwitz, 2009).

In a laboratory experiment, Duchon et al. (1988) compared the maximum force at suggested vacuum pressures (550–600 mmHg) prior to detachment for different types of vacuum devices. They found that the average force of traction exerted before detachment ranged from 18 to 20 kg. This result is interesting to bear in mind when one considers the older data of Wylie, who estimated the average tractive force required for delivery of infants was 15.95 kg for a primigravida and 11.33 kg for a multipara (Wylie, 1963; Feingold et al., 1988) (Fig. 15.2).

The original vacuum device developed in the 1950s by the Swedish obstetrician Dr. Tage Malmström was a disc-shaped stainless steel cup attached to a metal chain for traction. Because of technical problems and lack of experience with this instrument, vacuum devices did not gain popularity in the United States until the introduction of the disposable cups in the 1980s. There are two main types of disposable cups, which can be made of plastic, polyethylene, or silicone. The soft cup is a pliable, funnel or bell-shaped cup, which is the most common type used in the United States. The rigid cup is a firm mushroom-shaped cup (M cup) similar to the original metal disc-shaped cup and is available in three sizes.

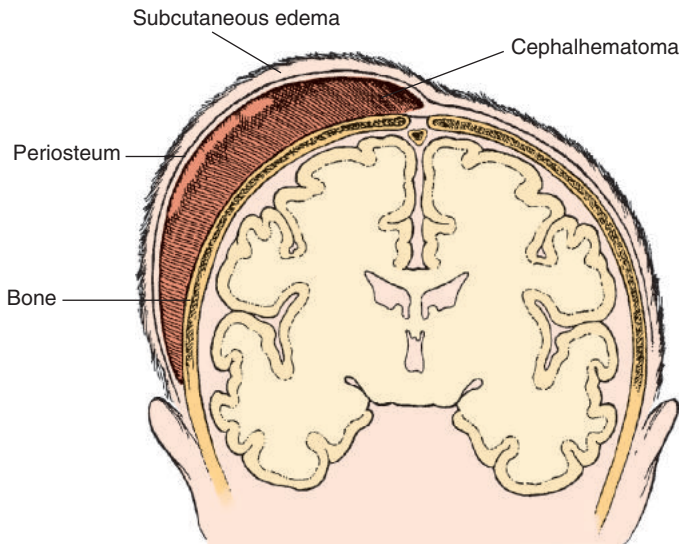
A metaanalysis of 1375 women in nine trials comparing soft and rigid vacuum extractor cups demonstrated that soft cups were more likely to fail to achieve a vaginal delivery, because of more frequent detachments (odds ratio [OR] 1.65, 95% confidence interval [CI] 1.19–2.29), but were associated with fewer scalp

injuries (OR 0.45, 95% CI 0.15–0.60) and no increased risk of maternal perineal injury (Hillier and Johanson, 1994). For example, the risk of scalp laceration with the rigid Kiwi OmniCup (Clinical Innovations, Murray, Utah) was reported to be 14.1%, compared with 4.5% using a standard vacuum device ($P = .006$). These and other authors concluded that hand-held soft bell cups should be considered for more straightforward occiput-anterior deliveries, and that rigid M cups should be reserved for more complicated deliveries, such as those involving larger infants, significant caput succedaneum (scalp edema), occiput-posterior presentation, or asynclitism.

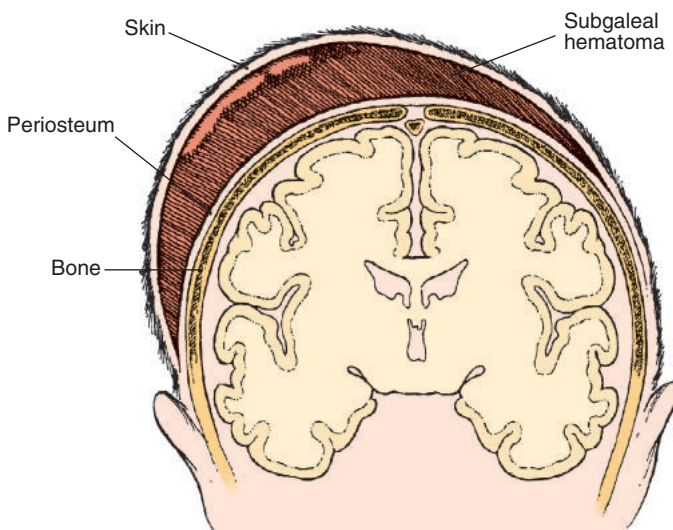
The vacuum extractor is used in the United States, but is not free from neonatal injury. Other than superficial scalp lacerations or abrasions, the use of the vacuum has been associated with cephalhematoma and subgaleal hemorrhages.

Cephalhematoma occurs when the force created by the vacuum results in the rupture of diploic or emissary vessels between the periosteum and outer table of the skull; this fills the potential space that exists between the two with blood. Although cephalhematomas are often cosmetically alarming, they are limited to traveling along one cranial bone, because the firm periosteal attachments limit further extravasation of blood across suture lines. Thus large amounts of blood cannot usually collect in this space, and serious neonatal compromise from this bleeding is rare. In a randomized trial of continuous and intermittent vacuum application, Bofill et al. examined factors associated with increasing the risk of cephalhematoma; it was found that only asynclitism and traction time were independently related to this complication (Hartley and Hitti, 2005). There was a clear relationship between increasing time of vacuum application (up to 6 minutes) and cephalhematoma. Interestingly, Hartley and Hitti did not find a significant independent association of neonatal injury with continuous versus intermittent vacuum, or with decreasing gestational age or increasing birth weight. Furthermore, the number of detachments was not correlated with cephalhematoma. These results were further corroborated by Teng et al., who conducted a prospective observational study of 134 vacuum extractions and found that only increasing total duration of vacuum application was associated with neonatal injury (Feingold et al., 1988). Metaanalysis of randomized trials comparing vacuum to forceps extractions showed that vacuums are more likely to fail to deliver the baby and lead to increased rates of cephalhematoma and retinal hemorrhage (Fig. 15.3) (Vacca, 2007).

Subgaleal hemorrhage poses much more risk for the neonate. It occurs when emissary veins above the skull and periosteum rupture, with blood dissecting through the loose tissue underlying the cranial aponeurosis, unimpeded by suture lines. A tremendous amount of blood, potentially the entire neonatal blood volume (approximately 250–350 mL), can fill this space and thus can compromise the neonate's condition (Hillier and Johanson, 1994). Much of the literature about this rare complication of vacuum extraction was published in the 1970s and early 1980s, with few recent studies to detail associated risk factors. Plauche, in his classic paper published in 1979 on vacuum-related neonatal injury, identified only 18 cases of subgaleal hematomas among 14,276 cases of vacuum-assisted births, in contrast to a mean incidence of cephalhematoma of 6% (Keith et al., 1988). These morbidity estimates are derived from data that are approximately 30–40 years old; nevertheless, Teng et al. noted an incidence of cephalhematoma of 8%, and 0.7% for subgaleal hemorrhage in their more recent investigation, which agrees well with Plauche's estimates (Herbst and Källén, 2008). The incidence in the United States in the last two decades is unknown as there was no specific CPT code to



• **Fig. 15.3** A cephalhematoma is a hemorrhage that occurs under the periosteum of the skull and is thus confined to a defined space with limited capacity for expansion. (Adapted from Gilstrap LC, Cunningham FG, Hankins GDV, et al. *Operative Obstetrics*, ed. 2. Stamford, CT: Appleton and Lange; 2002.)



• **Fig. 15.4** A subgaleal hematoma spreads along subcutaneous soft tissue planes and has no immediate barrier to expansion, creating the potential for significant neonatal hemodynamic compromise. (Adapted from Gilstrap LC, Cunningham FG, Hankins GDV, et al. *Operative Obstetrics*, ed. 2. Stamford, CT: Appleton and Lange; 2002.)

distinguish subgaleal hemorrhage from cephalhematoma or other forms of minor scalp injury until after 2010.

A study from Australian investigators evaluated 37 cases of subgaleal hemorrhage at a single tertiary care center accrued over a period of 23 years, with an estimated prevalence of 1.54/10,000 total births. The finding was that this complication occurred most often in primigravidae, and that a large proportion of these infants (89.1% compared with 9.8% of the general control population) had an attempted vacuum extraction (Fig. 15.4) (Chadwick et al., 1996). Failed vacuum extraction has been identified as a risk factor for subgaleal hemorrhage when comparing infants born after a

successful vacuum extraction (OR 7.3, 95% CI 5.5–9.7) (Ahlberg et al., 2016).

The choice of which instrument to use, forceps or vacuum, is usually determined by the obstetric care provider, depending on the skill level and experience with either method. There have been several randomized trials exploring the instrument used for operative vaginal delivery (Meniru, 1996; O'Grady et al., 2002; Menacker and Martin, 2008; Prapas et al., 2009). The Cochrane Library has pooled the results from 10 randomized trials comparing neonatal morbidity and successful vaginal delivery between these two devices (Roberts et al., 2002). This analysis found that the vacuum was more likely than forceps to fail (OR 1.69, 95% CI 1.31–2.19) and was associated with a greater likelihood of cephalhematoma (OR 2.38, 95% CI 1.68–3.37) and retinal hemorrhage (OR 1.99, 95% CI 1.35–2.98). However, the overall serious complication rate was low, and there was no difference in long-term morbidity between groups (Johanson and Menon, 2000).

A significant problem with the use of vacuum extraction is the difficulty some providers experience in abandoning the procedure and opting for cesarean delivery. One of the best cohort studies found that 82% of successful vacuum extractions occurred with the first three pulls (Murphy and Koh, 2003). When more than three pulls were necessary, signs of trauma were present in 45% of babies. Without clear progress toward vaginal delivery, as evidenced by progressive descent with each pull, attempts at further vaginal birth are not supported by the literature.

Shoulder Dystocia

Shoulder dystocia is arguably the most dreaded complication in obstetrics. The problem posed by this entity is that although it is highly anticipated, it is unpredictable and can appear despite the most cautious measures taken to prevent it. *Shoulder dystocia* is defined as the delivery of the fetal head with an impaction of the fetal shoulder girdle against the pubic symphysis, making subsequent delivery either difficult or impossible without performing auxiliary delivery maneuvers. In some cases the posterior shoulder may be lodged behind the sacral promontory – a bilateral shoulder dystocia.

Once shoulder dystocia occurs, a series of maneuvers – which have never been tested in a prospective fashion, because of the sporadic and unpredictable nature of this complication – are used to resolve it. The first step to resolving shoulder dystocia is usually the McRoberts maneuver, which consists of hyperflexing the maternal thighs onto the abdomen. This maneuver flattens the pubic symphysis and sacral promontory and facilitates delivery of both the anterior and posterior shoulders. The only prospective randomized study of prophylactic McRoberts maneuver in anticipation of shoulder dystocia showed no benefit to this strategy (Beall et al., 2003). If the McRoberts maneuver is unsuccessful, the next intervention is typically applying suprapubic pressure to remove the anterior shoulder from its impacted state behind the pubic symphysis. If these two maneuvers fail, either rotational maneuvers or extraction of the posterior fetal arm are usually tried. The Woods screw rotational maneuver or the Rubin rotational maneuver is used in an attempt to rotate the infant's shoulders to relieve the impaction of the shoulder against the pubic bone. Alternatively, delivery of the posterior arm can be accomplished by inserting the operator's hand into the vagina and grasping the fetal wrist in the posterior arm and guiding it through the vaginal introitus. It is often necessary to perform an episiotomy to have sufficient room in the vagina to accomplish this maneuver. An alternative maneuver

to fetal manipulation is the all-fours position, or Gaskin maneuver. With this maneuver, the mother is moved from the lithotomy position to a hands and knees position. Next, the posterior fetal shoulder, which is now at the 12 o'clock position, is delivered with gentle downward traction. If the dystocia continues unresolved, the Zavanelli maneuver (cephalic replacement) can be performed. After the fetal head is rotated from occiput transverse to occiput anterior, it is flexed and pushed back in the birth canal, and the child is delivered by emergent cesarean section. McRoberts maneuver, suprapubic pressure, or both will relieve shoulder dystocia in more than 50% of instances. Cephalic replacement should be rarely necessary.

In the past few years greater attention has been focused on enhanced practitioner training for shoulder dystocia by means of simulation. Prior to the development of high-fidelity models for shoulder dystocia training, many obstetric birth attendants had never successfully performed maneuvers other than the McRoberts maneuver and suprapubic pressure. Draycott et al. (2008) published data showing that the annual compulsory multidisciplinary training of all attending obstetric physicians and nursing staff in Southmead Hospital in the United Kingdom reduced the incidence of fetal injury and brachial plexus injury within 3 years of introduction. Grobman et al. (2011) found similar reduction in brachial plexus injury recognizable at the time of birth after establishment of a standard protocol and simulation training of providers. Inglis et al. (2011) found similar results when shoulder dystocia training was introduced. At the current time, multidisciplinary team training with a standard protocol appears to offer the best strategy to minimize brachial plexus injury once shoulder dystocia has occurred.

The prevalence of shoulder dystocia varies depending on the population studied and the presence of various risk factors known to predispose women to this obstetric emergency. Estimates range from 0.2% to 1% in a low-risk population to 20% in higher-risk groups (Feingold et al., 1988; Hartley and Hittii, 2005; Herbst and Källén, 2008; Ali and Norwitz, 2009). Maternal obesity, fetal macrosomia, history of prior shoulder dystocia, and maternal diabetes mellitus are the most common associated variables, but are not of sufficient prognostic power to be clinically useful in predicting shoulder dystocia (Herbst and Källén, 2008; Ali and Norwitz, 2009).

Because shoulder dystocia has the potential to cause significant neonatal morbidity and mortality, efforts have been made to predict its occurrence; unfortunately, no clinical guidelines have been clinically tested or proved. Ultrasound examination is commonly used in patients with suspected fetal macrosomia or diabetes to detect large birth weight infants who might be more likely to suffer shoulder dystocia. Third-trimester sonographic examination has an accuracy of $\pm 10\%$ – 15% in the prediction of fetal weight and is thus not highly reliable (Feingold et al., 1988; Keith et al., 1988; Hillier and Johanson, 1994). In addition, if ultrasound examination were completely reliable, the fetal weight cutoff that would prompt an elective cesarean section has not yet been determined.

If practitioners use ultrasound to evaluate estimated fetal weight (EFW), then two strategies have been suggested to avoid shoulder dystocia: labor induction or cesarean section. A recently published randomized clinical trial in France based on ultrasound evaluation of EFW has clarified the risks and benefits of labor induction to avoid shoulder dystocia and subsequent fetal injury (Boulvain et al., 2015). In this trial 800 patients with EFWs greater than the 95th percentile for gestational age were randomized to either induction of labor or observation at 37–39 weeks' gestation. At study conclusion, shoulder dystocia was reduced from 8% to 4% (OR 0.47,

CI 0.26–0.86). Predetermined composite of fetal injury was also reduced from 6% to 2% (CI 0.15–0.71). There were, however, no brachial plexus injuries or deaths in either group. In addition, the occurrence of cesarean delivery was similar in both groups (28% induced vs 32% expectant management: OR 0.89, CI 0.72–1.09). Currently in the United States, labor induction prior to 39 weeks is an adverse quality metric and has financial consequences for both the hospital and obstetric provider. Whether this indication will gain acceptance as an exception to this quality metric remains to be determined.

Rouse et al. (1996) elaborated further on the use of ultrasound to prevent shoulder dystocia. In their decision analysis, they showed that if one chose to perform an elective cesarean section for all women without diabetes but who had sonographically predicted macrosomia (EFW >4000 g), 2345 cesarean sections would need to be performed to prevent one permanent brachial plexus injury. If the 4500-g cutoff were selected, 50% more cesarean deliveries would be needed to prevent one permanent brachial plexus injury. In the mother with diabetes, if one chose a cutoff of 4500 g or greater, 443 cesareans would need to be done to prevent one permanent injury, a tradeoff that most practitioners now believe is acceptable. The conclusions from this decision analysis have been borne out by several other investigators who have established that the risk of nerve injury certainly increases with rising birth weight, but the large number of macrosomic infants who have a normal, spontaneous vaginal delivery without sequelae does not justify a policy of elective cesarean for macrosomia alone in a nondiabetic population (Loucopoulos and Jewelewicz, 1982; Lipitz et al., 1989; Meniru, 1996; O'Grady et al., 2002; Roberts et al., 2002; Pondaag et al., 2004; Menacker and Martin, 2008). Some believe that an EFW of greater than 5000 g provides an acceptable cutoff in nondiabetic patients to offer elective cesarean.

The ideal management of shoulder dystocia would minimize the occurrence of permanent fetal injury or death. In the best systematic review of brachial plexus injury to date, the risk of permanent brachial plexus impairment, if recognizable at birth, was 15%–20% (Pondaag et al., 2004). In another study, a prospective investigation evaluated the natural history of recovery following a birth-related brachial plexus injury of infants referred to a tertiary care, multidisciplinary neurologic center. Enrollment required identification of injury in the newborn period, initial evaluation at the center between 1 and 2 months of age, and lack of antigravity movement in the shoulder or elbow persisting until 2 weeks of age. In this group of children subject to ascertainment bias (as those injuries resolving before 2 weeks of age would not have been included in the results), complete neurologic recovery was documented in 66%, 20% had minimal impairment, and 14% had persistent, severe weakness (Learman, 1998).

At present there is no universally accepted method to prevent shoulder dystocia. Previous studies have shown that operative vaginal delivery, especially vacuum delivery, of a fetus suspected to have macrosomia either clinically or sonographically could increase the risk of shoulder dystocia (Benedetti and Gabbe, 1978). Thus it seems wise to avoid difficult forceps or vacuum delivery if a patient is thought to have an infant weighing more than 4000 g, especially if she has diabetes or a past history of shoulder dystocia.

Prospective studies have shown that brachial plexus injury is related to the extent of provider-applied traction to the fetal head and neck (Mollberg et al., 2007). However, there is a large variation in the provider assessment of traction used in deliveries of infants with brachial plexus recognizable at birth (Mollberg et al., 2007). Several studies evaluating simulated shoulder dystocia measured

traction forces applied by the birth attendant (Allen et al., 1991; Crofts, 2007; Deering et al., 2011). Provider experience, gender, and body habitus were not associated with the amount of force applied during delivery. Family medicine providers applied more force than obstetrics/gynecology providers. A significant number of all providers (19/47, 40%) applied greater than 100 newtons to the fetal neck, a suggested but not proven threshold for brachial plexus injury (Deering et al., 2011).

Currently, delivery training emphasizes that only axial traction be applied to the fetal neck, which should minimize stretch on the brachial plexus during delivery. Unfortunately, some infants are injured with minimal or no force being applied to the fetal neck, which suggests that some injuries occur as a result of a combination of labor and the standard techniques used to deliver all infants and are therefore not provider-dependent (Deering et al., 2011).

The documentation of the events surrounding shoulder dystocia is important, as is the discussion of current status and future status of an infant delivered with a birth injury after shoulder dystocia. The recurrence risk of shoulder dystocia is approximately 10%–15%, which is similar to the risk of shoulder dystocia with a known fetal weight of 4500 g. Both obstetric and pediatric providers should debrief a shoulder dystocia event immediately after the delivery. Regardless of whether fetal injury has occurred, it is optimal for both obstetric and pediatric providers to discuss the delivery events and subsequent newborn treatment plans if necessary with the mother before discharge. The diagnosis of shoulder dystocia is an obstetric diagnosis made at the time of delivery, which is based upon delivery findings as defined above. This is not a diagnosis that should be made by the pediatric team or based on observations of the neonate after delivery.

Vaginal Breech Delivery

Three to four percent of all infants at term will present in the breech position at the time of delivery (Hickok et al., 1992). There are three main types of breech presentations: the footling breech has one (single footling) or both (double footling) lower extremities presenting; the frank breech has both thighs flexed, but legs extended; and the complete breech has both thighs and legs flexed. The vaginal delivery of a singleton footling breech carries attendant risks of cord prolapse and head entrapment, and the consensus among obstetricians is that this presentation should be delivered by cesarean section (unless the fetus is a second twin: see later discussion on [Twin Delivery](#)). The frank breech has a lower risk of these adverse events occurring and thus could potentially be delivered vaginally. The complete breech presentation will convert to frank or footling during labor, and the appropriate management scheme for delivery depends on which leading fetal part will descend.

The mechanics of vaginal breech delivery are as follows. The frame of reference for the presenting part is the sacrum (i.e., sacrum anterior, posterior, or transverse). In the absence of urgent fetal indications, the singleton breech is allowed to deliver passively with maternal expulsive efforts until the infant has been delivered past the umbilicus. At this point the legs are gently reduced, and the trunk and body are gently rotated to bring the sacrum anteriorly. With the appearance of the scapula below the maternal symphysis, the arms are then delivered by gently sweeping them across the chest. Every effort is then made to keep the neck from extending during the delivery of the after-coming head; this is accomplished during delivery of the body by an assistant exerting suprapubic pressure on the fetal head to keep it flexed. Once the body has

delivered, the delivery of the head is accomplished by either the Mauriceau–Smellie–Veit maneuver or with Piper forceps directly applied to the fetal vertex. In the Mauriceau–Smellie–Veit maneuver one hand extends along the posterior neck and occiput and applies pressure to prevent hyperextension, while the other hand gently applies downward traction against the maxilla to flex the head forward as the head is delivered. A free video demonstrating the vaginal breech delivery is available online at the WHO Reproductive Health Library: <http://apps.who.int/rhl/videos/>.

The feasibility of vaginal breech delivery and its safety have been the subject of much debate throughout the past half century. With the advent of safe, expedient cesarean delivery in the United States, many obstetricians have favored cesarean section as the method of choice for management of the breech presentation at term. However, even in institutions with availability for expedient cesarean deliveries, there are situations such as precipitous delivery, out-of-hospital delivery, severe fetal anomalies, fetal death, or a mother's preference for vaginal birth that make it essential for clinicians to maintain the skills needed for a vaginal breech delivery. The literature to support this point of view has produced conflicting conclusions, and its interpretation is consequently difficult. Unfortunately, there are only two randomized trials that have explored the question of which delivery route is best for the term singleton frank breech fetus (Collea et al., 1980; Hannah et al., 2000).

Collea et al. (1980) randomized 208 women with a singleton frank breech presentation at term to vaginal delivery or cesarean section; they found a low overall risk of permanent birth injury or neonatal morbidity in the vaginal delivery group, although the incidence of neonatal morbidity was higher in the vaginal delivery group. Of note, a majority of conditions listed as morbidities (hyperbilirubinemia, meconium aspiration, mild brachial plexus injury) had resolved by hospital discharge. In addition, decreased neonatal morbidity with cesarean section was offset by a striking increase in higher maternal risk in the operative group. It must be remembered that this study was published in 1980, and standards of maternal and neonatal care have changed dramatically since then.

Hannah et al. (2000) published a large, multicenter, multinational trial that randomized 2088 women at 121 centers in 26 countries to planned vaginal birth or planned elective cesarean section. Criteria for enrollment were frank or complete term singleton breech with no evidence of fetal macrosomia. The investigation was halted when preliminary results showed that there was significantly increased neonatal mortality and severe morbidity in the vaginal breech arm compared with the cesarean arm (5.0% vs 1.6%). This conclusion was not altered by the experience of the delivering obstetrician or maternal demographic factors such as parity and race. Maternal morbidity between both groups was comparable. The difference in outcome was even more striking in countries such as the United States, with a low national perinatal mortality rate (5.7% vs 0.4%).

Criticisms of this study are that the patients enrolled did not have computed tomography (CT) pelvimetry performed, which in some institutions is standard practice before considering a vaginal breech delivery. Furthermore, subjects did not have continuous fetal monitoring, but rather intermittent fetal auscultation every 15 minutes. In addition, the capability of various centers to perform emergent cesarean sections differed markedly, and this could have potentially affected the neonatal morbidity and mortality rate. Nevertheless, it is unlikely that another large study will ever be performed to examine this issue, and the ultimate results are difficult

to dispute given the excellent study design and adequate sample size.

The Term Breech Trial Collaborative Group published outcomes of children after planned cesarean birth versus planned vaginal birth for breech presentation at term. In this analysis they followed 923 of the 1159 enrolled subjects (79.6%) to 2 years of age. The risk of death or neurodevelopmental delay was no different between the planned cesarean section versus the planned vaginal groups (14 children [3.1%] vs 13 children [2.8%]; relative risk [RR] 1.09, 95% CI 0.52–2.30) (Hannah et al., 2000).

In addition, there are several large retrospective series describing neonatal outcomes with the vaginal approach, most of which suggest that vaginal delivery in carefully selected patients carries a low risk of long-term neonatal morbidity and mortality (Hillier and Johanson, 1994; Diro et al., 1999; Herbst and Källén, 2008; Vlemmix et al., 2014; Vistad et al., 2015). A recent metaanalysis assessing the risks of planned vaginal breech delivery versus planned caesarean section for term breech birth included studies between 1993 and 2014 and included 27 articles with a total sample size of 258,953 women and reported the relative and absolute risks of perinatal mortality and morbidity in relation to mode of delivery. They reported that the absolute risks of perinatal mortality, fetal neurologic morbidity, birth trauma, a 5-minute Apgar score less than 7, and neonatal asphyxia in the planned vaginal delivery group were 0.35%, 0.7%, 0.7%, 2.4%, and 3.3%, respectively. They concluded that perinatal mortality and morbidity in planned vaginal breech delivery was significantly higher than with planned caesarean delivery, which is consistent with the aforementioned authors (Berhan and Haileamlak, 2016). The truly interesting point raised by all these investigators is that, aside from the issue of cesarean versus trial of labor, singleton breech infants regardless of mode of delivery have an increased risk of morbidity compared with their vertex counterparts. Breech infants had higher incidences of neonatal intensive care unit (NICU) admissions, eventful hospital courses, hip dislocation, and traumatic morbidity (soft tissue trauma, fracture, facial nerve paralysis, and brachial plexus palsy). Thus both the obstetrician and pediatrician must be aware that the infant in breech presentation requires careful attention upon birth for the presence of these potential factors. Furthermore, the risk of developmental hip dysplasia is increased in infants born in breech presentation. The American Academy of Pediatrics (AAP) has a clinical practice guideline for early detection of developmental dysplasia of the hip in these infants (AAP, 2000).

Diro et al. (1999) evaluated 1021 term singleton breech deliveries occurring at their institution over a 4-year period. Infants with a clinically adequate pelvis and frank breech presentation with an EFW less than 3750 g were allowed a trial of labor. They found an overall cesarean rate of 85.6%; however, for women allowed to deliver vaginally, the success rate, defined as vaginal delivery, was 50% (19 of 38 patients) for nulliparous women and 75.8% (116 of 153 patients) for multiparous women. The length of NICU stay was higher for the group delivered vaginally (17.4% vs 12.1%; $P = .036$), but major morbidities between operative and vaginal delivery were not significantly different. Long-term outcome was not evaluated. Of note, the women in this cohort had pelvic dimensions evaluated clinically, and not by X-ray or CT pelvimetry, as has been performed in other studies.

Norwegian investigators examined the neonatal outcomes of singleton term breech deliveries from 1991 to 2011. They evaluated 30,681 singleton term breech deliveries identified using the Medical Birth Registry in Norway. They compared the planned vaginal delivery with the planned cesarean delivery across two time periods:

January 1991 to November 2000 and November 2000 to December 2011. They identified an increase in cesarean delivery from 34.4% to 51.3% across these study periods. They also found that early neonatal mortality in the first 0–6 days after delivery declined from 0.10% to 0.04% ($P = .04$). During the second time period, 30.7% of term breech presentations delivered vaginally. There were eight deaths in the planned vaginal and four in the planned cesarean groups, although the difference was not statistically significant (OR 2.11, 95% CI 0.64–7.01). Neonatal outcomes were significantly worse in the planned vaginal delivery groups across both time periods (Vistad et al., 2015).

The delivery of the vaginal breech is an emotional issue; physicians trained in the art of the vaginal breech delivery maintain that for an appropriately selected candidate, vaginal breech delivery has acceptable neonatal risk and has the advantage of sparing the mother significant operative morbidity. Proponents of cesarean delivery further state that the level of resident training in the art of the singleton vaginal breech delivery has markedly diminished, with most graduating senior residents having performed few such births. Nonetheless, many practitioners will be required to assist in vaginal birth of a breech infant in unplanned situations. The acquisition of skills necessary to competently perform this procedure may need to be learned and practiced with simulation-based training, because the opportunities for training in most residency programs are few. ACOG has recommended that the decision regarding the mode of delivery should depend on the experience of the healthcare provider. However, cesarean delivery will be the preferred mode of delivery for most physicians because of the diminishing expertise in vaginal breech delivery (ACOG, 2006). As will be discussed later in Multifetal Delivery, this statement does not apply to the vaginal delivery of a nonvertex second twin.

Multifetal Delivery

With the advent of in vitro fertilization and the sophisticated assisted reproductive technologies, the incidence of multifetal pregnancies has dramatically increased, particularly higher-order multiples. The incidence of twin gestations in patients undergoing in vitro fertilization is currently 20% in the United States (Scholten, 2015), and it is 1%–3% for higher-order multiples. As a result, the frequency of twin gestation in the United States has increased from 1/53 infants in 1980 to 1/30 infants in 2009 (Martin et al., 2013); twins now account for 3% of births. Of these, 80% are dizygotic and 20% are monozygotic. This 3% of births accounts for 17% of preterm births and approximately 25% of infants of low birth weight and very low birth weight. The perinatal mortality rate of twins is sevenfold that of singletons, of which a small fraction is due to problems during labor and at delivery. The mode of delivery for twins is well delineated by several studies, and the issues surrounding the choice of vaginal birth versus cesarean delivery are outlined in the following sections (Smith et al., 2005).

Twin Delivery

Vertex–Vertex

Approximately 40% of twin gestations will be in a vertex–vertex presentation prior to delivery (Arabin and Kyvernitakis, 2011). It is almost universally accepted that the appropriate method of delivery is vaginal if both twins are vertex. The first infant is delivered like a singleton infant. The second infant is delivered in a similar fashion, but care must be taken not to rupture membranes before the head is well engaged, because this may increase the risk of

cord accident. Of note, the delivery of the second twin does not necessarily occur immediately after the first.

Vertex–Nonvertex

Vertex–nonvertex presentation occurs 38.4% of the time prior to delivery (Arabin and Kyvernitakis, 2011). The first twin is usually delivered vaginally. The options for delivery of the second twin are as follows: cesarean section, breech extraction, or attempts at external cephalic version and vertex delivery of the second twin if successful. The optimal delivery choice for the second twin has been the subject of much controversy. Many obstetricians claim that cesarean section is the safest approach to the nonvertex twin, whereas others claim that vaginal delivery affords equivalent neonatal outcome, sparing the mother from an unnecessary surgical procedure (Usta et al., 2005). There is only one randomized trial including 60 twin deliveries of planned vaginal versus cesarean section in this situation. Maternal morbidity and hospital stay were increased in the surgical group, but there were no differences in neonatal outcome (Rabinovici et al., 1987).

Hogle et al. (2003) performed a metaanalysis to determine whether a policy of planned cesarean or planned vaginal birth is preferable for twins. They found only four studies with a total of 1932 infants that met their inclusion criteria. There were no significant differences in maternal morbidity, perinatal or neonatal mortality, or neonatal morbidity between the two groups. They did find significantly fewer low 5-minute Apgar scores in the planned cesarean group, principally because of a reduction among breech first twins. They concluded that, if twin A is vertex, “there is no evidence to support planned cesarean section for twins.” In contrast, Smith et al. (2005) published a retrospective cohort study of 8073 twin births after 36 weeks’ gestation in Scotland between 1985 and 2001. There was a death of either twin in two of 1472 (0.14%) deliveries by planned cesarean and in 34/6601 (0.52%) deliveries by other means ($P = .05$; OR for planned cesarean 0.26, 95% CI 0.03–1.03). They concluded that planned cesarean may reduce the risk of perinatal deaths of twins at term by 75% despite the lack of statistical significance in outcomes between the two groups. The data also suffer from the fact that 30 of the 36 deaths were in second twins, and there were no data regarding fetal presentation (Smith et al., 2007).

There are several large cohort studies examining the issue of feasibility and safety of total breech extraction of the nonvertex second twin. These studies have almost unanimously reached the similar conclusion that the neonatal outcome for nonvertex second twins delivered vaginally is similar to the vertex first twin, but is not statistically different from those second twins delivered by cesarean section, regardless of birth weight or gestational age (Feingold et al., 1988; Keith et al., 1988; Hillier and Johanson, 1994; Hartley and Hitti, 2005; Herbst and Källén, 2008; Ali and Norwitz, 2009). Hartley and Hitti (2005) conducted a retrospective analysis of birth certificates and fetal and infant death certificates for 5138 twin pairs selected from those born in Washington State from 1989 to 2001. They concluded that if prompt vaginal delivery of twin B does not occur, the benefits of vaginal delivery for twin A might not outweigh the risks of distress and low Apgar scores in twin B and vaginal plus cesarean delivery for the mother (Hartley and Hitti, 2005).

The available body of evidence supports attempts at vaginal delivery of the nonvertex second twin. Of course the responsible obstetrician must choose a management plan most compatible with his or her experience and training. For those not versed in the techniques of successful vaginal breech extraction, cesarean

delivery might be a more prudent plan. As in the case of the singleton vaginal breech, simulation training may play a role in the acquisition and maintenance of skills for safe vaginal breech birth (Deering et al., 2006).

In addition to cesarean delivery and total breech extraction, there is the option of external cephalic version (i.e., attempting to turn a nonvertex fetus to vertex by abdominal manipulation). Studies have shown that this option is associated with a higher failure rate at successful vaginal delivery and other complications (such as cord accident and malpresentation not amenable to vaginal delivery) when compared with primary breech extraction or cesarean section (Hillier and Johanson, 1994; Learman, 1998).

If the vaginal approach is chosen, once the first twin is delivered the obstetrician inserts a hand into the uterine cavity and, under sonographic guidance if necessary, finds the feet of the second twin. Once the feet are firmly grasped, they are brought down into the vagina, and the membranes are then ruptured. Traction is applied to the fetus along the pelvic curve; once the body has been delivered through the introitus, delivery of the arms, shoulders, and after-coming head proceed in a fashion similar to that of a singleton breech.

Nonvertex–Nonvertex

The most uncommon combination for presenting position at birth is nonvertex–nonvertex, which occurs 19.1% of the time (Arabin and Kyvernitakis, 2011). Because of the theoretical risk of interlocking twins, as well as the recent data showing the greater morbidity for the singleton vaginal breech (see the preceding discussion), cesarean section is the recommended choice for delivery of the nonvertex–nonvertex presentation.

Monochorionic, Monoamniotic Twins

Monochorionic, monoamniotic twins share a single intraamniotic space and thus have a higher risk of cord and extremity entanglement during the course of delivery. It is commonly accepted that the optimal mode of delivery is a planned cesarean section before labor ensues.

Higher-Order Multiple Gestations

Most perinatologists would suggest cesarean delivery for triplets and higher-order multiples (Lipitz et al., 1989). Although this practice is common, the data mandating cesarean delivery are far from conclusive.

A Dutch study compared the outcomes of triplets delivered vaginally and abdominally at two institutions (Learman, 1998). One hospital favored cesarean section, whereas, at another, trial of labor was offered to all appropriate candidates. The success of vaginal delivery was relatively high (34 of 39 women [87%]). There was a higher incidence of neonatal mortality and postdelivery depression (as estimated by Apgar score) in the hospital favoring operative delivery compared with the vaginal delivery group. The biases inherent in this study are obvious, although the reported findings have been corroborated by several other reports from single institutions that offer trial of vaginal delivery to triplet gestations (Meniru, 1996; O’Grady et al., 2002; Menacker and Martin, 2008). Vintzileos et al. (2005) attempted to estimate the risks of stillbirth and neonatal and infant deaths in triplets, according to mode of delivery; they used the “matched multiple birth” data file that was composed of triple births that were delivered in the United States during 1995 through 1998 and found that 95% of all triplets were delivered by cesarean delivery. Vaginal delivery (all vaginal)

was associated with an increased risk for stillbirth (RR 5.70, 95% CI 3.83–8.49) and neonatal (RR 2.83, 95% CI 1.91–4.19) and infant (RR 2.29, 95% CI 1.61–3.25) deaths. They concluded that cesarean delivery of all three triplet fetuses is associated with the lowest neonatal and infant mortality rate and that vaginal delivery among triplet gestations should be avoided (Vintzileos et al., 2005). Most of the data on triplet births consist of small cohort studies and not randomized trials, and the possibility of type II or beta errors exists in the interpretation of many of these studies (Feingold et al., 1988; Keith et al., 1988). Thus delivery of triplet gestations vaginally while not an unreasonable approach has nearly disappeared from the practice of modern obstetrics in favor of routine cesarean delivery. Currently, quadruplets and other higher-order multiples are usually delivered by cesarean section (Ron-El et al., 1981).

Vaginal Birth After Cesarean: Neonatal Concerns

The overall cesarean delivery rate in the United States increased 60% from 1996 to 2009, from 20.7% to 32.9%. Since 2009, the rate has declined slightly to 32.7% in 2013, and nearly one-third of births continue to be delivered by cesarean each year (Hamilton, 2014). Surgery carries the maternal risks of increased blood loss, prolonged hospital stay, and longer recovery time compared with vaginal delivery. During the 1980s to 1990s, efforts were made to encourage women to attempt a trial of labor after a prior cesarean delivery because the success rates of a VBAC are reasonable, varying from 60% to 80%, depending on the indications for the prior cesarean delivery (Mozurkewich and Hutton, 2000).

Whereas VBAC rates remain relatively high in the United Kingdom at 33% (range 6%–64%), the rates are decreasing rapidly in the United States, from a high of 28.3% in 1996 to less than 1% in 2006 (Fang and Zelop, 2006; Caughey, 2009). This decrease occurred primarily because VBAC can result in uterine dehiscence, in which the prior scar asymptotically separates or, more seriously, uterine rupture occurs. A full discussion of VBAC, the studies supporting its safety, and the controversies surrounding its feasibility is beyond the scope of this chapter, and the interested reader is urged to consult *Williams Obstetrics*, 24th ed, for further details. This discussion instead focuses on neonatal risks from VBAC, particularly from its most dreaded complication, uterine rupture.

Studies have uniformly shown a risk of uterine rupture with VBAC on the order of 0.5%–1% (Feingold et al., 1988; Caughey, 2009). A large, retrospective study evaluated 20,095 women with a history of prior cesarean delivery and found that rupture risk was 0.16% if the woman elected for a repeat cesarean delivery; 0.52% if VBAC occurred as a result of spontaneous labor; 0.77% if labor was induced without prostaglandins; and 2.5% if labor was induced with prostaglandins (Feingold et al., 1988). Thus VBAC carries the lowest risk if labor is spontaneous. However, it is currently a well-accepted practice to induce and augment labor during a VBAC using mechanical dilation and/or oxytocin as the risk of uterine rupture remains less than 1%. The use of prostaglandins is not recommended during any portion of the labor process in patients with a prior uterine scar because of the increased risk of uterine rupture.

There are few large, well-designed studies specifically evaluating neonatal rather than maternal outcomes after VBAC. Kamath et al. (2009) performed a retrospective cohort study of 672 women who had one prior cesarean section and then underwent a trial of labor. They found that infants born by cesarean delivery had higher

rates of admission to the NICU (9.3% compared with 4.9%) and higher rates of oxygen supplementation for delivery room resuscitation (41.5% compared with 23.2%).

Yap et al. (2001) retrospectively evaluated 38,027 deliveries occurring at a single tertiary care institution and found 21 cases of uterine rupture; 17 occurred after a history of a prior cesarean delivery. The two neonatal deaths that occurred were a result of either prematurity (23-week-gestation fetus) or multiple congenital anomalies. All liveborn infants were discharged from the hospital without neurologic sequelae. Thus the ultimate neonatal outcome despite uterine rupture was favorable. However, all deliveries occurred in a tertiary care institution with readily available obstetric anesthesiologists, neonatologists, and obstetricians. Most deliveries after diagnosis of rupture occurred within 26 minutes.

A third group of investigators retrospectively identified 99 cases of uterine rupture occurring over a period comprising 159,456 births (Hillier and Johanson, 1994). Thirteen of these ruptures occurred before the onset of labor. There were six neonatal deaths, but four of these occurred in women with uterine rupture at admission and thus were never given a trial of labor. There were five cases of perinatal asphyxia, but once again it is not detailed whether these occurred in women allowed a trial of labor or in those who had ruptured on presentation to the hospital. Moreover, many of these women had an undocumented prior scar, which in some institutions would warrant an elective repeat cesarean section. The aforementioned study evaluating 20,095 women with a prior cesarean delivery and their subsequent risk of rupture found a neonatal mortality of 5.5% (Lydon-Rochelle et al., 2001). However, because this was a population-based study, it was not specified whether these deliveries occurred in tertiary care institutions with the capability of performing emergent operative rescue procedures in the event of uterine rupture.

Finally, Fang and Zelop (2006) reviewed all of the literature to date with regard to adverse neonatal outcomes after VBAC and found that the combined rates of intrapartum stillbirth and neonatal death were not statistically different between those electing trial of labor and those electing repeated cesarean section. Thus the true neonatal risk of VBAC, especially in the event of uterine rupture, cannot be precisely estimated at the current time. There are no studies adequately evaluating long-term outcomes of surviving infants after uterine rupture (Fang and Zelop, 2006).

It appears that the risk of uterine rupture after a prior cesarean delivery is low, but this risk increases when labor is induced with prostaglandins. It is appropriate to offer women VBAC, but they must be counseled carefully about the potential risk of uterine rupture. Careful documentation of the informed consent and labor management must be completed. Moreover, VBAC should occur in delivery facilities capable of rapidly performing an emergent cesarean section, because this improves the likelihood of minimizing adverse neonatal sequelae.

Cord Accidents

The term *cord accident* usually refers to adverse events affecting the fetus that occur as a result of a problem with the umbilical cord. This heterogeneous term encompasses umbilical cord prolapse, in which the cord delivers through the cervix and compression by a fetal part results in a significantly increased risk of asphyxia; it also includes such entities as cord entanglements or “true knots,” which can lead to fetal compromise.

The incidence of such events is not clearly known, because the diagnosis is often one of exclusion. One large population-based

study compared 709 cases of cord prolapse occurring among 313,000 deliveries to matched controls and found that low birth weight, male sex, multiple gestations, breech presentation, and congenital anomalies all increased the risk of umbilical cord prolapse (Ali and Norwitz, 2009). Not surprisingly, cord prolapse was associated with a high neonatal mortality rate (10%), which was reduced if cesarean rather than vaginal delivery was performed.

The standard of care in cases of cord prolapse is to proceed immediately with cesarean section as quickly as possible while an assistant elevates the presenting fetal part with a hand in the vagina to prevent compression of the umbilical cord. It is also of paramount importance to have appropriate pediatric support available at the time of delivery, because the newborn is likely to be depressed and require resuscitation.

As a clinical entity, cord accident (or in utero compromise), secondary to entanglement of the umbilical cord, is difficult to understand. Often in cases of in utero fetal demise (IUFD), a diagnosis or cause of fetal death is never found. It is tempting to attribute the demise to an event that compromises umbilical blood flow to the developing fetus, but the literature on this subject is scarce. HersHKovitz et al. (2001) identified 841 cases of true knots from a population of 69,139 deliveries (for a prevalence of 1.2%) and in a case-controlled study found that grand multiparity (>10 deliveries), chronic hypertension, history of genetic amniocentesis, male gender, and umbilical cord prolapse were all independently associated with true knots of the umbilical cord. The presence of a true knot was associated with both IUFD and a greater likelihood of cesarean delivery (HersHKovitz et al., 2001).

Suggested Readings

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16

Obstetric Analgesia and Anesthesia

MARK D. ROLLINS AND MARK A. ROSEN

KEY POINTS

- Labor results in significant pain for many women that is individualized, dynamic, and unpredictable.
- Although the effects of obstetric analgesia and anesthesia on the fetus and neonate are typically benign, there is potential for adverse neonatal effects.
- During pregnancy, labor, and delivery, women undergo fundamental changes in anatomy and physiology that affect all organ systems, significantly alter pharmacokinetic and pharmacodynamic responses to many drugs commonly used in anesthesia, and have important implications for anesthetic administration.
- Opioids are the most commonly used systemic medications for labor and delivery, but are administered with limitations on both dose and timing because they readily cross the placenta and are associated with a risk of neonatal respiratory depression in a dose-dependent fashion.
- Neuraxial analgesia (epidural, spinal, and combined spinal–epidural techniques) is widely used and the most effective method for labor analgesia. Epidural labor analgesia is a catheter-based technique that provides continuous analgesia during labor using administration of medication into the epidural space. Neuraxial anesthesia does not affect the length of the first stage of labor or increase the risk for cesarean delivery but has been associated with an increased use of forceps or vacuum for vaginal delivery.
- For the patient without an epidural catheter, spinal anesthesia is the most common regional anesthetic technique used for cesarean delivery. Use of general anesthesia for cesarean delivery is typically reserved for situations where neuraxial anesthesia is contraindicated or emergent delivery is needed.

Labor results in significant pain for many women that is individualized, dynamic, and unpredictable. Documented attempts to help relieve labor pain date back to early recorded history, but primitive efforts were mainly based on ineffective techniques of distraction, counter-stimulation, suggestion, or use of herbal remedies. Modern techniques for labor analgesia and obstetric anesthesia, essential for operative delivery, provide an effective and safe alternative to women seeking pain-free childbirth. Although the effects of obstetric analgesia and anesthesia on the fetus and neonate are typically benign, there is potential for adverse effects. This chapter introduces some of the scientific background and clinical techniques used in providing obstetric analgesia and anesthesia, as well as some of the potential maternal and neonatal complications.

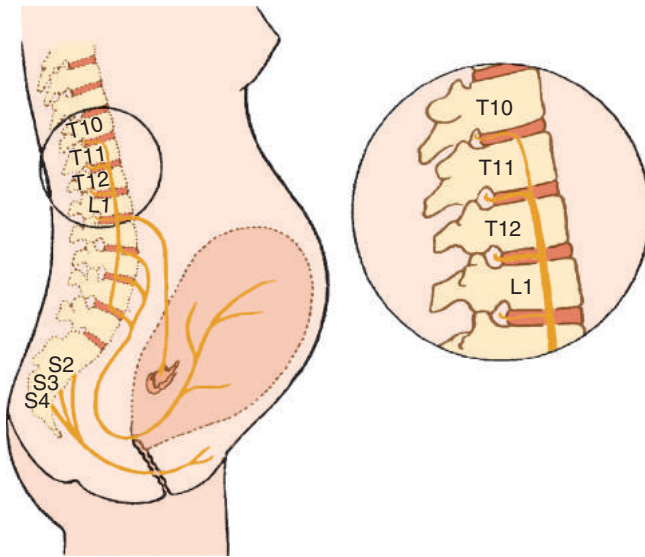
History of Obstetric Anesthesia

Modern obstetric anesthesia began in Edinburgh, Scotland, on January 19, 1847, when a professor of obstetrics, James Young Simpson, used diethyl ether to facilitate child delivery 3 months after Morton's historic demonstration of the anesthetic properties of ether at the Massachusetts General Hospital in Boston ([Longfellow and Wagenknecht, 1956](#)).

During the second half of the 20th century, anesthesiologists made significant advances in techniques and improved safety for delivering labor analgesia. Hingson and Edwards (1943) developed the continuous caudal catheter that preceded development of the epidural catheter. Virginia Apgar (1953) initially proposed a simple neonatal scoring system as a guide for evaluating the effects of obstetric anesthesia and later as a guide for neonatal resuscitation. Other early pioneers in the emerging specialty of obstetric anesthesia were Gertie Marx (1958), Sol Shnider (1963), and John Bonica (1967). They helped to characterize the normal changes in maternal physiology related to pregnancy, confirm the safety and efficacy of obstetric analgesia, determine the effects of these techniques on uterine blood flow and placental transfer of anesthetic agents, and evaluate the effects of these techniques and agents on newborn well-being.

Anatomy of Labor Pain

Contraction of the uterus, dilatation of the cervix, and distention of the perineum cause pain during labor and delivery. Somatic and visceral afferent sensory fibers from the uterus and cervix travel with sympathetic nerve fibers to the spinal cord ([Fig. 16.1](#)). These fibers pass through the paracervical tissue and course with the hypogastric nerves and the sympathetic chain to enter the spinal cord at nerve roots in the lower thoracic and upper lumbar segments (T10 to L1). During the first stage of labor (cervical dilation), the majority of painful stimuli are the result of afferent nerve impulses from the lower uterine segment and cervix, as well as contributions from the uterine body causing visceral pain (poorly localized, diffuse, and usually described as “a dull but intense aching”). The pain increases in severity as the first stage progresses. These nerve cell bodies are located in the dorsal root ganglia of levels T10 to L1. During the second stage of labor (pushing and expulsion), afferents innervating the vagina and perineum cause somatic pain (well localized and typically described as “sharp”). These somatic impulses travel primarily via the pudendal nerve to dorsal root ganglia of



• **Fig. 16.1** Parturition Pain Pathways. Nerves that accompany sympathetic fibers and enter the neuraxis at the T10, T11, T12, and L1 spinal levels carry afferent pain impulses from the cervix and uterus. Pain pathways from the perineum travel to S2, S3, and S4 via the pudendal nerve. (From Miller RD, Pardo MC. *Basics of Anesthesia*. 6th ed. Philadelphia: Elsevier Saunders; 2011.)

the nerve roots in the sacrum (S2 to S4). Pain during this stage is caused by distention and tissue ischemia of the vagina, perineum, and pelvic floor muscles, associated with descent of the fetus into the pelvis and delivery. Neuraxial analgesic techniques that block levels T10 to L1 during the first stage of labor must be extended to include S2 to S4 for effective pain relief during the second stage of labor.

Changes in Maternal Physiology and the Implications

During pregnancy, labor, and delivery, women undergo fundamental changes in anatomy and physiology. These alterations are caused by changing hormonal activity, biochemical shifts associated with increasing metabolic demands of a growing fetus, placenta, and uterus, and mechanical displacement by an enlarging uterus (Cheek et al., 2002; Parer et al., 2002).

Maternal Circulatory System

Hypotension can occur when a pregnant woman is in the supine position because of compression of the vena cava by the gravid uterus. Significant aortoiliac artery compression occurs in 15%–20% of parturients and vena caval compression is universal, often as early as 13–16 weeks' gestation. Therefore supine positioning is avoided during anesthetic administration in the second and third trimesters. Significant lateral tilt is used in all cesarean deliveries and frequently during labor analgesia to help preserve uterine blood flow. Vena caval compression also contributes to lower extremity venous stasis and increased risk of thromboembolus.

Cardiac output increases during pregnancy, reaching an output 50% greater than the prepregnant state by the third trimester. During labor, maternal cardiac output increases during the first and second stages, reaching an additional 40% above prelabor values in the second stage (Robson et al., 1987). Each uterine

contraction results in the autotransfusion of 300–500 mL of blood back into the maternal central circulation. The greatest increase in cardiac output occurs immediately after delivery, when values can increase as much as 75% above predelivery levels. This abrupt increase in cardiac output is secondary to the loss of aortic caval compression, autotransfusion from the contracted uterus, and decreased venous pressure in the lower extremities (Kjeldsen, 1979).

Physiologic (dilutional) anemia of pregnancy occurs as a result of a greater increase in plasma volume (45%) than in red blood cell volume (20%) by term. Average blood loss at delivery – approximately 500 mL for vaginal delivery and 1000 mL for cesarean delivery – is well tolerated because of the expanded blood volume of pregnancy and autotransfusion (about 500 mL) from the contracted uterus after delivery (Cheek et al., 2002).

Maternal Airway and Respiratory Systems

During pregnancy, the maternal airway has significantly increased mucosal edema and tissue friability throughout the pharynx, larynx, and trachea. These changes make laryngoscopy and intubation more challenging. In addition, the presence of comorbidities such as preeclampsia, upper respiratory tract infections, and the active pushing and increased venous pressure during the second stage further exacerbate airway tissue edema (Munnur et al., 2005).

At term, minute ventilation is increased approximately 50%, oxygen consumption is increased by more than 20%, and functional residual capacity is decreased by 20%. The combination of these changes (increased oxygen consumption and decreased oxygen reserve) results in a state promoting rapid desaturation during periods of apnea. The changes in both airway and respiratory physiology during pregnancy make ventilation and intubation more difficult and increase the potential for complications with induction of general anesthesia. A current multi-institutional database of adverse obstetric anesthesia events notes that rates of failed intubation are approximately 1:533, although none of the ten failed obstetric intubations in the study resulted in maternal mortality (D'Angelo et al., 2014).

Maternal Gastrointestinal System

The gravid uterus increases intragastric pressure and causes the stomach and esophagus to reposition, resulting in decreased competence of the esophageal sphincter. Elevated progesterone and estrogen levels further reduce esophageal sphincter tone. Consequently, most pregnant women experience symptoms of gastric reflux (Marrero et al., 1992). Furthermore, gastric emptying is delayed by active labor and administration of opioids. Delayed gastric emptying and decreased competence of the esophageal sphincter cause an increased risk of pulmonary aspiration with induction of general anesthesia, which has important implications for airway management that are discussed in detail in the General Anesthesia section.

Uterine and Fetal Circulation

Uterine weight and blood flow increase throughout gestation from approximately 100 mL/min before pregnancy to approximately 700 mL/min (10% of cardiac output) at term gestation, with 50%–80% of the uterine blood flow perfusing the intervillous space (placenta) and 20%–50% supporting the myometrium. Uterine vasculature has limited autoregulation and remains (essentially) maximally dilated under normal conditions during pregnancy.

Maternal uterine blood flow decreases as a result of either reduced uterine arterial perfusion pressure or increased arterial resistance. Decreased perfusion pressure can result from systemic hypotension secondary to reduced cardiac preload from hypovolemia, aortic caval compression, or significant decreases in vascular resistance from the initiation of neuraxial anesthesia or induction of general anesthesia. Uterine perfusion pressure can also decrease from increased uterine venous pressure associated with vena caval compression (e.g., supine position), uterine contractions (particularly uterine hypertonus by oxytocin hyperstimulation), or a significant increase in intra-abdominal pressure (pushing during second stage or seizure activity). Despite these potential effects, phenylephrine (α -adrenergic) is useful for treating maternal hypotension secondary to neuraxial anesthesia, and it has been demonstrated to result in less fetal acidosis and base deficit compared with treatment with ephedrine (primarily β -adrenergic) in many clinical trials (Lee et al., 2002; Ngan Kee et al., 2009; Smiley, 2009). If treated promptly, transient maternal hypotension does not lead to fetal depression or neonatal morbidity.

Analgesic Options for Labor and Vaginal Delivery

The pain of labor is highly variable and described by many women as severe. Factors influencing the patient's perception of labor pain include duration of labor, maternal pelvic anatomy in relation to fetal size, use of oxytocin, parity, participation in childbirth preparation classes, fear and anxiety about childbirth, attitudes about and experience of pain, and coping mechanisms. The choice of analgesic method resides primarily with the patient. The medical condition of the parturient, stage of labor, urgency of delivery, condition of the fetus, and availability of qualified personnel are also factors. Many different techniques are available to alleviate labor and delivery pain, and none appears to increase the risk of cesarean delivery (Wong et al., 2005; American College of Obstetrics and Gynecology [ACOG], 2006; Sng et al., 2014; Practice Guidelines for Obstetric Anesthesia, 2016).

Analgesia refers to pain relief without loss of consciousness; regional analgesia denotes partial sensory blockade in a specific area of the body, with or without partial motor blockade. Regional anesthesia is the loss of sensation, motor function, and reflex activity in a limited area of the body. General anesthesia results in the loss of consciousness, and the goals for providing general anesthesia typically also include, amnesia, analgesia, and skeletal muscle relaxation.

Techniques for labor analgesia must be safe for both mother and fetus and individualized to satisfy the analgesic requirement and desires of the parturient; they also must accommodate the changing nature of labor pain and the evolving, varied course of labor and delivery (e.g., spontaneous vaginal delivery, instrumentally assisted vaginal delivery, and cesarean delivery). The current approaches to pain relief are outlined in [Box 16.1](#).

Nonpharmacologic Analgesia

There are a variety of nonpharmacologic techniques for labor analgesia. Many seem to reduce labor pain perception, however most lack the rigorous scientific methodology for the useful comparison with pharmacologic methods. Although data are limited, acupuncture, acupressure, transcutaneous electrical nerve stimulation, relaxation, and massage all demonstrate a modest analgesic

• BOX 16.1 Techniques for Labor Analgesia

Nonpharmacologic methods

Systemic opioids

Inhaled nitrous oxide

Regional techniques

Epidural

Spinal

Combined spinal–epidural

Paracervical block

Pudendal block

benefit during labor (Arendt and Tesmer-Tuck, 2013). Other techniques such as hypnosis and intradermal water injections have not been shown to be more effective than placebo. A metaanalysis reviewing the effectiveness of a support individual (e.g., family member, doula) noted that parturients with a support individual used fewer pharmacologic analgesia methods and had a decreased length of labor, less dissatisfaction, and a lower incidence of operative deliveries (Hodnett et al., 2012).

Systemic Medications

Opioids are the only commonly used systemic medications for labor and delivery, but are administered with limitations on both dose and timing because they readily cross the placenta and are associated with a risk of neonatal respiratory depression in a dose-dependent fashion. Although pain relief from the administration of systemic opioids is frequently inadequate for the duration of labor, this option can be beneficial for short-term analgesia, particularly in early labor. Opioids are inexpensive, easy to administer, and do not require a trained anesthesia provider. However, they have a high rate of maternal side effects (sedation, respiratory depression, dysphoria, nausea, pruritus), can decrease fetal heart rate (FHR) variability and fetal movements, and carry a potential risk of neonatal respiratory depression and changes in neurobehavior. Systemic administration of opioids at doses that are safe for mother and newborn provides some pain relief but does not have the analgesic efficacy of regional techniques. Systemic opioids are recommended for administration in small doses, with minimization of repeated dosing to reduce the accumulation of drug and metabolites in the fetus. Larger doses would risk excessive maternal sedation, maternal respiratory depression, loss of protective airway reflexes, newborn respiratory depression, and impairment of both early breastfeeding and newborn neurobehavior.

Opioids differ in pharmacokinetics, pharmacodynamics, method of elimination, and the presence of active metabolites, but all readily cross the placental barrier through passive diffusion. Systemic opioids are most useful for patients with minimal to moderate pain, precipitous labor, or contraindications to neuraxial blockade, such as a coagulopathy.

Meperidine remains the most widely used opioid worldwide. Maternal half-life of meperidine is 2–3 hours, with the half-life in the fetus and newborn being significantly greater and more variable at values between 13 and 23 hours (Kuhnert et al., 1979). In addition, meperidine is metabolized to an active metabolite (normeperidine) that can significantly accumulate after repeated doses. With increased dosing and a shortened time interval between dose and delivery, there is greater neonatal risk of decreased Apgar scores, lowered oxygen saturation, prolonged time to sustained respiration, abnormal neurobehavior, and more difficulty initiating successful breastfeeding (Nissen et al., 1997).

Morphine was used more frequently in the past, but currently is rarely used. Like meperidine, it has an active metabolite (morphine-6-glucuronide) and a prolonged duration of analgesia (3–4 hours). The half-life is longer in neonates compared with adults, and it produces significant maternal sedation.

Fentanyl is a synthetic opioid with a short duration of action (approximately 30 minutes), no active metabolites, and a ratio of fetal to maternal plasma concentrations of approximately 1:3. In small intravenous (IV) doses of 50–100 µg over an hour there were no significant differences in Apgar scores, respiratory depression, or neurobehavior scoring compared with newborns of mothers who did not receive fentanyl (Rayburn, Rathke, et al., 1989). In addition, a comparison of equianalgesic doses of IV fentanyl compared with IV meperidine demonstrated a decreased frequency of maternal nausea, vomiting, and prolonged sedation in the fentanyl group (Rayburn, Smith, et al., 1989). In addition, neonates whose mothers received meperidine required naloxone more often compared with the fentanyl-exposed infants.

Remifentanyl, an ultra-short-acting opioid rapidly metabolized by nonspecific serum esterases, is significantly metabolized by the fetus, with umbilical artery:vein ratios of approximately 0.3 (Kan et al., 1998). Remifentanyl administered by a patient-controlled analgesia (PCA) device is an analgesic option for women who have contraindications to neuraxial blockade. The primary benefit of choosing this rapidly metabolized drug is to minimize opioid-related side effects on the neonate. Remifentanyl can be used effectively for labor with PCA dosing, but it is difficult to achieve satisfactory analgesia without significant potential of maternal respiratory depression, and therefore requires more intensive monitoring and nursing. Consequently, it is typically reserved for patients with contraindications to epidural anesthesia (Van de Velde and Carvalho, 2016). In a prospective randomized controlled trial comparing the effectiveness of epidural analgesia with a remifentanyl PCA with optimized settings, epidural analgesia was significantly more effective than PCA with regard to labor pain; more sedation and hemoglobin desaturation were noted during remifentanyl analgesia, but there was no difference between groups in fetal and neonatal outcomes (Volmanen et al., 2008). A more recent equivalence trial performed between remifentanyl PCA and epidural analgesia found remifentanyl was inferior to epidural analgesia for satisfaction of pain relief and pain relief scores (Freeman et al., 2015).

Inhaled Nitrous Oxide

The use of nitrous oxide is widespread in Canada, Australia, Scandinavia, the UK, and other parts of the world, and recently its use has increased in the United States (Likis et al., 2014). Nitrous oxide is a weak analgesic but can provide satisfactory pain relief and anxiolysis for some parturients. It is inhaled intermittently in a 50% mixture with oxygen and provides satisfactory labor analgesia for some women, but is a much less effective analgesic compared with epidural analgesia. Side effects are minimal, with nausea, dizziness, and drowsiness among the most common. Uterine contractility is not affected and the degree of maternal cardiovascular and respiratory depression is very mild. At a 50% concentration (without coadministration of opioids or other sedatives), nitrous oxide is insufficient to cause unconsciousness or loss of protective airway reflexes. Nitrous oxide provides a safe analgesic option for laboring women when administered with appropriate equipment by trained personnel, which is essential to ensure safety (i.e., limiting the nitrous oxide concentration, avoiding administration of a hypoxic mixture, avoiding coadministration of other agents)

(Camann et al., 2015). It can be used during the first, second, or third stage of labor. The effects of nitrous oxide are quickly reversed with discontinuation because of its rapid respiratory elimination, and it does not cause neonatal depression, regardless of duration of administration (Rosen, 2002a). When administered with appropriate scavenging equipment, there does not appear to be concern regarding occupational exposure. Despite its historical use, rigorous scientific studies are lacking to adequately assess its overall efficacy, safety, and long-term effects on the fetus and newborn (King and Wong, 2014).

Neuraxial (Regional) Analgesia

Neuraxial analgesia, including epidural, spinal, and combined spinal–epidural (CSE) techniques, is the most widely used method for labor analgesia in the United States (Bucklin et al., 2005). Neuraxial techniques typically involve epidural and spinal administration of local anesthetics and often the coadministration of epidural and spinal opioid analgesics. Administration of other adjuvants, such as epinephrine and clonidine, also can decrease the dose of local anesthetics required for analgesia but such adjuvants are used much less frequently than opioids and do not appear to offer a significant advantage (Aveline et al., 2002; Polley et al., 2002).

Neuraxial Local Anesthetics

Local anesthetics reversibly block nerve impulse conduction via voltage-gated sodium channels. Their chemical structures are secondary or tertiary amines, which are weak bases. Local anesthetics differ in their onset, peak plasma concentration, potency, and duration based on their lipid solubility, protein binding, site of injection, and concentration. Vascular absorption of local anesthetics limits the safe dose that can be administered. Bupivacaine and ropivacaine are the most commonly used local anesthetics for labor analgesia and are extremely safe when appropriately dosed for epidural or intrathecal administration. An accidental, large intravascular dose of any local anesthetic can result in significant maternal morbidity or mortality.

As with all drugs, placental transfer is determined by molecular size, lipid solubility, protein binding, charge, and maternal drug concentration. Local anesthetics are highly lipid soluble and have a low ionized fraction. However, the lower pH of the fetus has the potential to increase the fraction of ionized molecules, decrease lipid solubility, and result in ion trapping. Therefore in an acidotic fetus, higher concentrations of local anesthetic can accumulate (ion trapping) and result in decreased neonatal neuromuscular tone. If a direct intravascular or intrafetal injection of local anesthetics occurs, significant toxicity and depression can develop, signified by bradycardia, ventricular arrhythmia, and severe cardiac depression with acidosis.

Neuraxial Opioids

Epidural administration of lipid-soluble opioids (e.g., sufentanil, fentanyl) provides rapid analgesia equivalent to that of systemic administration but remains inferior to that of dilute concentrations of local anesthetics. When lipid-soluble opioids are coadministered with local anesthetics in the epidural space, they decrease the total local anesthetic dose required and lower the minimum local anesthetic concentration needed to achieve adequate labor analgesia (Lyons et al., 1997; Buyse et al., 2007). The most common maternal

side effects of conventional doses of epidural fentanyl or sufentanil are pruritus and nausea. Although typical doses used for labor analgesia do not adversely affect the neonate, the potential for respiratory depression is a function of the amount and timing of epidural opioid administered because much of the opioid administered in the epidural space crosses to the maternal plasma.

Subarachnoid (i.e., spinal, intrathecal) opioid injections provide effective maternal labor analgesia. Analgesic effects of spinal opioids are more potent than epidural or systemic administration, but are of limited duration (≤ 2 hours). Spinal fentanyl or sufentanil administration is often performed as part of a CSE technique (discussed in the following section). Intrathecal opioids are often combined with small doses of local anesthetic (e.g., 2.5 mg bupivacaine), decreasing the dose of opioid needed and the incidence of pruritus and nausea (Wong et al., 2000). Use of high-dose intrathecal opioids (e.g., sufentanil 7.5 μg) is associated with increased risk of fetal bradycardia and severe pruritus, even without the presence of hypotension (Van de Velde, 2005). The mechanism for fetal bradycardia is uncertain, but may be from uterine hyperactivity following the rapid analgesia. A systematic review of studies comparing intrathecal opioids with other methods of labor analgesia noted an increase in fetal bradycardia (odds ratio [OR] 1.8, 95% confidence interval [CI] 1.0–3.1) and increased maternal pruritus (relative risk [RR] 29.6, 95% CI 13.6–64.6), but no increased risk of cesarean delivery because of FHR abnormalities (Mardirossoff et al., 2002).

Neuraxial Techniques for Labor Analgesia

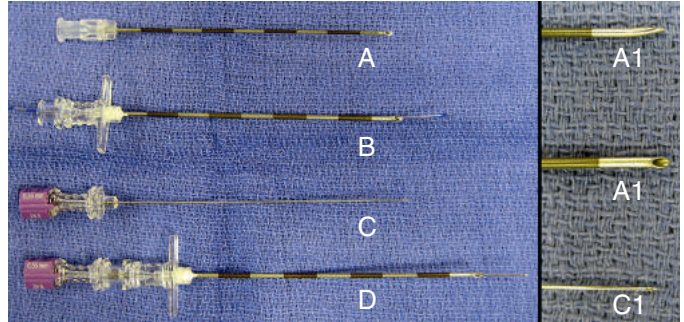
Neuraxial techniques represent the most effective form of labor analgesia, and they achieve the highest rates of maternal satisfaction (Declercq et al., 2007). The patient remains awake and alert without sedative side effects, maternal catecholamine concentrations are reduced, hyperventilation is avoided, cooperation and capacity to participate actively during labor are facilitated, and predictable analgesia can be achieved, superior to the analgesia provided by all other techniques.

Epidural Analgesia

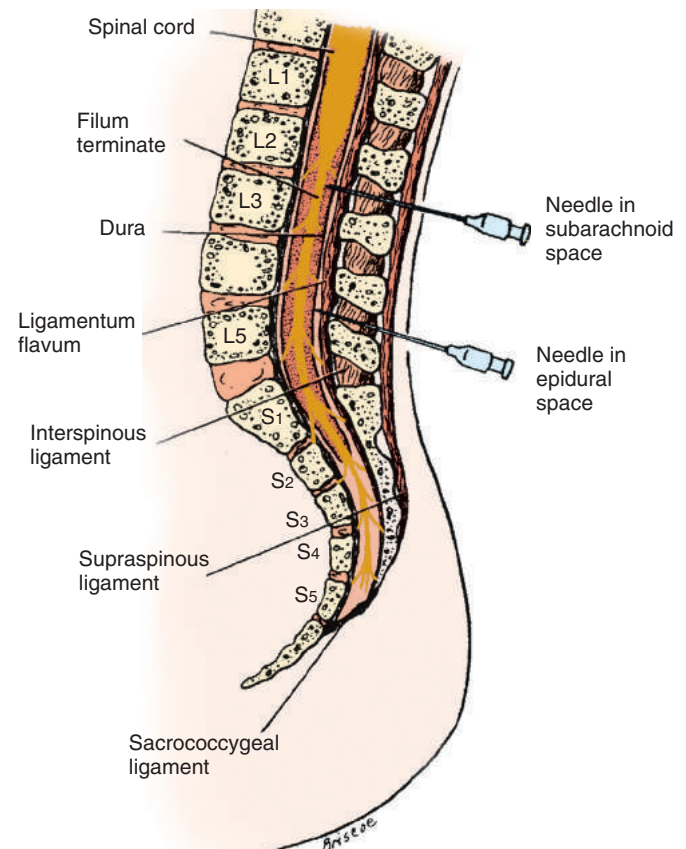
Epidural labor analgesia is a catheter-based technique that provides continuous analgesia during labor through the administration of medication into the epidural space. A catheter is inserted through a specialized needle (Fig. 16.2) that is directed between vertebral spinous processes in the back (typically in the lower lumbar segments, L3 to L5). The needle traverses the skin and subcutaneous tissues, supraspinous ligament, interspinous ligament, and the ligamentum flavum and is advanced into the epidural space (Fig. 16.3). The tip of the needle does not penetrate the dura, which forms the boundary between the intrathecal or subarachnoid space and the epidural space. To locate the epidural space, a tactile technique called “loss of resistance” is used. The tactile resistance noted with pressure on the plunger of an air-filled or saline-filled syringe dramatically decreases as the tip of the needle is advanced through the ligamentum flavum (dense resistance) into the epidural space (no resistance). The epidural space has an average depth of approximately 5 cm from the skin. Once the needle is properly positioned, a catheter is inserted through the needle, and the needle is removed. The catheter is secured and analgesia is achieved by administration of local anesthetics, opioids, or both (see [Neuraxial Local Anesthetics](#) and [Neuraxial Opioids](#) sections above) and maintained throughout the course of labor and delivery. The catheter

can also be used for administration of operative anesthesia (cesarean delivery) and postoperative analgesia, when necessary.

After catheter placement, a “test dose” of lidocaine (e.g., 45 mg) and epinephrine (e.g., 15 μg) is administered. If the catheter is inadvertently placed into an epidural vein, the epinephrine causes transient maternal tachycardia and the lidocaine can cause maternal tinnitus and metallic taste. If the catheter is inadvertently positioned



• **Fig. 16.2** Photograph of Typical Needles and Catheters Used for Neuraxial Analgesia and Anesthetic Techniques. (A) Epidural needle (18-gauge Tuohy) with magnification of tip shown in frontal and side views at right (A1). (B) Epidural needle (Tuohy) with catheter inserted through needle. (C) Spinal needle (24-gauge Whitacre) with magnification of tip shown at right (C1). (D) Spinal needle inserted through epidural needle for use in combined spinal-epidural technique.



• **Fig. 16.3** Schematic Diagram of Lumbosacral Anatomy Showing Needle Placement for Subarachnoid and Lumbar Epidural Blocks. (From Rosen MA, Hughes SC, Levinson G. Regional anesthesia for labor and delivery. In: Hughes SC, Levinson G, Rosen MA (eds). *Shnider and Levinson's Anesthesia for Obstetrics*. 4th ed. Baltimore: Lippincott Williams & Wilkins; 2002:123-148.)

into the subarachnoid space, the small dose of lidocaine results in the rapid onset of profound maternal analgesia and significant lower extremity weakness. By test-dosing the catheter, possible disastrous complications such as total spinal anesthesia or maternal cardiovascular collapse are significantly minimized.

Epidural medications can be administered in bolus doses or by continuous infusion. Patient-controlled epidural anesthesia (PCEA) is a delivery technique allowing the patient to self-administer small boluses of epidural analgesics with or without a background infusion. Studies comparing PCEA with continuous infusion technique have found decreased local anesthetic requirements, less anesthesia provider intervention, equivalent or improved patient satisfaction, equivalent or decreased motor blockade, and no significant differences in effects on the fetus or neonate (Practice Guidelines for Obstetric Anesthesia, 2016). Using PCEA delivery with a background infusion compared with PCEA alone further improves labor analgesia, reduces the need for clinician boluses, and does not increase maternal or neonatal adverse effects (Heesen et al., 2015).

More recently, programmed intermittent epidural bolus (PIEB) delivery provides automated fixed epidural boluses at scheduled intervals. Similar to continuous infusions, PIEB can be used alone or with a PCEA dosing technique. Recent studies suggest that PIEB slightly reduces local anesthetic usage, improves maternal satisfaction, and decreases the need for rescue boluses when compared with a continuous infusion technique (George et al., 2013; McKenzie et al., 2016).

Dilute solutions of bupivacaine or ropivacaine are typically chosen for epidural labor analgesia, while more potent concentrations of lidocaine or chloroprocaine are often administered for cesarean delivery. As an example of the difference in concentrations, bupivacaine solutions of 0.1% or less are typically used for labor analgesia, while 0.5% bupivacaine would be needed to produce adequate surgical anesthesia for a cesarean delivery. Dilute solutions of local anesthetics minimize the motor blockade and preserve the perception of pelvic pressure with descent of the fetus during labor. Most practitioners routinely use low concentrations of local anesthetics with coadministration of an opioid (e.g., 2 µg/mL fentanyl) for its synergistic effect.

Effects on the Progress of Labor and Rate of Operative Delivery

The use of epidural analgesia has been associated with a prolonged second stage of labor and increased rates of assisted and cesarean delivery. A 2011 systematic review of the literature found that neuraxial analgesia is associated with a 20-minute longer mean duration of the second stage and an increased rate of instrumented vaginal delivery, when compared with the use of systemic opioids for labor analgesia (RR 1.42, 95% CI 1.28–1.57, 23 trials, 7935 women) (Anim-Somuah et al., 2011). This increase in the duration of the second stage is not harmful to the mother or infant and does not dictate intervention with the presence of a reassuring fetal status and ongoing labor progress (ACOG, 2003). The use of epidural analgesia has no effect on the rate of cesarean delivery (Anim-Somuah et al., 2011). Although it was previously believed that placement of an epidural early in labor (<4 cm dilation) increased cesarean delivery rates, randomized controlled clinical trials comparing women receiving either systemic opioids or neuraxial analgesia in early labor (both spontaneous and induced), demonstrated no difference in rates of cesarean delivery (Wong et al., 2005; Sng et al., 2014). Consequently, both the ACOG and the American Society of Anesthesiologists guidelines recommend

that maternal request is sufficient justification for epidural analgesia, and should not depend on an arbitrary amount of cervical dilation (ACOG, 2006; Practice Guidelines for Obstetric Anesthesia, 2016).

Spinal Analgesia

In parturients without epidural analgesia, spinal analgesia can be administered in the second stage of labor near the time of anticipated delivery. A small dose of a local anesthetic, opioid, or both is injected into the subarachnoid space. This dose of local anesthetic is far less than that used for spinal anesthesia for cesarean delivery, and it has minimal effects on motor nerve function. Compared with epidural analgesia, it has the benefits of a more rapid onset, a lower failure rate, and is technically easier and quicker to perform. It has the significant disadvantage of a finite effective duration (approximately 90 minutes), but can be extremely useful for certain circumstances such as forceps-assisted delivery for a woman without epidural analgesia.

Combined Spinal–Epidural Analgesia

CSE labor analgesia is a variation of neuraxial analgesia that combines the lumbar epidural technique and spinal analgesia, using an intrathecal dose to initiate analgesia. After placement of the epidural needle, but before insertion of the epidural catheter, a spinal needle is passed through the indwelling epidural needle (see Fig. 16.2), puncturing the dura, and a small dose of local anesthetic or opioid is administered. Segmental analgesia results more rapidly than with epidural administration of local anesthetics. In a systematic review comparing CSE with epidural labor analgesia, CSE analgesia had a faster onset of effective analgesia (especially in spread to sacral roots), but was associated with more pruritus (Simmons et al., 2012). No differences were seen for mode of delivery, maternal hypotension, postdural puncture headache rate, or need for blood patch.

Contraindications and Complications of Neuraxial Techniques

Certain conditions contraindicate neuraxial procedures: these include patient refusal, infection at the needle insertion site, significant coagulopathy, hypovolemic shock, increased intracranial pressure from mass lesion, and inadequate provider expertise. Other conditions such as systemic infection, neurologic disease, and mild coagulopathies should be evaluated on a case-by-case basis. Human immunodeficiency virus infection is not a contraindication to regional technique in the pregnant patient (Hughes et al., 1995).

Rare, but life-threatening, complications can result from administration of regional anesthesia. Current rates of injury secondary to epidural catheter placement include epidural abscess/meningitis (1:63,000), epidural hematoma (1:250,000), and serious neurologic injury (1:36,000) (D'Angelo et al., 2014). Other serious complications are from accidental IV or intrathecal injections of local anesthetics. An unintended bolus of IV local anesthetic causes dose-dependent consequences ranging from minor side effects (e.g., tinnitus, perioral tingling, mild blood pressure and heart rate changes) to major complications (e.g., seizures, loss of consciousness, severe arrhythmias, cardiovascular collapse). The severity depends on the dose, type of local anesthetic, and preexisting condition of the parturient. Measures that minimize the likelihood of accidental intravascular injection include careful aspiration of the catheter before injection, test dosing, and incremental administration of therapeutic doses.

A “high spinal” (or total spinal) can result from an unrecognized epidural catheter placed subdural, migration of the catheter during its use, or an overdose of local anesthetic in the epidural space (i.e., high epidural). Both high spinals and high epidurals can result in severe maternal hypotension, bradycardia, loss of consciousness, and blockade of the motor nerves to the respiratory muscles. A recently published repository of serious complications in over 250,000 obstetric anesthetics noted high neuraxial block (1:4300), respiratory arrest (1:10,000), and anesthesia-related cardiac arrest (1:128,000) (D’Angelo et al., 2014).

Treatment of complications resulting from both intravascular injection and high spinal is directed at restoring maternal and fetal oxygenation, ventilation, and circulation. Intubation, vasopressors, fluids, and advanced cardiac life support algorithms are often required. In any situation of maternal cardiac arrest with unsuccessful resuscitation, the fetus should be delivered by surgical hysterotomy if the mother is not resuscitated within 4 minutes of the arrest. This guideline for emergent cesarean delivery increases the chances of survival for both the mother and neonate (Katz et al., 1986; Jeejeebhoy et al., 2015).

A variety of less severe complications such as inadequate analgesia, headache, or hypotension can occur with neuraxial blockade. The retrospective rates of inadequate epidural analgesia or inadequate CSE analgesia requiring catheter replacement were 7% and 3%, respectively, at a US academic center (Pan et al., 2004). The rate of accidental dural puncture during epidural catheter placement is 1.5%, and approximately half of these will result in a severe headache (Choi et al., 2003). These post-dural puncture headaches are typically managed with analgesics or a blood patch if necessary. Hypotension (decrease in systolic blood pressure greater than 20%) secondary to sympathetic blockade is the most common complication of neuraxial blockade for labor analgesia, with rates of approximately 14% (Simmons et al., 2012). Prophylactic measures include left uterine displacement and hydration. Although standards for timing, amount, and hydration fluid remain controversial, dehydration should be avoided. There is no significant difference in rates of hypotension following spinal anesthesia if a preload or coload of IV crystalloid or colloid is administered (Banerjee et al., 2010; Tawfik et al., 2014; Practice Guidelines for Obstetric Anesthesia, 2016). Hypotension treatment consists of further uterine displacement, IV fluids, and vasopressor administration. Small boluses of either phenylephrine or ephedrine can be used to treat hypotension. Although ephedrine (primarily β -adrenergic) was historically used, phenylephrine (primarily α -adrenergic) is associated with less fetal acidosis (Ngan Kee et al., 2009; Butwick et al., 2015). If treated promptly, maternal hypotension does not lead to fetal depression or neonatal morbidity.

A rise in core maternal body temperature and fever is associated with labor epidural analgesia. Although it was originally believed that all women who had epidural analgesia gradually increased their core temperature, more current studies suggest that only about 20% of women who receive epidural labor analgesia develop a fever and the remaining 80% have no increase in core body temperature (Arendt and Segal, 2013). Although the etiology of the maternal temperature rise remains uncertain, an association with noninfectious inflammation mediated by proinflammatory cytokines is supported most consistently in the literature. It is not associated with a change in white blood cell count or with an infectious process, and treatment is not necessary (Arendt and Segal, 2013). In addition, the fever associated with epidural labor analgesia does not increase the incidence of neonatal sepsis and need not affect neonatal septic work-up.

Other potential side effects from neuraxial blockade during labor include pruritus, nausea, shivering, urinary retention, motor weakness, and a prolonged block.

Paracervical Block

A paracervical block is infrequently used to provide pain relief during the first stage of labor. Analgesia is not as profound as with epidural or spinal regional block, and the duration of analgesia is short (45–60 minutes). The technique consists of submucosal administration of local anesthetics immediately lateral and posterior to the uterocervical junction, which blocks transmission of pain impulses at the paracervical ganglion. Complications from systemic absorption or transfer of local anesthetic can occur and there is a rare occurrence of direct fetal trauma or injection. A paracervical block is associated with a 15% rate of fetal bradycardia based on a metaanalysis of studies (Rosen, 2002b). The bradycardia is usually limited to less than 15 minutes, and treatment is supportive.

Pudendal Block

A pudendal block is infrequently used to provide pain relief during the second stage of labor at the time of delivery. In most centers this technique is used when epidural or spinal techniques are unavailable. A sheathed needle is guided to the vaginal mucosa and sacrospinous ligament just medial and posterior to the ischial spine. Injection of local anesthetic blocks sensation of the lower vagina and perineum. Although the technique can provide analgesia for vaginal delivery or uncomplicated instrumental delivery, the rate of failure is high (Nikpoor and Bain, 2013). Additional complications include systemic local anesthetic toxicity, ischiorectal or vaginal hematoma, and, rarely, fetal injection of local anesthetic.

Anesthesia for Cesarean Delivery

In the United States, the vast majority of cesarean deliveries are performed with neuraxial anesthesia. It offers the advantages of less anesthetic exposure to the neonate, has the benefit of an awake mother at the delivery, allows for placement of neuraxial opioids to decrease postoperative pain, and avoids the risks of maternal aspiration and difficult airway associated with general anesthesia. However, the use of general anesthesia is sometimes required if regional anesthesia is contraindicated (e.g., coagulopathy, hemorrhage) or for emergent deliveries (e.g., fetal bradycardia, uterine rupture). Benefits of general anesthesia compared with regional anesthesia include a secure airway, controlled ventilation, rapid and reliable onset, and potential for less hemodynamic instability.

Epidural Anesthesia for Cesarean Delivery

Epidural anesthesia is an excellent choice for surgical anesthesia when an indwelling, functioning labor epidural catheter is in place. Epidural anesthesia provides the ability to titrate the desired level of anesthesia and extend the block time if needed. The volume and concentration of local anesthetic agents used for surgical anesthesia are larger than those used for labor analgesia. Typically the anesthesiologist attempts to provide a dense block from the T4 level to the sacrum. This technique might not completely alleviate the visceral pain and pressure sensation associated with peritoneal manipulation, and adjuvant drugs are occasionally

necessary. Epidural block failure rates for cesarean delivery following use of a labor epidural are known to be greater in the urgent setting compared with elective cases and range between 1.7% and 19.8% (Carvalho, 2012). In some cases conversion to general endotracheal anesthesia may be required. Antiemetics are frequently given to decrease nausea and vomiting associated with the cesarean delivery and hemodynamic effects from the dense neuraxial blockade. Epidural morphine is typically administered near the end of the procedure to decrease postoperative pain for up to 24 hours.

Spinal Anesthesia for Cesarean Delivery

For the patient without an epidural catheter, spinal anesthesia is the most common regional anesthetic technique used for cesarean delivery. The block is technically easier than epidural placement, more rapid in onset, and more reliable in providing surgical anesthesia from the midthoracic level to the sacrum (Riley et al., 1995). The risk of profound hypotension is greater with spinal anesthesia than with epidural anesthesia because the onset of the sympathectomy is more rapid. However, this risk can be nearly eliminated by avoidance of aortocaval compression, prehydration, and appropriate use of vasopressors. Data suggest that spinal anesthesia can be used safely for patients with preeclampsia (Hood and Curry, 1999). A typical spinal anesthetic could consist of hyperbaric bupivacaine with morphine added to decrease postoperative pain. A hyperbaric solution facilitates appropriate local anesthetic spread in the cerebrospinal fluid to a position near T4. The duration of a single-shot spinal anesthetic is variable, but normally provides adequate surgical anesthesia for greater than 90 minutes. In selected circumstances, the use of a CSE technique offers the advantage of a spinal anesthetic, with rapid onset of a dense block and the ability to administer additional local anesthetic through the epidural catheter if the procedure lasts for an extended time.

Local Anesthesia

Although cesarean delivery can be performed with local infiltration, it is accompanied with considerable discomfort to the woman and risks the possibility of local anesthetic overdose. Most obstetricians are not trained to perform the technique. However, local tissue infiltration is useful in rare circumstances, such as acute fetal distress without an available anesthesia provider.

General Anesthesia

Use of general anesthesia for cesarean delivery is typically reserved for situations where neuraxial anesthesia is contraindicated or emergent delivery is needed. Based on data from the period 1997–2002, the RR of general anesthesia is 1.7 times that of neuraxial anesthesia, with two-thirds of the mortality associated with general anesthesia caused by intubation failure or induction problems (Hawkins et al., 2011). Appropriate airway examination, preparation for unanticipated events, and familiarity with techniques and the algorithm for difficult intubation are critical for providing a safe general anesthetic. A current multi-institutional database of adverse obstetric anesthesia events notes that rates of failed intubation are approximately 1:533, although none of the ten failed obstetric intubations in the study resulted in maternal mortality (D'Angelo et al., 2014).

After denitrogenation of the lungs (i.e., preoxygenation), general anesthesia is induced by rapid-sequence administration of an IV

induction agent, followed by a rapidly acting muscle relaxant. The trachea is intubated with a cuffed endotracheal tube, and a surgical incision is made after confirmation of tracheal intubation and adequate ventilation. Anesthesia is maintained by administering inhaled halogenated agents (e.g., sevoflurane), as well as benzodiazepines, opioid analgesics, propofol, nitrous oxide, and additional muscle relaxants if needed. These additional intravenous agents are normally administered after the baby is delivered to avoid placental transfer to the neonate. Before delivery of the baby, the primary anesthetic for the incision and delivery is the IV induction agent, because there is little time for uptake and distribution of the inhaled agents into the mother or fetus (Dwyer et al., 1995). If intubation attempts fail, the cesarean delivery may proceed if the anesthesiologist communicates that it is possible to reliably ventilate the mother's lungs with either a facemask or a laryngeal mask airway (Practice Guidelines for Obstetric Anesthesia, 2016). Halogenated agents are often partially replaced with other anesthetic agents following delivery to decrease the adverse effects on uterine muscular tone (atony).

Induction Agents

Anesthesiologists use a variety of agents to rapidly induce unconsciousness. Among the most common are propofol, etomidate, and ketamine. Each agent represents a different biochemical class, and each has specific advantages and cardiovascular effects.

Propofol action is rapid in onset with an umbilical artery to umbilical vein (UV) ratio of 0.7 (Dailland et al., 1989). Propofol administration has no significant effect on neonatal behavior scores, with typical induction doses of 2.5 mg/kg; however, larger doses (9 mg/kg) are associated with newborn depression (Gregory et al., 1990). Why there is a lack of neonatal effects is unclear, but may be caused by redistribution into maternal vascular-rich tissue beds, first-pass metabolism by the neonatal liver, additional dilution by the fetal circulation, and higher fetal brain water content.

Etomidate also has a quick onset of action because of its high lipid solubility. It rapidly crosses the placenta, and rapid hydrolysis and redistribution results in a relatively short duration of action. Unlike propofol, etomidate has minimal effects on the cardiovascular system, can cause involuntary muscle tremors, has higher rates of nausea and vomiting, and can increase the risk of seizures in patients with decreased thresholds. At typical induction doses (0.3 mg/kg), etomidate administration can cause decreased neonatal cortisol production (<6 hours), but the clinical significance remains uncertain (Crozier et al., 1993).

Ketamine, a structural analogue to phencyclidine, is an analgesic, hypnotic, and amnestic with minimal respiratory depressive effects. In contrast to propofol, sympathomimetic characteristics of ketamine increase arterial pressure, heart rate, and cardiac output through central stimulation of the sympathetic nervous system. Similar to etomidate, it is an appropriate choice for a patient in hemodynamic compromise. No neonatal depression is noted with typical induction doses (Little et al., 1972). Larger doses can increase uterine tone and reduce uterine arterial perfusion. Even in low doses, ketamine has profound analgesic effects.

Nitrous Oxide

Inhaled nitrous oxide is often used as part of maintenance for general anesthesia because of its minimal effects on maternal hemodynamics and uterine tone. Alone, it is insufficient to provide adequate anesthesia for cesarean delivery. It rapidly crosses the placenta with increasing UV to maternal artery ratios of 0.37 in the first 2–9 minutes, increasing to 0.61 at 9–14 minutes (Karasawa

et al., 2003). The effects of nitrous oxide on the neonate are not significant. Additional information about nitrous oxide is found in the section on Analgesic Options for Labor and Vaginal Delivery: *Inhaled Nitrous Oxide*.

Inhaled Halogenated Anesthetics

Isoflurane, sevoflurane, and desflurane are all halogenated hydrocarbons that differ in chemical composition, physical properties, biotransformation, potencies, and rates of uptake and elimination. In clinical use, specialized vaporizers deliver these volatile liquid agents, so that the inhaled concentrations can be carefully titrated by anesthesiologists because of the relatively profound cardiovascular effects and potential for uterine muscle relaxation. These agents are important components of general anesthesia for cesarean delivery, but readily cross the placenta. Placental transfer of inhalation agents is rapid because these are nonionized, highly lipid-soluble substances of low molecular weight, which cross the placenta to the fetus by diffusion along a concentration gradient, much like oxygen and carbon dioxide. The concentrations of these agents in the fetus depend directly on the concentration and duration of anesthetic in the mother. Although general anesthesia is often utilized when emergent cesarean delivery is required, neonatal depression is more likely due to the previously compromised fetus than the impact of anesthesia. A depressed fetus will probably become a depressed neonate. A Cochrane review of 16 studies comparing neuraxial blockade with general anesthesia in otherwise uncomplicated cesarean deliveries found that “no significant difference was seen in terms of neonatal Apgar scores of six or less and of four or less at five minutes and need for neonatal resuscitation” (Afolabi and Lesi, 2012). The authors concluded that there was no evidence to show that neuraxial anesthesia was superior to general anesthesia for neonatal outcome.

Recent experimental animal studies have demonstrated neuronal apoptosis in the developing brain when a variety of agents are administered to induce and maintain general anesthesia (Loepke and Soriano, 2008; Istaphanous and Loepke, 2009). Implications for the fetus and neonate from the extremely brief anesthetic exposures associated with cesarean delivery are currently unknown because of a lack of human studies and difficulties extrapolating animal study methodology to humans.

The “induction-to-delivery” interval is not as important in neonatal outcome as the uterine “incision-to-delivery” interval, during which uterine blood flow can be compromised and fetal asphyxia can occur. A long induction-to-delivery time can result in a neonate who is lightly anesthetized but should not be associated with causing asphyxia. If inhaled anesthetics are administered to the mother for a prolonged period, neonatal anesthesia, evidenced by flaccidity, cardiorespiratory depression, and decreased tone, can be anticipated (Moya, 1966). In such cases, the infant would be lightly anesthetized and should easily respond to basic, supportive treatment measures focused on effective ventilation; cardiopulmonary resuscitation should not be necessary. Ventilation will allow elimination of the inhalation anesthetic by the infant’s lungs since the two factors that govern recovery from inhaled anesthesia are the rate of delivery of anesthetic back to the lungs from body tissues (by cardiac output) and the rate of fall of the alveolar anesthetic concentration through exhalation (by ventilation). Rapid improvement of the infant should be expected; otherwise, a search for other causes of depression is imperative. For these reasons, it is critical that clinicians experienced with neonatal ventilation be present at cesarean deliveries during which the mother receives

general anesthesia and the time from skin incision to delivery is expected to be prolonged (e.g., known percreta, large fibroids), or maternal condition necessitates an atypical induction and maintenance of anesthesia. A discussion of the operative and anesthetic plan by the neonatologist, obstetrician, and anesthesiologist is crucial for optimizing the outcome of neonates in these situations.

Neuromuscular Blocking Agents

Succinylcholine remains the skeletal muscle relaxant of choice for obstetric anesthesia, because of its rapid onset and short duration of action. In appropriate doses (1–1.5 mg/kg), intubating conditions are achieved within 45 seconds. Because it is highly ionized and poorly lipid soluble, only small amounts cross the placenta. Side effects include increased maternal potassium levels and myalgias, and succinylcholine is a known trigger agent for malignant hyperthermia in susceptible individuals. This depolarizing neuromuscular blocking agent is normally hydrolyzed in maternal plasma by pseudocholinesterase and usually does not interfere with fetal neuromuscular activity. Although pseudocholinesterase activity is decreased in pregnancy, neuromuscular blockade by succinylcholine is not significantly prolonged. If large doses are given (2–3 mg/kg), it results in detectable levels in umbilical cord blood, but extreme doses (10 mg/kg) are needed for the transfer to result in neonatal neuromuscular blockade.

Rocuronium is a rapid-acting, nondepolarizing neuromuscular blocker that is an acceptable alternative to succinylcholine. It provides adequate intubating conditions in approximately 60–90 seconds in appropriate doses. Unlike succinylcholine, it has a much longer duration of action, decreasing maternal safety in the event the anesthesiologist is unable to intubate or ventilate the patient. It has the benefit of not being a triggering agent of malignant hyperthermia or elevating serum potassium levels.

During the operation, nondepolarizing neuromuscular blocking agents can be titrated to improve operating conditions. Under normal circumstances, the poorly lipid-soluble, highly ionized, nondepolarizing neuromuscular blockers (i.e., rocuronium, vecuronium, cisatracurium, pancuronium) do not cross the placenta in amounts significant enough to cause neonatal muscle weakness (Kivalo and Saarikoski, 1972). This placental impermeability is only relative, however, and neonatal neuromuscular blockade can occur when large doses are given (Older and Harris, 1968).

The diagnosis of neonatal depression secondary to neuromuscular blockade can be made on the basis of the maternal history (e.g., prolonged administration of neuromuscular blockers, history of atypical pseudocholinesterase), the response of the mother to neuromuscular blocking drugs, and the physical examination of the newborn. The paralyzed neonate has normal cardiovascular function and good color, but lacks spontaneous ventilatory movements, has muscle flaccidity, and shows no reflex responses. The anesthesiologist can place a nerve stimulator on the neonate and demonstrate the classic signs of neuromuscular blockade. Treatment consists of ventilatory support for up to 48 hours until the neonate excretes the drug. Reversal of nondepolarizing relaxants with cholinesterase inhibitors may be attempted (e.g., neostigmine, 0.06 mg/kg), but adequate ventilatory support is the mainstay of treatment. Concomitant administration of an anticholinergic (e.g., atropine, glycopyrrolate) is normally necessary to prevent severe bradycardia from muscarinic side effects of the increased acetylcholine.

Summary

This chapter serves as a general overview of the changes in maternal physiology during pregnancy and briefly discusses options and techniques for both labor analgesia and anesthesia for cesarean delivery. Its purpose is to allow the pediatrician and neonatologist a better understanding of the decisions and concerns of the anesthesiologist and the implications of his or her interventions.

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17

The Human Genome and Neonatal Care

C. MICHAEL COTTON AND JEFFREY C. MURRAY

KEY POINTS

- In the 50 years since the identification of the double helix, and the 15 years since the publication of the almost complete sequence of the human genome, the amount of information about the human genome, its construction, and how it is regulated has exploded.
- Thousands of individuals have had their entire genomes or exomes sequenced and shared, along with corresponding phenotype information. This comprehensive genome, exome, and linked phenotype information enhances our ability to identify genetic links with previously unexplained structural and functional disorders among newborns.
- The rapidity of adoption of new technology and informatics is challenging clinicians to keep up with the rapidly expanding understanding of the genome and how it impacts patients and their families.

Within 50 years of the discovery of the double helix structure of DNA by Watson and Crick, the Human Genome Project achieved the major milestone of identifying an almost complete sequence of the approximately 3 billion nucleotides contained in the human haploid genome (International Human Genome Consortium, 2001; Venter et al., 2001). Since the publication of these single consensus genomes, advances in sequencing technology have enabled the reporting of in excess of 100,000 complete genomes and the characterization of millions of genetic variants in millions of individuals. These developments have advanced our understanding of gene function and regulation and have led to the direct consumer use of genetics and the discovery of the genetic contributors to thousands of common and rare diseases.

Linking genetic variations to disease is a key step to advancing understanding of pathophysiology and human development that will lead to better treatment and management strategies. The potential impact of personal genotype–phenotype information, now widely characterized as one component of precision medicine, is as worthy of concerted collaborative efforts and study as are the exciting advances in knowledge of pathophysiologic and developmental biology aspects of disease (Collins and Varmus,

2015). Newborn medicine has not seen the degree of inclusion of the genome as has cancer and other common adult medicine disorders such as type 2 diabetes and macular degeneration. However, genetic sequencing and informatics analysis technologies and approaches are now being widely used in the diagnosis of newborn birth defects and metabolic disorders, and while it may someday be applied to newborn screening directly, a host of technical and ethical issues will first need resolution (Botkin et al., 2015; Botkin and Rothwell, 2016; Lantos, 2016).

This chapter reviews key concepts about the human genome, particularly its components and structure and certain types of variations. We review the evolving use of genome-focused tools by clinicians and researchers trying to identify novel causative genes for fetuses and neonates with suspected monogenic conditions. We also discuss evolving information and use of genomic testing to identify associations between genetic variations and the complex disorders associated with preterm birth, focusing on intraventricular hemorrhage (IVH) and bronchopulmonary dysplasia (BPD). Finally, we discuss how the massive amount of genomic information emerging from thousands of accumulating sequenced individuals challenges us to question how best to use this information to provide better prenatal, perinatal, and neonatal clinical care.

History of Mapping and Sharing the Genome

The Human Genome Project has been an international effort from its beginnings and had critical predecessors in the human gene mapping (HGM) meetings focused on identifying the chromosomal location of normal and disease-causing genetic variants. The community established by the HGM meetings provided an infrastructure that enabled the more comprehensive sequence-based maps to be developed in the wake of the HGM meetings (Watson and Cook-Deegan, 1991). The international effort continues, with some countries conducting population-based studies applying whole genome or exome sequencing to tens of thousands of individuals (Manolio et al., 2015). By participating in collaborative efforts, clinicians and researchers have now published studies of comprehensive views of genetic variation for hundreds of thousands of individuals with type 2 diabetes, breast cancer, or normal traits

such as height or birth weight. These collaborative international efforts have created the framework for one of the greatest successes of the Human Genome Project—that is, information generated relevant to the human DNA sequence and its variation held in public trust and with open access to the scientific community through accessible databases and analytic platforms that now number in the dozens (NCBI Resource Coordinators, 2016).

One major hub for clinicians that contains both clinical and scientific descriptors of genetic findings that is searchable by disease, phenotype, or genetic variant is the website Online Mendelian Inheritance in Man (OMIM)—a comprehensive catalogue, updated daily, of now more 15,000 genes and diseases with some degree of association, including the ever-growing total of more than 4000 validated single gene disorders. The online version, www.ncbi.nlm.nih.gov/omim/, a compendium of human genes and phenotypes, also provides links to multiple other online resources that provide information about genetic variants, including their associations with disease, genetic conservation across species, the differences in variant prevalence between populations of different ancestries, predicted variations in protein structures relative to genetic variations, proteins, and classification of genes in thousands of different biological pathways (<http://omim.org/help/external>). The Clinical Sequencing Exploratory Research Consortium, funded by the National Human Genome Research Institute and the National Cancer Institute, is among the newest collaborative efforts accumulating whole genome and exome sequences and studying their role within the practice of medicine. The consortium is exploring analytic and clinical validity and utility, as well as the ethical, legal, and social implications of sequencing via multidisciplinary approaches (Green et al., 2016).

Defining the Genome and Genomics

While genetics as a discipline is focused on the study of single genes and their effects, genomics is defined as the comprehensive study of the functions and interactions of all the genes in the genome. The genes of eukaryotic organisms are divided into exons and introns. Exons are regions of DNA that are “expressed” or translated into protein and can include regulatory signals for how much of that gene product is made and in what tissue. Introns are intervening regions that are not translated into protein. The “exome” is the collective term for all of the exons. Despite their apparent importance and influence as being the “source code” for the proteins that allow us to function, exons account for less than 2% of the DNA in the entire genome. Regions outside of the formal coding exons are increasingly recognized for playing critical roles in how and when the genes themselves are expressed and functioning. The Human Genome Project quantified the total number of base pairs (bps; approximately 3 billion), and defined genes to approximately 30,000, and provided more information about how genomic DNA might be classified. The very definition of what a “gene” is changes as we learn more about distant regulatory elements, DNA modifications, chromatin structure, gene–gene interactions, alternative splicing and exon skipping, post-translational modification, and a host of other factors that determine what a gene does and is (Guttmacher and Collins, 2002; US Department of Energy, 2013).

In the years since the description of the primary human sequence, a deeper understanding of the nature of the DNA and its sequence and its regulation, and definitions of exons and introns, has developed. Interestingly, we have learned that approximately 8% of the intronic base sequences are the products of human endogenous

retroviruses. The segments are DNA-based copies of their own ribonucleic acid (RNA) genetic material inserted over millennia in the human genome (Wildschutte et al., 2016). Even before the sequencing of the complete human genome, we knew that there were regulatory regions of the genome close to exons, sometimes called *promotor regions*. These are sites where transcription factors can bind to “turn on” or “turn off” a gene’s expression. The regulatory region is usually a different set of bps than the actual transcription start site where transcription of messenger RNA (mRNA) is initiated (Geer and Messersmith, 2002). Understanding how noncoding, nonregulatory region sequences play a role in human health and disease will be an important area of study for decades to come.

As our understanding of the genome has expanded, we have learned that many more RNAs and proteins can be made from the DNA that underlies what we previously thought were single genes. While approximately 30,000 physically discrete genes are known, the principle of alternative splicing—i.e., mRNA composed of different exons from the same gene—increases the estimate of the number of proteins possible from 30,000 to well over 100,000. The basic principle is that mRNA can be transcribed from different exons within the same gene to make different proteins. One important example is in the developing neuronal system, in which over 90% of multi-exon genes are alternatively spliced throughout development (Wang et al., 2008; Mitchell, 2011; Norris and Calarco, 2012; Shi et al., 2012; Gazina et al., 2015).

Mitochondrial DNA

Mitochondria are the energy-producing organelles present in thousands of copies within each cell. Each mitochondrion has its own genome, distinct from the nuclear genome, and is thought to have arisen from incorporation of bacterial DNA by a eukaryotic cell. Although the mitochondrial genome is approximately 16,500 bps in length, there is a wide range of disorders, some lethal, associated with variation in the mitochondrial sequence. In addition, because mitochondria reside in the cytoplasm and are not found in sperm, they have a unique pattern of maternal-only inheritance, in which mothers pass their mitochondria to all of their offspring, with their daughters in turn passing that on to subsequent generations and with no passing of mitochondrial DNA from males to their children. The knowledge that variants in mitochondrial DNA can cause disease, plus the progress made in techniques and technology for molecular and cellular manipulation, has led to embryonic mitochondrial transplantation, where “healthy” mitochondria are transplanted into the cytoplasm of an early-stage embryo identified to have a lethal mitochondrial variant (Richardson et al., 2015; Committee on the Ethical and Social Policy, 2016).

Types of Variation in the Human Genome

Single Nucleotide Polymorphisms

Single nucleotide polymorphisms (SNPs) were the first commonly characterized contributors to human genetic variation in the molecular age. Prior to that, only visible chromosomal variants and amino acid variants found in proteins comprised the known genetic variations in humans, and these were limited in number and were prohibitively expensive to characterize at a population level. SNPs are specific nucleotide sites in the human genome where it is possible to have two (or even three or four) different nucleotides at a specific position on a chromosome. For example, there might be either a Thymine or a Guanine at a specific site. These sites in the genome

where variations occur are common, with up to 1% of the 3 billion bps of the human DNA sequence being potentially variable between any two individuals, resulting in tens of millions of SNPs across the genome. Most variation is found across all human populations, although some variants appear to be highly population-specific or ancestry-specific. Chip-based DNA genotyping allows the genotyping assays of greater than 1 million SNPs simultaneously on one individual at a cost approximating US\$100. Examples of these genome-wide studies of diseases of premature neonates are described later in the chapter. Known SNPs are catalogued in the online public-domain resource the Single Nucleotide Polymorphism database (dbSNP: <http://www.ncbi.nlm.nih.gov/snp>). Most SNPs appear to not be disease-causing themselves, although they may lie adjacent to DNA changes that do contribute to disease predisposition and can be detected using the phenomena of linkage disequilibrium (Christensen and Murray, 2007).

Copy Number Variants

A relatively recent discovery in human genetic variation has been the importance of copy number variants (CNVs) as contributors to human inherited disorders. Although both small and large deletion and duplication events of the human DNA sequence have been known since the 1970s, based on cytogenetic banding, only recently has the widespread role that these play in disease been recognized. CNVs can range in size from a few nucleotides to thousands, or occasionally hundreds of thousands, or millions of bps in length, resulting in the possibility that two healthy people could have complete genomes with total numbers of bps that differed by millions (Redon et al., 2006). These variations may contain one or multiple genes that can exist in two, three, or more copies arrayed in tandem at particular chromosomal positions. When these tandem arrays of largely identical sequences align themselves during meiosis, there is occasionally a misalignment that can result in the deletion or duplication of one or more of the copies. This event in turn can create a range of the number of copies present from zero to many (Zhang et al., 2009). When functional genes or functional regulatory elements are contained within the copied or deleted element, the amount of gene product made may be increased or decreased from a reference level. Early examples in which CNVs contributed to disease include the DiGeorge syndrome 22q deletion and deletions associated with spinal muscular atrophy and Charcot-Marie-Tooth disease. New microdeletion syndromes are being characterized with great precision using array-based DNA analysis that has rapidly replaced the traditional karyotype as the first line of chromosomal analysis. Other rearrangements leading to congenital anomalies, developmental delay, or both, include the 15q11-q13 defects seen in Prader-Willi syndrome and Angelman syndrome. The origin of the rearrangement, either the maternal or paternal chromosome with the CNV, determines which of the two phenotypes emerge (Prader-Willi if the paternal-derived chromosome has the variant, and Angelman if the deletion is in the inherited maternal chromosome) (Morrow, 2010). Finally, in areas such as autism, it is clear that these microdeletion or duplication events are a contributing explanation for the sometimes sporadic, as well as familial nature, of these disorders. Their contribution to human genetic disease is now suggested to be in excess of 10% of all variant-contributed disease. Despite their importance, many technical and clinical issues related to their identification and meaning remain unresolved (Ali-Khan et al., 2009). Additionally, their widespread presence creates clinical challenges in determining whether they are causal or merely coincidental in clinical cases (Martin et al., 2015).

Chromosomal microarrays, which include assessments of insertions, duplications, and deletions across the entire genome as well as some allelic variants strongly associated with disease, have become the most commonly used clinical test to identify CNVs (Watson et al., 2014).

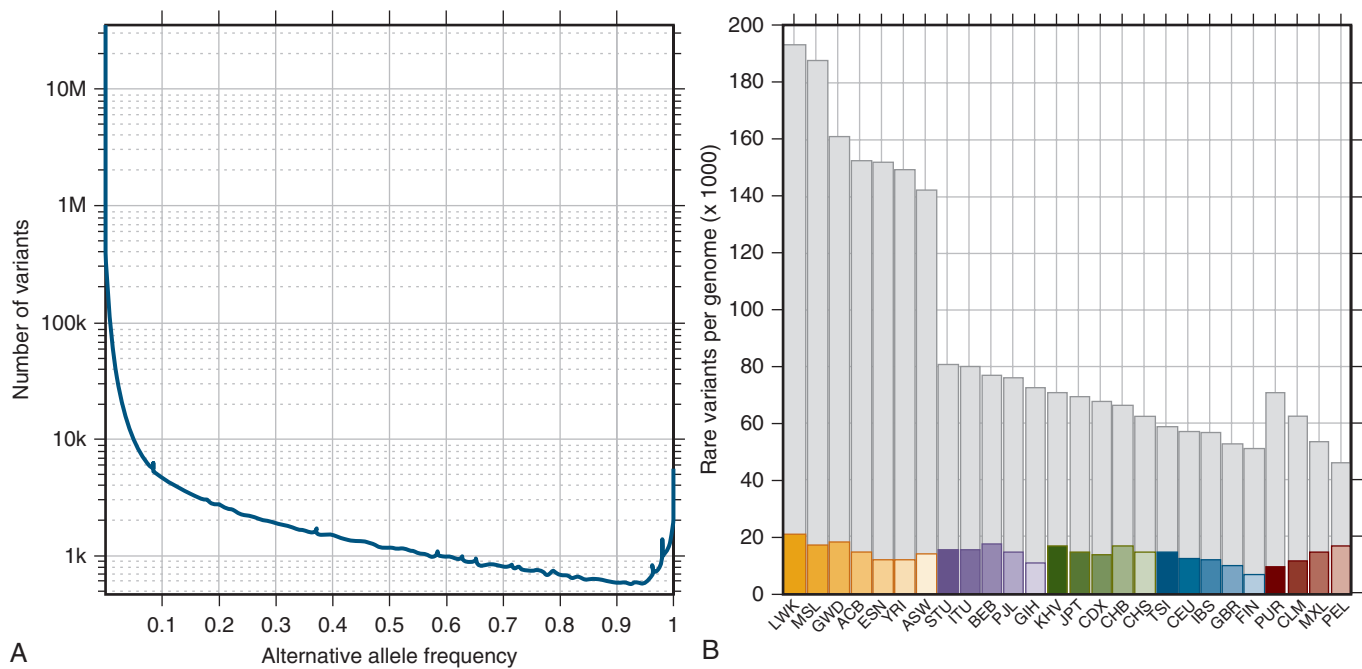
Current Variant Counts

In the years since completion of the human genome map and with progress being made with sequencing, the number of identified variants in the map has exploded. As recently as 2015, in results from whole genome sequencing of over 2500 individuals from 26 populations of varying ancestry, over 88 million variants have been identified. Almost 85 million are SNPs, 3.6 million are insertion/deletions, and 60,000 are structural variants. Interestingly, the vast majority of SNPs occur rarely. Over 60% of the autosomal variants identified across all individuals had population frequencies of less than 0.5%. Many of these were observed to be much more common in at least one specific geographically defined ancestry group. A “typical” single individual is likely to have 4–5 million variants, with most being SNPs and short insertion/deletions, with only 1%–5% of these (40,000–200,000) being “rare variants” with a frequency of less than 0.5% in the population. Of these, most are inherited from parents, and 50–100 are *de novo* (Veltman and Brunner, 2012). Most of these variants do not appear to be linked with disease, either by being predicted as protein-truncating variants, or peptide-sequence altering variants, or variants overlapping known promotor regions, or other functional genomic regions, but if they do fall into one of these categories, they can lead to disordered development or disease (The 1000 Genomes Project Consortium, 2015) (Fig. 17.1).

Regulation of Gene Expression

In addition to variations in genomic DNA, gene expression can also be modified by molecular structures outside of genomic DNA. The key components of these epigenetic modifiers are direct biochemical modifications to genomic nucleotides, the proteins that form the core around which DNA wraps (histones), and short strands of noncoding RNA such as microRNA (miRNA; Bird, 2007). Methylation of DNA nucleotides can either interfere with or block transcription-factor binding to DNA or can recruit compounds that bind only to methylated DNA and suppress transcription (Robertson and Wolffe, 2000; Bird, 2007). During embryogenesis, methylation depends on the supply and enzymatic transfer of methyl groups to genomic DNA, which requires energy, suggesting links between methylation and nutrition. In animal models, variation in methylation has been observed with changes in the environment, diet, and the presence or absence of a nurturing environment (Weaver et al., 2004; Desai et al., 2015). Histones can also be methylated and acetylated, and differential lysine methylation or acetylation leads to repression or activation of gene transcription (Desai et al., 2015). Modification in methylation of histones has been associated with obesity and type 2 diabetes (Jufvas et al., 2013). The classic example of epigenetic modification in humans comes from studies of 60-year-old survivors prenatally exposed to starvation during the Dutch Hunger Winter (1944–1945) and their siblings born in less-stressed years. Epigenetic modifications were noted in methylation of whole blood *IGF2* gene, two obesity-related non-imprinted genes (*TNF*, *leptin*) as compared with their unexposed, same-sex siblings (Heijmans et al., 2008).

MiRNAs are unique short strands (18–25 nucleotides) of transcribed, single-strand RNA that bind to mRNA and inhibit



• **Fig. 17.1** Figure of Variant Counts: Where's the Variation? Variant counts. (A) The number of variants within the phase 3 sample as a function of alternative allele frequency. (B) The average number of detected variants per genome with whole-sample allele frequencies of less than 0.5% (gray bars), with the average number of singletons indicated by colors. (From The 1000 Genomes Project Consortium, Auton A, Brooks LD, et al. A global reference for human genetic variation. *Nature*. 2015; 526: 68–74.)

efficient translation of the targeted, complementary mRNA into protein. They have been estimated to comprise 1% of all the genes in humans as well as other animals. Over 1900 have been characterized and listed on mirbase.org, <http://mirbase.org/> (Kozomara and Griffiths-Jones, 2011; Tétreault and De Guire, 2013). One miRNA can suppress hundreds of different mRNAs and, overall, may impact transcription of up to one-third of the genes in the human genome (De Guire et al., 2013). They have been described among all plants and animals, and even some unicellular organisms, with highly conserved structures (Lewis et al., 2005; Mendell, 2008; Stefani and Slack, 2008). Interestingly, they can be extruded from cells and travel freely in blood and are found in multiple biologic fluids (Cortez et al., 2011; Tétreault and De Guire, 2013). Early work characterized their importance in tumorigenesis, through mechanisms such as inhibition of tumor suppression pathway genes or apoptosis inhibition, or, as in the originally described association between miRNA with B-cell leukemia, deletion of the miRNA sequence allowed overexpression of an antiapoptotic factor that led to B-cell leukemia (Cimmino et al., 2005; Petrocca et al., 2008). They have been implicated as important in neuronal, muscle, lung, heart (size, structure, and conductance), and immune cell development, as knock-out or mutations of specific miRNAs lead to birth defects, cardiac arrhythmias, severe lung hypoplasia, and ventricular septal defects (Stefani and Slack, 2008; Ventura et al., 2008; Tétreault and De Guire, 2013).

Connecting Genes to Diseases

Identification of Disease-Related Genes

One hundred and one years prior to the sequencing of the human genome, Archibald Garrod noted that alkaptonuria seemed to occur at high prevalence among children born to parents who

were first cousins. From this, he hypothesized that this dark urine effect was probably a disorder of metabolism inherited according to Mendel's laws of heredity, the first disorder in humans attributed to what would become genetics. He also noted that the chemical basis for darkening the urine could be a sign of human biologic diversity, one that could be harmless, but other such inherited traits might be manifest as lethal disease. He coined the term *inborn errors of metabolism* to describe diseases caused by errors and variations in chemical pathways (Garrod, 1902; Scriver, 1996). Over the next 100+ years, particular facts about DNA and its impact on human development and disease have come to light, including, at approximately the midpoint between Garrod and the Human Genome Project, the identification of the double helix as the structure that allows for transcription and translation (Watson and Crick, 1953).

Clinicians caring for neonates in the intensive care nursery often hypothesize that genetics or the interaction of the individual neonate's genome and environment must contribute to structural anomalies, metabolic derangements, and the common, complex disease phenotypes noted among newborns and prematurely born neonates. Currently we estimate that 5%–10% of newborns have congenital or chromosomal disorders. For decades, 20% of infant deaths in the United States have been attributed to chromosomal and congenital anomalies. Of these, only a small minority will have an identified syndrome or identified genetic cause (Sowards, 1999; Mathews and MacDorman, 2007; Carmichael, 2014). In addition to the deaths readily attributable to congenital defects or chromosomal abnormalities, deaths attributed to sudden infant death syndrome are more and more often associated with genetic variations identified through sequencing and subsequent testing of variants in model systems, potentially adding substantially to the number of deaths attributable to genetic variation (Van Norstrand and Ackerman, 2010). In addition to mortality risk,

newborns with complex congenital anomalies and chromosomal disorders who survive beyond the first days and weeks are vastly overrepresented among hospitalizations in pediatric wards and intensive care units. These children are hospitalized more often and have hospitalizations that are longer and more expensive than children without underlying chromosomal anomalies or congenital defects (McCandless et al., 2004; Colvin and Bower, 2009; Jama-Alol et al., 2014; Razzaghi et al., 2015). As clinicians begin to understand the complex interactions between the entire genome and the environment and work to optimize use of the sequencing tools, informatics, and genomic analysis strategies that are emerging, these estimates may increase. As more genetic variants are linked with disease, our hope is that our ability to provide care based on better understanding of the pathophysiology of the disease and susceptibility to complications, such as respiratory infections, that arise from awareness of the genetic variations at the heart of the problem will guide preventive and treatment strategies that will improve outcomes (Beaulieu et al., 2012).

One of the primary benefits of the reference human DNA sequence is our ability to move quickly from finding the location of a gene on a chromosome to identifying the specifics of that gene and the disease-causing mutations. Gene mapping approaches to human gene identification have been in use since the late 1970s, and the first successes were the identification of single-gene disorders, sometimes termed *monogenic* or *Mendelian* because their inheritance patterns follow the traditional modes of autosomal dominant, autosomal recessive, and sex linked.

There are three primary methodologies for gene identification (Altshuler et al., 2008). The first involves linkage studies using large families with genetic disorders or many small families with the same disorder than can be studied and their data pooled. These linkage-based approaches can provide a relatively well-defined chromosomal location for single-gene conditions and has led to successful gene-finding for cystic fibrosis, neurofibromatosis, and hundreds of additional, mostly rare, conditions. This approach can also be applied to common but genetically complex traits for which there are no simple inheritance patterns. This technique may require hundreds of small families, and the resultant gene localization is imprecise. A second method for gene localization can make use of small chromosome rearrangements, such as balanced translocations or small deletions or duplications that result in a phenotype for a known disorder. In these cases, the location of the chromosomal rearrangement immediately suggests that a gene at or near that rearrangement is etiologic and can be used to again directly search for evidence of a specific gene and mutation in that region. A third approach involves an unbiased search across the entire genome, utilizes whole genome or exome sequencing, and applied “filters” that narrow the number of potential variants based on assessing frequency of variants among normal, inheritance patterns, and “in silico” predictions of protein changes that result from the variant in the genetic code (Davis et al., 2014; Francescatto and Katsanis, 2015).

Making a Diagnosis in the Human Genome Era

In infants suspected of having a genetic disease, knowing the etiology can inform subsequent care, both for treatment and prevention of problems associated or anticipated (Coulter et al., 2011; Willig et al., 2015). The broadening knowledge of disorders linked with variants (such as those listed in OMIM), the increasing availability, and decreasing cost of sequencing since completion of the Human Genome Project have increased the use of extensive sequencing

and have led to questions of when extensive sequencing should be carried out relative to more familiar testing including karyotypes and chromosomal microarrays (Stavropoulos et al., 2016).

Traditionally, genetic testing in infants suspected of having a genetic variation-related condition has started with a chromosomal karyotype, or fluorescence in situ hybridization (FISH) testing for aneuploidy, or other chromosomal anomalies such as chromosomal rearrangements and large gains and losses in chromosomal material. The tests sometimes were used to confirm readily apparent family history and clinical picture suspicions that fit a known syndrome or disorder. Clinicians would also order a chromosomal microarray first when the clinical picture and history do not provide support for a known single gene (e.g., cystic fibrosis, Meckel–Gruber syndrome, etc.) or environmental exposure (fetal alcohol, congenital rubella, etc.) or when a known chromosomal disorder such as one of the aneuploidies (trisomy 18 or 21, etc.) is suspected. In many cases the mother may have already had an analysis carried out as part of prenatal screening that may also inform the diagnostic strategy. CNVs can identify high-resolution gains and losses in genetic material, including insertions and deletions (indels) (Miller et al., 2010). Chromosomal microarrays are used to assess infants with multiple anomalies and may identify up to 20% of potentially causative genetic variations (specific variants at a single nucleotide or insertions, deletions, or CNVs that have previously been associated with disease phenotype). Much progress has come as the chromosomal microarray has evolved to include oligonucleotide and single nucleotide probes that identify both single nucleotide variants as well as CNVs (including microduplication and microdeletion syndromes with high sensitivity and specificity) (Miller et al., 2010). While microarrays have greatly improved, the sequence of karyotype, FISH, and microarray is now in some transition, as cost and time to complete whole exome and whole genome sequencing are both rapidly decreasing.

Applying Exomes and Whole Genome Sequences in Clinical Management

First-tier tests such as karyotypes and chromosomal microarrays identify only 20%–25% of genetic variations likely to be contributing to developmental delay, congenital malformations, metabolic disorders, or other problems of extreme phenotype suspected to be genetically related. The addition of whole exome and whole genome sequencing is gathering additional information that is increasing the rate of identified likely causative variants (Lee et al., 2014; Yang et al., 2014; Stavropoulos et al., 2016). The tools of whole exome and even whole genome sequencing are now being applied clinically in cases where a genetics cause is suspected but where CNV analysis or the clinical presentation does not provide a specific answer. While these applications are costly and still generate discussion about when and how to apply them, the trend currently would seem to favor their increasingly widespread application to cases in the nursery and beyond (Shashi et al., 2014; Petrikin et al., 2015). If costs of sequencing drop below the \$100 range and the time to complete sequence and informatics filtering to identify DNA variants drops from weeks and months to a day or two, sequencing may become very widespread. That said, cost and time for genotyping and analysis to identify rare, potentially causative variants will not be the only considerations for this application of sequencing. The ethical issues of incidental findings arising from whole genome and whole exome sequencing will need to be addressed. For example, whole exome sequencing can disclose risks mostly applicable only in adult life (*BRCA1* mutation risks

for breast cancer, for example) that will be far in the future for the neonate tested but of potential immediate impact for the parent who is a likely carrier but may be unaware of (and potentially uninterested in knowing) this risk. Guidelines for responses to these incidental or secondary findings in neonates are evolving (Bortkin et al., 2015; Bortkin and Rothwell, 2016; Lantos, 2016).

The first clinical report of whole exome sequencing leading to a clinical diagnosis was a 15-month-old boy with failure to thrive and an inflammatory bowel disease–appearing phenotype. Multiple diagnostic tests had failed to identify a specific etiology, so his providers completed exome sequencing. The sequencing identified over 10,000 variants, but “filtering” to identify variants that were nonsynonymous, rare (occurring in less than 1% of the population), and predicted to cause changes in proteins that could alter function, narrowed the list of candidates to one novel, hemizygous missense mutation in the X-linked inhibitor of an apoptosis gene with a known central role in proinflammatory response and bacterial sensing (Wortheley et al., 2011). Since this first case, thousands of whole exome and whole genome sequences have been completed, and groups are reporting improved rates of finding likely causative variants (Yang et al., 2013, 2014).

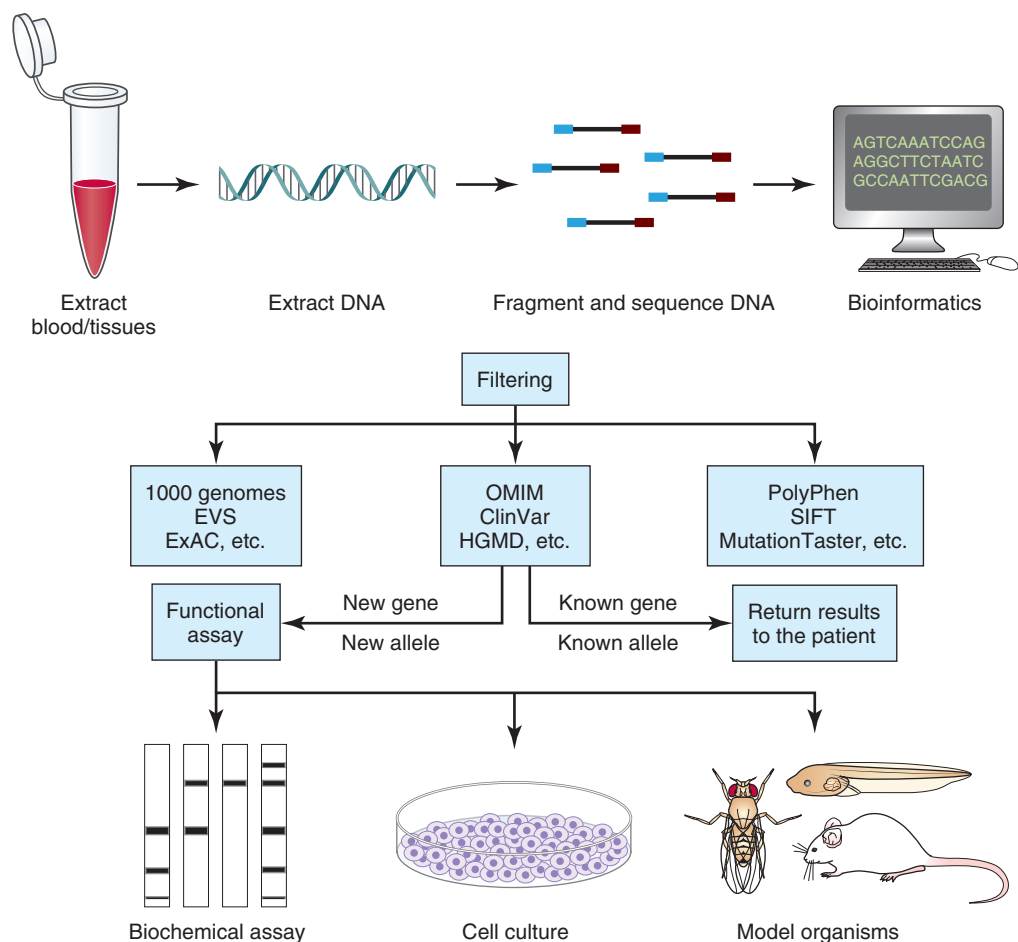
For clinicians, the processes used and standards applied to define *de novo* variants discovered by extensive sequencing as “causative” are of primary importance, as presence of causative alleles will likely drive healthcare and reproductive decisions. These processes and standards to determine functional relevance or causation are evolving and nonuniform. However, the process can be summarized as sequencing, followed by identification of all variants from reference sequences, and then filtering to a much smaller number of variants that are rare, known to be disease-causing, or are likely, through prediction modeling, to alter function. The process has been helped by informatics and collaboration. In the simplest case, some mutations identified in whole genome or whole exome sequencing can be identified as disease causing or disease linked by comparing with databases such as OMIM, ClinVar, and the Human Gene Mutation Variant database. In cases where no mutations are identified that have been previously linked with disease, exome or whole genome sequence variants can be assessed for their population frequency by comparison with thousands of normal healthy individuals’ sequences in the 1000 Genomes and Exome Variant databases, with the assumption that disease-causing variants are likely to be extremely rare or never reported in the populations catalogued in the publicly available datasets. The prediction of protein function alterations for the rare or *de novo* variants identified by sequencing can be predicted by tools such as PolyPhen, SIFT, and Mutation Taster. The American College of Medical Genetics and Genomics (ACMG) recommends use of specific standard terminology—*pathogenic*, *likely pathogenic*, *uncertain significance*, *likely benign*, and *benign*—to describe variants identified in genes that cause Mendelian disorders and provides a system for classifying variants into these five categories based on criteria using typical types of variant evidence (e.g., population data, computational data, functional data, segregation data) (Richards et al., 2015). The next level of prediction of causality, beyond the epidemiologic and genetic–protein structural predictions, comes with validation using *in vivo* models such as zebrafish, flies, and rodents (Francis et al., 2015) (Fig. 17.2).

Whole exome and whole genome sequencing are becoming a clinical reality in the nursery for newborns with congenital anomalies and extreme phenotypes suspected to be caused by genetic variants. The next step could be the inclusion of sequencing of every newborn as part of newborn screening. The goal of newborn screening is

to test for multiple (currently ~ 50 in most states) disorders that benefit from early detection and intervention. With rapid sequencing and filtering through available sequencing data to identify variants in genes already known to be linked with disease, this approach could provide prediction and diagnosis for thousands of conditions that could benefit from early detection and intervention or, for known lethal conditions, allow for earlier implementation of holistic care emphasizing quality of life and parental and family bonding choices to be made (Petrikin et al., 2015).

The rapid sequencing approach has been tested utilizing the “50 hour genome” in a select group of infants and their parents in an intensive care nursery. Success of this approach relies on much more than simply the sequencing and filtering of the genome. Clinicians had classified the neonate’s presenting clinical features in specific Human Phenotype Ontology terminology. This phenotype information was then mapped to informatics tools, either local or publicly available, and then a rank-order list of over 5000 monogenic disorders was matched with the neonate’s phenotype. Sequencing results were compared with reference sequences to identify insertions, deletions, and variations in sequence. This first-level filter identified several million variants, which were further filtered to identify those with population frequency less than 1% and categorized to American Society of Human Genetics criteria 1–3, which are (1) high likelihood of being pathogenic, (2) likely pathogenic, or (3) unknown significance. The next step was to match the neonate’s phenotype with the variant’s likely causative phenotype. Likely causative variants were then further scrutinized by a panel of experts, and the likely variants that emerged were confirmed by independent resequencing. In the first report of 35 ill infants, all less than 4 months old, 20 (57%) had causative variants identified. Average age at testing was 26 days, and median days to a confirmed, reported diagnosis was 23. Most (65%) were *de novo* variants. Almost half of the infants diagnosed by whole genome sequence lacked phenotypic traits that would have predicted the variant being causative, suggesting either that the phenotype had not yet developed or that there was phenotypic pleiotropy or variant phenotype with the genotype (Willig et al., 2015). PhenomeCentral is a web portal (<https://phenomecentral.org>) where clinicians and rare disease scientists can meet and exchange phenotypic and relevant genetic information from exome sequences or candidate gene testing. It includes information from several rare and undiagnosed disease programs worldwide (Buske et al., 2015).

Two sources of challenge, dilemma, or opportunity are readily identifiable with genome-wide or exome-wide testing. First is what to do with the variants of unknown significance that were also identified. The conventional approach is to not report these results to family but to maintain and continuously check these variants for disease associations as more sequences and phenotypes are accumulated worldwide and disease-variant links can be established. Currently, more than 20 novel disease gene discoveries or substantive phenotype expansions of previously known gene–phenotype associations are reported each month (Petrikin et al., 2015). The second dilemma is whether or not to inform parents of sequence variants in genes that are not related to the conditions that led to sequencing but have been previously identified as likely to be causative of preventable or manageable disease. These variants, previously termed *incidental* or *secondary variants*, have now been classified by the ACMG as secondary variants. There are 56 genes at present identified. The current guidance from the ACMG is that a qualified genetics healthcare professional should obtain written informed consent for the sequencing. The process should



• **Fig. 17.2** Next-Generation Sequencing Linking Variants With Disease. Next-generation sequence workflow complemented with functional studies. Upon consent of family members, collection of either blood or tissue is performed, followed by extraction of DNA. The DNA is then fragmented, and a sequence library is subjected to massively parallel sequencing. Resulting reads of the DNA sequences are then processed and curated and compared against existing human genetic databases to identify possible candidate variants that have previously been associated with human genetic disease. The identification of genes or alleles not previously implicated in human genetic disease requires functional assays to test variants' pathogenicity. EVS, Exome variant server; ExAC, Exome Aggregation Cexomonsortium; HGMD, Human Gene Mutation Database; OMIM, online Mendelian inheritance in man. (Modified from Francescatto L, Katsanis N. Newborn screening and the era of medical genomics. *Semin Perinatol.* 2015; 39: 617–622.)

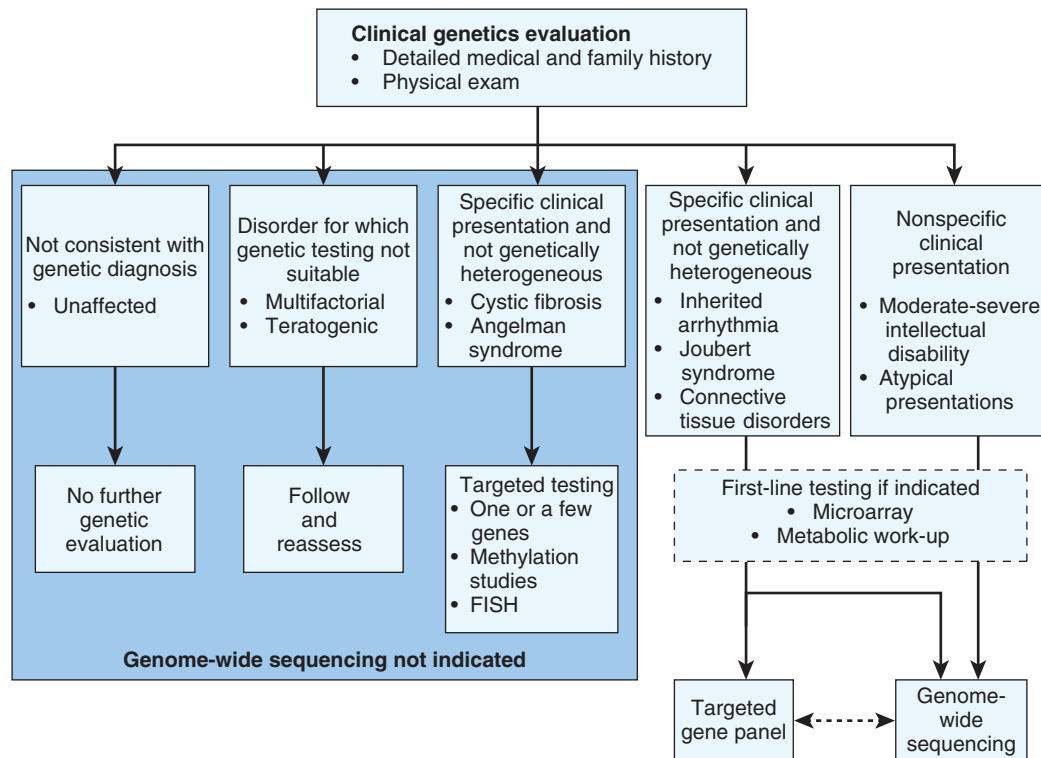
include information about uncertainty around interpretation of results, especially with rare variants in previously undescribed genetic associations and the potential for the impact that findings may have on other family members. As for secondary findings, ACMG recommends that parents be given the choice to opt out of notification of these findings, for their child and for themselves (as sequencing to diagnose neonatal conditions often includes sequencing of the neonate and both parents) (ACMG Board of Directors, 2015).

The Canadian College of Medical Geneticists has also provided guidance on genome-wide sequencing use for identifying suspected monogenic disease. Their Clinical Indications Working Group lists aspects of familial history and clinical conditions that are likely to be identified via a genome-wide or exome-wide sequencing approach (Table 17.1). They also provide guidance on which type of disorders warrant consideration of the sequencing approach early in the diagnostic work-up (Fig. 17.3). Their guidance regarding secondary or incidental findings for children is that incidental results that reveal risk for a highly penetrant condition that is medically actionable during childhood should be reported to the parents.

TABLE 17.1 Characteristics Suggesting Likelihood of Monogenic Disease or Facilitating Interpretation of Genome-Wide Data

| | |
|-------------------------|---|
| Family history | Similar affected individuals Recognizable inheritance patterns Consanguinity |
| Phenotype | Severity Specificity of clinical presentation (e.g., neuropathy, seizures, metabolic disease) |
| Clinical interpretation | Careful phenotyping (detailed physical exam, imaging studies, blood chemistries) Normal chromosomal microarray and other relevant laboratory testing Exclusion of acquired causes such as infection |

From Boycott K, Hartley T, Adam S, et al. Canadian College of Medical Geneticists. The clinical application of genome-wide sequencing for monogenic diseases in Canada: Position Statement of the Canadian College of Medical Geneticists. *J Med Genet.* 2015; 52: 431–437.



• **Fig. 17.3** Example of a Decision Aid to Facilitate the Diagnostic Evaluation of Patients With Rare Disease of Suspected Monogenic Etiology From the Canadian College of Medical Geneticists. Decision aid to facilitate the diagnostic evaluation of patients with rare disease of suspected monogenic etiology. This decision aid highlights where genome-wide sequencing may prove useful in the evaluation process. The conditions listed in each box are representative examples only. For specific clinical presentations associated with genetic heterogeneity, the decision regarding the use of a targeted panel versus genome-wide sequencing is dependent on a number of factors, including the availability of the testing options and the yield of such panels. Patients with negative targeted gene panels may benefit from subsequent clinical genome-wide sequencing. Conversely, consideration of a targeted panel subsequent to uninformative clinical genome-wide sequencing would be dependent on the depth of coverage achieved in the latter instance. *FISH*, Fluorescence in situ hybridization. (Adapted from Boycott K, Hartley T, Adam S, et al. Canadian College of Medical Geneticists. The clinical application of genome-wide sequencing for monogenic diseases in Canada: Position Statement of the Canadian College of Medical Geneticists. *J Med Genet.* 2015; 52: 431–437.)

A child's risk for adult-onset genetic conditions should not be communicated unless (1) the parents request the disclosure and (2) disclosure could prevent serious harm to the health of a parent or family member—determined on an individual, case-by-case basis. The Canadian guidance is that there is no obligation to re-contact children after they become adults to let them know of potentially later-onset monogenic diseases (Boycott et al., 2015).

Complex Common/Multigenic Diseases of Newborns

In addition to the diseases and disorders directly linked to genetic variants, there are strong indications that genetic variations contribute to risks of the common complex diseases associated with prematurity, including retinopathy of prematurity, necrotizing enterocolitis, sepsis, IVH, and BPD (Bhandari and Gruen, 2015). These indications include higher prevalence of the disorder among monozygotic compared with dizygotic twins. There is also variation in risk associated with ancestry (Jo, 2014).

Efforts to identify genetic links with the major morbidities of prematurity were based on testing SNPs in “candidate genes” that

were best-guess genes based on their assumed contributions to the morbidities of inflammation, coagulation, vascular development, or extreme phenotypes noted in Mendelian diseases (Hartnett et al., 2014; Bhandari and Gruen, 2015; Ment et al., 2015). While some of these investigations identified associations with *P* values of less than .05 between presence of the minor allele (the less frequently appearing variant allele in the population) and the morbidity, the studies were frequently limited by sample size, major differences between case and control populations, and uncertainty about defined phenotypes. Prior experience indicated that replication of such associations was unlikely, and identification of genetic links with complex common diseases such as macular degeneration and type 2 diabetes in adults was being uncovered by assessing large populations with genome-wide genotyping and testing for associations (Ioannidis et al., 2001; Wellcome Trust Case Control Consortium, 2007).

As previously noted, finding genetic variants associated strongly with relatively common complex traits is difficult. The challenge derives at least in part because variants in different genes may each contribute to the odds of developing the disease, and each gene's contributions may depend on interaction with environmental

contributors. This heterogeneity creates substantial difficulties in finding and confirming that any single gene or variant plays a role in the disease of interest. One approach to rapidly screen millions of relatively common (occurring in more than 1% of the population) variants for association with disease is called *genome-wide association study*, or *GWAS*. The GWAS approach has been enabled by (a) the growing number of sequenced individuals of different ancestral backgrounds which allows quantification of variant frequency, (b) advances in technology allowing for relatively inexpensive and rapid genotyping of millions of genotypes from samples collected from thousands of cases and controls, and (c) advances in statistical techniques which allow researchers test associations between genotyped and imputed variants and the disease phenotype of interest (Manolio et al., 2009).

The prerequisite to the comprehensive search for variation was the development of the human haplotype map, or “HapMap” (<http://hapmap.ncbi.nlm.nih.gov/>). The HapMap project developed a comprehensive understanding of the relationship of SNPs across multiple human ancestral groups that included Europe, Africa, and Asia (International HapMap Consortium, 2007). In essence, haplotypes are groupings of SNPs that travel together, i.e., when the locus for SNPA is an “A,” the locus for SNPB is also an A, and the locus for SNPC is a “G.” This AAG combination is termed a *haplotype*. HapMap is an essential component of a GWA study because it provides a comprehensive reference listing of the relationships of SNPs and CNVs to enable their careful characterization in case and control populations with enhanced power by taking advantage of the human genome’s patterns of linkage among SNPs. By looking for evidence of DNA sequence variation or allelic or haplotype variation in which one allele is found significantly overrepresented in a case compared with a control population, there is a strong suggestion that the etiologic gene and variant lie in the vicinity of the surrogate or marker gene allele.

One challenge of the GWA study is that while it can identify one or more loci associated with the disease, implementation of a GWA requires expensive technology and large (usually numbering in the thousands), well-phenotyped case and control populations. Thousands of cases and controls must be available to have sufficient power to detect the small effects seen. A second caveat is that the loci found usually have low relative risks (RRs) or odds ratios (ORs) so that the clinical effect of any one identified locus is very small (Hardy and Singleton, 2009). However, collections of loci can have a combined substantial impact, and even a low OR may identify a new biologic pathway that could provide great insights into etiology and treatment (Hirschhorn, 2009). Besides detecting disease-associated genetic risk factors, these studies also contribute to knowledge of the genetics of normal trait variation, such as height and skin color. Using GWAs, more than 50 genes were found to have a role in factors such as height determination (Hirschhorn and Lettre, 2009). However, although identical twin studies tell us that height is almost entirely genetically determined across a broad range of environmental variation, the genetic findings to date explain only a small amount of the contributors to height. There is still much to learn in regard to normal trait variation and the ability to make predictions about a child’s future physical traits or cognitive behavioral range. New approaches beyond GWA are still critically needed to find this large amount of unexplained genetic contributors, but these may possibly be addressed with sequencing of the whole genome or whole exome (Manolio et al., 2009).

Results of GWA studies that tested millions of SNPs for association with the phenotypes IVH and BPD have been published (Hadchouel et al., 2011; Ambalavanan et al., 2015; Ment et al.,

2015). For BPD, one SNP in one gene (*SPOCK2*) has been identified as having variants associated with BPD, and within the same study, the association was as strong among white subjects as African subjects (Hadchouel et al., 2011). A second GWA study which utilized the State of California’s newborn screening blood spots could not replicate the *SPOCK2* associations and did not identify any other SNPs associated with BPD with P values less than 10^{-6} (Wang et al., 2013). Most recently, Ambalavanan et al. reported on a cohort of 1000 extremely low birth weight infants and was also not able to replicate the *SPOCK2* associations in the US cohort; however, in pathways analysis, 77 biologic pathways were associated with BPD, including the microRNA miR-219 pathway, which had the strongest association with severe BPD. When mild BPD and severe BPD were analyzed separately, the pathways segregated almost completely (only three were shared). There were also differential associations noted among different racial and ethnic groups (Ambalavanan et al., 2015). In addition to genome-wide testing for associations between SNPs and BPD, one group tested associations between CNVs and BPD and found no associations that reached genome-wide significance levels (Hoffmann et al., 2014). In another cohort, associations between CNVs in three regions were associated with BPD. Fifteen of the 21 genes represented in these regions differ in expression between the pseudoglandular and canalicular phases of lung development (Ahmad et al., 2016). If the analysis is expanded to the wider level of the entire exome, one group has reported assessment of 26 premature neonates with severe BPD and identified unique rare variants in *NOS2* (inducible nitric oxide synthase), matrix metalloproteinase 1 (*MMP1*), *CRP* (C-reactive protein), *LBP* (lipopolysaccharide-binding protein), and the toll-like receptor (*TLR*) family (Carrera et al., 2015). The group from California that completed the GWA study and CNV analysis has also reported results of exome sequencing on 50 BPD-affected and unaffected premature neonates and identified 258 genes with rare nonsynonymous mutations in patients with BPD. The identified genes with variants included genes involved in collagen tissue organization and lung structural development (Li et al., 2015). The findings of the GWA study, exome, CNV, and pathways analyses strongly suggest that the heritability of BPD risk is complex and is linked to uncommon variation in genes involved in one or more pathways involved with any of the complex components of lung organ development. Many more infants will have to be consistently assigned phenotypes and have sequencing completed in order to identify the most common pathways implicated in development of BPD.

Variants in three coagulation genes have been frequently studied for their associations with IVH in preterm infants. The candidate genes are the factor V Leiden (*F5*) variant, polymorphisms of the methylene-tetrahydrofolate reductase gene (*MTHFR*), and the prothrombin 20210G>A variant (*F2*). They have had interesting, but not always replicated, associations. In addition, variants in *COL4A1* have been associated with severe IVH in preterm and term born neonates and were found to be clustered in families; however, in larger cohorts, these associations have not always been replicated. In addition to BPD, severe IVH is the only other major morbidity of prematurity to be tested with unbiased GWA. Over one million SNPs were assessed for GWA with severe IVH in a well-phenotyped cohort. A significant 10-SNP haplotype in the region on chromosome 1 between the genes *GM140* (also known as *LINC01699*, long intergenic non-protein coding RNA 1699) and *CACNA1E* (calcium channel, voltage dependent, R type and alpha 1E subunit) was determined to be strongly linked with severe IVH, but the association was not replicated in a small cohort (64 cases, 226 controls) (Ment et al., 2015).

While these studies show promise in identifying regions in the genome linked with significant morbidity and interactions among gene variants in biologic pathways, the consistent message is that the discovery of compelling and actionable genetic links with the complex common morbidities of prematurity will not be simple. While we have found rare variants linked with very specific phenotypes—for example in severe respiratory distress syndrome and progressive pulmonary disease—the best chance to identify variants that contribute to a significant number of premature neonates who develop a major morbidity remains the assembly of very well-phenotyped study cohorts, accurate genotyping, and a willingness among investigators to share data for testing and replication of findings.

Guidelines and Recommendations for Integration of Genomics Into Practice

With the rapid introduction of whole exome and whole genome sequences into clinical medicine, clinical geneticists and generalist pediatricians and neonatologists, as well as families and numerous other caregivers, are faced with an enormous amount of information that provides a limited amount of specific actionable data and a much vaster amount of information of unclear meaning. In an effort to give some guidance, stakeholders from a variety of disciplines and perspectives met and developed guidelines for how to apply the current (2016) state of genomics in clinical medicine. In the current clinical setting, since these fields move so rapidly, it is important to maintain close collaborations between caretaker clinicians such as neonatologists or other pediatricians and clinical/molecular geneticists who can provide optimal guidelines on the best tests to order in any challenging situation and how to interpret the sometimes complex results.

Prenatal Identification of Genetic Disorders

The application of array and sequencing technologies has also revolutionized the field of prenatal testing in recent years with noninvasive prenatal diagnosis now becoming the norm for identification of aneuploidies (Evans et al., 2016). Ultrasound and maternal serum testing continue to be important methods applied at a population level to screen for those structural abnormalities (e.g., neural tube defects, omphalocele, cleft lip/palate, congenital heart disease, renal anomalies, etc.) that in many cases are developmental disruptions without a known gene specific cause. Beyond the detection of aneuploidies that rely on the presence of fetal DNA in the maternal circulation, it has even become possible, currently as a research tool, to carry out whole genome sequencing of the fetus to detect single-gene defects (Kitzman et al., 2012). As with newborn screening by whole genome sequencing, the sequencing of a fetus's whole exome is equally fraught with ethical and social issues beyond the mere technical challenges that are now being surmounted. The future use of these approaches will depend on both solving the technologic challenges and developing consensus views on the best ethical approaches (Tabor et al., 2012).

The Future

As technologies advance, genetics is increasingly moving from advances in diagnostics to therapeutics. Cell-based therapies,

advances in bone marrow transplantation, direct treatment of metabolic disorders, and mutation-specific interventions for cystic fibrosis and Duchenne muscular dystrophy have all provided hope to families with a child in whom diagnosis, but not curative therapy, was once the rule. Now a new technology, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated protein-9 nuclease (Cas9) (Sternberg and Doudna, 2015), holds out a new promise that targeted gene editing may be possible to apply on an individual basis. This approach, first associated with an immune function found in bacteria, may enable defective genes to be readily cut out and replaced by the normal DNA sequence. However, as with any new advance, clinicians need to walk the line between enthusiasm and hope promised by the new discoveries and the realities of how long it can take to move from the lab to the clinic in applying new findings to ensure they are safe, effective, and ethically applied. Nonetheless, the experience of the last several decades of human genome research holds even greater promise for the future.

Suggested Readings

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Prenatal Diagnosis

EDITH Y. CHENG

KEY POINTS

- All pregnant women have the option to undergo prenatal screening/diagnosis for genetic conditions and/or birth defects.
- Specific indications for genetic counseling and prenatal diagnosis options include a history of chromosome abnormality, Mendelian genetic disorder, or metabolic disorder; increased risk for neural tube defect; abnormal maternal serum screening test; or a fetal anomaly suspected/diagnosed on ultrasound.
- Successful prenatal diagnosis requires a known condition associated with a structural abnormality visible in the fetus, a biochemical abnormality in amniotic fluid/amniocytes, or a known molecular mutation.
- Preimplantation genetic screening/diagnosis selects out embryos with a genetic condition or aneuploidy and transfers either chromosomally normal embryos or embryos without the at-risk genetic condition.

Background

In the late 1970s, only two programs comprised the paradigms in prenatal screening/diagnosis: (1) elevated maternal serum alpha fetoprotein (MSAFP) to screen for neural tube defects (NTs) and (2) maternal age over 35 years at delivery to screen for Down syndrome (DS). Neither program used imaging routinely, and only second trimester amniocentesis for fetal karyotyping was available. Today, more than 45 years later, genomic discoveries and advances in fetal imaging technology present women with many prenatal screening and/or diagnostic options for the evaluation of their fetus for birth defects and/or genomic abnormalities and, in some cases, provide an opportunity for in utero treatment. Early diagnosis of birth defects broadens the scope of management options and allows preparation for delivery and postnatal support, all of which have improved viability and outcome of serious birth defects that in previous decades would be termed prenatally as “lethal anomalies.” The care of the mother–fetus dyad is now multidisciplinary, through partnerships between maternal–fetal medicine, neonatology, pediatric surgery, and pediatric subspecialists. The goal of this chapter is to discuss the breadth of prenatal diagnosis to illustrate the complexity of the technology and choices, recognize their benefits, accuracy, and limitations, and understand their impact in the care of pregnancies with genetic disorders or fetal anomalies.

Principles of Prenatal Screening and Diagnosis

Screening is the systematic application of a test to identify individuals at high risk for an asymptomatic, well-defined, severe medical condition with an established incidence, for which identification would lead to prevention and/or treatment. The screening test should be cost effective, simple and safe, readily available and accessible, and should have a well-defined performance. An accurate diagnostic test should be available to confirm or refute the screen positive result. In prenatal screening programs, there should be timely transfer of test results, counseling, respect for the ethical and cultural values and decisions of patients, and full discussion of all options if the suspected condition is confirmed.

Diagnosis of a suspected condition in an at-risk fetus requires a known diagnosis for which the condition is associated with a detectable abnormality either within the fetus and/or the fetal tissues. This could be in the form of a structural abnormality readily seen on ultrasound (that appears at the appropriate developmental stage of the system affected), a cytogenetic abnormality, a biochemical abnormality in the amniotic fluid or in cultured amniocytes, a genomic duplication or deficiency identified on microarray analysis, or a known mutation associated with the condition. For example, a mother with autosomal dominant achondroplasia has a 50% chance of having an affected child. Knowing that in an affected fetus the long bones do not demonstrate growth deceleration until after 24 weeks' gestation, a normal growth ultrasound at 20 weeks' gestation would not be reassuring, but a normal growth ultrasound at 30 weeks' gestation would essentially exclude the diagnosis in her fetus. Conversely, if she had a known mutation for achondroplasia, genetic testing of chorionic villi retrieved at 13 weeks' gestation or amniocytes retrieved by amniocentesis at 16 weeks would inform the status of the fetus before the condition is visible prenatally.

Invasive Prenatal Diagnostic Procedures

Midtrimester Genetic Amniocentesis

The term *amniocentesis* refers to the procedure of removing amniotic fluid under ultrasound guidance from the uterus. It is performed for many reasons, including determination of fetal lung maturity

for decisions regarding delivery of a late preterm infant or measurement of bilirubin in the amniotic fluid as a surrogate for fetal anemia in pregnancies complicated by Rhesus D (RhD) isoimmunization. Between 15 and 20 weeks' gestation, amniocentesis is performed, predominantly for prenatal diagnosis. The amniotic fluid contains desquamated cells from fetal skin, bladder, and the gastrointestinal tract, which serve as sources for cytogenetic and enzymatic/biochemical studies. DNA can also be extracted from these cultured amniocytes for genomic studies. Proteins such as alpha fetoprotein (AFP) and acetylcholinesterase are measured to confirm an NTD suspected on ultrasound.

The benefits of second trimester amniocentesis include its large international clinical experience of over 40 years, the standardization of culture and cytogenomic techniques, which decrease the culture failure rate to 0.1%, and its diagnostic accuracy, broad availability, and relative safety (Winsor et al., 1999). Based predominantly on data obtained in the 1980s and 1990s, when second trimester amniocentesis was widely performed, the incidence for minor complications such as cramping and leakage of fluid immediately after the procedure was collectively about 1%, while the incidence of significant complications such as chorioamnionitis and/or miscarriage was 0.25%–0.5% (Centers for Disease Control and Prevention, 1995).

The only large randomized study of complications associated with second trimester amniocentesis, completed on 4606 low-risk women (aged 25–34 years), observed a pregnancy loss rate of 1.7% in the study group after amniocentesis compared with 0.7% in the control group; in this study however, an 18-gauge needle was used (Tabor et al., 1986). The procedural-related risk was revisited through the multicenter First Trimester and Second Trimester Evaluation Risk (FASTER) trial in 2004, which observed a loss rate after second trimester amniocentesis (and before 24 weeks' gestation) of 0.06% or 1/1600 (Eddleman et al., 2006). The earlier and higher reported fetal loss rate likely reflects the nuances that contribute to the safety of any procedure that requires technical expertise: the 16-year period between 1990 and 2006 was a time when noninvasive screening with cell-free DNA (cfDNA) was not available, and prenatal diagnosis for trisomy 21 and other fetal anomalies required invasive procedures. In the United States, maternal serum screening for DS with a screen positive rate of 5% was universally recommended and broadly adopted. Ultrasound guidance, use of a 22-gauge needle, and a large volume of patients at a referral institution allowed practitioners to maintain their technical skills. Each institution should calculate its own complication rate. In 2008, Odibo and colleagues reported on a single center's 16-year experience and identified a fetal loss rate of 0.13% or 1/769 (Odibo et al., 2008). A 2015 metaanalysis of miscarriage after amniocentesis in more than 42,000 women who had the procedure—compared with 138,000 who did not—estimated the procedural loss rate to be approximately 0.11% or 1/900 (Akolekar et al., 2015). These data support that midtrimester amniocentesis is safe when the procedure is performed by experienced providers in large volume referral centers. Thus the current estimated procedural risk discussed with patients is approximately 0.1%–0.3 %.

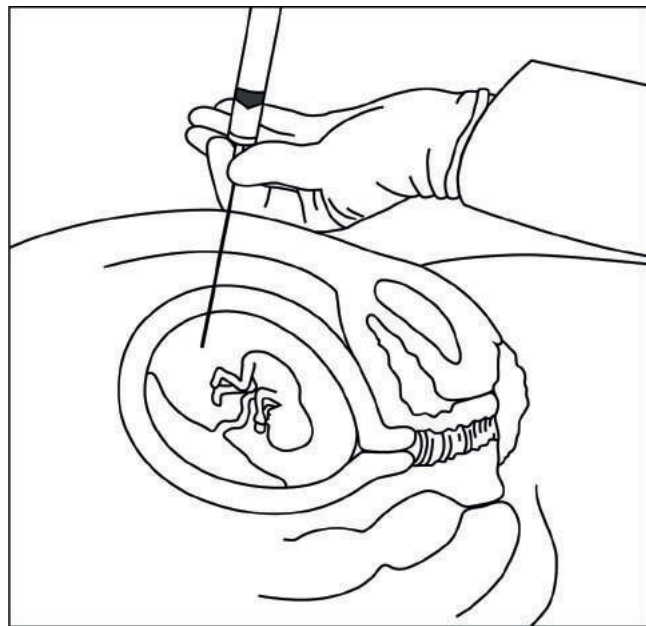
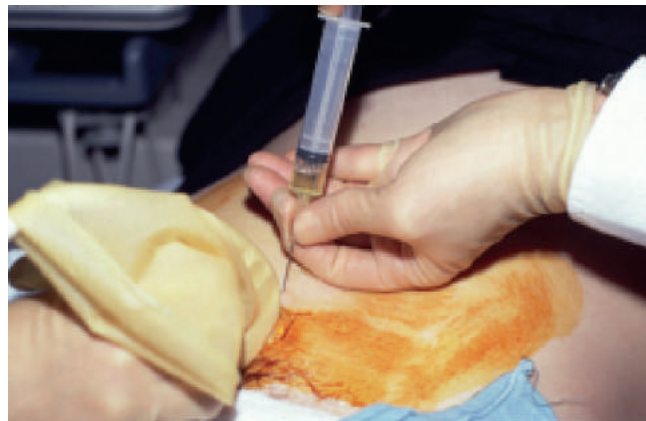
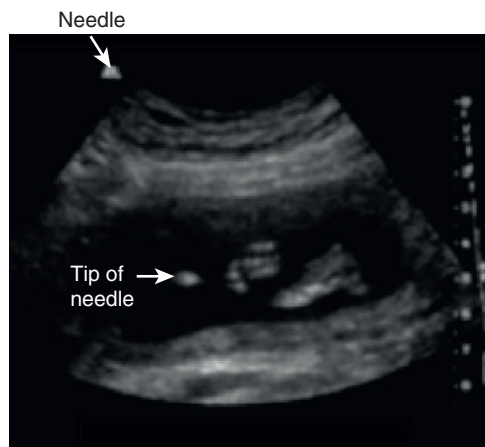
Early amniocentesis, performed between 11 and 14 weeks' gestation, was briefly explored in the late 1990s. The only large prospective study, the Canadian Early and Mid-Trimester Amniocentesis Trial, randomized 4334 women to early amniocentesis versus midtrimester amniocentesis and observed a higher pregnancy loss rate, more rupture of membranes, more culture failures, and greater procedural difficulty in the early amniocentesis group (The Canadian Early and Mid-Trimester Amniocentesis Trial [CEMAT] Group,

1998; Johnson et al., 1999). An unanticipated observation was a 1.3% incidence of club feet when early amniocentesis was performed between 11 and 13 weeks' gestation, compared with 0.1% after midtrimester amniocentesis.

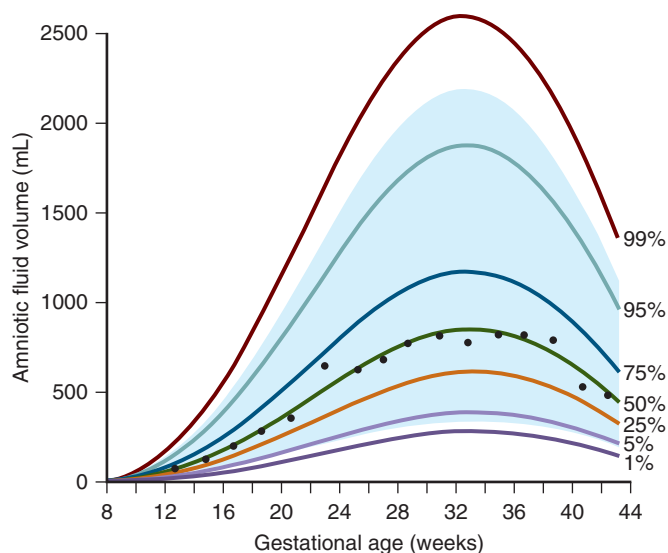
The timing of second trimester amniocentesis is based on a number of factors. The uterus prior to 15 to 16 weeks of gestation is still within the maternal pelvis and not easily accessible (Fig. 18.1). At midtrimester the volume of amniotic fluid is about 150–300 mL, with the fetal kidneys beginning to produce urine (Fig. 18.2). There is uncertainty about the rate of fetal urine production in early pregnancy, but at 25 weeks' gestation, the fetal urine output appears to be about 110 mL/kg per 24 hours. For fetal karyotyping and genomic studies, approximately 20–40 cc of amniotic fluid is necessary to obtain an adequate number of amniocytes for culture in order to provide results with appropriate accuracy and confidence. While midtrimester amniocentesis has not been associated with permanent structural or functional consequences to the exposed fetuses, the association of early amniocentesis with a 10-fold increase in club feet demonstrates the importance of an adequate amount of amniotic fluid at this developmental stage for normal orthopedic development of the fetus. Midtrimester amniocentesis performed later, at 18 to 22 weeks' gestation, is technically easier, and there is less concern for removing 40 cc of amniotic fluid. However, cases requiring complex molecular testing may involve weeks of analysis, thus extending completion of testing late in pregnancy and potentially limiting management options.

Chorionic Villus Sampling

Chorionic villus sampling (CVS) involves the aspiration of the chorion frondosum either transabdominally or transcervically between 10 and 13 weeks' gestation (Fig. 18.3). Trophoblasts and mesenchymal core cells of the chorionic villi provide actively growing cells for karyotype and genomic analyses and biochemical/enzymatic studies. In contrast to second trimester amniocentesis, mosaicism (the finding of two or more cell lines with a different chromosome constitution—usually trisomy) occurs in about 1%–2% of cases (Goldberg and Wohlferd, 1997). Because the cell types represent both extraembryonic and embryonic tissue, resolution of a mosaic CVS result requires identification of the source of the chromosome abnormality by completing an amniocentesis and, in some cases, fetal blood sampling for karyotype. As a result of CVS, the developmental processes of *confined placental mosaicism*, *trisomic rescue*, and *uniparental disomy* were discovered (Kalousek and Vekemans, 1996). Depending on the placental cell lineage from which the chromosome abnormality was derived, confined placental mosaicism could result in (1) generalized mosaicism affecting both the placenta and fetus; (2) mosaicism in the placenta only and a diploid/chromosomally normal fetus; (3) chromosome abnormality confined to the placenta with a chromosomally normal fetus; or (4) chromosomally normal placenta and a mosaic fetus. Trisomic rescue refers to the process by which the zygote began as a trisomic conceptus and, through postzygotic loss of the extra chromosome, became diploid while the placenta remained mosaic or completely abnormal. The clinical consequence of trisomic rescue is chromosome dependent because some genes require the presence of both maternal and paternal copies to express a normal phenotype. In the case of chromosome 15, for example, if CVS demonstrated mosaicism for trisomy 15 and genetic amniocentesis demonstrated that the fetus was diploid for chromosome 15, studies to confirm biparental inheritance must be completed to predict a normal fetal



• **Fig. 18.1** Midtrimester Genetic Amniocentesis Under Ultrasound Guidance.



• **Fig. 18.2** Volume of Amniotic Fluid Across Fetal Gestation. (Adapted from Bruce RA, Wolf EF. Normal amniotic fluid volume changes throughout pregnancy. *Am J Obstet Gynecol.* 1989;161:382–388.)

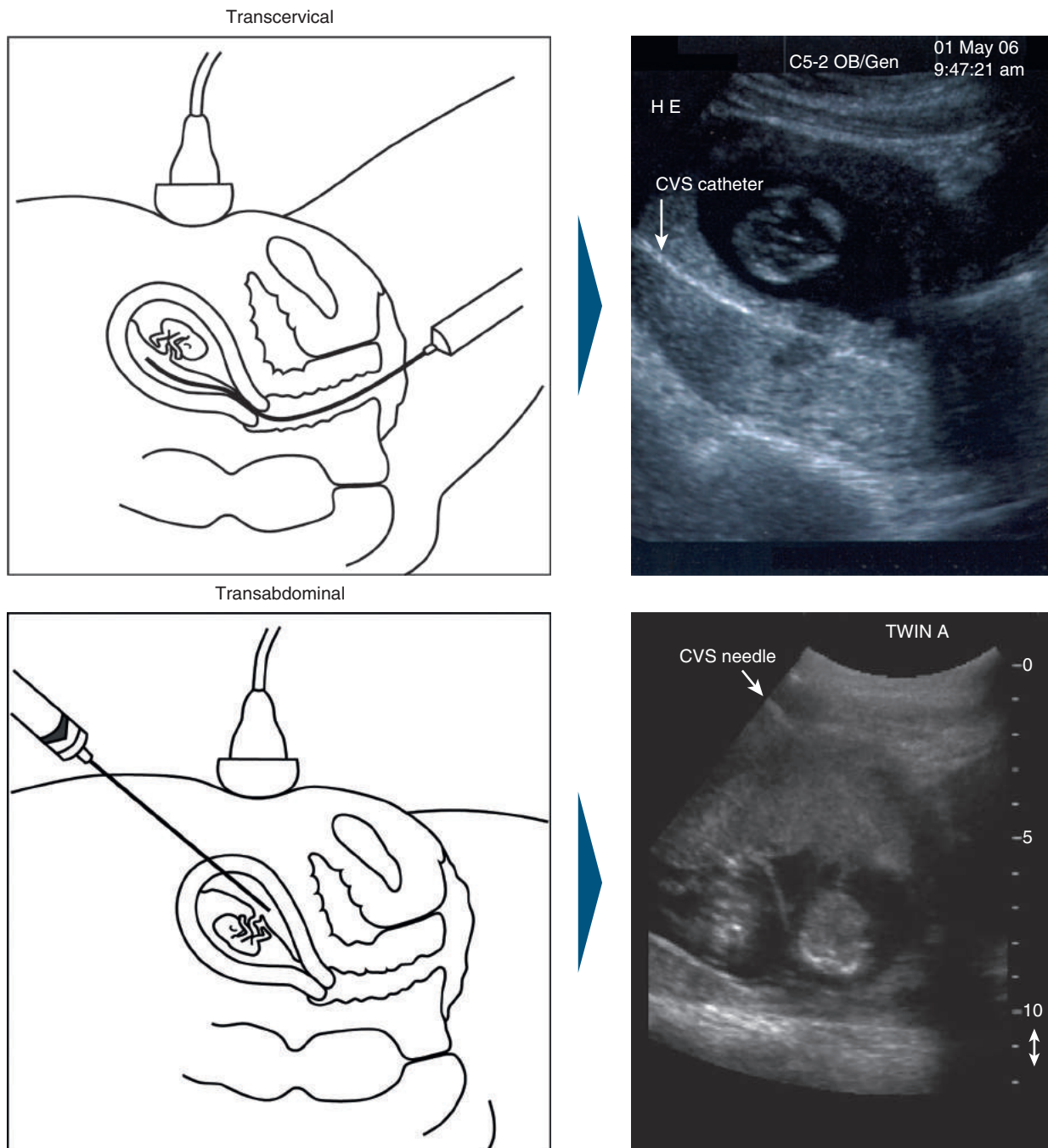
phenotype. However, if the fetus contained two maternal copies of chromosome 15, it would be predicted to have Prader–Willi syndrome or Angelman syndrome if it had two paternal copies of chromosome 15.

CVS is associated with a pregnancy loss rate of about 1/500 (Akolekar et al., 2015). It requires operator expertise and continuous ultrasound guidance. Maternal cell contamination studies are completed to discriminate between female fetal results and contamination from maternal cells. CVS allows for early diagnosis at a time when the privacy of the pregnancy can still be maintained and should be considered if complex diagnostic strategies (requiring time) are anticipated. Because CVS is dependent on operator expertise within a small gestational age window, it is not readily accessible to all patients.

Characteristics of midtrimester amniocentesis and CVS are compared in Table 18.1.

Percutaneous Umbilical Cord Blood Sampling

Fetal cordocentesis or percutaneous umbilical cord blood sampling (PUBS) was introduced in 1985 (Daffos et al., 1985). The ease of DNA-based testing for genetic conditions has essentially replaced



• **Fig. 18.3** Transcervical and Transabdominal Chorionic Villus Sampling Under Ultrasound Guidance. CVS, Chorionic villus sampling.

PUBS as a diagnostic tool in prenatal diagnosis. Today, the sampling of fetal blood is most commonly used for the diagnosis of fetal anemia or thrombocytopenia. As a therapeutic tool, it is used for in utero transfusion of blood or platelets and, rarely, for administration of antiarrhythmic medications for the treatment of fetal tachyarrhythmias. The procedure is completed under continuous ultrasound guidance with a 22-gauge spinal needle placed into the umbilical vein and can be performed beginning at approximately 18 weeks' gestation and subsequently throughout the remainder of the pregnancy. Before 18 weeks' gestation, the fetal umbilical vein may be too small although our group has completed a successful transfusion of a 16-week hydropic fetus due to Kell isoimmunization;

in this type of urgent situation, direct transfusion into the fetal heart is also possible. Exanguination (if the cord is lacerated), periumbilical vein hematoma in Wharton jelly, preterm rupture of membranes, preterm labor, or placental abruption are some of the complications of PUBS. The procedure requires operator expertise and the risk of the procedure is about 1%.

Genetic Testing of the Fetus

Fetal karyotyping still has a role in prenatal diagnosis and can be completed on chorionic villi, amniocytes, and fetal blood. Interphase fluorescent in situ hybridization (IFISH) with small

TABLE 18.1 Characteristics of Midtrimester Amniocentesis and Chorionic Villus Sampling for Prenatal Diagnosis

| | CVS | Amniocentesis |
|---|-------------|---------------|
| Gestational age at procedure | 10–13 weeks | 15–20 weeks |
| Miscarriage rate | 1/500 | 1/500–1/800 |
| Culture mosaicism | 1%–2% | 0.1% |
| Turnaround time | 7–10 days | 7–14 days |
| Diagnostic accuracy | >99% | >99% |
| IFISH | + | + |
| Karyotype | + | + |
| Microarray | + | + |
| Neural tube defect screening ^a | – | + |

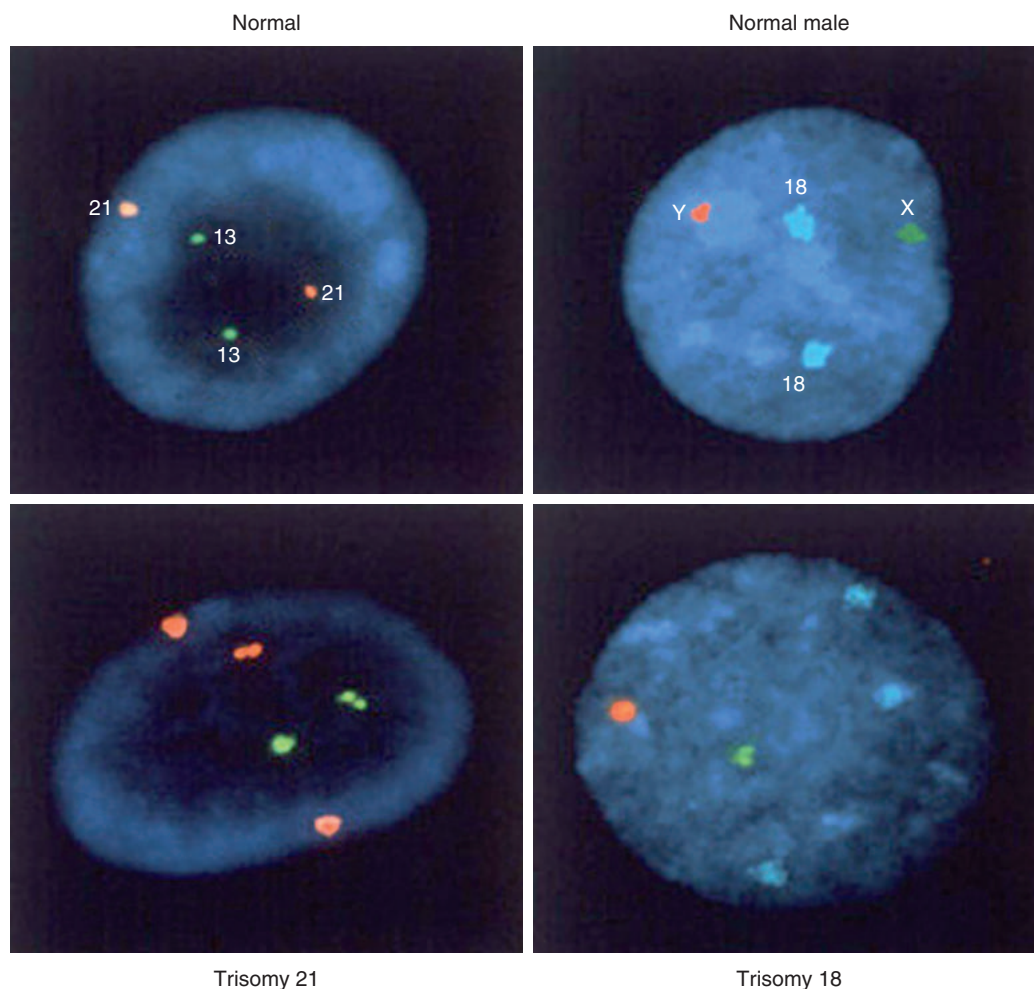
^aWomen who undergo CVS will need to have midtrimester maternal serum screening for maternal serum alpha fetoprotein.

CVS, Chorionic villus sampling; IFISH, interphase fluorescent in situ hybridization.

chromosome-specific DNA probes (10–300 kb) is used for rapid confirmation of suspected aneuploidy in uncultured amniocytes (Fig. 18.4). Historically, IFISH has been used for identification of extra chromosome material and specific submicroscopic gene deletions associated with genetic disorders. Today, this cytogenetic tool has been largely replaced by microarray analyses that expose genomic deficiencies and duplications that are smaller than those previously identified by IFISH. In cases in which a genetic condition is not associated with a known mutation, biochemical and enzymatic/functional studies of cultured amniocytes may be the only means for prenatal diagnosis for the condition in question.

Microarray Technology

Chromosomal microarray (CMA) technologies are bridges between cytogenetics and molecular genetics. They have greater resolution than traditional cytogenetics and are platforms designed to measure DNA regions for gains or losses across the entire genome simultaneously. The utility of microarray technology has been thoroughly evaluated in the postnatal and adult population and now replaces the standard banded karyotype that geneticists have relied on since the 1970s. CMA is now the first-line diagnostic test for individuals with autism spectrum disorder and unexplained birth defects and/



• **Fig. 18.4** Interphase Fluorescent In Situ Hybridization on Uncultured Amniocytes. The hybridization process takes approximately 24 hours after which the signals are counted for diagnosis.

or cognitive delay (Miller et al., 2010). Compared with standard karyotyping, CMA detects a causative genomic imbalance in an additional 10%–15% of these patients. In 2010, the American College of Medical Genetics issued practice guidelines for array-based technology in clinical medical genetics and supported CMA as the first-line test for the investigation of individuals with developmental delay and/or congenital anomalies (Manning and Hudgins, 2010).

In December 2013, The American College of Obstetricians and Gynecologists and the Society for Maternal–Fetal Medicine issued a joint committee opinion on the use of CMA analysis in prenatal diagnosis (American College of Obstetricians and Gynecologists Committee on Genetics, 2013; South et al., 2013). In a large multicentered trial supported by the Eunice Kennedy Shriver National Institute of Child Health and Human Development, CMA was found to perform just as well as standard karyotyping in the diagnosis of common aneuploidies in fetal samples (from CVS or amniocentesis) from women undergoing prenatal diagnosis (Wapner et al., 2012). In pregnancies with normal ultrasounds that had been referred for standard indications such as advanced maternal age or positive screening for DS, CMA yielded clinically relevant information in an additional 1.7% of pregnancies. However, in pregnancies with abnormal ultrasounds and a normal karyotype, CMA identified clinically significant genomic changes in an additional 6% of pregnancies, although it did not identify triploidy and balanced translocations. The added value of prenatal microarray analysis is further supported by DeWit and colleagues who, in a systematic review of the literature up to 2013, reported genomic imbalances in 3.1%–7.9% of fetuses with an isolated ultrasound abnormality and normal karyotype (DeWit et al., 2014). The value of microarray analysis is also seen in the genetic evaluation of stillbirths. Reddy and colleagues found that microarray analysis yielded more relevant genetic information than karyotyping in term stillbirths with or without anomalies, even after correcting for aneuploidy (Reddy et al., 2012).

Although there is no consensus regarding the type of array platforms used in prenatal diagnosis, *high-density single nucleotide polymorphism (SNP) arrays* are now becoming the platform of choice, as they allow for greater in-depth coverage of the genome to reveal smaller duplications and deficiencies. In addition, SNP arrays have the advantage of detecting long continuous stretches of DNA homozygosity (identical DNA sequences between a pair of chromosomes). These findings could reveal uniparental disomy, as seen in rare genetic conditions such as Angelman syndrome, as part of the work-up for confined placental mosaicism, or could uncover consanguinity.

Because the mechanisms causing genetic conditions are diverse and complex, no microarray platform will be completely diagnostic. Genetic conditions not associated with a relative change in DNA sequences will not be identified. Depending on the platform used, low level mosaicism and genomic regions not represented by the platform may not be detected. Tetraploidy may not be detected. Other mechanisms of genetic disease such as point mutations, methylation disorders, and mitochondrial disorders cannot be uncovered using microarray technology. In addition, as the resolution of the technology improves, secondary and incidental findings, both with and without known clinical implications, will arise as the genome is searched. Copy number variants not previously reported in established databases are referred to as *variations of unknown significance*. For these reasons, women should have pretest and posttest genetic counseling to discuss expectations and limitations of microarray testing in their pregnancy.

Exome Sequencing

Exome sequencing focuses on targeted sequencing of the protein coding regions of the genomic DNA and shows promise as a new tool in gene discovery for complex diseases and for facilitating the accurate diagnosis of individuals with unsolved Mendelian conditions. Yang and colleagues presented convincing data on 250 patients with suspected but undiagnosed genetic conditions in which exome sequencing was performed (Yang et al., 2013). Eighty-six mutated alleles were discovered and considered highly likely to be causative in 62 of the 250 patients, achieving a 25% molecular diagnostic success rate.

Application of exome sequencing in prenatal diagnosis is increasing as families and clinicians wish to uncover a genetic explanation for observed fetal anomalies. In a 2015 publication describing 24 fetuses with ultrasound abnormalities, exome sequencing provided a definitive genetic diagnosis in five fetuses (Drury et al., 2015). Currently, exome sequencing is used as a research tool and/or as a “second tier” test for phenotypes for which no diagnosis has been made using conventional microarray platforms.

Noninvasive Prenatal Screening

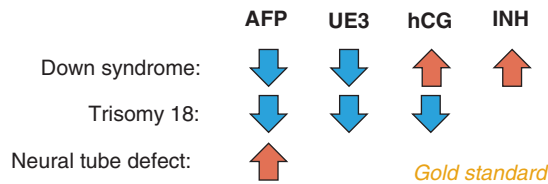
Maternal Serum Screening

Second trimester maternal serum screening for abnormal fetal conditions in the 1970s preceded ultrasound and focused on identifying fetal NTD through elevated MSAFP. An elevated MSAFP level of 2.5 multiples of the median (MoM) or greater generally signals a pregnancy that should undergo further evaluation. Because AFP is a circulating protein in the fetus, any disruption in the integrity of the fetal body, such as an open NTD, gastroschisis, or omphalocele and some dermatologic disorders, will be associated with elevations of AFP in maternal serum and amniotic fluid. Other considerations for elevated MSAFP are incorrect dating of the pregnancy, multiple gestation, impending fetal demise, and history of vaginal bleeding.

In 1984, screening for DS and trisomy 18 because of low MSAFP was added (Merkatz et al., 1984). Other analytes, human chorionic gonadotropin (hCG), unconjugated estriol (UE3), and inhibin-A (INH), were subsequently added to improve the detection rate (DR) for DS (Fig. 18.5). The patterns of serum analyte levels for NTD, DS, and trisomy 18 are illustrated in Fig. 18.6. Trisomy 18 has a unique pattern in that all of the analytes are low; however, INH is not measured because it does not increase the discriminatory value of this pattern for trisomy 18. Analyte levels are reported in MoM to standardize the gestational age dependency of the interpretation of the levels. Likelihood ratios (LRs) are calculated for each analyte based on a comparison of the proportion of affected and unaffected pregnancies with the same MoM value. The final risk or adjusted risk (AR) is then a product of the patient's background risk ($BR = \text{maternal age-related risk}$) and the four LRs generated by the four second trimester analytes (MSAFP, hCG, UE3, INH) ($AR = BR \times LR1 \times LR2 \times LR3 \times LR4$). For example, a 30-year-old woman has an a priori risk of 1/900 for DS at term. If she completed a second trimester of the four serum analytes (also known as the Quad Screen), and the LRs for MSAFP, UE3, hCG, and INH were 1.7, 10.5, 2.0, and 1.0, respectively, then her AR for DS at term would be 1/25, similar to someone who is 45 years or older. A DS risk of 1/270 at midtrimester or greater is considered a positive screen, and additional testing should be offered.

- 1970's:** High AFP for NTD
1980's: Maternal age for DS
1984: Low MSAFP + maternal age
1988: Triple screen
1990: **1st trimester screening**
 — Nuchal thickness (NT)
 — PAPP-A
 — β -hCG
1992: **Quad screen** (Inhibin A)
1999: **Integrated screen**
 — 1st/2nd trimester

• **Fig. 18.5** Evolution of Maternal Serum Screening for Neural Tube Defect and Down Syndrome. *AFP*, Alpha fetoprotein; β -*hCG*, β -human chorionic gonadotrophin; *DS*, Down syndrome; *MSAFP*, maternal serum alpha fetoprotein; *NT*, nuchal thickness; *NTD*, neural tube defect; *PAPP-A*, pregnancy-associated plasma protein-A.



• **Fig. 18.6** Serum Analyte Patterns for Down Syndrome, Trisomy 18, and Neural Tube Defect. Dimeric inhibin-A is not interpreted for trisomy 18 because of the unique analyte pattern for trisomy 18 and does not increase the detection rate for trisomy 18. *AFP*, Alpha fetoprotein; *hCG*, human chorionic gonadotrophin; *INH*, inhibin-A; *UE3*, unconjugated estriol.

In the first trimester, three additional markers, fetal nuchal thickness (NT) measured by ultrasound, maternal serum pregnancy-associated plasma protein-A (PAPP-A), and *hCG* are used to calculate LRs to adjust the patient's risk. The combination of all the components of the first and second trimester screening elements, known as the Integrated Screen, provides the highest DR for DS when a 5% screen positive rate is used (Malone et al., 2005). However, many options for screening are available depending on gestational age at entry to prenatal care, access to first trimester ultrasound for NT evaluation, and patient desire for information at different stages of her pregnancy (American College of Obstetricians and Gynecologists, 2007). Components of the screening options for DS are outlined in Table 18.2.

Prenatal Fetal Imaging

First trimester NT or thickness refers to the measurement of the normal subcutaneous fluid-filled space at the back of the fetal neck between 10 and 14 weeks' gestation. In normal fetuses, the maximal thickness increases with increasing gestational age as defined by the crown rump length (CRL). An increased NT is associated with DS and is now used in conjunction with maternal age and first and second trimester maternal serum analytes to provide the highest DR for DS (Malone et al., 2005). An LR is generated for NT measurement based on CRL and incorporated into the AR calculation. Other chromosome abnormalities, fetal anomalies, and poor pregnancy outcomes are also observed with increasing NT (Souka et al., 2005) (Fig. 18.7).

NT measurement is obtained by transabdominal imaging of the pregnancy, and its success as a screening tool depends on the accurate procurement of images for measurement, which is

TABLE 18.2 Screening Options for Down Syndrome and Their Detection Rates

| Screening Test | Detection Rate (%) |
|---|--------------------|
| First Trimester^a | |
| • NT measurement alone | 64–70 |
| • NT, PAPP-A, <i>hCG</i> | 82–87 |
| Second Trimester^b (Serum) | |
| • Triple screen (<i>AFP</i> , <i>hCG</i> , <i>UE3</i>) ^c | 69 |
| • Quad screen (<i>AFP</i> , <i>hCG</i> , <i>UE3</i> , inhibin-A) | 81 |
| Integrated Screening Options | |
| • First and second trimester serum only | 85–88 |
| • NT + first and second trimester serum | 94–96 |
| Other Options | |
| • Stepwise sequential screen | 95 |
| If first trimester positive screen, offer diagnostic test | |
| If first trimester negative screen, continue as Integrated Screen | |
| • Contingent/first trimester only | 88–94 |
| If first trimester positive, offer diagnostic test | |
| If first trimester negative, no further screening | |

^aFirst trimester = 11–14 weeks' gestation.

^bSecond trimester = 15–22 weeks' gestation.

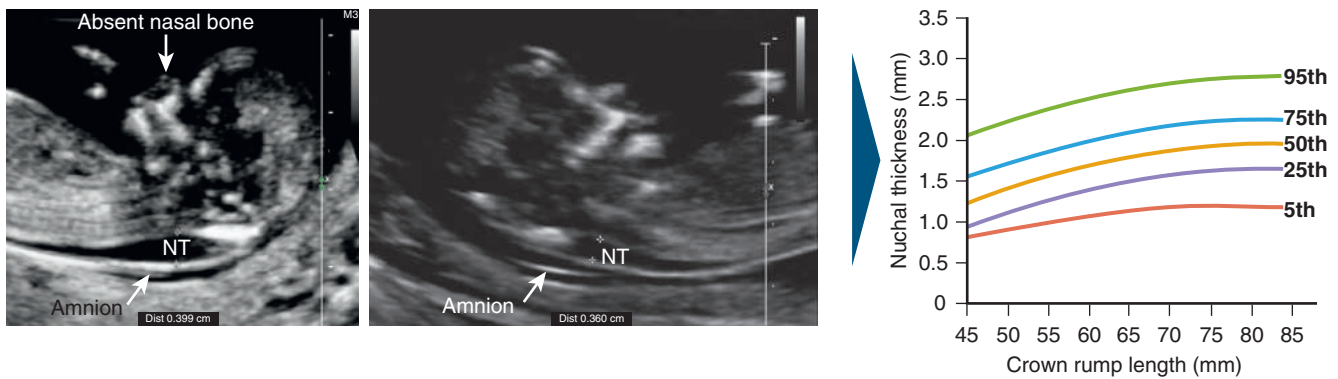
^cNo longer standard of care in United States.

AFP, Alpha fetoprotein; *hCG*, human chorionic gonadotrophin; *NT*, nuchal thickness; *PAPP-A*, pregnancy-associated plasma protein-A; *UE3*, unconjugated estriol.

dependent on the size and position of the fetus, maternal habitus, and operator performance. Operator performance is audited for quality following formal training and certification of competency. The FASTER trial and the Fetal Medicine Foundation study observed the highest success rates in obtaining NT measurements at the first patient visit when the CRL was between 45 and 84 mm, corresponding to 11 weeks 3 days and 14 weeks 2 days of gestation respectively (Malone et al., 2004). The additional benefit of completing the ultrasound in this gestational age window is the opportunity to confirm pregnancy viability and identify other fetal anomalies such as anencephaly, abdominal wall defects, and possibly congenital heart defects.

Other ultrasound findings in the first trimester fetus that may be associated with DS include *absent* or *hypoplastic nasal bone*, *abnormal ductus venosus blood flow*, and *tricuspid regurgitation*. Initially thought promising, the role of absent or hypoplastic nasal bone as an independent marker for DS in a general low-risk population screening program is unclear, because of ethnic variation in nasal bone size and the difficulty in obtaining acceptable images (Malone et al., 2004). However, the nasal bone is absent in 70% of DS fetuses, 55% of trisomy 18 fetuses, and 35% of trisomy 13 fetuses (Cicero et al., 2001). Both abnormal flow of the ductus venosus and tricuspid regurgitation in the first trimester have been associated with fetuses at high risk for chromosome abnormalities (Matias et al., 1998a; Matias et al., 1998b; Huggon et al., 2003). Because these studies were completed by experienced sonographers and were performed on high-risk fetuses only, this information may not be applicable to a general low-risk population.

The scope of information regarding *second trimester ultrasonography for aneuploidy screening and diagnosis of fetal anomalies* is too



| Nuchal thickness | Chromosomal defects | Normal karyotype | | |
|------------------|---------------------|------------------|---------------------------|----------------|
| | | Fetal death | Major fetal abnormalities | Alive and well |
| <95th %ile | 0.2% | 1.3% | 1.0% | 97% |
| 95th–99th %iles | 3.7% | 1.3% | 2.5% | 93% |
| 3.5–4.4 mm | 21.1% | 2.7% | 10.0% | 70% |
| 4.5–5.4 mm | 33.3% | 3.4% | 18.5% | 50% |
| 5.5–6.4 mm | 50.5% | 10.1% | 24.2% | 30% |
| >6.5 mm | 64.5% | 19.0% | 46.2% | 15% |

• **Fig. 18.7** Interpretation of Nuchal Thickness and Relationship to Gestational Age and Risk for Fetal Outcomes. NT, nuchal thickness. (Adapted from Souka AP, Von Kaisenberg CS, Hyett JA, Sonek JD, Nicolaides KH. Increased nuchal thickness with normal karyotype. *Am J Obstet Gynecol.* 2005;192:1005–1021.)

broad and complex to cover in this chapter. In the United States, prenatal diagnosis units follow the practice guidelines developed in conjunction with the American College of Radiology, the American College of Obstetricians and Gynecologists, and the Society of Radiologists in Ultrasound (*American Institute of Ultrasound in Medicine*, 2013). The following discussion summarizes some key concepts in prenatal diagnosis and second trimester imaging of the fetus.

In addition to an increased first trimester NT, DS fetuses can exhibit other abnormalities in the second trimester that would adjust the patient's a priori risk. [Table 18.3](#) provides an overview of the common ultrasound markers and their LR's (*American College of Obstetricians and Gynecologists*, 2016). Note that some of these markers signal other pathologic processes. Therefore it is important to consider the entire breadth of diagnoses for some of these markers. Calculation of DS AR based on markers can be performed at <http://perinatology.com/calculators2.htm> (Age Adjusted Ultrasound Risk Assessment).

Accurate identification of fetal NTD can be readily accomplished in the second trimester ultrasound. This is especially important for maximizing management options for the pregnancy in light of the availability of in utero fetal surgery for repair of the defect (*Adzick et al.*, 2011). The “lemon” sign representing scalloping of the frontal bones and the “banana” sign representing obliteration of the cisterna magna with distortion of the cerebellum due to herniation of the hindbrain (Chiari malformation) are the two most sensitive and specific findings for an open NTD. Observation of these cranial defects should initiate a detailed survey of the fetal spine. Mild ventriculomegaly in the second trimester is variably observed in only 70% of cases. The location and extent of the defect are important for

TABLE 18.3 Ultrasound Markers Associated With Aneuploidy and Down Syndrome

| Marker | Likelihood Ratio |
|---|---------------------------------|
| Cystic hygroma (first trimester) | >50% aneuploid, Turner syndrome |
| Isolated echogenic intracardiac focus (second trimester) | 1.4–1.8 for DS |
| Renal pelviectasis ≥ 4 mm (20 weeks' gestation) | 1.5–1.6 for DS |
| Echogenic bowel ^a | 5.5–6.7 for DS |
| Ventriculomegaly (10–15 mm) | 25 for DS |
| Isolated choroid plexus cyst(s) (≤ 20 weeks' gestation) | No association |
| Short femur length (< 2.5 %ile for gestational age) | 1.1–2.2 for DS |

^aGrade 3 = density same as bone.
DS, Down syndrome.

anticipation of postnatal bowel and bladder function, ambulation, neurocognitive development, and consideration for in utero fetal surgery. Amniocentesis is recommended to confirm an open defect by demonstrating elevated amniotic fluid AFP levels and presence of acetylcholinesterase and to exclude fetal genomic abnormalities, which are found in 10% of fetuses with an open NTD (*Sepulveda et al.*, 2004). Eligibility for fetal surgery includes a chromosomally normal fetus diagnosed between 19 weeks 0 days and 25 weeks 6

days with an isolated myelomeningocele between T1 and S1, with evidence of hindbrain herniation in a nonobese (body mass index <35) healthy woman with no history or risk factor for preterm delivery. Among children who underwent in utero surgery for repair of spina bifida between 19 and 25 weeks' gestation under the Management of Myelomeningocele Study (MOMs trial), there was a 50% reduction in the need for a ventricular shunt, less Chiari malformation, and improved motor skills, with twice as many children walking independently at 30 months compared with the postnatal surgery group (Adzick et al., 2011).

First and second trimester ultrasounds readily diagnose *anencephaly*, *fetal gastroschisis*, and *fetal omphalocele*. The gestational age at which nuchal thickness (NT) screening is performed is especially efficient in excluding these conditions. Early diagnosis of fetal anencephaly maximizes management options and decreases maternal complications. Large gastroschises are readily suspected during the evaluation of the NT. Fetal omphaloceles may be ambiguous in the first trimester because of the physiologic herniation of the midgut into the base of the umbilical cord before 12 weeks' gestation. After 12 weeks, the gut returns to the abdominal cavity; persistence of a midline abdominal wall sacular defect with the umbilical cord arising from the apex of the sac and abdominal contents in the sac is diagnostic of an omphalocele. In contrast, gastroschisis is typically paraumbilical to the right of the cord insertion with free floating eviscerated bowel in the amniotic fluid. It is important to differentiate between these two anatomic defects; gastroschisis is generally isolated and rarely associated with fetal chromosome abnormalities. However, this condition is associated with a high rate of intrauterine growth restriction, oligohydramnios, and intrauterine fetal demise; prenatal care pathways for surveillance may be helpful in optimizing the fetus for postnatal interventions. In contrast, omphaloceles are strongly associated with other pathologic processes including other structural anomalies and genomic or syndromic conditions. Amniocentesis is recommended to exclude an underlying genomic imbalance that could impact postnatal interventions and prognosis. Fetuses with gastroschisis tolerate labor and vaginal delivery well, while fetuses with large omphaloceles are delivered by cesarean section because of the risk of rupture during vaginal delivery.

Prenatal skeletal dysplasia should be suspected when the long bone measurements are at or less than the 5th percentile or more than three standard deviations below the mean for gestational age (Krakow et al., 2009). Concurrent abnormalities such as fractures (osteogenesis imperfecta types II–IV), shape of the skull (thanatophoric dwarf), or decreased mineralization (hypophosphatasia) may direct the diagnosis. However, the diagnosis of most nonlethal congenital skeletal dysplasias will not be made until after birth or even into adulthood. Thus prenatal diagnosis of skeletal dysplasias centers around the probability of lethal pulmonary hypoplasia, which directs the prenatal and postnatal management of the pregnancy.

To date, two-dimensional ultrasound remains the standard imaging modality for prenatal screening and diagnosis. Three-dimensional ultrasound is helpful in visualizing any external structural abnormalities such as cleft lip/palate and spina bifida. The use of fetal magnetic resonance imaging is increasing and is most helpful in evaluating fetal intracranial abnormalities suspected on ultrasound.

Cell-Free DNA (Noninvasive Prenatal Testing)

cfDNA analysis in maternal plasma is used in noninvasive prenatal testing (NIPT) with greatest focus on prenatal detection of

aneuploidy for chromosomes 13, 18, 21, and X. In pregnancy, approximately 3%–20% of the total cfDNA in maternal plasma is derived from the pregnancy (Lo et al., 1997; Lun and Chiu, 2008). Although many refer to this as “free fetal DNA,” the DNA is in fact derived *primarily from the placenta*, which serves as a surrogate for the fetus and is cleared from the maternal circulation within hours after delivery (Lo et al., 1997). The molecular principle behind NIPT is the measurement of relative amounts of circulating free placental DNA compared with the amount of circulating free maternal DNA. In other words, a pregnancy at 14 weeks affected with DS will show an excess of DNA fragments for chromosome 21, assuming that the mother does not herself have DS or is not mosaic for DS.

The positive predictive value (PPV) using cfDNA screening in women aged 40 years is 87% for DS, 68% for trisomy 18, and 57% for trisomy 13; for women aged 25 years, the PPV for DS was 33%, 13% for trisomy 18, and 9% for trisomy 13 (Meck et al., 2015). Wang and colleagues also evaluated the test performance of cfDNA screening in high-risk pregnancies and found a poor performance for sex chromosome aneuploidy, with a PPV of only 39%, because the sex chromosome aneuploidy may reflect the mother rather than the fetus (Wang et al., 2015). False-positive and false-negative results may be related to fetal mosaicism, vanishing twin, confined placental mosaicism, unsuspected maternal chromosome abnormality, and unsuspected maternal malignancy (Amant et al., 2015). For these reasons, the American College of Obstetricians and Gynecologists and the Society for Maternal–Fetal Medicine strongly recommend that NIPT should “be an informed patient choice after pretest counseling and should not be part of routine prenatal laboratory assessment.” They further recommend that “a patient with a positive test result should be referred for genetic counseling and should be offered invasive prenatal diagnosis for confirmation of test results” (American College of Obstetricians and Gynecologists, 2015).

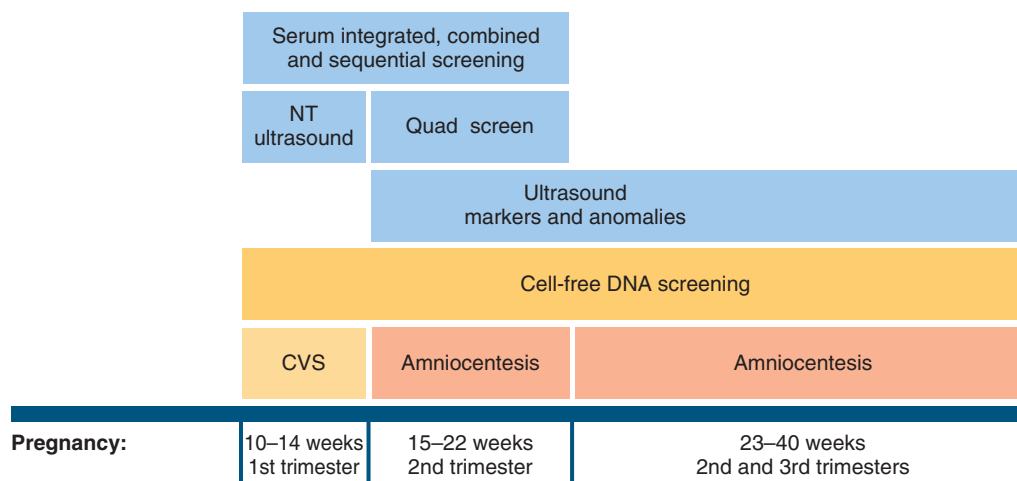
Other considerations in the performance of cfDNA screening include its dependency on gestational age and “fetal fraction.” Low fetal fraction (defined as less than 4% fetal DNA in maternal serum) is inversely associated with maternal body mass index (Table 18.4) (Wang et al., 2013; Norton et al., 2015). In a study of 222,384 pregnancies, Wang and colleagues identified that at 10 to 11 weeks' gestation, the median percentage of fetal DNA in maternal serum is about 10% and increases at a rate of 0.1% until

TABLE 18.4 Relationship Between Maternal Weight and Adequate Fetal Fraction (>4%) in Maternal Serum

| Maternal Weight (kg) | Adequate Fetal Fraction (%) ^a |
|----------------------|--|
| <70 | >99 |
| 71–80 | 99 |
| >90–100 | 96 |
| >100–110 | 95 |
| >140 | 71 |

^aAssuming ≥4% fetal fraction.

Adapted from Wang E, Batey A, Struble C, Musci T, Song K, Oliphant A. Gestational age and maternal weight effects on fetal cell-free DNA in maternal plasma. *Prenat Diagn*. 2013;33:6662–6666.



• **Fig. 18.8** Prenatal Screening and Testing Options During Pregnancy. NT, nuchal thickness; CVS, chorionic villus sampling.

21 weeks' gestation; after 21 weeks, the rate of increase is 2% per week (Wang et al., 2013). Two percent of pregnancies during the 10 to 21 gestational-week period had less than 4% "fetal" cfDNA (Wang et al., 2013). In a secondary analysis, of a large randomized comparison of cfDNA screening and traditional first trimester screening with NT measurement and maternal serum analytes in low-risk women, Norton and colleagues discovered that among women in whom there were no results on cfDNA testing, the prevalence of aneuploidy was 2.7% (1/38), which is significantly higher than expected (1/236, 0.4%) for this low-risk group of women (Norton et al., 2015). Specifically, for women with a "fetal" fraction less than 4%, 4.7% (9/192) had aneuploidy, although six of these cases were detected by standard screening. Nonetheless, the high prevalence of aneuploidy in noninformative cases (due to low "fetal" fraction) may reflect aberrant biology of the aneuploid placenta.

cfDNA screening currently offers reliable information about the three most common aneuploidies. However, in a recent review of 220 chromosome abnormalities identified prenatally between 2009 and 2014, cfDNA testing did not detect about half of the clinically significant chromosome abnormalities that were found either by karyotyping or microarray (Shani et al., 2016). More importantly, 79% of the abnormalities were from pregnancies with abnormal serum screening and/or abnormal ultrasound findings. These findings underscore the fact that current cfDNA technology is a screening tool and does not have the breadth and depth to offer the scope of genomic information about the fetus with multiple complex anomalies that could be obtained through CVS or genetic amniocentesis. Although some companies are offering testing for selected microdeletion syndromes, these tests have not been validated clinically and should not be chosen for diagnosis of a fetus at known risk.

In summary, noninvasive prenatal screening has expanded to cover the entire course of pregnancy, allowing directed diagnosis of several common chromosomal conditions without invasive testing (Fig. 18.8). Points to consider when counseling for NIPT are found in Box 18.1. The next frontier in genomic medicine and prenatal diagnosis is clinical application of noninvasive sequencing of the entire prenatal genome for prenatal diagnosis (Fan et al., 2012; Kitzman et al., 2012).

• BOX 18.1 Noninvasive Prenatal Testing Counseling: Key Points

- Fetal fraction is inversely associated with maternal body mass index resulting in noninformative results.
- Performance of test not established for multiple gestations.
- Tests only for abnormal DNA quantities associated with chromosomes 13, 18, 21, and sex chromosomes.
- Does not test for other genetic conditions or genomic imbalances.
- No clinical validation for common microdeletion syndromes.
- Does not replace CVS or amniocentesis for direct analysis of genomic abnormalities.
- Confirmatory tests should be completed following a positive NIPT result, especially if there are no ultrasound abnormalities.
- Offers noninvasive testing at late gestations.
- Does not screen for neural tube defects: ultrasound and MSAFP should still be offered between 15 and 20 weeks.
- Family history should be obtained to determine if NIPT is the appropriate test for the patient.
- If patient presents in the first trimester, consider obtaining ultrasound for NT before offering NIPT.
- Genetic counseling should be provided to all patients considering NIPT.

CVS, Chorionic villus sampling; MSAFP, maternal serum alpha fetoprotein; NIPT, noninvasive prenatal testing; NT, nuchal thickness.

Preimplantation Genetic Diagnosis/Screening

Preimplantation Genetics

Preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS) require in vitro fertilization (IVF) technology and can be considered a form of prenatal diagnosis. Knowledge of the genetic mechanisms and risks for fetal anomalies as a function of the IVF and PGD/PGS processes is important as these women undergo prenatal screening with ultrasound, maternal serum screening, or NIPT. For the neonatologist, this background information may influence, for example, the suspicion that a newborn may have Beckwith–Wiedemann syndrome, since this syndrome

is associated with pregnancies achieved through IVF technologies. Women of advanced maternal age who achieve pregnancies using their own eggs often undergo PGS of the embryos for aneuploidy. Confirmatory testing by CVS or amniocentesis is recommended, but few women elect to undergo an invasive procedure that risks a much desired pregnancy. NIPT offers these women an intermediate solution for “confirmation” of a euploid fetus. However, knowledge of the number of embryos transferred and concordance with the number of viable fetuses seen on ultrasound are important if NIPT is used; an embryonic demise of a transferred twin could result in discordant NIPT results. Unique to IVF pregnancies, regardless

of whether embryos have undergone PGD or PGS, is the concern for genetic conditions and or birth defects as a result of imprinting errors or other mechanisms that may disrupt the normal developmental processes of the early embryo. Maternal serum screening for aneuploidy is not as robust in twin gestations as it is in singleton pregnancies. NIPT has not been validated in multiple gestations and is currently not recommended ([American College of Obstetricians and Gynecologists, 2015](#)). Thus ultrasound in some cases becomes the most reliable prenatal screening test for multiple gestations. Definitive prenatal diagnosis of twins discordant for birth defects still requires CVS or amniocentesis.

Summary

This exciting period of discoveries and advancements in prenatal diagnosis offers women a broad choice of studies to investigate the health of the fetus. For couples at high risk for a known condition, the option of PGD/PGS or DNA-based prenatal diagnosis offers them hope and a chance for a healthy baby. For fetuses with life-threatening structural anomalies, such as hypoplastic left heart syndrome, prenatal diagnosis offers preparation for delivery at a high-risk center where neonatal support and subspecialty intervention

offer the neonate a chance for survival. In these efforts, we have educated our patients and ourselves, for in the last 40 years we have had a window to peek into the developmental processes of the human embryo through imaging and genomics. Now, we have the potential to understand the action of developmental genes over time in both normal and abnormal conditions, along the fetal–pediatric–adult continuum.

Suggested Readings

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- American College of Obstetricians and Gynecologists. Committee Opinion 640: cell-free DNA screening for fetal aneuploidy. *Obstet Gynecol.* 2015;126:e31-e37.
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19

The Dysmorphic Infant

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KEY POINTS

- A genetics evaluation should be considered for a patient in the setting of multiple anatomic anomalies, known maternal exposure to a teratogen, a history of familial disorders, increased carrier frequency or ethnic risk, or multiple pregnancy losses.
- The essential parts of a genetic evaluation include the medical history, family history, dysmorphism examination, literature review, and diagnostic testing.
- The best clues are the rarest and are generally the most helpful in establishing a differential diagnosis.
- The advancement of techniques utilized in genetic testing, such as chromosomal microarrays and next-generation sequencing, has greatly improved the ability to make a genetic diagnosis.
- In cases in which there is no clear diagnosis, prognosis and treatment should be determined according to the organ systems involved and the extent of their impairment.

Genetic disorders have a major impact on public health, as indicated by several large epidemiologic studies (Table 19.1) (Scriver et al., 1973; Hall et al., 1978; McCandless et al., 2004). Genetic factors may also contribute to more than two-thirds of the conditions prompting admission to a children's hospital (McCandless et al., 2004). Early identification of the genetic nature of a given condition may then help to appropriately focus resources for providing better care to these individuals. It is therefore critical to implement a systematic approach to evaluating a dysmorphic or malformed newborn. This chapter outlines such a general approach.

The clinical geneticist incorporates the following five essential tools in the evaluation of a child suspected of having a primary genetic disorder:

- History: prenatal, birth, and medical
- Pedigree analysis or family history
- Specialized clinical evaluation that includes a detailed dysmorphism examination
- Comprehensive literature search
- Genetic laboratory analyses (e.g., karyotype, chromosomal microarray, sequencing)

History

Prenatal

A complete gestational history should be generated, including results of prenatal testing such as maternal serum and noninvasive

prenatal screening, ultrasonography, and diagnostic genetic testing through chorionic villus sampling or amniocentesis (Box 19.1). The maternal age at conception should be documented, because the risk of chromosomal anomalies such as nondisjunction rises with maternal age. It is important to identify prenatal exposures to infection and medications, maternal habits such as alcohol and drug use, maternal chronic illnesses such as maternal diabetes, and pregnancy-related complications. An additional significant historical component involves the presence of abnormal levels of amniotic fluid. Oligohydramnios (decreased amniotic fluid volume) can be associated with either a fluid leak or a genitourinary abnormality, whereas polyhydramnios (excess amniotic fluid volume) can be seen in fetuses with neuromuscular disease or gastrointestinal malformations.

It is also important to identify exposure to environmental agents that might act as teratogens. Teratogens are environmental agents that may cause structural and functional diseases in an exposed fetus. Each teratogen may have a characteristic expression pattern, with a specific range of associated structural anomalies and dysmorphic features. Specific effects and the extent of those effects depend on the time of exposure, duration, and dosage as well as interactions with maternal and genetic susceptibility factors. In general, more severe effects are typically correlated with exposure early in the pregnancy and with more extensive (i.e., higher dose) exposure. The list of well-documented human teratogens is short and includes such substances as alcohol, thalidomide, warfarin, trimethadione, valproate, isotretinoin, and hydantoin. If history of an exposure is documented, an effort should be made to identify the developmental time and level of exposure. This information is critical, because the counseling and calculation of recurrence risk for a given malformation are vastly different if environmental exposures are involved.

Birth

Another important component of the gestational history is obtaining information on fetal activity, size, and position. Often the mother's subjective impressions can be further confirmed by examining obstetric records of the perinatal period. A history of hypotonia may be further supplemented by reports of poor fetal movements and breech presentation. Perinatal information including gestational age, fetal position at delivery, the length of labor, type of delivery, and any evidence of fetal distress, such as passage of meconium, are all relevant data (Box 19.2). Apgar scores, the need for resuscitation, birth parameters (weight, length, and head circumference), any malformations seen at birth, and all abnormal test results should be noted.

• BOX 19.1 Elements of Prenatal History for the Dysmorphic Newborn

Maternal Health

Age

Disease: diabetes, hypertension, seizure disorder

Mode of Conception

Natural

Assisted reproductive technologies

Fertility medications

In vitro fertilization

Intracytoplasmic sperm injection

Gamete intrafallopian transfer

Artificial insemination

Exposures

Medications

Alcohol

Environmental agents

Infections (gestational age at exposure)

Prenatal Testing

Ultrasonography (gestational age performed)

Maternal serum screening

Chorionic villus sampling, amniocentesis, noninvasive prenatal screening and indications

• BOX 19.2 Elements of Perinatal and Birth History for the Dysmorphic Newborn

- Fetal activity
- Delivery
- Type (e.g., indication for cesarean section)
- Gestational age
- Fetal presentation
- Apgar scores, history of distress, or resuscitation
- Growth parameters
- Malformations noted

• BOX 19.3 Elements of Pedigree Analysis and Family History for the Dysmorphic Newborn

Identification of relatives with:

- Congenital anomalies (especially those similar to probed)
- Mental retardation

Photographs (objective evidence)

Parental reproductive history

- Pregnancy losses (gestational ages)
- Infertility

Medical histories of primary relatives

Ethnic origin

Consanguinity

TABLE 19.1 Genetic Disorders in Pediatric Hospital Admissions

| Genetic Disorders | Montreal (1973) | Seattle (1978) | Cleveland (2004) |
|-----------------------------|-----------------|----------------|------------------|
| Chromosome, single gene (%) | 7.3 | 4.5 | 11 |
| Polygenic (%) | 29 | 49 | 60 |
| Nongenetic disorders (%) | 64 | 47 | 29 |
| Total number of admissions | 12,801 | 4115 | 5747 |

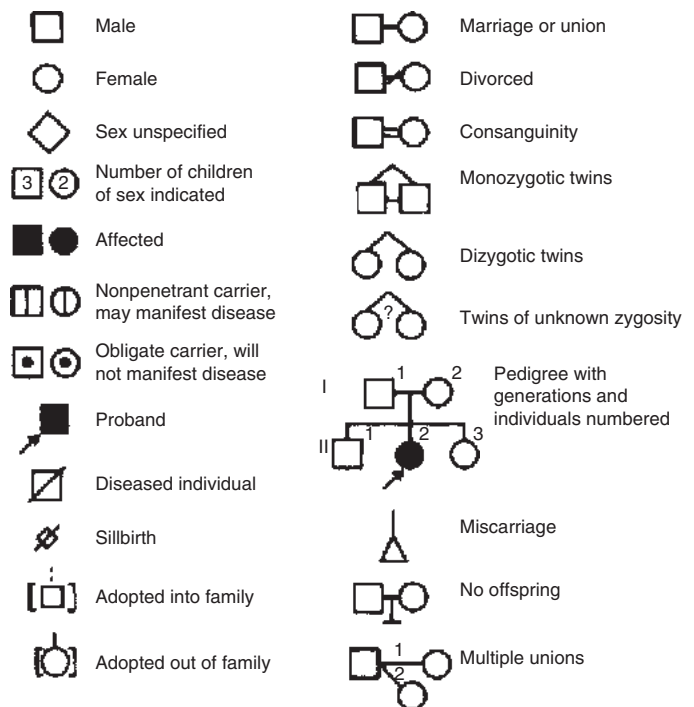
Data from Hall JG, Powers EK, McIlvane RT, Ean VH. The frequency and financial burden of genetic disease in a pediatric hospital. *Am J Med Genet.* 1978;1:417–436; McCandless SE, Brunger JW, Cassidy SB. The burden of genetic disease on inpatient care in a children's hospital. *Am J Hum Genet.* 2004;74:121–127; and Scriver CR, Neal JL, Saginur R, Clow A. The frequency of genetic disease and congenital malformation among patients in a pediatric hospital. *Can Med Assoc J.* 1973;108:1111–1115.

Medical

A full review of the medical issues of the child should include the baby's general health, test results, identification of any chronic medical issues, and need for hospitalization. Evaluation of growth, review of systems, developmental assessment, and notation of unusual behaviors can also provide important clues to a diagnosis.

Pedigree Analysis and Family History

A critical part of any genetic evaluation is the family history (Box 19.3); this is best accomplished by creating a three-generation pedigree, which is a schematic diagram depicting familial relationships using standard accepted symbols (Fig. 19.1). This formal



• **Fig. 19.1** Symbols commonly used for pedigree notation. (From Nussbaum RL, McInnes RR, Willard HF (eds). *Thompson and Thompson's Genetics in Medicine*, 7th ed. Philadelphia, PA: WB Saunders; 2007:117.)

record can also be used to summarize positive responses elicited during the interview. Special attention should be paid to ethnic origins of both sides of the family, consanguinity, and any first-degree relatives with similar malformations to those of the patient being evaluated, also known as the *index case*, *proband*, or *propositus*. An extended family history should be used to identify relatives with

congenital anomalies, developmental abnormalities, physical differences, or sudden death. Often photographs can provide clear objective evidence of a descriptive history.

Reproductive histories, especially of the parents, should be elicited. Specifically, questions should be asked about infertility, miscarriages, and stillbirths. The occurrence of more than two first-trimester miscarriages increases the probability of finding a balanced translocation in one parent (Campana et al., 1986; Castle and Bernstein, 1988). A balanced translocation is a rearrangement of genetic material such that two chromosomes have an equal exchange without loss or gain of material. There are typically no associated clinical features with such a rearrangement. However, when chromosomes align to recombine for meiosis in the sperm or egg, this exchange produces a risk of unequal distribution and an unbalanced translocation in the resulting fetus. In this case, there would be aneuploidy for part of a chromosome. It has been estimated that 25% of stillbirths exhibit single or multiple malformations, and in at least half of these cases there is a genetic etiology for the malformations. Couples with two or more pregnancy losses should undergo routine chromosome analysis or karyotyping. When possible, chromosome analysis such as a karyotype or microarray should be performed on the stillborn fetus or products of conception (ACOG Committee Opinion No. 581, 2013).

Obtaining a formal family history is helpful in discovering information that is often critical to making a diagnosis. Positive responses may help to discern a Mendelian pattern of inheritance for a given genetic disorder. For example, a disease affecting every generation, with both males and females involved, such as Marfan syndrome, displays an autosomal dominant pattern of inheritance. A pattern of X-linked recessive disease, such as hemophilia, instead shows affected males related through unaffected or minimally affected females; transmission in this pattern should not occur from father to son.

Physical Examination for Dysmorphology

A congenital malformation can be described as a “morphologic defect of an organ, part of an organ, or larger region of the body resulting from an intrinsically abnormal developmental process” (Jones et al., 2013). The term *dysmorphology* was introduced by Dr. David Smith in the 1960s to describe the study of human congenital malformations (Smith, 1966; Aase, 1990). This study of “abnormal form” emphasizes a focus on structural errors in development with an attempt to identify the underlying genetic etiology and pathogenesis of the disorder.

In a landmark study, Feingold and Bossert (1974) examined more than 2000 children to define normal values for a number of physical features. These standards were devised as screening tools to objectively identify children with differences possibly attributable to a genetic disorder. Important measurements include head circumference, inner and outer canthal distances, interpupillary distances, ear length, ear placement, internipple distances, chest circumference, and hand and foot lengths. Other graphs and measurements using age-appropriate standards can be found in compendia such as the *Handbook of Physical Measurements* (Gripp et al., 2013).

The assessment should begin with newborn growth parameters that can reflect the degree of any prenatal insult. Measurements such as height, weight (usually reflecting nutrition), and head circumference should be plotted on newborn graphs. Gestational age-appropriate graphs should be used for premature neonates. It is often helpful to express values that are outside the normal range

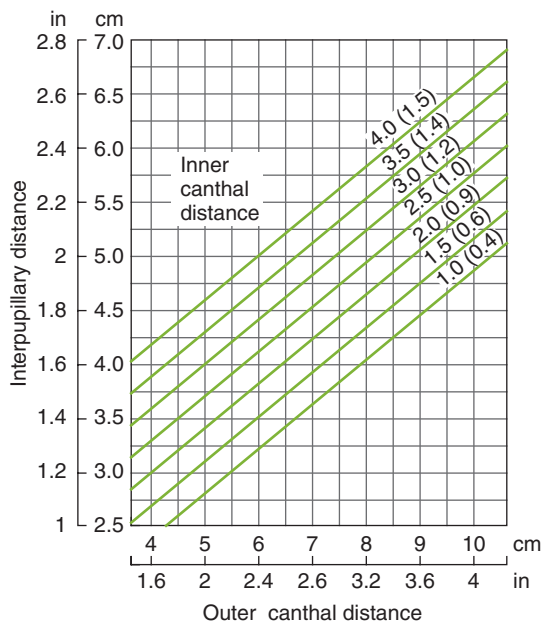
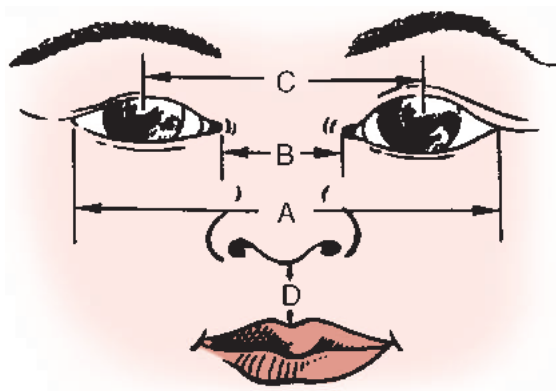
as 50th percentile for a different gestational age or in the number of standard deviations (SDs) above or below the mean. For example, a full-term baby with microcephaly may have a head circumference of less than the fifth percentile for 38 weeks. This degree of microcephaly could be stated more clearly if described as a specific number of SDs below the mean (e.g., -3 SD below the mean).

A complete physical examination should include assessment of patient anatomy for features varying from usual or normal standards. This assessment can often provide clues to embryologic mechanisms. The data obtained should then be interpreted using comprehensive standard tables that are available for these purposes. Special attention to familial variants should be given.

The shape and size of the head and fontanels should be noted as well as the cranial sutures, with assessment for evidence of craniosynostosis or an underlying brain malformation. Any scalp defects should also be documented. The shape of the forehead, appearance of the eyebrows (such as synophrys), and the texture and distribution of hair should be noted. The spacing of the eyes, or canthal measurements (Fig. 19.2), the interpupillary distance (Fig. 19.3; see also Fig. 19.2), palpebral fissure lengths (Fig. 19.4), presence or absence of colobomata and epicanthal folds, and determination of whether the palpebral fissures are turned upward or downward are components of the morphological examination of the eyes. Examination of the ears should include a search for preauricular and postauricular pits and tags, and assessment of the placement (Fig. 19.5), length (Fig. 19.6), and folding of the ear is important. Ear development occurs in a temporal frame similar to that of the kidneys during embryogenesis, and external ear anomalies can be associated with renal anomalies. Evaluation of the nose should cover the shape of nasal tip, the alae nasi, presence of anteverted nares, the length of the columella, and patency of the choanae. The mouth and throat are examined for the presence of a cleft lip or palate; the shape of the palate and uvula is noted, and the presence of unusual features, such as tongue deformities, lip pits, frenula, and natal teeth, are recorded. A small retrognathic or receding chin, which can be a part of several syndromes or an isolated finding, should be noted. The neck is inspected for excess nuchal folds or skin and for evidence of webbing. Any bony abnormalities in the neck should prompt an evaluation of the cervical vertebrae to confirm cervical and airway stability.

Evaluation of the chest and thorax involves lung auscultation and cardiac examination. Abnormal findings should prompt a consultation with a pediatric cardiologist and appropriate echocardiographic or invasive studies as needed. External measurements include determining the internipple distance and its ratio with respect to the chest circumference (Fig. 19.7). The abdominal examination is focused on determining whether organomegaly is present, a finding often associated with an inborn error of metabolism. The umbilicus should also be examined, with any hernias and the number of vessels present in the newborn cord being noted. A two-vessel cord, in which only a single artery is present, can be associated with renal anomalies. The genitourinary examination concentrates on determining whether anomalies such as hypospadias, chordee, cryptorchidism, micropallus, and ambiguous genitalia are present. These external anomalies may be associated with internal anomalies involving the upper urinary tract as well. The anus is examined for evidence of tags, its placement, and its patency.

The back should be assessed, especially for the shape of the spine and any associated defects, such as myelomeningocele. These defects prompt further radiologic evaluation to assess for potential functional limitations. In addition, a sacral dimple or hair tuft at

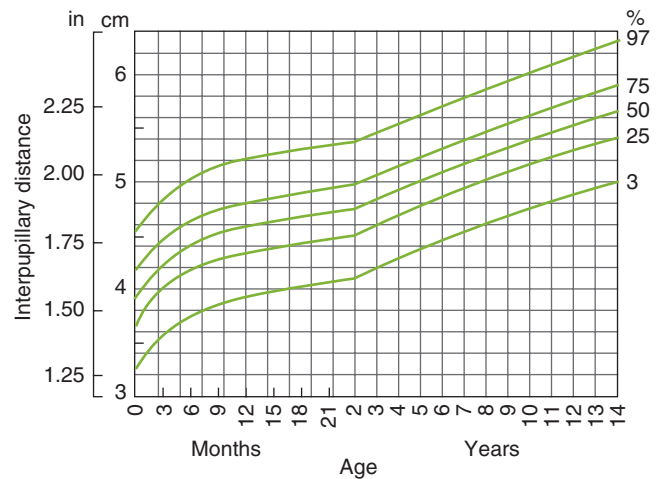


• **Fig. 19.2** Canthal Measurements. Various eye measurements are depicted (top). A indicates the outer canthal distance, B indicates the inner canthal distance, and C indicates the interpupillary distance (IPD), which is difficult to measure directly. The IPD can be determined using the graph at the bottom or with the Pryor formula: $IPD = (A - B) 2 + B$. (From Feingold M, Bossert WH. Normal values for selected physical parameters: an aid to syndrome delineation. *Birth Defects Orig Artic Ser.* 1974;10:1–16.)

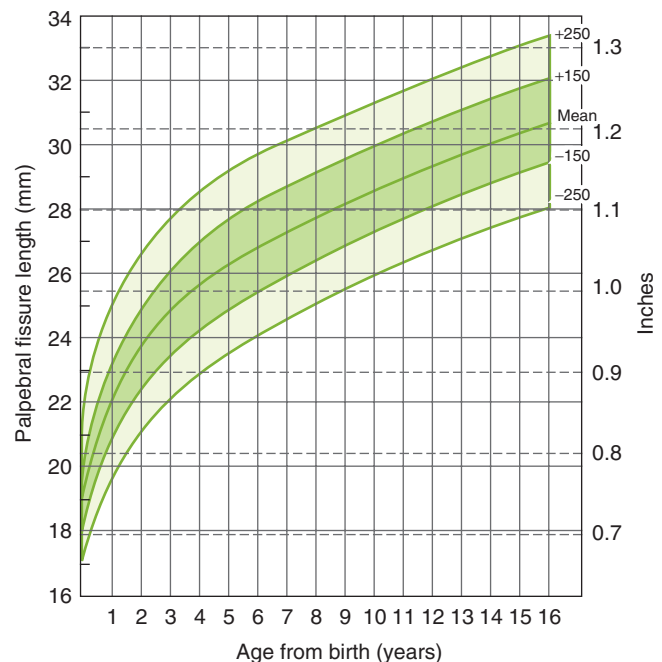
the base of the spine should be noted, because either could signify developmental abnormalities in the underlying neural tissue.

Minor anomalies are often manifested in the extremities. Gross differences in the hands and feet include polydactyly (more than five digits), and whether the extra digits are located in a preaxial (first digit) or postaxial (fifth digit) position should be noted. Syndactyly (fusion of the digits), clinodactyly (incurving of the digits), and hand and foot lengths, which should be expressed as a percentile measured on age-appropriate graphs (Figs. 19.8–19.9), are important to document. Often these data can provide important clues to a unifying syndrome.

Dermal ridge patterns, or dermatoglyphics, are formed on the palms and soles early in embryonic life, and they vary considerably among individuals. This variation can be inherited and can be influenced by disturbances to the development of the peripheral limb buds. Environmental exposures and chromosomal aberrations can greatly affect the formation of these structures and are reflected



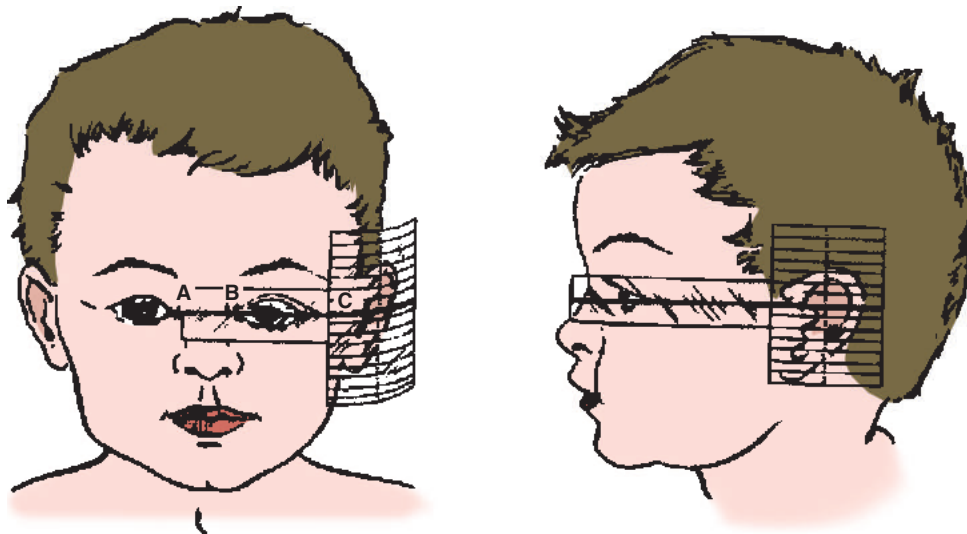
• **Fig. 19.3** A Nomogram for Interpupillary Distance at Different Ages for Both Sexes. (From Feingold M, Bossert WH. Normal values for selected physical parameters: an aid to syndrome delineation. *Birth Defects Orig Artic Ser.* 1974;10:1–16.)



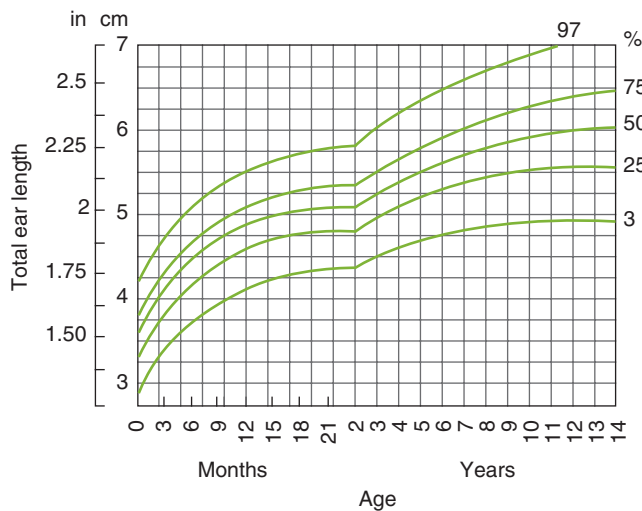
• **Fig. 19.4** A graph of palpebral fissure length from birth to age 16 years for both sexes. (From Hall JG, Allanson JE, Gripp KW, Slavotinek A. *Handbook of Physical Measurements.* New York, NY: Oxford University Press; 2007.)

by the dermatoglyphic pattern of an individual. Each of the distal phalanges has one of three basic dermal ridge patterns: arches, whorls, or loops (Fig. 19.10). The predominance of a single pattern can be an associated feature of a genetic disorder. For example, the occurrence of arches on eight or more digits is a rare event but is frequently encountered in children with trisomy 18 (Table 19.2).

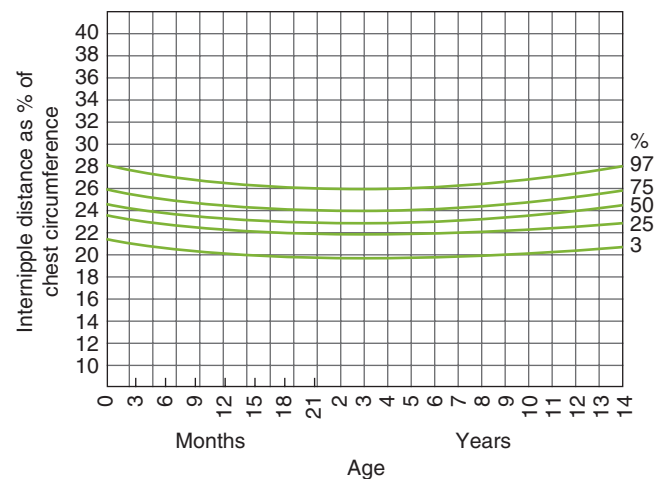
Deltas, or triradii, form at the convergence of three sets of ridges on the palm. This junction is where the hypothenar, thenar, and distal palmar patterns converge. There are typically no triradii in the hypothenar area of the palm, but when patterning is present or is large, a distal triradius arises, which is found in only 4% of normal Caucasian individuals but in 85% of patients with trisomy



• **Fig. 19.5** Ear Placement. Using the medial canthi (A and B) as landmarks, one draws a central horizontal line and extends it to a point (C) on the side of the face. Ears attached below this line are considered low set.



• **Fig. 19.6** Graph showing various percentiles for ear length plotted against age. (From Feingold M, Bossert WH. Normal values for selected physical parameters: an aid to syndrome delineation. *Birth Defects Orig Artic Ser.* 1974;10:1–16.)



• **Fig. 19.7** The internipple distance as a percentage of the chest circumference plotted against age for both sexes. (From Feingold M, Bossert WH. Normal values for selected physical parameters: an aid to syndrome delineation. *Birth Defects Orig Artic Ser.* 1974;10:1–16.)

TABLE 19.2 Dermatoglyphic Patterns Associated With Specific Dysmorphic Disorders

| Dermatoglyphic Pattern | Associated Disorders |
|------------------------|--|
| Excess arches | Trisomy 13, trisomy 18, Klinefelter syndrome (47,XXY), deletion 5p (cri du chat), fetal phenytoin exposure |
| Excess ulnar loops | Trisomy 21 |
| Excess whorls | Smith–Lemli–Opitz syndrome, Turner syndrome (45,X), 18q deletion |

21 (Down syndrome). A single transverse palmar crease is found in 4% of controls but in more than half of patients with trisomy 21 (Fig. 19.11) and in even greater proportions in patients with other trisomies. The hallual area of the foot, located at the base of the big toe, also has a dermal ridge pattern, usually a loop or whorl. A simple pattern or open field in this region is found in less than 1% of controls but in more than 50% of patients with Down syndrome (Fig. 19.12). This unusual dermal pattern is also associated with hypoplasia of the hallual pad and a wide space between the great and second toes in these patients.

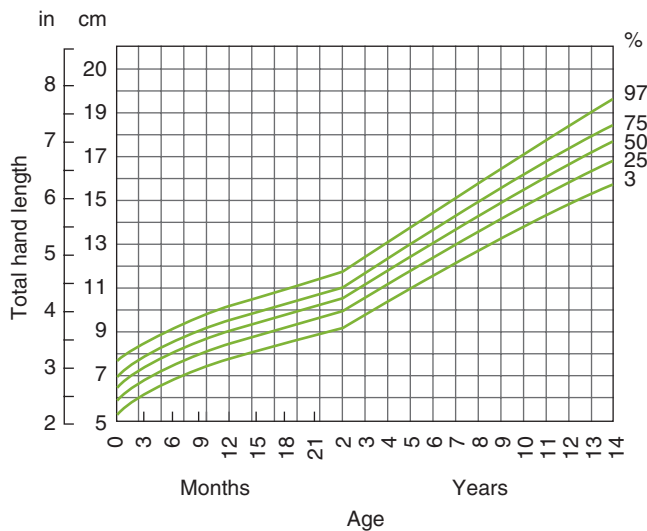
An examination of the skin is also important, since phakomatoses or skin manifestations can herald the presence of an underlying disorder (Smithson and Winter, 2004). Examples are café-au-lait spots (associated with neurofibromatosis type I) and ash leaf spots (associated with tuberous sclerosis and detected with the use of a Wood lamp). Irregular pigmentation can be suggestive of

chromosomal mosaicism, in which the different skin pigmentation patterns represent a different, mixed chromosomal composition. Hemangiomas and other skin diseases are also noteworthy.

Finally, a careful neurologic examination with input from a specialist is often warranted in the child with multiple anomalies, because the neurologic status is often the most reliable prognostic indicator. Evaluation of tone, feeding, unusual movements, and the presence of seizure activity are critical pieces of diagnostic information.

Adjunct Studies

An exhaustive physical examination often reveals differences that require further evaluation for diagnostic, prognostic, and treatment



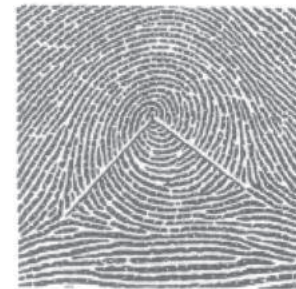
• **Fig. 19.8** The total hand length plotted against age for both sexes. (From Feingold M, Bossert WH. Normal values for selected physical parameters: an aid to syndrome delineation. *Birth Defects Orig Artic Ser.* 1974;10:1–16.)



Simple Arch

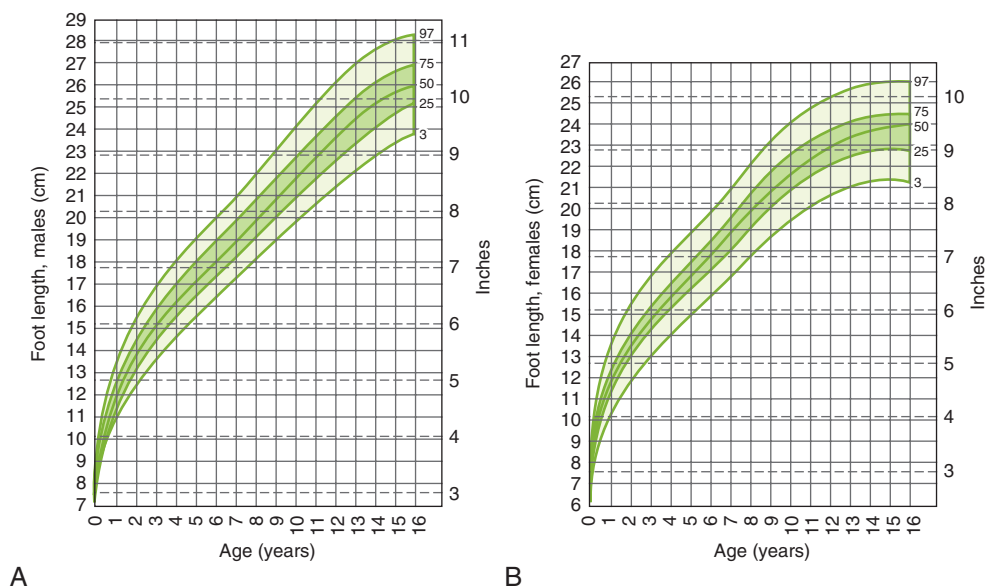


Loop



Whorl (Spiral)

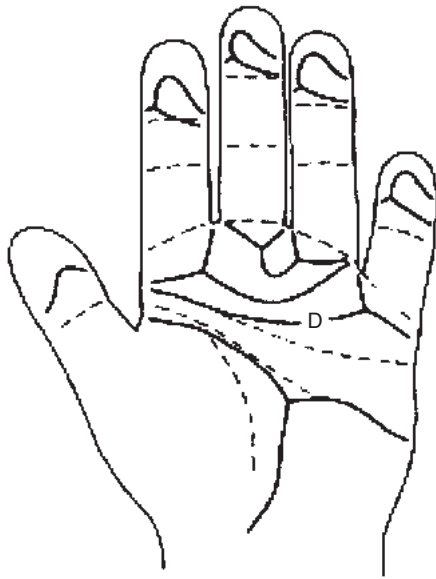
• **Fig. 19.10** Basic fingerprint patterns (dermatoglyphics). (From Holt SB. *The Genetics of Dermal Ridges*. Springfield, IL: Charles C Thomas; 1968.)



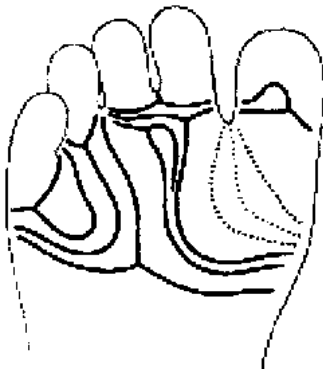
A

B

• **Fig. 19.9** Total foot lengths plotted against age for boys (A) and girls (B). (From Hall JG, Allanson JE, Gripp KW, Slavotinek A. *Handbook of Physical Measurements*. New York, NY: Oxford University Press; 2007.)



• **Fig. 19.11** The Patterns on the hand of a patient with Down syndrome depicting the palmar crease (D). (From Holt SB. *The Genetics of Dermal Ridges*. Springfield, IL: Charles C Thomas; 1968.)



• **Fig. 19.12** The distal sole of the foot of a patient with Down syndrome, depicting the characteristic “open field” hallucal pattern. (From Holt SB. *The Genetics of Dermal Ridges*. Springfield, IL: Charles C Thomas; 1968.)

• BOX 19.4 Adjunct Studies in the Evaluation of the Dysmorphic Newborn

- Investigation of internal malformation
- Assessment of neurologic function
- Identification of organ systems involved
- Ultrasonography and/or magnetic resonance imaging
- Brain imaging
- Electroencephalography as indicated
- Electromyography as indicated
- Prognosis
- Treatment and intervention

purposes (Box 19.4). Poor feeding or cyanosis may lead to detection of internal organ malformations on echocardiogram or abdominal ultrasonography. Differences in head shape suggest the need for skull radiographs, three-dimensional computed tomography, or magnetic resonance imaging (MRI) of the brain. A disproportionality of the limbs prompts a skeletal survey and bone age measurement.

• BOX 19.5 Underlying Mechanisms of Malformation

Syndrome

Pathogenetically related pattern of anomalies

Sequence

Pattern of anomalies derived from a presumed or known previous anomaly or mechanical disturbance

Association

Nonrandom occurrence of multiple anomalies

Field Defect

Disturbance of a developmental field leading to a pattern of anomalies

It is prudent in children with anomalies involving multiple systems to obtain the input of relevant specialists. This step is often essential to medical decision making and for planning interventions. Abnormal neurologic findings should prompt a consultation with a trained specialist and interpretation of studies such as head ultrasonography, brain MRI, brainstem auditory evoked responses, and electroencephalogram for seizure activity. Muscle dysfunction might result in the ordering of electromyography, muscle ultrasound, or nerve conduction studies. Visual involvement requires a fundoscopic examination by an experienced pediatric ophthalmologist, and sometimes studies of visual evoked responses or electroretinograms are needed to predict visual prognosis. Often there are well-characterized genetic disorders that have a specific pattern of abnormal findings in these highly specialized studies. Even when a unifying diagnosis is reached, there is often variation in the clinical phenotype, and determining the patient's prognosis on a system-by-system basis is typically the most appropriate and accurate way to proceed.

Literature Review

The occurrence of malformations can fit into one of several categories (Box 19.5). The next step toward reaching a diagnosis is to analyze the data generated from the evaluation and attempt to categorize the findings. A syndrome is a “collection of anomalies involving more than one developmental region or organ system” (Aase, 1990). The word itself means a “running together” or “pattern of multiple anomalies thought to be pathogenetically related.” Therefore a given congenital anomaly may be an isolated defect in an otherwise normal individual or part of a multiple malformation syndrome. Furthermore, the primary malformation itself can determine additional defects through an interrelated cascade of physical and functional processes; if ensuing malformations are related to one primary defect, factor, or event, a pathogenetic sequence has occurred. A classic example is the Pierre Robin sequence or constellation, consisting of a small recessed jaw, midline U-shaped cleft palate, and relatively large and protruding tongue. The primary anomaly is the small jaw, which does not allow adequate room for the tongue and displaces it superiorly. The displaced tongue prevents closure of the palatine shelves, causing the cleft palate. The recurrence risk with this isolated occurrence is negligible. However, one should also remember that such sequences can also be part of a larger constellation of findings that does fit into a syndrome, as Pierre Robin sequence can when it is part of the recurrent 22q11.2 deletion syndrome or Stickler syndrome (associated with mutations in one of several collagen genes). The recurrence

risk for an affected individual passing on these particular syndromes to their children is 50% (autosomal dominant inheritance).

In addition, a cluster of several malformations that are not developmentally related can occur in a nonrandom fashion called an *association* that may appear without characteristic dysmorphic features. One such statistically nonrandom association of defects consists of *vertebral defects*, *anal atresia*, *cardiac defects*, *tracheo-esophageal fistula*, *esophageal atresia*, *renal dysplasia*, and *limb anomalies* (VACTERL). It should be noted that not all features need to be present and that the extent of involvement of each system is widely variable. Although there have been various candidate genes and chromosomal regions described in some patients with VACTERL association, there is no common genetic etiology known at this time (Chen et al., 2016). CHARGE syndrome (*coloboma*, *heart disease*, *atresia choanae*, *retarded growth and development*, *genital anomalies*, and *ear anomalies/deafness*) was considered an association until the *CHD7* gene was identified to cause approximately 60%–70% of the cases (Lalani et al., 2006). Additionally, pathogenic variants in the *SEMA3E* gene have been described in a small number of patients (Lalani et al., 2004).

These associations often manifest as sporadic rather than familial occurrences. Because they are not clearly related by a common etiology or pathogenesis, associations are not considered syndromes and do not technically constitute a diagnosis. Instead, they are a recognition of a statistically significant association of features. It is important to remember that many of these same anomalies can occur as features of chromosomal aneuploidy or other syndromes. Syndromic malformations tend to occur in more than one developmental field. A field defect or complex is a set of primary malformations in a developmental field that originates from a single or primary abnormality in embryonic development (see Box 19.5).

When generating a differential diagnosis of malformations that might occur together, the evaluator must also consider structures that may appear abnormally formed but in fact are structures that underwent normal development and then received some insult that distorted their true form (Box 19.6). For example, a *deformation* describes the abnormal form, shape, or position of a part of the body that was caused by mechanical forces. Examples are clubfoot, hip dislocation, and craniofacial asymmetry; they can result from intrinsic (embryonic) or extrinsic (intrauterine) mechanical forces that alter the shape or position of an organ or part that had already undergone normal differentiation. Deformations are estimated to occur in 2% of births, and such factors as fetal crowding from

the presence of multiple fetuses and uterine malformations, as well as oligohydramnios, and a face presentation during delivery can cause them.

Along similar lines, a *disruption* describes a “morphologic defect of an organ, part of an organ, or larger region of the body resulting from the extrinsic breakdown of, or an interference with, an originally normal developmental process” (Aase, 1990). The classic example of a disruption is entanglement of the fetus in amniotic bands. Amniotic bands are ribbons of amnion that have ruptured in utero and cause disruptions of normal developmental processes in the fetus, either through physical blockage or interruption of the blood supply or by entangling and tearing of developing structures. This effect is seen most often with digits and limbs, and remnants of the bands, or constriction marks, can frequently be seen at birth. If the fetus should swallow a band, a cleft palate might result; this etiology is a very different etiology from that of cleft palate occurring as a primary malformation. Recurrence risk counseling of the parent would be very different in these two scenarios.

Dysplasias occur when there is “an abnormal organization of cells into tissue(s) and its morphologic results” (Aase, 1990). Dysplasia tends to be tissue specific rather than organ specific (e.g., skeletal dysplasia) and can be localized or generalized.

In summary, structural or morphologic changes identified at birth can occur during intrauterine development as a result of malformations, deformations, disruptions, or dysplasia. However, approximately 90% of deformations undergo spontaneous correction. Malformations and disruptions often require surgical intervention when possible. Dysplasias are typically not correctable, and the affected individual experiences the clinical effects of the underlying cell or tissue abnormality for life (Table 19.3).

After the history and physical evaluation are complete, a cross-reference of two or more anomalies is useful to generate a differential diagnosis. When the rest of the neonate’s physical and history findings are added, the possibilities can often be narrowed down to a few entities that may be amenable to diagnostic testing. If multiple anomalies are present, it is usually best to start with the least common. As Aase (1990) has stated, “The best clues are the rarest. The physical features that will be the most helpful on differential diagnosis are those infrequently seen either in isolation or as part of syndromes. Quite often, these are not the most obvious anomalies or even the ones that have the greatest significance for the patient’s health.” Cross-referencing is usually best accomplished by using published compendia of malformation syndromes. These compendia have been supplemented by databases that are accessible online (i.e., GeneReviews, Online Mendelian Inheritance in Man, PubMed, Simulconsult, Phenomizer, etc.). The availability of such tools allows the cross-referenced features to be compared easily with those of other described syndromes that may include similar

• BOX 19.6 Processes Leading to Altered Form or Structure

Deformation

Abnormal form resulting from mechanical forces

Disruption

Morphologic defect caused by interference with a previously normal developmental process

Dysplasia

Altered morphology because of abnormal organization of cells into a given tissue

TABLE
19.3

Examples of Morphologic Differences

| | |
|--------------|--|
| Malformation | Cardiac septal defects, cleft lip |
| Deformation | Clubfoot |
| Disruption | Amniotic bands |
| Dysplasia | Localized: hemangioma |
| | Generalized (skeletal): achondroplasia |

malformations. This systematic review produces a differential diagnosis for the constellation of features described and identifies references to pertinent literature.

The recognition of patterns of genetic entities involves the comparison of the proband with the examiner's personal experience of known cases and a search of the literature. Multiple anomalies may be causally related, occur together in a statistically associated basis, or occur together merely by chance. Diagnosis of a genetic disorder relies heavily on the ability of the clinician to suspect, detect, and correctly interpret physical and developmental findings and to recognize specific patterns. Accurate diagnosis of a syndrome in a child is important to the identification of major complications and their treatment if possible. It is also crucial for long-term management of patients and for parental counseling about recurrence in future offspring.

Specialized Laboratory Tests

In sorting through the multiple possibilities listed, the geneticist uses one other important tool – the availability of highly specialized cytogenetic and molecular genetic testing, including:

- Karyotype
- Fluorescence in situ hybridization
- Chromosomal microarray (see Chapter 20)
- Molecular analysis, such as sequencing (single gene, panel, exome, genome), methylation testing (e.g., for Beckwith–Wiedemann, Prader–Willi, or Angelman syndromes), or polymerase chain reaction–based techniques (e.g., for congenital myotonic dystrophy or fragile X syndrome)

The standard karyotype, or analysis of stretched and stained chromosome preparations usually taken from a peripheral blood sample, can often confirm a suggested diagnosis or explain a set of major malformations not classically encountered together. Further description of specific chromosomal abnormalities is addressed in Chapter 20; it is sufficient to note that multiple malformation syndromes can result from large visible chromosome rearrangements that lead to deletion or addition of genetic material (aneuploidy). These rearrangements can involve an entire arm of a chromosome or can be submicroscopic, requiring further special testing. It has become the standard of care in several centers to offer more specialized molecular testing, such as a chromosome microarray, as an adjunct to or instead of karyotype analysis. In general, microarray-based methods are currently focused on detecting copy number changes (smaller deletions or duplications not detectable by a karyotype) and can be performed in a targeted or genome-wide fashion (Emanuel and Saitta, 2007). Individual gene sequencing or next-generation sequencing of gene panels, an exome (sequencing of the exons of the genes), or a genome (sequencing the entirety of an individual's genetic makeup) can also be performed. It can be useful to inquire with a geneticist or genetic counselor for the availability of specific gene mutation testing that may be clinically available or performed on a research basis. GeneTests and the Genetic Testing Registry are Internet databases of laboratories

worldwide that provide such services (<https://www.genetests.org>; <https://www.ncbi.nlm.nih.gov/gtr/>).

Diagnosis

There are cases in which, after a detailed examination, exhaustive literature search, and genetic testing, no unifying diagnosis is evident. Aase (1990), a dysmorphologist, advises, “Don't panic! The absence of a diagnosis may be distressing to the diagnostician and the family, but it is much less dangerous than the possibility of assigning the wrong diagnosis with the risk of erroneous genetic and prognostic counseling and possibly hazardous treatment.” Therefore in cases in which there is no clear diagnosis, prognosis and treatment should be determined according to the organ systems involved and the extent of their impairment. In addition, when the newborn has a severe, untreatable impairment or the patient's condition is critical, it may be prudent to offer and obtain consent for a full postmortem examination by an experienced pediatric pathologist. A skin sample, and sometimes blood, can be taken from the expired fetus (or newborn prior to death) for establishing a cell line or for extracting DNA for future testing. Information gained from such investigations may often become relevant for family members, including the parents, allowing one to provide accurate recurrence risk counseling and perhaps offer prenatal testing of a new pregnancy. Such information can often help to provide closure for the family as well.

When should a genetics evaluation be considered? The following clinical situations prompt a further genetic evaluation and counseling by a specialist:

- Multiple anatomic anomalies
- History of maternal exposure to teratogens
- Familial disorders
- Increased carrier frequency or ethnic risk
- Multiple pregnancy losses

As described previously, if a birth defect is identified in the presenting patient or proband, especially if the defect is associated with other anatomic anomalies, short stature, or developmental delays, the features of a specific genetic syndrome may be present. A known history of maternal exposure to a potential teratogen would also be an indication for consultation. Conditions appearing to be familial, a family history of hereditary disorders involving malformation of a major organ, or major physical differences such as unusual body proportions, short stature, or irregular skin pigmentation would warrant genetic investigation. Mental retardation, blindness, hearing loss, or neurologic deterioration in multiple family members suggests a genetic etiology. Likewise, a strong family history of cancer or a defined ethnic risk for specific disorders such as Ashkenazi Jewish heritage and its association with a higher carrier frequency for Tay–Sachs disease would be an indication for genetic evaluation. The occurrence of multiple pregnancy losses would also raise the suspicion of a genetically influenced cause and indicate the need for further investigation and counseling.

Summary

Diseases with underlying genetic bases have significant effects on health care and its delivery. An appreciation of these entities, coupled with an organized, systematic evaluation, can help to define the

nature of a given disorder and aid in the development of the optimal plan of treatment and care for the patient.

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20

Chromosome Disorders

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KEY POINTS

- Early identification of the genetic nature of a given condition in a patient can aid in treatment and help to identify resources for providing better health care to these individuals.
- In counseling the family of a newborn in whom Down syndrome has been diagnosed, it is important to include the organ systems affected in the baby and the severity of each malformation when one is defining a prognosis. The wide variability of the phenotype should be emphasized, with a care plan tailored to the needs of the individual patient.
- Although the prognosis in trisomy 13 and trisomy 18 is extremely poor, there is emerging evidence that various interventions can improve the survival and quality of life for the child and the family, and this should be discussed with the parents during a prenatal or postnatal visit.
- The use of chromosomal microarrays allows the identification of smaller chromosomal deletions and duplications that are largely undetectable by standard karyotyping techniques and should be considered in the genetic evaluation of a newborn with multiple congenital anomalies.
- Noninvasive prenatal screening is a relatively new technology that analyzes the cell-free fetal (placental) DNA fraction circulating in maternal peripheral blood. This testing is accepted as an initial screen for select aneuploidy conditions, and if an abnormality is identified, then diagnostic testing such as karyotyping or chromosomal microarray analysis of amniotic cells is recommended.

It has been estimated that 3% of newborns have a major structural anomaly that will affect their quality of life. Although most of these patients have a single malformation, 0.7% of infants have multiple major anatomic malformations. In an additional 2%, a major anomaly is discovered by the age of 5 years. Early identification of the genetic nature of a given condition can aid in treatment and help to identify resources for providing better health care to these individuals. This chapter will focus on genetic disorders and syndromes with underlying chromosomal abnormalities that typically manifest themselves in the newborn period. In addition, it will discuss the shift in genetic evaluation and diagnosis toward the use of microarray-based and next generation sequencing diagnostic techniques.

Human Karyotype

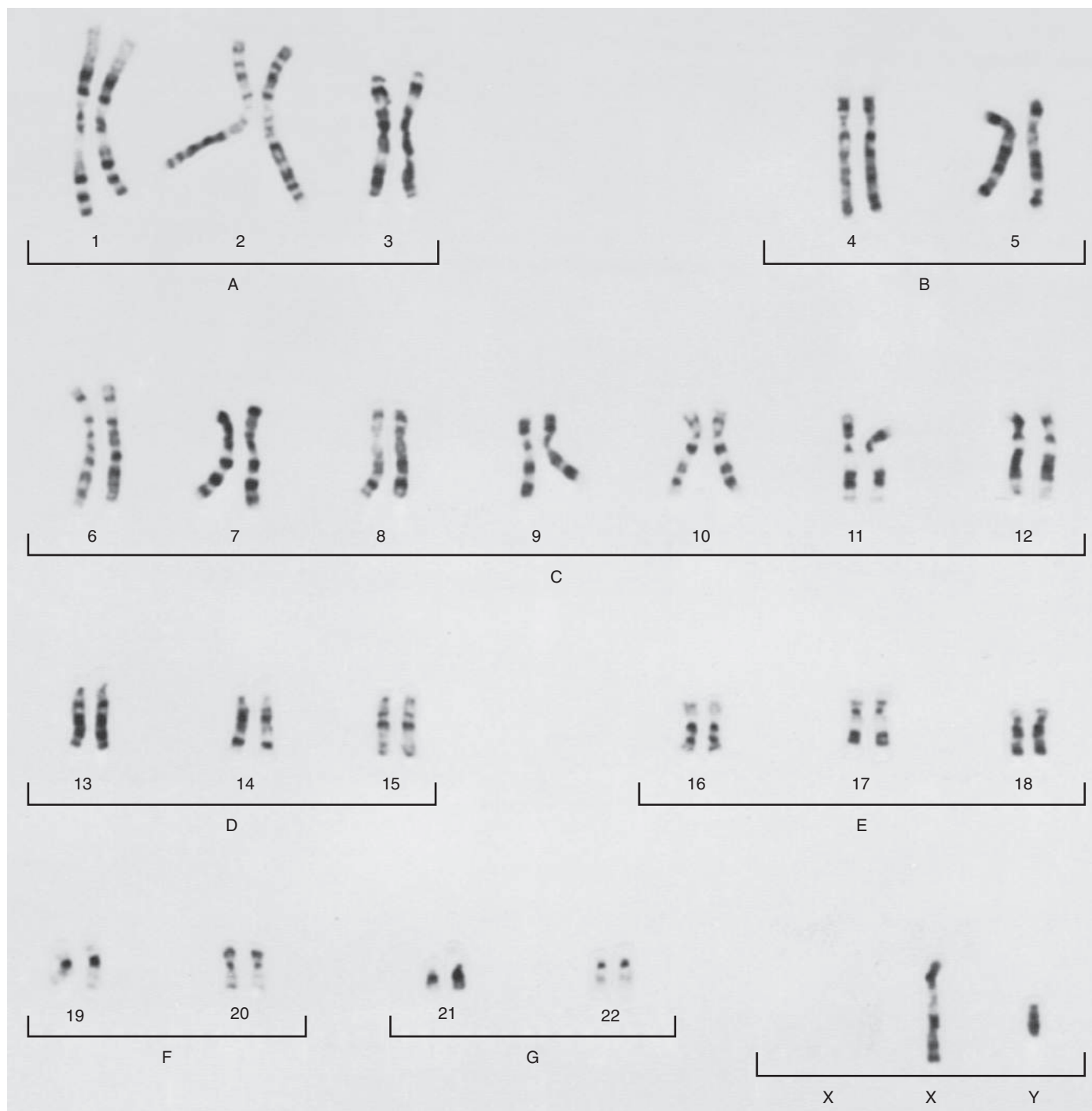
Chromosomes consist of tightly compacted DNA whose structure is maintained by association with histones and other proteins.

When treated and stretched, chromosomes from dividing cells can be visualized under the light microscope as linear structures with two arms joined by a centromere. The short arm is designated the *p arm* and the long arm is designated the *q arm*. The ends of the *p* and *q* arms are known as *telomeres*. Human chromosomes were first visualized in 1956 (Lejeune and Turpin, 1960), and each pair shows a distinctive size, centromeric position, and staining or banding pattern after treatment with special dyes, allowing it to be identified and classified. In standard international cytogenetic nomenclature, each chromosome is identified by a number, in general from largest to smallest. This presentation, or karyotype (Fig. 20.1), normally consists of 46 chromosomes, with 22 pairs of autosomes and one set of sex chromosomes – two X chromosomes for females (46,XX) and an X chromosome and a Y chromosome for males (46,XY).

Karyotype analysis is performed in cells undergoing mitosis, or cell division, in which the chromosomes condense and can be stained and visualized. Thus cells that can be stimulated to divide and grow in culture, such as peripheral blood lymphocytes, skin fibroblasts, and amniocytes, are typically used. Cells from bone marrow and chorionic villi are normally undergoing rapid cell division and can also be karyotyped successfully. Historically, several different staining methods have been described. However, G-banding (Giemsa staining) is the standard cytogenetic method used. This technique permits a resolution of at least 400 bands among all the chromosomes and can be adapted to allow high-resolution analysis of up to 800 bands to analyze structural rearrangements as small as 5–10 million base pairs or 5–10 megabase (Mb) pairs.

Gamete formation, either spermatogenesis or oogenesis, is accomplished by a process known as *meiosis*. In the first part of meiosis (meiosis I), homologous chromosomes align as pairs and cross over, exchanging genetic material, also known as *recombination*. In this stage, reduction division, the recombined pairs separate and the typical diploid content (46 chromosomes) of the cell is reduced by half to a haploid complement of 23 chromosomes. In the next stage, meiosis II, the chromosomes separate, similar to mitosis (Nussbaum et al., 2007). The full diploid state of the cell will be restored at the time of fertilization.

An imbalance of genetic material, or aneuploidy, occurs from a net loss or gain of genetic material during sperm or egg formation or less commonly during the initial divisions of the embryo. This missing or extra genetic material can be small pieces or parts of chromosomes or an entire chromosome itself. The classic recognizable aneuploidy syndromes involve trisomy (three copies of a full chromosome) such as those of chromosomes 13, 18, and 21 or monosomy (only a single copy) of a complete chromosome, such



• **Fig. 20.1** G-Banded Human Male Karyotype. The 46 chromosomes are arranged in 23 pairs, each with a specific banding pattern.

as the X chromosome. Trisomies in particular can occur from nondisjunction, a failure of normal chromosome separation. In such cases, a pair of homologs does not separate in meiosis, and one daughter cell receives both homologs of that pair, and the other cell receives none. This event can occur in either stage of gamete division, meiosis I or meiosis II. Most human meiotic nondisjunction arises during oocyte formation, specifically in maternal meiosis I. Nondisjunction of meiosis I is especially pronounced in trisomies of the acrocentric chromosomes (13, 14, 15, 21, and 22) and in XXX trisomy (MacDonald et al., 1994;

Zaragoza et al., 1994). The occurrence of meiotic nondisjunction increases significantly with maternal age. Therefore prenatal karyotyping from amniocentesis or chorionic villus sampling (CVS) is offered to women aged 35 years or older (Hook and Cross, 1982). Noninvasive prenatal screening is a relatively new technology that analyzes the cell-free fetal DNA fraction circulating in maternal peripheral blood. This screen uses sequencing-based approaches to assess the copy number of specific chromosomes. It is commonly being performed as an initial screen for select aneuploidy conditions. If there is a high risk of a chromosomal abnormality in the fetus,

diagnostic testing such as karyotyping or chromosomal microarray analysis of amniotic cells is recommended (Hardisty and Vora, 2014).

Nondisjunction can also occur in mitosis, with uneven division of genetic material during early embryonic cell division. This can result in two cell lines: one trisomic lineage that is potentially viable and one monosomic line. If this event occurs after the first postzygotic division, cells with a normal chromosome complement may also exist with cells containing an aneuploid complement, as a *mosaic* chromosome constitution. This could be a possible mechanism for the occurrence of mosaic Down syndrome, where a certain percentage of the cells in the patient have three chromosomes 21, while the rest of the cells have the expected chromosome complement containing two chromosomes 21. Mosaicism is also seen with sex chromosome aneuploidies, most notably mosaic Turner syndrome, where a subset of the cells examined show a 45,X complement and another population of cells from the patient may have a normal 46,XX or XY complement.

Partial aneuploidy may result from several mechanisms, such as rearrangements of material between nonhomologous chromosomes that can occur in the gametes of a balanced translocation carrier. The carrier parent has no net loss or gain of genetic material and is usually phenotypically normal; however, the offspring are at increased risk of an unbalanced rearrangement and its phenotypic consequences. Attention has also been focused on deletion syndromes caused by the loss of genetic material from several chromosomes (e.g., 1p-, 4p-, 5p-), with a resulting, often recognizable, phenotype. Other chromosome microdeletions have been mechanistically tied to aberrant recombination due to the presence of segmental duplications, or large “blocks” or segments of DNA that contain chromosome-specific repetitive sequences (Emanuel and Shaikh, 2001). The highly homologous repeats can mediate misalignment and nonallelic homologous recombination between two homologs. Segmental duplications are present in regions of the genome prone to rearrangements, such as the pericentromeric regions of 7q11, 15q11, 17q11, and 22q11, leading to the phenotypes seen in Williams–Beuren syndrome, Prader–Willi or Angelman syndrome, Charcot–Marie–Tooth disease or hereditary neuropathy with liability to pressure palsies, and the recurrent 22q11.2 deletion syndromes, respectively (Emanuel and Saitta, 2007). The use of chromosomal microarrays has allowed the identification of smaller chromosomal rearrangements (deletions and duplications) that are submicroscopic and largely unrecognized by standard karyotyping techniques. The use of high-resolution microarrays in infants with multiple congenital anomalies has, in many cases, led to the identification of a specific genotype, with clinical investigations then further defining the associated phenotype (Bejani and Shaffer, 2008).

Trisomies

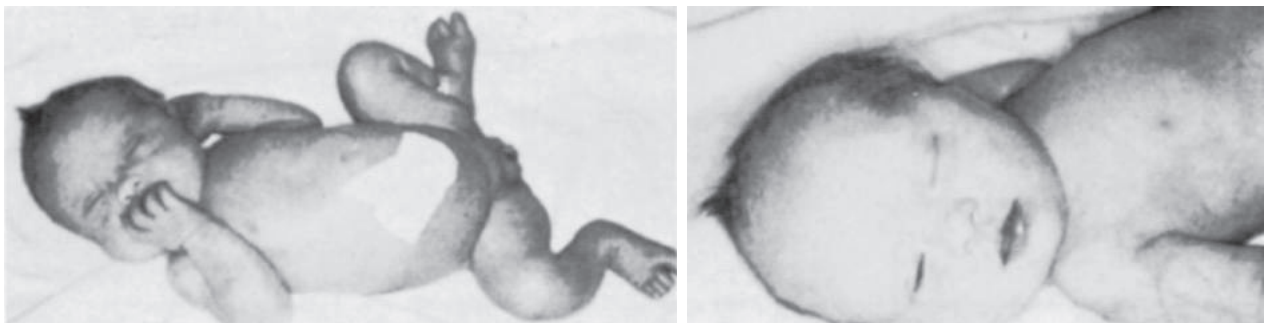
Down Syndrome (Trisomy 21)

Lejeune and Turpin (1960) demonstrated that trisomy of human chromosome 21 caused the constellation of findings recognized as Down syndrome (Fig. 20.2). This chromosome disorder was the first to be described and is the most common viable autosomal trisomy, occurring in approximately 1 in 700–800 live births (Hook, 1992). The vast majority (>90%) occur secondary to meiotic nondisjunction, and a pronounced maternal age effect is encountered. Approximately 3%–5% of cases are caused by a translocation that could be either *de novo* or inherited from a balanced translocation-carrier parent that subsequently becomes unbalanced and trisomic in the baby. Typically, the translocated chromosome 21 rearranges with another acrocentric chromosome, often chromosome 14, resulting in a Robertsonian translocation. Mitotic nondisjunction, or mosaic Down syndrome, has been demonstrated in approximately 3% of cases as well, with variable features ranging from normal to a typical Down syndrome phenotype.

Clinical Features

It is the more common occurrence of Down syndrome in babies of older mothers that led to the recommendation for prenatal karyotyping for advanced maternal age (>35 years) at the time of conception, with samples typically obtained by amniocentesis after 15 weeks' gestation, or CVS at 10–12 weeks' gestation. Maternal serum analyte testing is recommended for prenatal screening purposes for all pregnant women, with results showing low alpha fetoprotein, low unconjugated estriol, and elevated total human chorionic gonadotropin levels. Noninvasive prenatal screening is also accepted as an initial study for fetal aneuploidy (Committee Opinion 640, 2015). Associated ultrasonographic findings for Down syndrome, including a cardiac defect, shortened long bones, underdeveloped fetal nasal bone, nuchal translucency or thickening, echogenic small bowel, and duodenal atresia (“double-bubble” sign), may be seen in 50%–60% of fetuses.

Most patients with Down syndrome, if it is not diagnosed prenatally, are usually recognized at birth because of the typical phenotypic features. The constellation of physical findings associated with Down syndrome consists of brachycephaly, the presence of a third or confluent fontanel, upward-slanted palpebral fissures, epicanthal folds, Brushfield spots in the irises, flattened nasal root, small posteriorly rotated ears with overfolded superior helices, prominent tongue, short neck with excess nuchal skin, single palmar creases, brachydactyly, fifth-finger clinodactyly, exaggerated gap between the first and second toes, open field hallux pattern, and



• **Fig. 20.2** Newborn with Down syndrome (trisomy 21) illustrating some of the characteristic facial features, including upward-slanting palpebral fissures and a flat facial profile.

hypotonia (see Fig. 20.2). Often the physical features conform to an easily distinguishable phenotype, but in some cases prematurity or ethnic variations can make a clinical diagnosis less straightforward. An immediate karyotype is indicated to confirm the diagnosis and its mechanism (e.g. trisomy, translocation) and as preparation for recurrence risk counseling for the family. Malformations involving many organ systems have been described in Down syndrome, and whether the diagnosis is known prenatally or determined in the newborn period, several clinical investigations are warranted when this diagnosis is suggested (Bull, 2011). The most common malformation is congenital heart disease (seen in approximately 50% of cases), which may require surgical intervention. Atrioventricular canal defects are often encountered (mean of 40%), although ventricular septal defects (VSDs), atrial septal defects (ASDs), tetralogy of Fallot, and patent ductus arteriosus (PDA) are all described in the disorder. An echocardiogram is indicated in all cases, and medical and surgical interventions for cardiac lesions are routine. Gastrointestinal malformations, especially duodenal atresia (2%–5%), in addition to Hirschsprung disease and less frequently encountered conditions, such as esophageal atresias, fistulas, and webs throughout the tract, have been described. It is critical to carefully monitor the baby's feeding and bowel function before considering discharge from the nursery.

Although growth parameters can be in the range of 10%–25% at birth, significantly decreased postnatal growth velocity is encountered in these patients. Separate growth curves have been devised for patients with Down syndrome (Fernandes et al., 2001), because growth retardation involving height, weight, and head circumference has been well documented. However, the most recent health supervision guidelines for patients with Down syndrome recommend that patients be assessed on the basis of the World Health Organization or Centers for Disease Control and Prevention growth curves (Bull, 2011). An initial ophthalmologic evaluation is also indicated in the first few months of life and then annually, because strabismus, cataracts, myopia, and glaucoma have been shown to be more common in children with Down syndrome. In addition, hearing loss of heterogenous origin is present in approximately half of patients, with middle ear disease contributing to this problem.

Spinal cord compression caused by atlantoaxial subluxation from ligamentous laxity and subsequent neurologic sequelae can be a complication of the disorder. Radiographs are obtained when there is concern for myopathic symptoms related to spinal cord compression (weakness, abnormal reflexes, incontinence, etc.). Physicians should be vigilant in evaluating the cervical spine, especially before administration of anesthesia. Other associated disorders that merit screening are hypothyroidism in approximately 5% of patients, often with the presence of thyroid autoantibodies. Initial evaluation occurs with newborn screening programs, followed by additional measurement of thyroid-stimulating hormone and free thyroxine levels at 6 months, 12 months, and then yearly thereafter. Bone marrow dyscrasias, such as neonatal thrombocytopenia, and transient self-resolving myeloproliferative disorders, such as leukemoid reaction, have been observed in the first year of life, and a complete blood count with differential should be performed at birth. An elevated rate of leukemia with a relative risk 10–18 times greater than normal up to age 16 years has been described. Acute nonlymphoblastic leukemia is seen at higher rates in congenital or newborn cases, but the distribution becomes similar to that of non-Down syndrome patients after age 3 years. Survival of patients with Down syndrome is shorter after a diagnosis of acute lymphoblastic leukemia than in diploid patients (Epstein,

2001). There is also an increased risk of iron-deficiency anemia, with recommended screening to include annual hemoglobin level measurement starting at 12 months of age then annually thereafter. If the hemoglobin level is low, then a complete blood count with iron studies should be performed.

Patients with Down syndrome demonstrate a wide range of developmental abilities, with highly variable personalities and behavioral phenotypes as well (Pueschel et al., 1991). Central hypotonia with concomitant motor delay is most pronounced in the first 3 years of life, as are language delays. Therefore immediate and intensive early intervention and developmental therapy are critical for maximizing the developmental outcome. A wide range of intelligence has been described, with conflicting data on genetic and environmental modifiers of outcome (Epstein, 2001). Seizure disorders occur in 5%–10% of patients, often manifesting themselves in infancy.

The most common causes of death in patients with Down syndrome are related to congenital heart disease, to infection (e.g., pneumonia) that is thought to be associated with defects in T-cell maturation and function, and to malignancy (Fong and Brodeur, 1987). Once medical and surgical interventions for the correction of associated congenital malformations are complete and successful, the long-term survival rate is good. However, less than half of patients survive to 60 years, and less than 15% survive past 68 years. Neurodegenerative disease with features of Alzheimer disease is encountered in most patients who are older than 40 years. The gene for amyloid precursor protein (*APP*) is on chromosome 21, and overexpression of this gene in the trisomic state leads to early-onset beta-amyloid plaques in the brain. Neurofibrillary tangles, cerebrovascular pathology, white matter pathology, oxidative damage, neuroinflammation, and neuron loss are also seen in the brains of patients with Down syndrome. Frank dementia is not typical, as there appear to be compensatory responses that delay the onset of dementia after the development of amyloid deposition (Head et al., 2016). Men with Down syndrome are almost always infertile, whereas small numbers of affected women have reproduced (Epstein, 2001).

In counseling the family of a newborn in whom Down syndrome has been diagnosed, it is important to include the organ systems affected in the baby and the severity of each malformation when one is defining a prognosis. Above all, the wide variability of the phenotype should be emphasized, with a care plan tailored to the needs of the individual patient.

Genetic Counseling

If a complete (full chromosome) or mosaic trisomy 21 is found, parental karyotypes are generally not analyzed, because the karyotypes are normal in virtually all cases. After having one child with Down syndrome, a mother's recurrence risk for another affected child is approximately 1% higher than her age-specific risk (Hook, 1992). This fact is especially significant in younger mothers, whose age-specific risks are low. If a de novo translocation resulting in Down syndrome is found, the recurrence risk is less than 1%. If the mother is found to carry a constitutional balanced Robertsonian translocation, the risk of another translocation Down syndrome fetus is approximately 15% at the gestational age when amniocentesis is offered and 10% at birth. However, if the father is the translocation carrier, the recurrence risk is significantly smaller, approximately 1%–2% (Epstein, 2001). Whereas array-based diagnostic techniques will identify the copy number change associated with the trisomy, structural rearrangements such as Robertsonian translocations are not readily detected. In this situation a karyotype will provide

information regarding the mechanism of the copy number change, which is needed for accurate recurrence risk counseling.

Trisomy 18 (Edwards Syndrome)

Trisomy 18 is encountered in 1 in 6000 live births and is associated with a high rate of intrauterine demise. It is estimated that only 5% of conceptuses with trisomy 18 survive to birth and that 30% of fetuses in whom trisomy 18 is diagnosed by second-trimester amniocentesis die before the end of the pregnancy (Hook, 1992). Findings on prenatal ultrasonography can raise suspicion for the disorder – growth retardation, oligohydramnios or polyhydramnios, heart defects, myelomeningocele, clenched fists, and limb anomalies. Diagnostic testing is recommended when prenatal ultrasonography findings are suggestive of this condition. Maternal serum screening can show low values for alpha fetoprotein, unconjugated estradiol, and total human chorionic gonadotropin.

Clinical Features

Phenotypic features present at birth consist of intrauterine growth restriction (1500–2500 g at term), small narrow cranium with prominent occiput, open metopic suture, low-set posteriorly rotated ears, and micrognathia with small mouth. Characteristic clenched hands with overlapping digits, excess of arches on dermatoglyphic examination, hypoplastic nails, and “rocker-bottom” feet or prominent heels with convex soles (Fig. 20.3) are also described. Additional malformations encountered in this syndrome include congenital heart disease (ASD, VSD, PDA, pulmonic stenosis, aortic coarctation), cleft palate, clubfoot deformity, renal malformations, brain anomalies, choanal atresia, eye malformations, vertebral anomalies, hypospadias, cryptorchidism, and limb defects, especially of the radial rays.

The prognosis in this disorder is extremely poor, with more than 90% of babies succumbing in the first 6 months of life and only 5% alive at 1 year old. Death is caused by central apnea, infection, and congestive heart failure. The newborn period is characterized by poor feeding and growth, typically requiring tube feedings. Universal poor growth and profound mental retardation



• **Fig. 20.3** Newborn with trisomy 18, showing prominent occiput, characteristic facial appearance, and clenched hands.

with developmental progress typically leveling at that of a 6-month-old infant (Baty et al., 1994) have been documented. Malignant tumors such as hepatoblastoma and Wilms tumor have been described in some survivors. There is emerging evidence that various interventions can improve the survival and quality of life for the child and the family, and this should be discussed with the parents during a prenatal or postnatal visit (Carey, 2012; Kosho and Carey, 2016).

Genetic Counseling

The typical estimate of the recurrence risk for trisomy 18 in a future pregnancy is a 1% risk over the maternal age-specific risk for any viable autosomal trisomy (Hook, 1992). Trisomy occurring from a structural rearrangement, such as a translocation, warrants parental karyotype analysis before the recurrence risk can be assessed.

Trisomy 13 (Patau Syndrome)

It has been estimated that approximately 2%–3% of fetuses with trisomy 13 survive to birth, with a frequency of 1 in 12,500 to 1 in 21,000 live births (Hook, 1992). As with other trisomies, abnormal noninvasive prenatal screening findings for advanced maternal age or the presence of fetal ultrasonographic findings may prompt diagnostic testing by CVS or amniocentesis that can result in a prenatal diagnosis of trisomy 13.

Clinical Features

Trisomy 13–associated midline malformations include congenital heart disease, cleft palate, holoprosencephaly, renal anomalies, and postaxial polydactyly (Fig. 20.4). In addition, microcephaly, eye



• **Fig. 20.4** Stillborn With Trisomy 13. The facial appearance is that of cebocephaly, which is associated with holoprosencephaly. There is an extra digit on the ulnar border of the right hand.

anomalies, and scalp defects can suggest the diagnosis. Brain malformations such as holoprosencephaly are found in more than half of patients with concomitant seizure disorders. Microcephaly, split sutures, and open fontanelles are encountered. A scalp defect (cutis aplasia) that has sometimes been mistakenly attributed to a fetal scalp monitor is specific to the disorder, being found in 50% of cases. Eye malformations, including iris colobomas and hamartomatous cartilage “islands,” can be seen on fundoscopic examination.

Congenital heart disease is present in approximately 80% of patients, usually VSD, ASD, PDA, or dextrocardia. Limb anomalies, such as postaxial polydactyly, single palmar creases, and hyperconvex narrow fingernails, are also seen. The fingers can be flexed or overlapped and can show camptodactyly. An increased frequency of nuclear projections in neutrophils, giving a drumstick appearance similar to that of Barr bodies, can also be found. This finding would be especially striking in males, in whom Barr bodies would not be expected.

As with trisomy 18, prognosis for the fetus with trisomy 13 is extremely poor, with 80% mortality in the neonatal period and less than 5% of patients surviving to 6 months old. Mental retardation is profound, and many patients are blind and deaf as well. Feeding difficulties are typical. Also similar to trisomy 18, various interventions can increase the survival of these infants and improve their overall quality of life. A discussion with the parents should be considered regarding these possibilities (Carey, 2012; Kosho and Carey, 2016).

Genetic Counseling

Recurrence risk data suggest that, as with trisomy 18, the chance that a woman will have a child with any trisomy after a pregnancy affected by trisomy 13 is rare. The estimated risk is 1% higher than the maternal age–related risk for the recurrence of any viable autosomal trisomy in a subsequent pregnancy.

45,X (Turner Syndrome)

In early embryogenesis, two active X chromosomes are required for normal development. Turner syndrome, a phenotype associated with loss of all or part of one copy of the X chromosome in a female conceptus, occurs in approximately 1 in 2500 female newborns. The 45,X karyotype or loss of one entire X chromosome accounts for approximately half of the cases. A variety of X chromosome anomalies—including deletions, isochromosomes, ring chromosomes, and translocations—account for the remainder of the causes. It is important to note that approximately 0.1% of fetuses with a 45,X complement survive to term; the vast majority (>99%) are spontaneously aborted. This fact underscores the requirement for both X chromosomes during embryonic development. Additional studies indicate that in approximately 80% of cases, it is the paternally derived X chromosome that is lost (Willard et al., 2001).

Clinical Features

There is wide phenotypic variability in patients with Turner syndrome. Features present at birth include short stature, webbed neck, craniofacial differences (epicanthal folds and high arched palate), hearing loss, shield chest, renal anomalies, lymphedema of the hands and feet with nail hypoplasia, and congenital heart disease. Typical cardiac defects include bicuspid aortic valve, coarctation of the aorta, valvular aortic stenosis, and mitral valve prolapse.

Growth issues, especially short stature, are the predominant concern in childhood and adolescence; the mean adult height of patients with Turner syndrome is 135–150 cm without treatment. Growth hormone therapy has been shown to increase final adult height, with the age of initiation of therapy not yet established, but it can be administered as early as 9 months (Bondy, 2007). Primary ovarian failure caused by gonadal dysplasia (streak gonads) can result in delay of secondary sexual characteristics and primary amenorrhea. Cyclic hormonal therapy should reflect the process of normal puberty (Bondy, 2007) to aid the development of secondary sex characteristics and menses as well as to help bone mass. Infertility, related to gonadal dysplasia, is typical and has been successfully treated with assisted reproduction techniques and donor oocytes. It is important to evaluate the patient for structural cardiovascular defects before pregnancy.

In terms of intellectual development, specific difficulties with spatial and perceptual thinking lead to a lower performance intelligence quotient; however, this syndrome is not characterized by mental retardation.

Triploidy (69,XXX or 69,XXY)

As its name implies, triploidy is a karyotype containing three copies of each chromosome. The mechanisms that lead to this state include fertilization of the egg by two different sperm (dispermy) and complete failure of normal chromosome separation in maternal meiosis. The vast majority of triploid fetuses are spontaneously aborted, accounting for up to 15% of chromosomally abnormal pregnancy losses. Live births of affected fetuses are rare, and reports of survival beyond infancy are only anecdotal. Mosaicism with combinations of diploid and triploid cells (mixoploid) has also been documented. Malformations, including hydrocephalus, neural tube defects, ocular and auricular malformations, cardiac defects, and 3–4 syndactyly of the fingers, are associated findings. In addition, the placenta is often abnormal, typically large, and cystic.

Deletion Syndromes

In addition to the aneuploid conditions described previously, partial monosomy of a chromosome can lead to a recognizable pattern of malformation. Three well-described syndromes that are associated with the deletion or loss of genetic material from the short, or p, arms of chromosomes 1, 4, and 5 are described. All these syndromes are associated with heterozygous deletions that involve the loss of many genes located in a specific region, or haploinsufficiency.

Chromosome 1p Deletion Syndrome (1p–)

Monosomy for the distal short arm of chromosome 1, or deletion of 1p36, has been associated with a constellation of clinical findings. A characteristic facies consisting of frontal bossing, large anterior fontanel, flattened midface with deep-set eyes, and developmental delay has been described (Fig. 20.5). Orofacial clefting, hypotonia, seizures, deafness, and cardiomyopathy are also noted.

This deletion syndrome is estimated to occur in approximately 1 in 10,000 live births, and it is the most frequently occurring subtelomeric deletion. Greater recognition of the phenotype and widespread use of chromosomal microarrays has led to improved diagnosis of this condition. Most deletions arise *de novo* in the patient, with approximately 3% being attributable to malsegregation of a balanced parental translocation. The size of the deletion differs, from submicroscopic (<5 Mb) to large, cytogenetically visible



• Fig. 20.5 Child with deletion of 1p.

deletions larger than 30 Mb. A correlation between the size of the deletion and the severity of clinical features is suggested.

Wolf–Hirschhorn Syndrome (4p–)

Distal deletions of the short arm of chromosome 4 are associated with a recognizable pattern of malformation. This syndrome is estimated to occur in 1 in 50,000 births and has features such as intrauterine growth restriction, microcephaly, midline structural defects such as cleft lip and cleft palate, cardiac septal defects, and hypospadias. The characteristic facial features are described as the *Greek helmet* facies, as evidenced by hypertelorism with epicanthi, a high forehead with a prominent glabella, and a beaked nose. Prominent, low-set ears are also seen. Hypotonia, failure to thrive, and developmental delay are common, with approximately 30% mortality in the first year of life. Many patients have lived well into childhood and even into adulthood, although profound growth impairment and mental retardation are typical and often accompanied by seizures.

The 4p deletions are typically cytogenetically visible on karyotype analysis, although small submicroscopic deletions have also been described. In cases where the clinical features are suggestive but the karyotype is not revealing, further cytogenetic analysis using specific 4p telomere fluorescence in situ hybridization (FISH) probes or more commonly a chromosomal microarray can be diagnostic. Microarray analysis also provides information about the relative size and gene content of the deleted region. More than 80% of 4p deletions arise de novo in the patient, with minimal risk of recurrence. In the 10%–15% of cases resulting from a translocation, analysis of parental samples clinically is indicated for appropriate recurrence risk counseling.

Cri du Chat Syndrome (5p–)

Partial monosomy of 5p is seen in approximately 1 in 50,000 live births and is associated with a multiple congenital anomaly syndrome

named for the unusual cry of the affected babies, described as similar to that of a cat, or *cri du chat*. The constellation of features associated with this disorder includes low birth weight, microcephaly, round face, hypertelorism or telecanthus, downward-slanting palpebral fissures, epicanthi, and broad nasal bridge. Hypotonia and cardiac defects are also seen, including ASD, VSD, and tetralogy of Fallot. Early issues include failure to thrive and pronounced developmental delay. The unusual cry usually resolves during infancy, and survival into adulthood is possible but is typically associated with severe mental retardation. Intensive therapy appears to provide some benefit, and more sensitive measures of cognition demonstrate clearly better receptive language skills than expressive language ability. Therefore children may understand more complex verbal language than their expressive skills might demonstrate (Cornish et al., 1999).

It is estimated that almost 100 genes are lost when the putative critical region from 5p15.2 to p15.33 is deleted (Shaffer et al., 2001; Zhang et al., 2005). Close to 90% of 5p deletions arise de novo in the affected child, incurring a minimal risk of recurrence (<1%). The remainder arise from malsegregation of a balanced translocation in a carrier parent, which would be associated with a 10%–15% risk of recurrence of an unbalanced karyotype in a future liveborn infant. Parental chromosome analysis is indicated for proper recurrence risk counseling.

Chromosomal Microarrays

In the past 10 years, characterization of variations of the genome that fall in the range between the single nucleotide and visible chromosomal changes—submicroscopic structural variants (typically less than 5 Mb) that cannot be seen by standard karyotyping—has been accomplished through the use of chromosomal microarrays (Bejjani and Shaffer, 2008; Miller et al., 2010; Martin and Warburton, 2015). Microarray-based methods are focused on detecting copy number changes, such as gains and losses of chromosomal material; however, structural rearrangements such as balanced

translocations or inversions are not detectable by this method. Microarray testing can be performed in either a targeted or more commonly a genome-wide fashion. Single nucleotide polymorphism arrays include probes based on known polymorphisms present in the human genome. Single nucleotide polymorphism-based arrays or dense oligonucleotide arrays have the advantage of detecting gains or losses of shorter stretches of the genome, because the probes for a given region can be densely arrayed, and the sensitivity for detecting alterations of that region is greatly enhanced. With a single test, microarrays can detect copy number variants in a genome-wide approach, revealing disorders usually identified by cytogenetic analysis and multiple individual FISH studies. Whereas microarray analysis can provide a robust and exceptional level of resolution from a diagnostic perspective, the major difficulty with the current interpretation of the results lies in assigning causality and clinical significance to the multiple alterations that are detected in each individual. Toward this end, the use of online databases (e.g., Database of Genomic Variants, <http://dgv.tcag.ca/dgv/app/home>) with information on normal variation in multiple ethnic populations and testing of unaffected parents remain standard approaches for discerning whether a copy number change is likely to cause disease.

Segmental Duplications and Microdeletion Syndromes

A greater appreciation of the complexity of the human genome and its structure has been afforded with the completion of the human genome sequence. This work has focused attention on regions of the genome that are prone to rearrangement. The presence of these regions appears to have a significant role in the origin of several genetic disorders (Emanuel and Shaikh, 2001; Emanuel and Saitta, 2007). These disorders result from inappropriate dosage of crucial genes in a given genomic segment via either structural mechanisms (deletion or duplication) or functional mechanisms (imprinting or uniparental disomy [UPD]). It has also been demonstrated that many of the regions prone to recurrent rearrangements have a common element: the presence of large, chromosome-specific segmental duplications. These repeat structures appear to mediate misalignment and aberrant crossover during recombination, resulting in rearrangements such as deletions and duplications (Fig. 20.6). Many of these large low copy repeat structures are localized to a single chromosome or within a single chromosomal band. Examples of such genomic disorders include hemophilia A (inversion of Xq28), Sotos syndrome (in which a number of patients have a deletion of 5q35), Smith–Magenis syndrome (deletion of 17p11.2), Charcot–Marie–Tooth disease (interstitial duplication on 17p12), and the reciprocal deletion of this same region of 17p12, leading to hereditary neuropathy with liability to pressure palsies, and a small percentage of patients with neurofibromatosis type I (deletion involving 17q11.2).

In this section we highlight several deletion syndromes that occur on chromosomes whose underlying genomic structure contains segmental duplications. These include Williams–Beuren syndrome (involving 7q11.2), Prader–Willi syndrome (PWS) or Angelman syndrome (involving an imprinted region of 15q11 through 15q13), and the recurrent 22q11.2 microdeletion syndrome (also known as DiGeorge or velocardiofacial syndrome). The latter is the most commonly occurring microdeletion syndrome in humans. It is important to note that in several of these microdeletion syndromes, reciprocal duplications of the exact same region may also occur

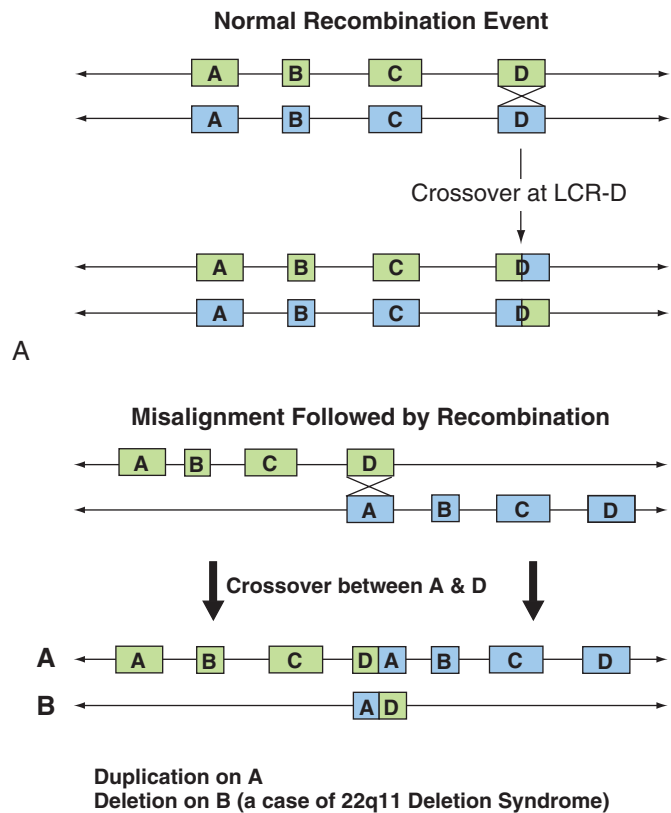


Fig. 20.6 (A) Alignment of low copy repeats before exchange. (B) Misalignment of low copy repeats before exchange can result in rearrangement. LCR, Low copy repeats.

(see Fig. 20.6) and can show a different phenotype than the deletion of the same region. In general, duplication syndromes cause fewer abnormalities and show a wider phenotypic variability, but they are often characterized by developmental delays with or without behavioral abnormalities.

Newly recognized recurrent microdeletion syndromes (e.g., 1q21.1, 3q29, 15q13.3, 16p11.2, and 17q21) were identified because of the increased use of chromosomal microarrays in the clinical and research settings. These regions are similarly flanked by segmental duplications, likely predisposing to their rearrangements. Many of the patients with these particular rearrangements do not receive a clinical diagnosis in the newborn period, since they may not present with significant anatomic malformations or dysmorphic features that prompt a genetic evaluation; however, they can present during childhood with variable clinical findings. Typically there is developmental delay, behavioral abnormalities (such as an autism spectrum disorder), or intellectual disability. Many of these syndromes are complicated because they have also been reported in unaffected family members, which proves to be a challenge when one is ascribing causality to the rearrangement.

When an abnormality is identified by microarray analysis, the genes located in the affected region can be identified. It is then possible to decide what role, if any, the affected genes have in the observed phenotype. For example, deleted genes could be identified that may predispose a patient to cancer because of a germline loss of one copy of a tumor suppressor gene (Adams et al., 2009), prompting careful surveillance for tumor formation. Therefore a genetic paradigm has developed where patients are initially

genotyped (genotype first), followed by evaluation of the altered genes in a given region to determine their possible effect on the future phenotype.

Williams–Beuren Syndrome (7q11.2 Deletion)

The estimated incidence of Williams–Beuren syndrome is 1 in 10,000 live births. The phenotype has a variable spectrum but usually consists of distinctive facies, growth and developmental retardation, cardiovascular anomalies, and occasionally infantile hypercalcemia (Fig. 20.7). Babies with Williams–Beuren syndrome usually show some degree of intrauterine growth restriction with mild microcephaly. Facial features include epicanthal folds with

periorbital fullness of subcutaneous tissues, flat midface, anteverted nostrils, long philtrum, thick lips, large open mouth, and stellate irises that may not be discernible at birth. Most infants have a cardiovascular abnormality; supraventricular aortic stenosis (SVAS) is the most commonly associated defect, seen in more than 50% of cases. Pulmonary artery stenosis is also often encountered. It is interesting to note that isolated SVAS can also exist as a separate autosomal dominant trait and has been shown to occur from mutations of the elastin gene (*ELN*), which is located within the deletion region on 7q11.2. Patients with Williams–Beuren syndrome are typically missing one copy of the elastin gene.

Hypercalcemia, which is manifested in approximately 10% of patients with this disorder, is severe and persists through infancy.



• **Fig. 20.7** Williams–Beuren Syndrome. (A) Neonate with a coarse face, periorbital fullness, wide mouth, and thick lips with decreased Cupid's bow. (B) Neonate profile showing periorbital fullness, flat nasal bridge with full tip, and prominent cheeks. (C) Infant with periorbital fullness, flat nasal bridge, thick lips with decreased Cupid's bow, pouty lower lip, and low-set, full cheeks. (D) Infant profile showing dolichocephaly (increased anteroposterior diameter of head), a higher nasal bridge than in the neonate, full nasal tip, pouty lower lip, long neck, sloping shoulders, and part of pectus excavatum.

Umbilical and inguinal hernias are also associated features. Issues in infancy include feeding and growth problems, with pronounced irritability and colicky behavior. Hoarse voice, strabismus, hypertension, and joint mobility restrictions may develop later in childhood. In terms of development, the typical mild to moderate mental retardation can be masked by relatively advanced language skills, although gross motor and visual–motor integration skills are especially affected. Attention-deficit disorders are common, and a characteristic outgoing personality is often described in affected children.

Many of the classic features of Williams–Beuren syndrome are not clearly discernible in the newborn period, but the diagnosis should be suggested in any child with SVAS, hypercalcemia, and facial features consistent with the disorder. The diagnosis can be confirmed quickly by chromosomal microarray analysis or FISH using probes specific for the deleted region of 7q11.2. Because the condition is typically sporadic and most deletions arise *de novo*, the risk of recurrence in subsequent pregnancies is minimal. An affected adult, however, would pass on the condition in an autosomal dominant manner, with a 50% risk of the disorder in his or her child.

22q11.2 Deletion Syndrome

A deletion of 22q11.2 has been identified in most patients with the classically termed conditions DiGeorge, velocardiofacial, and conotruncal anomaly face syndromes, leading to the realization that these clinical entities all reflect features of the same genomic disorder (McDonald-McGinn *et al.*, 2015). The list of findings associated with 22q11.2 deletion syndrome is extensive and differs by patient. Estimates indicate that 22q11.2 microdeletion syndrome occurs in approximately 1 in 1000 fetuses (Grati *et al.*, 2015). This disorder is the most common microdeletion syndrome occurring in humans and is a significant health concern in the general population.

The phenotype is characterized by a conotruncal cardiac anomaly and often aplasia or hypoplasia of the thymus and parathyroid glands. Most patients with a deletion can receive a diagnosis as newborns or infants with significant cardiovascular malformations,

including interrupted aortic arch type B, truncus arteriosus, or tetralogy of Fallot, along with functional T-cell abnormalities and hypocalcemia. In addition, facial dysmorphism may be present (Fig. 20.8), including hooded eyelids, hypertelorism, overfolded ears, bulbous nasal tip, a small mouth, and micrognathia. Since the initial report by DiGeorge in 1968, the spectrum of associated clinical features has been expanded to include anomalies such as palatal anomalies, vascular rings, feeding and swallowing problems, gastroesophageal reflux, renal agenesis, and hypospadias (McDonald-McGinn *et al.*, 1997). Before advances in the medical and surgical treatment of children with complex congenital cardiac disease and immune deficiencies, this disorder was associated with significant morbidity and mortality.

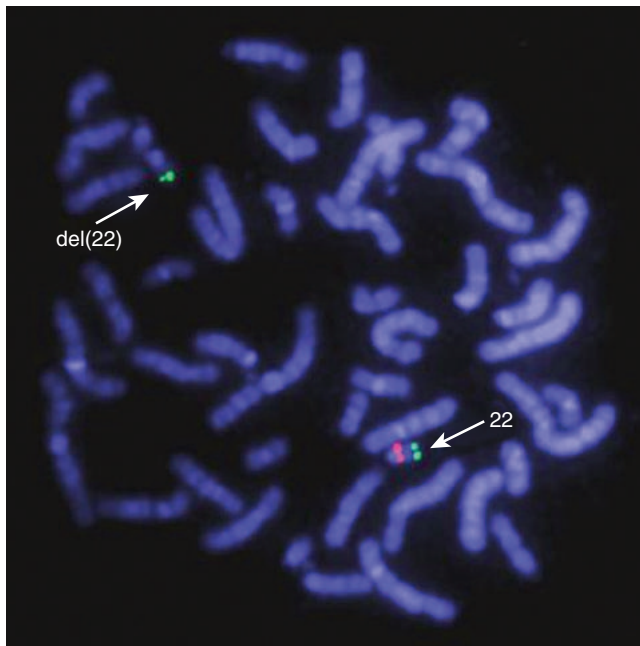
Developmental delays or learning disabilities have been reported in most patients with 22q11.2 deletion syndrome, and a wide range of developmental and behavioral findings have been observed in young children (Emanuel *et al.*, 2001). In the preschool years, affected children were most commonly found to be hypotonic and developmentally delayed with language and speech difficulties. Severe or profound retardation was not seen, and one-third of patients functioned within the average range (Bassett *et al.*, 2011).

The vast majority of patients (80%–90%) have the same large deletion, approximately 2.4–3 Mb, that is detected by FISH (Fig. 20.9) or chromosomal microarray. The deletion affects approximately 50 genes and 7 micro ribonucleic acids (McDonald-McGinn *et al.*, 2015). The size of the deletion remains unchanged when it is inherited from an affected parent. However, the phenotype can be widely variable, even within a family. Although smaller recurrent deletions that are half the size of the common deletion occur (1.5 Mb), a smaller size does not correspond to milder symptoms, making genotype–phenotype correlations difficult.

Most 22q11 deletions occur as *de novo* events, with less than 10% of them being inherited from an affected parent. The prevalence of these *de novo* 22q11.2 deletions indicates a high rearrangement rate within this genomic region that is related to the presence of recombination-permissive duplicated DNA sequences or segmental duplications in 22q11 (Emanuel and Shaikh, 2001; Emanuel and Saitta, 2007; Shaikh *et al.*, 2007).



• **Fig. 20.8** Facial differences associated with 22q deletion syndrome. A. Frontal view showing upslanting palpebral fissures, bulbous nose, and small chin. B. Profile view showing deep set eyes and overfolded superior helices.



• **Fig. 20.9** Fluorescence in situ hybridization study of a 22q deletion. The white arrows point to both copies of the 22nd chromosome. One chromosome 22 shows a hybridization pattern involving a control probe (green) and the 22q11.2 region probe (red). The other chromosome 22 shows a hybridization pattern of a control probe (green) and absent 22q11.2 region probe (probe), suggesting this region is deleted. (Courtesy of Beverly S. Emanuel.)

Additional Microdeletion and Microduplication Syndromes

As mentioned previously, several identified genetic syndromes have been described with the increased use of chromosomal microarrays (Slavotinek, 2008; Watson et al., 2014). These regions are frequently flanked by segmental duplications, which is the likely reason for their prevalence in diverse patient populations, as well as the fact that there are reciprocal rearrangements (deletion or duplication). Almost all of the patients with these more recently recognized deletions or duplications were not initially recognized on the basis of their clinical features but were instead ascertained by microarray analysis.

Deletions of 1q21.1 with a size of approximately 1.35 Mb have been seen in patients with variable presentations, including developmental and behavioral abnormalities, mild facial dysmorphism, and microcephaly (Brunetti-Pierri et al., 2008; Mefford et al., 2008). In 3q29 microdeletion syndrome, the deletion is approximately 1.6 Mb and was initially discovered (Willatt et al., 2005) in patients with mild to moderate mental retardation, microcephaly, and nonspecific facial dysmorphism. Duplications of this region have also been described in patients with developmental delays, mental retardation, and microcephaly (Lisi et al., 2008). Reciprocal duplications of the region deleted in Williams–Beuren syndrome (7q11.23) are characterized by distinctive facial features, congenital anomalies, and intellectual and developmental disabilities including poor expressive speech in contrast to the deletion of the same region (Mervis et al., 2015). The 15q13.3 region is distal to the more commonly known 15q11–q13 locus associated with Prader–Willi and Angelman syndromes. Patients with a 1.5-Mb deletion of 15q13.3 have variable phenotypes but typically manifest seizures or abnormal electroencephalograms (Sharp et al., 2008). A smaller

680-kilobase (kb) deletion has also been described in a range of patients with neurobehavioral phenotypes (Shinawi et al., 2009). Interestingly, this deletion is often maternally inherited, suggesting that there might be an imprinting mechanism involved. Deletions of 16p12.2 can manifest as developmental and intellectual disability, cardiac malformations, epilepsy, hearing loss, renal and genital anomalies (the latter in males), and cleft lip/palate (Girirajan et al., 2015). A recurrent 550-kb deletion of 16p11.2 has been discovered in approximately 1% of patients with autism (Weiss et al., 2008) and the reciprocal duplication associated with attention deficit–hyperactivity disorder and schizophrenia (McCarthy et al., 2009; Shinawi et al., 2010). Chromosome deletions of 17q21.31 of approximately 500–650 kb were also found in patients with developmental delays, learning disabilities, and variable facial dysmorphism. Mutations of *KANSL1*, located within the 17q21.31 deletion, can also be associated with the same phenotype (Koolen and de Vries, 2013).

Disorders of Imprinted Chromosomes

A growing recognition of mechanisms regulating gene expression has emerged in the last few decades. Two of the most exciting concepts with important clinical correlates are imprinting and UPD. The term *genomic imprinting* implies that a whole region of a chromosome or a group of genes in a given region is subject to a difference in their expression that depends on whether they reside on the maternally inherited or the paternally inherited chromosome. In these cases, a genetic disorder might manifest itself on the basis of whether the genomic region was inherited maternally or paternally. The genes in an imprinted region are not necessarily mutated, but they are epigenetically marked such that the cell can distinguish between the maternal and paternal copies and coordinate expression on the basis of that distinction. At the molecular level, it appears that differences in the methylation of the DNA, and its replication and regulation at the transcriptional level, appear to be involved in this mechanism. This mechanism has become an area of important research advancement, and there are now more than 200 known or predicted genes and chromosomal regions thought to be important in human disease (<http://www.geneimprint.com/site/genes-by-species>).

Prader–Willi Syndrome

It has been demonstrated that occasionally, instead of one copy of each chromosome being inherited from each parent, both copies of a given chromosome or chromosomal region can come from the same parent. This phenomenon, known as *uniparental disomy* or *UPD*, is associated with advanced maternal age. It becomes a significant issue when the chromosome involved is imprinted or has regions on it that are imprinted.

PWS involves the loss of activity from the paternally derived proximal long arm of chromosome 15 (15q11). This loss can occur through deletion or disruption of this region or through maternal UPD such that no paternal chromosome 15 is present (Nicholls et al., 1989). Newborns with PWS have pronounced central hypotonia, hyporeflexia, and a weak cry. The poor tone manifests itself as sucking and swallowing difficulties that can lead to failure to thrive and the need for feeding tubes in infancy. Facial differences that have been described include bifrontal narrowing, almond-shaped eyes, and a small, downturned mouth. Genitalia are often hypoplastic, with cryptorchidism being common in boys with this syndrome. The commonly reported small hands and feet are not

always demonstrated in the newborn. Strabismus and hypopigmentation relative to the family are also common.

A history of poor fetal activity during the pregnancy can often be elicited, especially if the mother has had prior pregnancies. Consistent with the hypotonia, breech presentation and perinatal insults are found more frequently than usual. The extreme hypotonia begins to abate in the first year of life, and motor development improves, although developmental delay is the rule, especially for gross motor skills and speech. The feeding improves in the first few years of life and gives way to often uncontrollable hyperphagia and obesity. This issue and other behavioral problems, including severe temper tantrums, obsessive–compulsive disorder, and autism spectrum disorders, are encountered throughout life. Most patients manifest mild to moderate mental retardation. Early diagnosis and recognition of the various medical issues along with preemptive implementation of behavioral therapy are essential components of the optimal management of these issues.

Deletions of the region critical in PWS have been demonstrated in up to 70% of patients. The deletion can be detected by a chromosomal microarray or FISH analysis using a probe specific for this region of chromosome 15. A small number of patients have a disruption of this area as the result of a chromosomal translocation. To date, no single gene in this region has been implicated as the cause, but five genes are known to have paternal-only expression of protein-encoding genes (*MKRN3*, *MAGEL2*, *NECDIN*, *SNURF*, and *SNRPN*). It has been noted, however, that patients who have PWS as a result of a deletion of the region are more likely to be hypopigmented. This feature has been attributed to deletion of a gene involved in pigmentation, *OCA2* (Rinchik et al., 1993). Recurrence risks are negligible in cases in which de novo deletions are found and sporadic occurrence is usually encountered.

Approximately 20%–25% of patients with PWS show maternal UPD that can be detected by means of a molecular assay designed to assess specific methylation differences between maternal and paternal alleles. Methylation analysis findings are abnormal in more than 99% of affected individuals but will not determine whether the cause is a deletion or maternal UPD. Further study is required if an abnormal methylation result is obtained. A maternal age effect has been demonstrated in UPD cases, and recurrence risks in families without deletions are estimated at 1 in 1000. In addition, this region of the genome is subject to regulation by imprinting. Large chromosome-specific segmental duplications are found in 15q11 and have been implicated in mediating the recurrent deletion of this genomic region (reviewed in Emanuel and Shaikh, 2001; Emanuel and Saitta, 2007).

Angelman Syndrome

Loss of genetic material from the 15q11 region from the maternal copy of chromosome 15 is associated with Angelman syndrome. Clinical features are not evident in the newborn period and infancy but include significant mental retardation, seizures, ataxic gait, tongue thrusting, inappropriate bursts of laughter, and facial differences, including protruding jaw, wide mouth, thin upper lip, and widely spaced teeth. The mental retardation and hypopigmentation overlap with the features of PWS but Angelman syndrome is a distinct entity.

Seventy percent to 75% of patients have a deletion of 15q11 that is detectable by FISH analysis. A small percentage (3%–5%) have evidence of paternal isodisomy (two paternal copies) of this region of chromosome 15, with no apparent maternal chromosome contribution. Unlike PWS, Angelman syndrome has been associated

with mutations in a single gene, *UBE3A* (which encodes an enzyme involved in the ubiquitin pathway of protein degradation), that has been detected in up to 10% of patients. In addition, mutations of an imprinting center locus on chromosome 15 are associated with 1%–2% of Angelman phenotypes (Dagli et al., 2015). Methylation analysis can be performed and will detect abnormalities in approximately 75%–80% of patients because of a deletion or UPD. If methylation analysis findings are normal but Angelman syndrome is still suspected, *UBE3A* sequence analysis should be considered. The vast majority of cases result from a sporadic event, and the risk of recurrence can be best evaluated once the genetic mechanism has been determined for a given patient.

Beckwith–Wiedemann Syndrome

Beckwith–Wiedemann syndrome affects approximately 1 in 14,000 newborns and manifests itself as an overgrowth syndrome in the neonatal period. The characteristic findings are macrosomia, abdominal wall defect, and macroglossia (Fig. 20.10). Affected babies are large for their gestational age with proportionate length and weight. Infants of mothers with diabetes also manifest macrosomia but are more likely to have a weight disproportionately greater than length. Advanced bone age is also noted in Beckwith–Wiedemann syndrome. Hemihypertrophy caused by asymmetric growth is common, as is visceromegaly of various organs, including the spleen, kidneys, liver, pancreas, and adrenal glands.

Other characteristic features of the syndrome are macroglossia, linear creases of the earlobe with indentations on the posterior helix, and severe hypoglycemia. Although the hypoglycemia responds quickly to therapy, it can be present for several months; therefore recognition of the condition and immediate therapeutic intervention are critical in these cases. The hypoglycemia resolves spontaneously with age, and the physical diagnostic features also become less prominent with age, making the diagnosis more difficult to ascertain.

Equally important is the establishment of routine ultrasonographic surveillance at regular intervals, because children with Beckwith–Wiedemann syndrome are at increased risk of malignant tumors, especially Wilms tumor. The estimated risk is as high as 8% for patients with hemihypertrophy. Many centers currently perform ultrasonography at 3-month intervals until the school-age years (approximately 8 years old). Monitoring of serum alpha fetoprotein levels at the same intervals until age 4 years has proved



• **Fig. 20.10** Macrosomic Infant With Macroglossia and Lax Abdominal Musculature. These findings are typical of Beckwith–Wiedemann syndrome. (From Viljoen DL, Jaquire Z, Woods DL. Prenatal diagnosis in autosomal dominant Beckwith–Wiedemann syndrome. *Prenat Diagn.* 1991;11:167–175.)

valuable, as several cases of hepatoblastoma have also been reported and detected with this adjunct study.

Although most cases of Beckwith–Wiedemann syndrome appear to arise de novo, up to 15% may be familial. In familial cases, the transmission is autosomal dominant, because of mutations of the *CDKN1C* gene in 40% of familial cases but only in 5%–10% of de novo cases. In addition, this region of the genome (11p15.5) appears to be imprinted such that the maternal allele is not usually expressed. The insulin-like growth factor type 2 gene (*IGF2*) is located in this region and encodes an important factor involved in fetal growth. Mutations causing overexpression of the paternal allele or underexpression of the maternal allele can result in an imbalance of expression leading to the overgrowth and tumor formation encountered in these patients. Paternal UPD has proved to be a mechanism involved in 10%–20% of sporadic cases of Beckwith–Wiedemann syndrome. A method for detecting methylation abnormalities at two distinct genetic loci within 11p15 accounts for approximately 60% of patients and is related to the overexpression of the *IGF2* gene. Therefore all the available testing methods combined can detect the cause in approximately 85% of patients with Beckwith–Wiedemann syndrome. The recurrence risk for future affected siblings or offspring of the proband depends on the specific genetic abnormality causing the disorder and can range from low (UPD, methylation abnormality) to as high as 50% (*CDKN1C* mutation).

Russell–Silver Syndrome

Russell–Silver syndrome presents in neonates with intrauterine growth retardation followed by postnatal growth deficiency. The

head size is usually normal, causing a relative macrocephaly that may have the appearance of hydrocephalus. Facial features can include a broad and prominent forehead, triangular-shaped face with a small chin, and downturned corners of the mouth. The fingers can show brachydactyly, camptodactyly, or more commonly fifth-finger clinodactyly. Other concerns involve limb-length discrepancy, delayed bone age, café au lait macules, hypospadias in males, developmental delays, diaphoresis, and hypoglycemia during the first 3 years of life. These patients are often examined by a geneticist as a toddler with growth retardation, proportionate short stature, and normal head circumference. When one is evaluating patients with growth retardation, it becomes important to know the prenatal and postnatal growth parameters, because they might provide a clue to the diagnosis of Russell–Silver syndrome.

The molecular mechanisms underlying the pathogenesis show that Russell–Silver syndrome is likely caused by abnormalities of imprinted genes. Maternal UPD of chromosome 7 is present in 7%–10% of patients, and the symptoms are likely caused by overexpression of the maternal *GRB10* gene, which suppresses the activity of various growth factor receptors. In approximately 35% of patients, imprinting abnormalities of 11p15.5 occur because of a loss of the paternally expressed *IGF2* gene, leading to decreased prenatal and postnatal growth. This finding contrasts with that for some patients with Beckwith–Wiedemann syndrome, in whom *IGF2* is overexpressed, causing increased growth. For that reason, patients with Russell–Silver syndrome do not have a significantly increased risk of neoplasia compared with patients with Beckwith–Wiedemann syndrome, and routine cancer surveillance protocols are typically not recommended.

Summary

This chapter has summarized the rapidly expanding field of chromosomal and genomic disorders, concentrating on those that commonly manifest themselves in the newborn period. The widespread development and clinical implementation of molecular cytogenetic techniques have allowed the identification of subtle rearrangements that were previously undetectable. These advances now enable the discernment of new syndromes in which the chromosomal anomaly may be defined before a characteristic phenotype is recognized. In addition, a greater understanding of

the role of segmental duplications and their effects on human genetic disorders as well as of the influence of mechanisms that regulate gene expression, such as imprinting, is emerging. The tremendous advances in genomics led by the completion of the Human Genome Project and the development of new molecular diagnostic tools present new challenges for clinicians to better diagnose, understand, and care for patients with genetic disorders and their families.

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21

Introduction to Metabolic and Biochemical Genetic Diseases

STEPHEN CEDERBAUM

KEY POINTS

- Inborn errors may present in a variety of ways.
- Inborn errors should be systematically considered when one is evaluating an ill newborn.
- Inborn errors often present with unique or characteristic physical findings and also laboratory findings that help in developing a differential diagnosis.
- Newborn screening can be integrated into the evaluation of inborn errors.
- DNA sequencing can be used in the evaluation of sick infants.

Inborn errors of metabolism or biochemical genetic disorders are one type of genetic disease that may be encountered in the neonatal period as an acute or more indolent illness. In these disorders, a mutation in a gene leads to an absent or defective gene product or enzyme and results in the accumulation of the precursor of the enzyme or a byproduct of it, a shortage of the product of the enzymatic reaction, or a combination of both. In reality the effects of many inborn errors on normal physiology are much more profound, causing many changes in gene expression and normal biochemical function. One example is a case of propionic acidemia in which interference with the mitochondrial respiratory apparatus is rarely measured except by the ascertainment of an elevated blood lactate level, but that may be much more frequent and represent only the most obvious and easily measured alteration.

Inborn errors of metabolism may be inherited by any genetic mechanism—autosomal dominant, autosomal recessive, sex-linked recessive—or through a mutation in the independently inherited mitochondrial genome (mitochondrial DNA), which leads to a circumstance in which the mother alone passes the abnormal DNA to all of her children, but the affected or carrier father passes to none of his offspring.

Most inborn errors are inherited as autosomal recessive conditions, with the carrier parents rarely expressing any obvious metabolic phenotype. A small minority are inherited in a sex-linked recessive or codominant manner, and they will be discussed in the context of their particular disease. Examples include ornithine transcarbamylase

deficiency (a urea cycle disorder) and Fabry disease (a lysosomal storage disorder), the latter not appearing in the neonatal period.

When viewed from the perspective of disease mechanism, most genetic disorders, whether single-gene disorders or disorders involving imbalance of chromosomal materials, could be considered to be inborn errors of metabolism. One or more changes in the DNA result in either altered gene expression or expression of a mutated gene, which then leads secondarily to an altered product of the reaction or reactions. We will not use this expansive and grandiose interpretation of inborn errors but will rather confine ourselves to the more traditional definition described in the first paragraph. Thus disorders such as cystic fibrosis and spinal muscle atrophy, considered inborn errors that require broader interpretation, will not be considered.

The advent of newborn screening in the early part of the 1960s for phenylketonuria (PKU) established a new paradigm for approaching inborn errors of metabolism and making the diagnosis and treatment before the symptomatic presentation and hence preventing rather than treating the condition. This approach has proved to be remarkably successful for PKU and congenital hypothyroidism, with few patients becoming intellectually disabled. In subsequent years the menu of tests expanded gradually, but it has greatly expanded in the last decade with the implementation of expanded newborn screening using tandem mass spectrometry technology, which allows ascertainment of a constellation of disorders. This expanded testing should alter the probability of, and the diagnostic testing for, inborn errors when incorporated into the diagnostic algorithms of the ill newborn. Moreover, the advancing technology permits some of the newborn dried blood spot to be used for a wider palette of tests, some of which may already be available. The consequences of this expansion have a downside for the neonatologist and the neonatal intensive care unit staff members. For a variety of reasons the sick and premature newborn, usually receiving intravenous alimentation and having immature or damaged organs, is far more likely to have a false positive newborn screening test result and require follow-up testing. It is important to recognize that a number of traditionally tested diseases are outside this class of disorders, such as hypothyroidism, congenital adrenal hyperplasia, cystic fibrosis, and hemoglobinopathies.

When one is considering inborn errors of metabolism, it is important to consider the molecular basis of mutation. Large

deletions of a gene are certain to eliminate enzymatic function and any residual gene product. Smaller deletions, especially if they remove one or more of the in-phase coding triplets, may permit a stable protein to be made and to function to some extent. Most small deletions, however, cause the synthesis of unstable and out-of-phase proteins that do not function and that have a short half-life within the cell. Some single-base-change mutations can introduce a stop codon, causing the synthesis of the polypeptide to halt abruptly and leave a nonfunctional enzyme. Single-base changes introducing a new amino acid differ in their effects from complete loss of activity to a lesser impact and finally to having no effect whatsoever. When one considers that the mutation test is then all modulated through the unique genetic background of the individual, there is no single final phenotype, severity, or time of onset for any genetic disorder. This variation must be considered when any diagnosis, genetic or not genetic, is considered.

In the period since the publication of the previous edition of this book, DNA sequencing has become a much more accessible and affordable diagnostic modality, and it is likely to increase in relevance and use in the near-term future. When the diagnosis is established by biochemical criteria, mutation analysis, done electively, is likely to enhance our understanding of the disorder in an individual patient and help to guide therapy. When one is confronted with a more challenging and ambiguous patient, DNA testing in the form of whole exome or whole genome sequencing is likely to become the genetic diagnostic modality of choice.

Classification of Inborn Errors of Metabolism

Each professional uses classification systems to permit effective reasoning as to possible causes of a symptom complex. A system for understanding inborn errors of metabolism is shown in [Box 21.1](#). Each group has common characteristics, modes of presentation, types of molecules involved, and tests that would be applied. Because the demarcation between the groups is not sharp, other systems can see them differently. This discussion is restricted to those disorders that may be symptomatic in the newborn period or in early infancy, whereas many severe disorders would be unlikely to be associated with neonatal disease and will be given less emphasis.

• BOX 21.1 Classification of Inborn Errors of Metabolism, 2007

Small-molecule disorders

- Amino acids
- Organic acids
- Sugars

Lysosomal storage disorders

- Mucopolysaccharides
- Sphingolipids
- Glycolipids

Energy metabolism disorders

- Oxidation disorders
- Fatty acid mobilization and metabolism disorders
- Glycogen storage diseases

Peroxisomal and membrane biogenesis disorders

Carbohydrate-deficient glycoprotein disorders

Cholesterol biosynthetic disorders

Disorders of biogenic amines, folate, and pyridoxine

Transport disorders

Purine and pyrimidine metabolism disorders

Receptor disorders

The disorders more commonly seen in the neonatal period are listed in [Box 21.2](#).

The first group consists of newborns with progressive lethargy, poor suck, neurologic deterioration, and often death. They have inborn errors of amino acids, the urea cycle, organic acids, or sugar metabolism. This group of patients is the product of normal pregnancies and deliveries and becomes ill after 36 hours of life, when the maternal circulation no longer cleanses the accumulating small molecules from the fetal or newborn blood, and the offending metabolites accumulate in intoxicating amounts ([Box 21.3](#)). Examples include maple syrup urine disease, methylmalonic and propionic acidemias, galactosemia, and ornithine transcarbamylase deficiency. The general characteristics are given in [Box 21.4](#); they are the disorders for which expanded newborn screening may lead to earlier detection and a more rapid diagnosis. These patients' condition is most likely to resemble sepsis, and they should be treated with antibiotics. Most disorders manifesting themselves acutely in the newborn period will be detected by the newborn screen, with only some urea cycle disorders and lactic acidoses likely to be missed by this screening panel. When diagnosed, these conditions are treated with dialysis, limitation of protein intake (except for galactosemia), fluid, and caloric support and some specific interventions. The association of identifying physical and laboratory characteristics and various disorders is listed in [Tables 21.1–21.2](#). The individual disorders are discussed in Chapters 22 and 23. When a diagnosis of a metabolic disorder appears

• BOX 21.2 Common Types of Inborn Errors of Metabolism With Newborn Presentation

- Amino acid disorders
- Organic acid disorders
- Disorders of ammonia metabolism
- Disorders of carbohydrate metabolism
- Disorders of gluconeogenesis or hypoglycemia
- Disorders of fatty acid oxidation
- Primary lactic acidoses (respiratory chain defects)
- Disorders of vitamin or metal metabolism
- Storage diseases (infrequently)
- Peroxisomal disorders
- Disorders of sterol metabolism
- Congenital defects in glycosylation

• BOX 21.3 Metabolic Diseases With Newborn Coma Secondary to Toxic Metabolite Accumulation or Mitochondrial Failure

- Galactosemia
- Inborn errors of ammonia metabolism
- Maple syrup urine disease
- Nonketotic hyperglycinemia
- Methylmalonic acidemia with or without homocystinuria
- Propionic acidemia
- Isovaleric acidemia
- Multiple carboxylase deficiency
- Glutaric aciduria type 2
- Fatty acid oxidation defects
- Primary lactic acidosis
- Pyruvate dehydrogenase deficiency
- Pyruvate carboxylase deficiency
- Mitochondrial respiratory chain or electron transport chain defects

• BOX 21.4 Characteristics of Small-Molecule Disorders

- High levels of metabolites in body fluids
 - Normal physical phenotype
 - Neonatal presentation
 - Periods of stability and instability
 - Considered to be intoxication disorders
 - Can often be treated by external manipulation
- Lysosomal storage disorders
- Usually born normally
 - Course is progressive, relentless, and indolent
 - Deposition of material seen clinically and microscopically
 - May be deforming
 - Cannot be addressed exogenously by dietary means
- Disorders of energy metabolism
- Mixed presentation between the first two categories
 - Can be catastrophic at presentation
 - May be present at birth or develop later
 - Usually progressive
 - May cause malformations
 - May have episodes of deterioration
 - Usually not treatable by dietary means
 - May be tissue specific or preferential

likely, tests for plasma amino acids, urine organic acids, plasma acylcarnitine, and plasma carnitine should be repeated, and ammonia and lactate levels should be determined.

The second major category of inborn errors is the lysosomal storage diseases. This group of disorders results from defective function of a catabolic hydrolase located in the lysosome that is generally responsible for breaking down complex glycosaminoglycans and sphingolipids that are products of normal cellular turnover (see [Box 21.4](#)). Unlike the small-molecule disorders in which the metabolites are found freely circulating in the body fluid compartments, these compounds accumulate intracellularly, are not removed by the maternal circulation, and are present in limited amounts in the body fluids. They most often cause no apparent symptoms in the newborn period or early infancy, because the pathologic metabolites accumulate slowly with time. Exceptions to this finding are the severe form of α -glucosidase deficiency or Pompe disease, the neonatal form of α -galactosidase deficiency, or Krabbe disease and galactosialidosis. These findings are discussed in Chapter 23, and some are listed in [Table 21.1](#). The disorders of mucopolysaccharides and glycolipids lead to the characteristic features pejoratively and inappropriately referred to as *gargoylism*, which consist of an exaggerated eyebrow, coarse-appearing facies, thick skin, hirsutism, and multiple abnormalities of the bones and joints seen on a radiograph. Attention to the disorder is often drawn by the hepatosplenomegaly. The metabolites are synthesized in the body and are not influenced by dietary intake.

The third important category of metabolic disorders is insufficient generation of energy by the mitochondrial machinery. These disorders can be caused by the inability to provide substrates such as glucose in glycogenoses; the inability to deliver substrate to the site of oxidation, such as the fatty acid and carnitine transport disorders; the inability to break down fatty acids in a stepwise fashion to provide reduced flavin adenine dinucleotide to be oxidized; or the deficient function of the mitochondrial respiratory pathway and energy-generating system itself. These disorders have characteristics in between those of the acute, small-molecule disorders and the storage disorders (see [Box 21.4](#)). They differ

• BOX 21.5 Signs and Symptoms of Inborn Errors in the Newborn

- Neonatal catastrophe (life threatening)
- Poor suck and feeding
- Gastrointestinal problems, vomiting
- Respiratory distress
- Cardiac failure
- Neurologic abnormalities: alertness, tone, seizures
- Organomegaly
- Ocular abnormalities
- Cutaneous changes

• BOX 21.6 Metabolic Diseases With Congenital Malformations or Dysmorphic Features

- Cholesterol biosynthetic disorders
- Peroxisomal disorders
- Glutaric aciduria type 2
- Primary lactic acidoses
- Congenital defects in glycosylation
- Lysosomal storage disorders
- Menkes disease

from the small-molecule disorders in the possible onset immediately at birth or before and from storage disorders in the generally normal physical features with hepatomegaly alone, a regular feature of glycogen storage disorders. The small-molecule and energy-generating disorders are discussed in Chapter 22, and the lysosomal storage disorders are discussed in Chapter 23.

Of the remaining groups that are encountered less frequently, the peroxisomal biogenesis disorders, carbohydrate-deficient glycoprotein disorders, and Smith–Lemli–Opitz syndrome (a cholesterol biosynthetic disorder) are discussed in Chapter 23. Other disorders are too infrequent to be considered in a general neonatology textbook. Disorders of biogenic amines are discussed in Chapter 22 and in greater depth in Chapter 65 on neonatal seizures, along with consideration of pyridoxine and folate disorders.

Signs and Symptoms of Inborn Errors

The limited symptomatic repertoire of the sick newborn is well established, but it is worth repeating. For this reason, the first thought when one is confronting a newborn in a deteriorating condition, with lethargy, poor suck, temperature instability, and neurologic abnormalities, is sepsis ([Box 21.5](#)). Most metabolic specialists have never confronted a sick newborn who has not had a “septic work-up” and who is not receiving standard antibiotics. The issue then becomes when to perform a metabolic work-up. The standard answer is that it should be performed when the neonatologist is concerned that the newborn in extremis does not fit the pattern that is expected from a child with sepsis or hypoxia. That threshold will differ by individual. Negative results of tests for infectious agents, a nonconfirmatory white blood cell count, hypoglycemia, unexpectedly severe acidosis, or hyperammonemia could be important triggers. Although dysmorphic features are not characteristic of inborn errors, there are some that may have subtle or occasionally pronounced abnormality on a physical examination; they are listed in [Box 21.6](#) and [Table 21.2](#).

TABLE 21.1**Unique or Characteristic Physical Findings in Inborn Errors (Major Examples)**

| Finding | Error | Finding | Error |
|----------------------------------|--|---|--|
| Hepatomegaly | Galactosemia Glycogen storage diseases Gluconeogenic defects Disorders of fatty acid oxidation and transport Mitochondrial respiratory or electron transport chain defects Hereditary tyrosinemia type 1 Urea cycle defects Peroxisomal defects Niemann–Pick disease type C Congenital defects in glycosylation | Retinitis pigmentosa | Mitochondrial respiratory or electron transport chain defects Sjögren–Larsson syndrome Peroxisomal disorders Abetalipoproteinemia |
| Hepatosplenomegaly | Gangliosidoses Niemann–Pick disease type C Mucopolysaccharidoses Wolman disease Ceramidase deficiency | Optic atrophy or hypoplasia | Pyruvate dehydrogenase complex deficiency Mitochondrial disorders Leigh disease Peroxisomal disorders |
| Macrocephaly | Glutaric acidemia type 1 Canavan disease | Corneal clouding or opacities | Mucopolipidoses Mucopolysaccharidoses Steroid sulfatase deficiency |
| Microcephaly | Mitochondrial respiratory or electron transport chain defects Leigh disease Methylmalonic acidemia with homocystinuria | Cataracts | Galactosemia Lowe syndrome Mitochondrial respiratory or electron transport chain defects Peroxisomal disorders Congenital defects in glycosylation |
| Coarse facial features | Gangliosidosis Mucopolipidoses Mucopolysaccharidosis type VII Sialidosis Galactosialidosis | Dislocated lens | Methionine synthetase deficiency Sulfite oxidase deficiency |
| Macroglossia | Pompe disease Gangliosidoses Mucopolysaccharidoses Mucopolipidoses | Bone or limb deformities or contractures | Storage, peroxisomal, or connective tissue disorders Inborn errors of cholesterol biosynthesis |
| Dystonia or extrapyramidal signs | Gaucher disease type 2 Glutaric acidemia type 1 Krabbe disease Crigler–Najjar syndrome Bioppter defects | Thick skin | Mucopolipidoses Gangliosidoses Mucopolysaccharidoses |
| Macular “cherry-red spot” | GM1 gangliosidosis Galactosialidosis Niemann–Pick disease type A Tay–Sachs disease (GM2 gangliosidosis) | Desquamating, eczematous, or vesiculobullous skin lesions | Acrodermatitis enteropathica Organic acidemias Early-onset forms of porphyria |
| | | Ichthyosis | Gaucher disease type 2 Steroid sulfatase deficiency |
| | | Alopecia | Multiple carboxylase deficiency |
| | | Steely or kinky hair | Menkes disease |
| | | Persistent diarrhea | Glucose galactose malabsorption Congenital lactase deficiency Congenital chloride diarrhea Sucrase isomaltase deficiency Acrodermatitis enteropathica Congenital folate malabsorption Wolman disease Galactosemia |

For discussion of specific disorders, see Chapters 22 and 23.

Modern neonatology has one tool that was previously unavailable: the expanded newborn screen. This screening will diminish the probability of many disorders, and the newborn screening follow-up hotline should be on the speed dial of every neonatal intensive care unit. The only acutely presenting disorders not ascertained by these studies are most hyperammonemias and lactic acidoses. These test results are available immediately in any tertiary or secondary care hospital. With a high level of concern and near normal

levels of lactate and ammonia, the standard battery of metabolic studies should be performed only when the index of suspicion for a metabolic disorder is particularly high and no alternative explanation for the poor condition of the patient is likely. When deemed necessary, these studies should include plasma amino acid levels, plasma acylcarnitine levels, and urinary organic acid levels. The abnormalities associated with individual disorders are discussed in Chapter 22.

**TABLE
21.2****Characteristic or Unique Laboratory or Diagnostic Testing Outcomes in Inborn Errors (Major Examples)**

| Outcome | Error | Outcome | Error |
|--|---|---------------------------------------|--|
| Metabolic acidosis with or without increased anion gap | Organic acidemias Maple syrup urine disease Fatty acid oxidation defects β -Ketothiolase deficiency Ketogenesis defects Disorders of pyruvate metabolism Mitochondrial respiratory chain or electron transport chain defects, including Leigh disease Galactosemia Glycogen storage disease type 1 Gluconeogenesis defects | Thrombocytopenia | Organic acidemias Pearson syndrome |
| Respiratory alkalosis | Urea cycle disorders | Anemia | Organic acid disorders Wolman disease Pearson syndrome Severe liver failure Galactosemia |
| Hyperammonemia | Urea cycle disorders Methylmalonic acidemia Organic acidemias Fatty acid oxidation disorders | Vacuolated lymphocytes or neutrophils | Lysosomal storage disorders |
| Ketosis | Organic acidemias Maple syrup urine disease Glutaric acidemia type 2 Ketogenesis defects Glycogen storage disease type 1 Gluconeogenesis disorders | Cardiomegaly | Pompe disease Barth syndrome Fatty acid oxidation defects Mitochondrial respiratory or electron transport chain defects Carbohydrate-deficient glycoprotein syndrome |
| Lactic acidosis | Mitochondrial respiratory or electron transport chain defects, including Leigh disease Pyruvate dehydrogenase complex deficiency Pyruvate carboxylase deficiency Organic acidemias Glutaric acidemia type 2 Fatty acid oxidation defects Ketogenesis defects Glycogen storage disease type 1 Gluconeogenesis disorders | Electrocardiographic abnormalities | Pompe disease (short PR interval, large QRS interval) Fatty acid oxidation disorders Mitochondrial respiratory or electron transport chain defects |
| Hypoglycemia | Hyperinsulinism Glycogen storage disease type 1 Gluconeogenesis disorders Maple syrup urine disease Glutaric acidemias Fatty acid oxidation defects Ketogenesis defects Galactosemia Severe liver failure Mitochondrial respiratory or electron transport chain defects | Ventricular hypertrophy | Pompe disease Organic acidemias Glutaric acidemia type 2 Fatty acid oxidation defects Mitochondrial respiratory or electron transport chain defects, including Leigh disease |
| Lipemia | Glycogen storage disease type 1 Lipoprotein lipase deficiency | Dysostosis multiplex | Gangliosidoses Mucopolysaccharidoses Mucopolipidoses Sialidosis |
| Positive urinary-reducing substances | Galactosemia Hereditary fructose intolerance Lowe syndrome | Stippled calcifications of patellae | Peroxisomal disorders Cholesterol biosynthetic defects |
| Discolored urine | Alkaptonuria Tryptophan malabsorption | Adrenal calcifications | Wolman disease |
| Leukopenia | Organic acidemias Glycogen storage disease type 1B Barth syndrome Pearson syndrome | Rhizomelia | Rhizomelic chondrodysplasia punctata |
| | | Hair abnormalities | Menkes disease Argininosuccinicaciduria |
| | | Basal ganglia lesions on MRI | Organic acidemias (later in life) Pyruvate dehydrogenase complex deficiency Mitochondrial respiratory or electron transport chain defects, including Leigh disease |
| | | Cerebellar atrophy or hypoplasia | Pyruvate dehydrogenase complex deficiency Mitochondrial respiratory or electron transport chain defects, including Leigh disease Carbohydrate-deficient glycoprotein syndrome |
| | | Agenesis of corpus callosum | Pyruvate dehydrogenase complex deficiency Pyruvate carboxylase deficiency Mitochondrial respiratory or electron transport chain defects |

MRI, Magnetic resonance imaging.

Emergency Treatment

An acutely ill child with an inborn error of metabolism is an emergency, and rapid rescue treatment is mandatory. When one is considering a differential diagnosis, special emphasis should be placed on disorders for which there is treatment, as opposed to those for which there is no treatment. As with all acutely ill patients, supportive care, including cardiorespiratory, hemodynamic status, fluids, and electrolytes, are the mainstays of treatment. Transfer from institution to institution should not be performed unless the patient's condition is stable and there is adequate vascular access for emergency treatment. Transfer to a tertiary care center with experience in caring for these children is desirable and should be performed as quickly as possible.

Virtually all disorders require a maximum source of calories (150 cal/kg is desirable, but 120 cal/kg is a minimum target) to prevent or diminish catabolism, and glucose is the most important part of this, especially in the absence of primary lactic acidemia or acidosis. As much lipid as is safe should be added to compensate for the caloric deficit.

Hemodialysis will remove most metabolites rapidly, but this is an extreme intervention in a newborn. It should be performed routinely in extreme and symptomatic hyperammonemia (ammonia levels of 400 mol/L or more), and many would consider dialysis in severe acidosis caused by acids other than lactate or ketones. Once a diagnosis has been established, specific therapy can be

initiated. It is important to emphasize that during this period of generic therapy, prolonged deprivation of exogenous protein or amino acids will cause endogenous protein breakdown and exacerbate the metabolic process. As a result, protein is added to the intravenous support fluids after 36–48 hours, beginning with 0.25–0.5 g/kg per day and increasing gradually to maintenance levels of 1.2–1.5 g/kg per day, pending a definitive diagnosis and assuming that it is tolerated.

Suggested Readings

- Online Mendelian Inheritance in Man, OMIM. McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, Md.) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, Md.), Available at <http://www.omim.org>.
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22

Inborn Errors of Carbohydrate, Ammonia, Amino Acid, and Organic Acid Metabolism

J. LAWRENCE MERRITT, II, AND RENATA C. GALLAGHER

KEY POINTS

- The establishment of universal newborn screening (NBS) for disorders of fatty acid, organic acid, and amino acid metabolism in the United States is a major public health accomplishment of the first decade of the 21st century.
- Early identification and treatment of affected newborns have decreased the morbidity and mortality associated with many of these conditions.
- Multiple disorders of intermediary and energy metabolism are *not* detected through NBS.
- The appropriate testing, including urine organic acid analysis, plasma acylcarnitine profile, and plasma amino acid analysis, should be performed in any symptomatic patient, even if the newborn screen result was “normal.”

Acute, life-threatening disease during the newborn period is a feature of many inborn errors of metabolism, including those of ammonia, carbohydrate, amino acid, fatty acid, ketone, and mitochondrial energy metabolism. Therefore it is critical that neonatologists are familiar with the clinical symptoms, laboratory findings, methods of diagnosis, and empiric—as well as specific—management of each of these classes of disease (Table 22.1). Importantly, while newborn screening (NBS) for some (but not all) disorders within each class has resulted in presymptomatic identification, allowing early institution of therapy and improved outcome for many affected individuals, clinical presentation before the development of symptoms may still occur because of environmental and biologic factors, as well as local NBS and follow-up protocols. Factors that prevent or delay identification by NBS include when the disease is not detected by NBS or is incompletely ascertained, when the newborn develops symptoms before the NBS result being reported, and when follow-up testing has not been completed (see Key Points). Thus familiarity with these diseases and their characteristic signs and symptoms is critical for early clinical recognition and the initiation of potentially life-saving empiric management (Table 22.2). Inborn errors of metabolism should also be considered in infants who develop symptoms outside of the immediate newborn period, as each of these disorders can have a later-onset presentation.

Carbohydrate Metabolism Disorders

Galactosemia

Elevated blood galactose, or *galactosemia*, is a result of a defect in one of three enzymes of the galactose metabolic pathway that converts galactose to glucose (Fig. 22.1). The disorder most clinically relevant in the newborn period is severe *galactose-1-phosphate uridylyltransferase* (GALT) deficiency. It is a cause of neonatal jaundice and coagulopathy and is life threatening. It is commonly referred to as “classic galactosemia.” This condition is the primary target of NBS for galactosemia. The other two enzyme defects that cause elevated blood galactose are *galactokinase* and *uridine diphosphate galactose-4-epimerase* deficiencies. Clinical features associated with each disorder are described later. In classic galactosemia, on the ingestion of lactose, a disaccharide of glucose and galactose, the substrate of the enzyme galactose-1-phosphate accumulates, as does galactose and the secondary metabolites galactitol and/or galactonate. Elevations of galactitol may cause characteristic “oil drop” cataracts that may be present at birth. The roles of the other metabolites in pathogenesis of the liver, kidney, brain, and ovarian dysfunction of severe GALT deficiency are not understood. They likely include the deficiency of galactose-1-phosphate conjugated to uridine, as well as toxicity of accumulating metabolites.

The frequency of classic galactosemia is estimated to be 1 in 60,000 to 1 in 75,000 births in the United States and Europe (Varela-Lema et al., 2016). There are also milder forms of unclear clinical significance, such as Duarte variant galactosemia, with enzyme activity of roughly 25% of wild type, which are frequently identified by abnormal NBS but are currently believed not to require treatment (Welling et al., 2017).

NBS methods for identification of classic galactosemia vary by state and include measurement of “total galactose” (galactose plus galactose-1-phosphate) and GALT enzyme activity (Pyhtila et al., 2015). Testing after an abnormal NBS may include DNA analysis to identify common mutations in the *GALT* gene. In states that measure analytes, children with galactokinase or epimerase deficiencies may be identified. These disorders will not be detected through screening for deficient GALT enzyme activity alone. The accuracy of NBS is dependent on information provided by the ordering

**TABLE
22.1****Presentations of Inborn Errors of Metabolism**

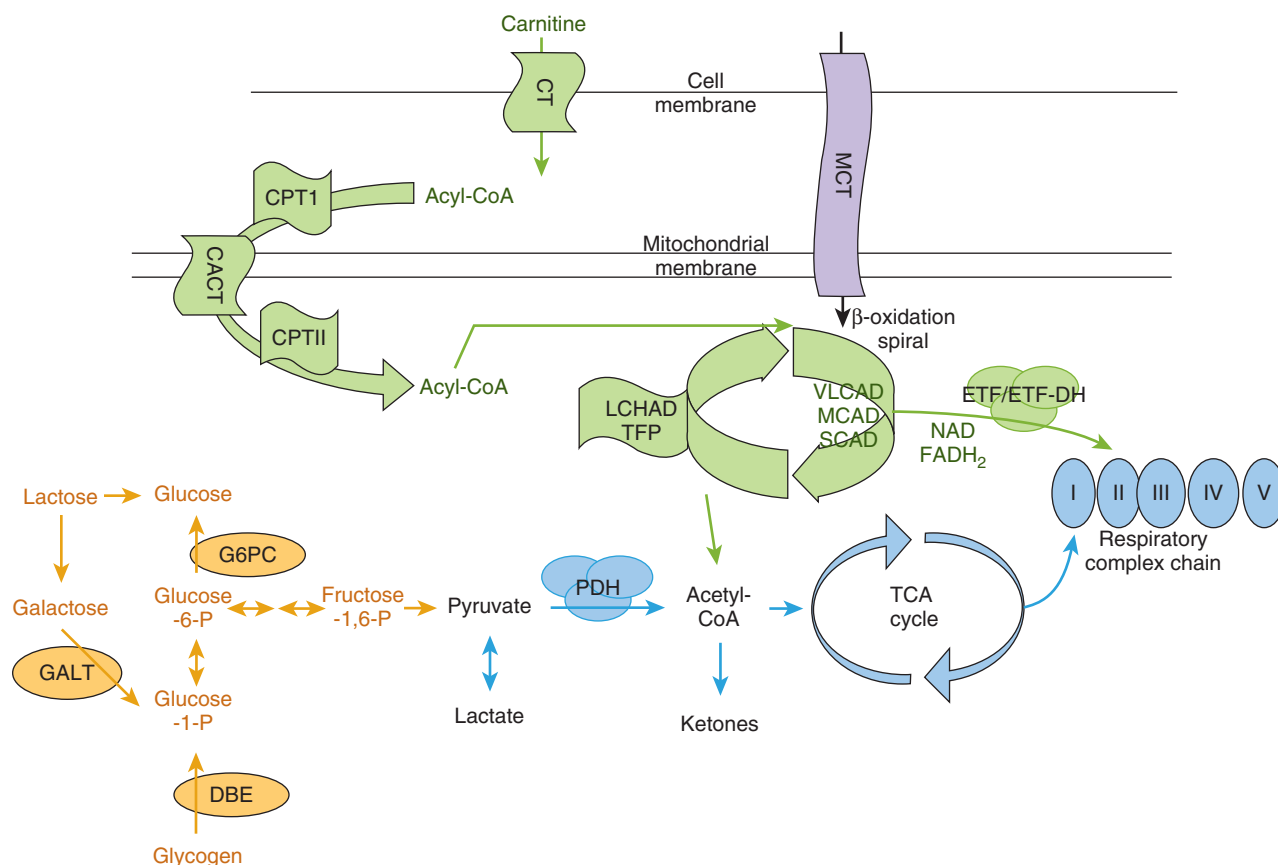
| | Newborn/ Early Onset | Acute Presentation | Chronic Presentation | Currently on Newborn Screening Panels |
|--|-------------------------|-----------------------|-------------------------|--|
| Carbohydrates | | | | |
| - Galactosemia | Yes | Yes | Yes | Yes |
| - Epimerase deficiency | Rare | Yes | Yes | Possibly |
| - Galactokinase deficiency | No | No | Yes | Possibly |
| - GSD Ia and Ib | Rare | Yes | Yes | No |
| - GSD II | Yes | Yes | Yes | Possibly |
| - GSD IV | Yes | Yes | Yes | No |
| - Hereditary fructose intolerance | No | Yes | Yes | No |
| - Fructose-1,6-bisphosphatase deficiency | No | Yes | No | No |
| Urea Cycle Disorders | | | | |
| - All types | Yes | Yes | Yes | Not all |
| - Transient hyperammonemia of the newborn | Yes | Yes | No | No |
| Aminoacidemias | | | | |
| - MSUD | Yes | Yes | Yes | Yes |
| - Tyrosinemia type 1 | No | Rare | Yes | Yes |
| - Nonketotic hyperglycinemia | Yes | Yes | Yes | No |
| - Cystathionine β -synthase deficiency | Rare | Yes | Yes | Yes |
| - Remethylation disorders | Yes | Yes | Yes | Possibly |
| - Phenylketonuria | No | No | Yes | Yes |
| Organic Acidemias | | | | |
| - Methylmalonic acidemia(s) | Yes | Yes | Yes | Yes |
| - Propionic acidemia | Yes | Yes | Yes | Yes |
| - Isovaleric acidemia | Yes | Yes | Yes | Yes |
| - Holocarboxylase synthase deficiency | Yes | Yes | Yes | Yes |
| - Biotinidase deficiency | No | Possibly | Yes | Yes |
| - Glutaric acidemia type 1 | Rare | Yes | Yes | Yes |
| Fatty Acid Oxidations Disorders | | | | |
| - MCADD | Yes | Yes | Yes | Yes |
| - VLCADD | Yes | Yes | Yes | Yes |
| - SCADD | No | Rare | Rare | Yes |
| - LCHADD and TFP | Yes | Yes | Yes | Yes |
| - CTD | Yes | Yes | Yes | Yes |
| - CPTI | Yes | Yes | Yes | Yes |
| - CACT | Yes | Yes | Yes | Yes |
| - CPTII | Yes | Yes | Yes | Yes |
| - MADD | Yes | Yes | Yes | Yes |
| Ketone Metabolism Disorders | | | | |
| - Mitochondrial acetoacetyl-CoA thiolase deficiency | Yes | Yes | Rare | Yes |
| - HMG-CoA lyase deficiency | Yes | Yes | Yes | Yes |
| - Succinyl-CoA 3-ketoacid-CoA transferase deficiency | Yes | Yes | Rare | No |
| Mitochondrial Disorders | | | | |
| - Primary lactic acidosis | Yes | Yes | Yes | No |
| - Pyruvate dehydrogenase complex deficiency | Yes | Yes | Yes | No |
| - Pyruvate carboxylase deficiency | Yes | Yes | Yes | No |
| - Electron chain deficiencies | Yes | Yes | Yes | No |
| - Leigh disease | Rare | Yes | Yes | No |
| - Pearson syndrome | Yes | Yes | Yes | No |
| - Barth syndrome | Yes | Yes | Yes | No |

CACT, Carnitine acylcarnitine translocase deficiency; CoA, coenzyme A; CPTI, carnitine palmitoyltransferase type I deficiency; CPTII, carnitine palmitoyltransferase type II deficiency; CTD, carnitine transporter deficiency; GSD, glycogen storage disorder; HMG, 3-hydroxy-3-methylglutaryl; LCHADD, long-chain acyl-CoA dehydrogenase deficiency; MADD, multiple acyl-CoA dehydrogenase deficiency; MCADD, medium-chain acyl-CoA dehydrogenase; MSUD, maple syrup urine disease; SCADD, short-chain acyl-CoA dehydrogenase deficiency; TFP, trifunctional protein deficiency; VLCADD, very long chain acyl-CoA dehydrogenase deficiency.

TABLE 22.2 Treatments for Inborn Errors of Metabolism

| | Dietary | Medications | Vitamin Supplementation | Other (Dialysis, Transplantation) |
|---|----------|-------------|-------------------------|-----------------------------------|
| Carbohydrates | | | | |
| - Galactosemia | Yes | No | Yes | - |
| - Epimerase deficiency | Yes | No | Yes | - |
| - Galactokinase deficiency | Yes | No | Yes | - |
| - GSD Ia and Ib | Yes | Yes | Yes | Liver, kidney Txp |
| - GSD II | Yes | Yes | No | - |
| - GSD IV | Yes | No | No | Liver, cardiac Txp |
| - Hereditary fructose intolerance | Yes | No | No | - |
| - Fructose-1,6-bisphosphatase deficiency | Yes | No | No | - |
| Urea Cycle Disorders | | | | |
| - All types | Yes | Yes | Yes | Liver Txp, HD |
| - Transient hyperammonemia of the newborn | No | Yes | No | HD |
| Aminoacidemias | | | | |
| - MSUD | Yes | No | Yes | Liver Txp, HD |
| - Tyrosinemia type 1 | Yes | Yes | No | Liver Txp |
| - Nonketotic hyperglycinemia | No | Yes | No | - |
| - Cystathionine synthase deficiency | Yes | Yes | Yes | - |
| - Remethylation disorders | Yes | Yes | Yes | HD |
| - Phenylketonuria | Yes | Yes | No | - |
| Organic Acidemias | | | | |
| - Methylmalonic acidemia | Yes | Yes | Some forms | Liver Txp, HD |
| - Propionic acidemia | Yes | Yes | No | Liver Txp, HD |
| - Isovaleric acidemia | Yes | Yes | Yes | HD |
| - Holocarboxylase synthase deficiency | Variable | No | Yes | - |
| - Biotinidase deficiency | No | No | Yes | - |
| - Glutaric acidemia type 1 | Yes | Yes | Yes | - |
| Fatty Acid Oxidations Disorders | | | | |
| - MCADD | Variable | Yes | No | - |
| - VLCADD | Yes | Yes | No | Cardiac Txp |
| - SCADD | No | Variable | No | - |
| - LCHADD and TFP | Yes | Yes | No | Cardiac Txp |
| - CTD | No | Yes | No | - |
| - CPTI | Yes | Yes | No | - |
| - CACT | Yes | Yes | No | - |
| - CPTII | Yes | Yes | No | - |
| - MADD | Yes | Yes | Yes | - |
| Ketone Metabolism Disorders | | | | |
| - BK thiolase deficiency | Yes | Yes | No | - |
| - HMG-CoA lyase deficiency | Yes | Yes | No | - |
| - SCOT deficiency | Yes | Yes | No | - |
| Mitochondrial Disorders | | | | |
| - Primary lactic acidosis | No | No | Some forms | - |
| - Pyruvate dehydrogenase complex deficiency | Yes | Yes | Yes | - |
| - Pyruvate carboxylase deficiency | No | Yes | No | - |
| - ETC defects | No | Variable | Some forms | - |
| - Leigh disease | No | Variable | Some forms | - |
| - Pearson syndrome | No | Yes | Yes | - |
| - Barth syndrome | Yes | Yes | Variable | Cardiac Txp |

BK, Beta-keto or mitochondrial acetoacetyl-CoA; CACT, carnitine acylcarnitine translocase deficiency; CoA, coenzyme A; CPTI, carnitine palmitoyltransferase I; CPTII, carnitine palmitoyltransferase II; CTD, carnitine transporter deficiency; ETC, electron transport chain; HD, hemodialysis; HMG, 3-hydroxy-3-methylglutaryl; LCHADD, long-chain acyl-CoA dehydrogenase deficiency; MADD, multiple acyl-CoA dehydrogenase deficiency; MCADD, medium-chain acyl-CoA dehydrogenase deficiency; SCADD, short-chain acyl-CoA dehydrogenase deficiency; SCOT, succinyl-CoA 3-ketoacid-CoA transferase; TFP, trifunctional protein deficiency; Txp, transplantation; VLCADD, very long chain acyl-CoA dehydrogenase deficiency.



• **Fig. 22.1** Overview of Metabolism of Carnitine Transport, Fatty Acid Oxidation, Glucose, TCA Cycle, and Mitochondrial Respiratory Chain. *CACT*, Carnitine acylcarnitine translocase; *CT*, carnitine transporter; *CPTI*, carnitine palmitoyltransferase I; *CPTII*, carnitine palmitoyltransferase II; *FADH₂*, flavin adenine dinucleotide; *DBE*, debranching enzyme; *GALT*, galactose-1-phosphate uridylyltransferase; *Acyl-CoA*, long-chain fatty acid attached to Coenzyme A; *LCHAD*, long-chain 3-hydroxy acyl-CoA dehydrogenase; *ETF/ETF-DH*, electron transfer flavoprotein/electron transfer flavoprotein dehydrogenase; these are deficient in *MADD*; *MADD*, multiple acyl-CoA dehydrogenase deficiency; *MCAD*, medium-chain acyl-CoA dehydrogenase; *MCT*, medium-chain triglyceride; *NAD*, nicotinamide adenine dinucleotide; *PDH*, pyruvate dehydrogenase; *SCAD*, short-chain acyl-CoA dehydrogenase; *TCA*, tricarboxylic acid; *TFP*, trifunctional protein; *VLCAD*, very long chain acyl-CoA dehydrogenase.

nursery, as transfused red blood cells may cause a false-negative NBS for galactosemia. (Transfusion of red blood cells will also impact detection of hemoglobinopathies.) Infants transfused before NBS require follow-up testing for galactosemia and hemoglobinopathies at least 4 weeks after transfusion. Also, infants who have not received a lactose-containing feeding (i.e., breast milk or nonsoy-based formulas) before screening may not have elevated galactose levels and may have false-negative NBS if the screening method is analyte based. False-positive NBS results can occur in hot weather if the screening method is enzyme activity, as the enzyme is denatured in heat. Mutation analysis and enzyme activity help identify neonates with classic galactosemia, who have a poorer prognosis than those with variant (nonclassic) galactosemia.

At presentation, total blood galactose levels may be elevated with elevated red blood cell galactose-1-phosphate (Gal-1-P) and urine galactitol levels. During this phase of severe hypergalactosemia, positive reducing substances will be present in urine, but these resolve within hours with dietary restriction of galactose. There may be factitious elevation of glucose in affected individuals, as measured by bedside glucometers (Ozbek et al., 2015). Following the initiation of treatment with a lactose-free diet, Gal-1-P levels decrease but rarely normalize, remaining elevated for the lifetime

of the patient. There is a target range for treatment, and maintenance of Gal-1-P levels in treatment range through dietary restriction of galactose is the current goal of short- and long-term dietary therapy (Welling et al., 2016).

Untreated classic galactosemia may present with severe multi-organ (liver and kidney) disease in the first days to weeks of life. The most common clinical symptoms are vomiting, poor feeding, failure to thrive, and lethargy, with jaundice and hepatomegaly on physical examination. About 10% of affected neonates develop sepsis, often due to *Escherichia coli*; other causative organisms include *Klebsiella* and *Enterobacter* (Berry, 1993). Characteristic laboratory findings are indirect hyperbilirubinemia and apparent liver failure with markedly elevated coagulation parameters out of proportion to the transaminase elevations. With continued lactose/galactose ingestion, liver disease can progress to cirrhosis with portal hypertension and splenomegaly. Renal Fanconi syndrome may also develop. Cataracts may develop in the first few weeks of life in untreated individuals, while some neonates are born with cataracts.

Management of the liver disease involves dietary restriction of galactose and supportive care. The coagulopathy resolves over several days. A lactose-free formula should be initiated as soon as classic

galactosemia is suspected as this can be life-saving. An early change to soy formula may mask the symptoms of the disease. With early dietary restriction, improvement has been seen in severe clinical presentations and in neonatal mortality (Varela-Lema et al., 2016). While growth and feeding return to normal, treated individuals may develop long-term complications including speech and language disorders, cognitive and learning problems, cataracts, primary ovarian insufficiency (in most females), tremor, dystonia, coordination problems, or severe ataxia (Varela-Lema et al., 2016). The cause of these complications is unknown, and they may not be improved by dietary restriction (Waisbren et al., 2012).

Patients with classic galactosemia are advised to continue a lactose-restricted diet for their entire lives, but this diet does have significant risk of calcium and vitamin D deficiencies, and these must be supplemented. Dairy and high galactose nondairy foods including fruits, legumes, and vegetables are restricted. Not all galactose is exogenous; there is some endogenous galactose production. Treatment compliance is monitored through frequent assessment of Gal-1-P levels. The restriction of galactose-containing fruits and vegetables may not be necessary lifelong (Van Calcar et al., 2014). The impact of treatment for Duarte variant versus no treatment is unclear, and clinical practice remains highly variable across the United States.

NBS has changed the clinical outcome of classic galactosemia and when results are provided as early as 3 to 4 days of life—before significant clinical symptoms—hospitalization is often avoided. A recent systematic review of NBS for galactosemia in Europe identified significant variability in galactosemia screening methods, cutoff values, and screening ages. Mortality ranged from 0%–100% with no agreement regarding treatment of variant forms, or timing of clinical follow-up, and evidence in favor of NBS was considered insufficient. The greatest confounder of the cost–benefit assessment of NBS was the false-positive testing rate and the effects of these test results on families (Varela-Lema et al., 2016).

Epimerase Deficiency Galactosemia

Uridine diphosphate-galactose 4'-epimerase (GALE) deficiency has several forms. Generalized GALE deficiency may present similarly to GALT deficiency when the enzyme is deficient in all tissues. Peripheral or intermediate forms of GALE deficiency are associated with deficient enzyme activity in red blood cells, with normal or only partially decreased enzyme activity in other tissues (i.e., white blood cells or fibroblasts). For the peripheral or intermediate forms, children receiving a normal, lactose-containing diet will not become symptomatic. GALE deficiency may be detected on newborn screen in states that test for classic galactosemia by analyte. These children have elevated Gal-1-P levels with normal GALT enzyme testing. Individuals with generalized disease can develop severe liver and renal disease if untreated, and they can be diagnosed with GALE deficiency through assessment of activity in red blood cells. GALE deficiency is treated identically to GALT deficiency, when symptomatic (Fridovich-Keil et al., 1993).

Galactokinase Deficiency

Galactokinase (GALK) deficiency causes elevated galactose but not Gal-1-P. Affected individuals also have elevated galactitol and may develop dense cataracts if untreated. GALK deficiency is also associated with pseudotumor cerebri, but the disease does not cause systemic effects (Bosch et al., 2002). As for GALE deficiency, GALK deficiency may be detected on newborn screen in states that test for classic galactosemia by analyte. GALT enzyme testing is normal. Cataracts may develop in the neonatal period, and early

treatment may improve or resolve cataracts if infants are treated with galactose restriction before 4–8 weeks of life (Hennermann et al., 2011). Lifetime dietary galactose restriction may be necessary but is milder than that required for GALT deficiency.

Glycogen Storage Diseases

Glycogen storage diseases (GSDs) are due to abnormalities in glycogen synthesis or utilization for energy production. They are divided into types primarily affecting the liver (types I, IIIb, IV, VI, and IX), the muscles (types II, V, and VII), or mixed (type IIIa and forms of IX). Hypoglycemia is often a presenting symptom of the liver-based GSDs. Most GSDs are inherited in an autosomal recessive manner, except GSD IXa and IXd (hepatic and muscle phosphorylase kinase deficiencies, respectively), which are X-linked. Severe GSD II is associated with an infantile cardiomyopathy and is most likely to be encountered in the neonatal intensive care unit.

Hepatic Glycogen Storage Diseases

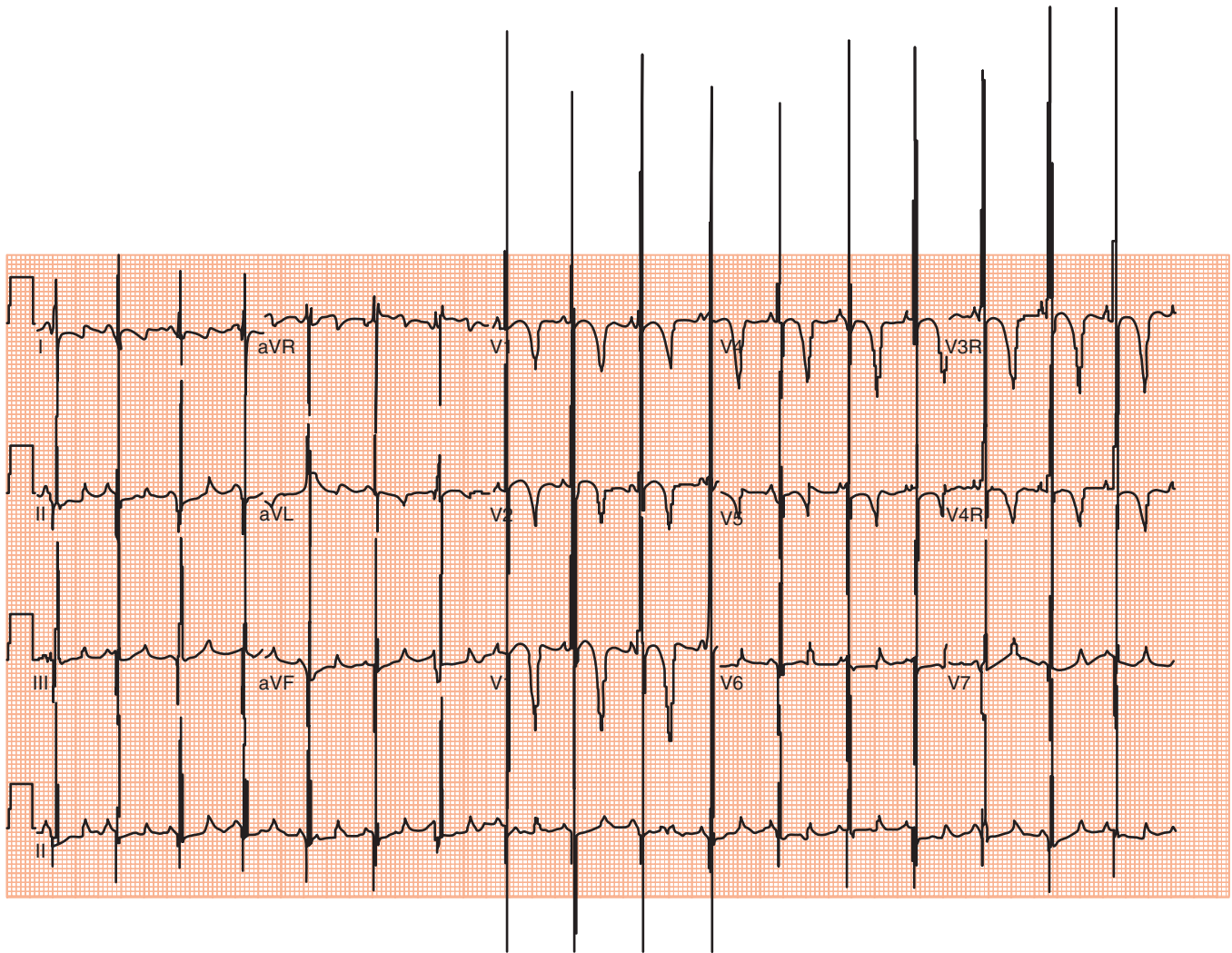
GSD I is due to glucose-6-phosphatase deficiency (von Gierke disease, Ia) or to deficient glucose-6-phosphate transport (Ib). Glucose-6-phosphatase plays a critical role in both glycogenolysis and gluconeogenesis (Fig. 22.1). The frequency of GSD type I is estimated to be 1 in 100,000 births, with 80% of cases being type Ia. The frequency is 1 in 20,000 in Ashkenazi Jews due to a founder mutation (Kishnani et al., 2014).

GSD I may not manifest in the neonatal period, as frequent newborn feeding may prevent symptomatic hypoglycemia and the development of hepatomegaly. Major clinical findings include failure to thrive, with an enlarged abdominal girth from hepatomegaly and hypoglycemia. Major laboratory findings are a rapid fasting hypoglycemia (typically within a few hours), with ketosis and lactic acidosis. Because of the high lactate level, individuals may be relatively asymptomatic from the hypoglycemia. Hypercholesterolemia, hypertriglyceridemia, and hyperuricemia may be seen in older individuals. Patients with GSD Ib additionally develop recurrent infections because of neutropenia and defective neutrophil function. Inflammatory bowel disease may develop in the first year of life. Diagnosis is now often confirmed through DNA sequencing of the *G6PC* (GSD Ia) and *SLC37A4* (GSD Ib) genes, although liver biopsy for enzyme activity may also be performed for diagnosis.

Therapy focuses on the prevention of hypoglycemia and resultant brain damage and growth failure, through frequent feedings and restriction of lactose and sucrose, as galactose and fructose derived from these feed into the blocked pathway (Kishnani et al., 2014). Continuous nasogastric feedings or boluses of uncooked cornstarch are essential at night and often during the day and result in improved glucose control and growth but do not completely correct other biochemical abnormalities. A comprehensive plan for treatment of intercurrent illnesses and emergencies is required. Neutropenia in type Ib may be treated with granulocyte colony-stimulating factor. Liver transplantation has been shown to improve metabolic control, fasting hypoglycemia, and growth (Boers et al., 2014). The disease also affects the kidneys including focal segmental glomerulosclerosis and progressive renal insufficiency.

Muscular Glycogen Storage Diseases

The most common and significant form of muscular GSD is type II, commonly called *Pompe disease* (*acid alpha-glucosidase deficiency* (abbreviated as GAA), also known as *acid maltase deficiency* or *lysosomal α -1,4-glucosidase deficiency*). This is the only GSD that is



• Fig. 22.2 Pompe Disease Electrocardiogram.

also a lysosomal storage disease (LSD), and it was the first identified LSD. Glycogen accumulates within the lysosome because of a defect in lysosomal-mediated degradation of glycogen. As for some other LSDs, enzyme replacement therapy has been developed for Pompe disease. This is the only current effective therapy for this disease.

Pompe disease has an estimated incidence of 1 in 40,000 in the Netherlands, based on the country's screening for three common mutations in newborn blood spots. The incidence ranges from 1 in 57,000 for late-onset disease to 1 in 138,000 for classic infantile disease (Ausems et al., 1999; Mechtler et al., 2012).

The classic infantile presentation of Pompe disease is hypotonia and hypertrophic cardiomyopathy. Creatine kinase, lactate dehydrogenase, and aspartate aminotransferase are elevated. The electrocardiogram is abnormal with a short PR interval and giant QRS complex in all leads, suggesting biventricular hypertrophy (Fig. 22.2). Late-onset presentations are of myopathy and have been diagnosed as early as the 2nd year of life. Diagnosis is made through the identification of decreased GAA activity in dried blood spots, fibroblasts, or muscle and confirmed via sequencing of the *GAA* gene (Zhang et al., 2006; Winchester et al., 2008). If the diagnosis is suspected, muscle biopsy can be avoided through blood spot and DNA testing but if performed will demonstrate vacuolar myopathy with glycogen storage within lysosomes and free glycogen

in the cytoplasm demonstrated by electron microscopy. The vacuoles are periodic acid-Schiff positive, digestible by diastase, and positive for acid phosphatase.

Decisions regarding which disorders are included on a state's NBS panel are made by each state, and some states now include Pompe disease. NBS in other countries has led to the initiation of early enzyme replacement therapy, which demonstrates improvements in cardiac size, muscle pathology, growth, and gross motor function in affected individuals but not in arrhythmias such as Wolff-Parkinson-White or in dysphagia or osteopenia (Chien et al., 2009; van Gelder et al., 2015). Long-term follow-up of early-treated individuals has demonstrated increased life span and increased ambulation with individuals not requiring mechanical ventilation (Chien et al., 2015). Gene therapy is being investigated with promising results in a mouse model of the disease (Falk et al., 2015; Todd et al., 2015).

Andersen disease, or GSD IV, is due to deficiency of glycogen branching enzyme, expressed in multiple tissues, and may manifest primarily as hepatic or muscular disease, with involvement of the heart and/or the nervous system. Two rare neuromuscular subtypes that present in the newborn period are the fatal perinatal neuromuscular subtype and the congenital neuromuscular subtype. The first presents with fetal akinesia sequence with polyhydramnios,

decreased fetal movement, fetal hydrops, and neonatal death or with hypotonia, muscular atrophy, arthrogryposis, and death in the neonatal period from cardiopulmonary failure (Magoulas and El-Hartab, 1993). The second presents with profound hypotonia, respiratory distress requiring mechanical ventilation, dilated cardiomyopathy, and death in early infancy (Escobar et al., 2012). The classic GSD IV subtype is the progressive hepatic subtype. Children are often normal at birth but develop failure to thrive, hypotonia, and potentially progressive liver dysfunction leading to cirrhosis and cardiomyopathy requiring liver and heart transplantation, respectively. Death may result from progressive cardiomyopathy despite liver transplantation. GSD IV is a rare autosomal recessive disorder, and diagnosis is confirmed through DNA sequencing of the *GBE1* gene or by detection of abnormal enzyme activity in muscle, liver, or skin fibroblasts.

Fructose Metabolism

The primary disorder of fructose metabolism is *hereditary fructose intolerance* (HFI). This is a rare autosomal recessive disorder triggered by ingestion of fructose, sucrose, or sorbitol, which may present clinically when infants are weaned from breast milk or formula and juice or fruit are added to the diet or when they receive a formula that contains fructose (Baker et al., 1993). Infants or neonates who are given sucrose solutions for pain relief during minor procedures may develop hypoglycemia, and a diagnosis of HFI should be considered in these cases. Clinical findings include pallor, lethargy, poor feeding, vomiting, loose stools, poor growth, hepatomegaly, and hypoglycemia, lactic acidemia, hyperuricemia, transaminase elevations, and positive urine reducing substances with ingestion of fructose. Renal tubular dysfunction may be present. Diagnostic testing consists of measuring enzyme activity in liver tissue and/or sequencing of *ALDOB*. Treatment includes elimination of fructose, sucrose, and sorbitol from the diet and medications. In practice, complete elimination of these can be quite difficult but is necessary for optimal outcome. Neonatal screening for HFI was investigated in 1996 in the United Kingdom, as early strict dietary treatment may avoid disease sequelae, but was not implemented (James et al., 1996).

Fructose-1,6-bisphosphatase deficiency is not a disorder of fructose metabolism. It is a disorder of gluconeogenesis, although as with other disorders of gluconeogenesis, therapy may include some limitation of dietary fructose. Patients may present in the newborn period with lactic acidosis and hypoglycemia when glycogen reserves are limited and then be clinically silent and present later (typically before 2 years of age) during times of fasting or following a fructose load. Acute crisis presents similarly to HFI and GSD Ia, as these are within the differential. This is a very rare disorder, with an estimated incidence between 1 in 350,000 and 1 in 900,000 and is autosomal recessive resulting from mutations in the *FBP1* gene (Lebigo et al., 2015).

Urea Cycle Disorders

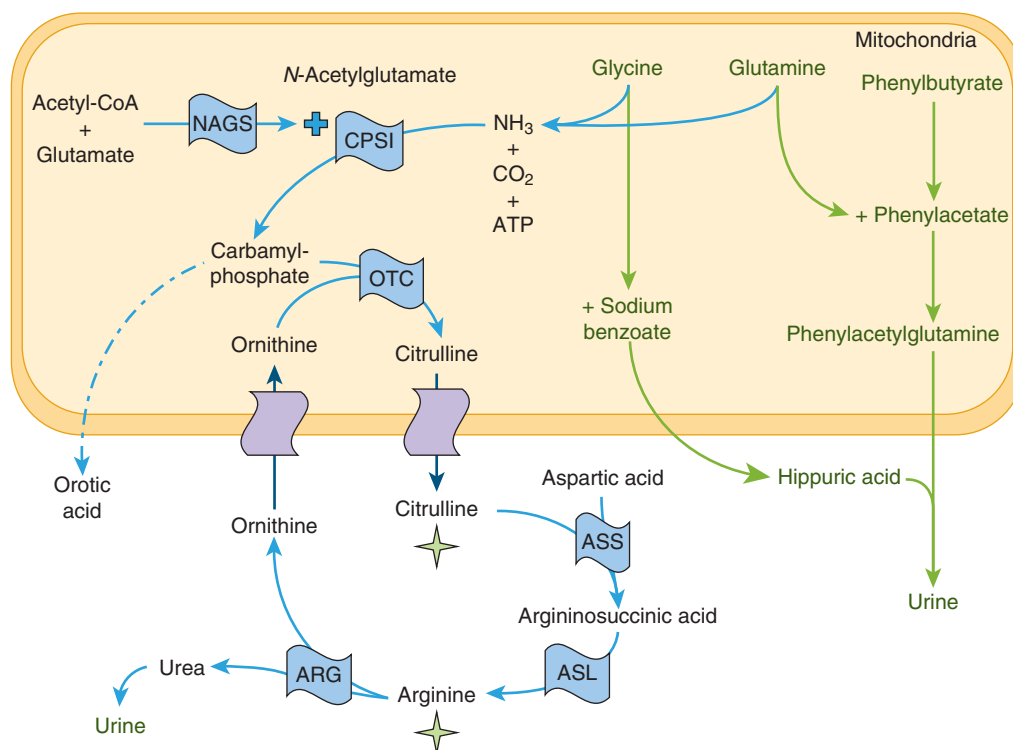
Removal of excess nitrogen is the function of the urea cycle. Urea cycle disorders (UCD) result from inhibition of the synthesis of urea from ammonia and classically manifest in the newborn period, although these may manifest at any age. Symptoms of hyperammonemia are provoked during episodes of protein catabolism (e.g., because of illness and poor oral intake) or dietary protein excess and are treated by controlling protein catabolism through dietary limitation of protein and removing offending toxic products (chiefly ammonia) with ammonia-scavenging medications or dialysis. UCDs

are frequently classified as proximal (mitochondrial) and distal (cytoplasmic). The three proximal UCDs are *N-acetylglutamate synthase deficiency* (NAGS), *carbamyl phosphate synthetase I* (CPSI) *deficiency*, and *ornithine transcarbamylase deficiency* (OTCD). The distal UCDs are *argininosuccinate synthetase deficiency* (known as *ASS deficiency* or *citrullinemia type I*, *CITI*), *argininosuccinate lyase deficiency* (also known as *argininosuccinic aciduria*, *ASA*), and *arginase 1 deficiency*. Additionally, disorders of the urea cycle include two of mitochondrial membrane transport. Deficiency of the mitochondrial ornithine transporter 1 is a cause of hyperammonemia, hyperornithinemia, and homocitrullinuria syndrome, and deficiency of the mitochondrial aspartate–glutamate transporter is a cause of citrullinemia type II or citrin deficiency. A defect of a plasma membrane transporter affects the renal tubular transport of cationic amino acids, including lysine, arginine, and the amino acids required for urea cycle function, and results in *lysine protein intolerance*, a multisystem disorder that only rarely manifests with neonatal hyperammonemia. Incidence estimates of all UCDs are 1 in 35,000 births, with OTCD, the most common UCD, estimated at 1 in 56,500 births (Summar et al., 2013). All UCDs are autosomal recessive except for OTCD, which is X-linked. Roughly 20% of heterozygote females manifest symptoms at some time in their life, and some of these present in the newborn period.

One complete turn of the urea cycle will produce a molecule of urea from two molecules of ammonia and one of bicarbonate. The nitrogen in ammonia is generated from the hepatic nitrogen pool of amino acids including glutamine, glutamate, and glycine. *N-acetylglutamate* is the product of the first enzyme in the cycle and is an essential activator of carbamyl phosphate synthetase I, which converts ammonia and bicarbonate into carbamyl phosphate. Ornithine and carbamyl phosphate are condensed by ornithine transcarbamylase to generate citrulline. Citrulline is combined with aspartate by argininosuccinate synthetase to create argininosuccinic acid. Fumarate is released from this by argininosuccinate lyase to create arginine. Urea is generated from arginine by arginase 1 and is excreted while ornithine reenters the urea cycle (Fig. 22.3).

Clinical symptoms in the newborn period are similar for all UCDs and are due to hyperammonemia. Severely affected newborns exhibit a progressive altered level of consciousness with drowsiness and lethargy progressing to unresponsiveness, beginning after 24 hours of life. Typical symptoms include poor feeding, vomiting, hyperventilation (caused by ammonia elevation and resulting in a primary respiratory alkalosis), and temperature instability. There may be peripheral circulatory failure that progresses to multiorgan failure. Marked hyperammonemia causes acute encephalopathy, leading to seizures, coma, and death if untreated. Ammonia levels should be checked in any infant with these symptoms, which may mimic sepsis or intestinal obstruction, and, if elevated, should be treated rapidly. Later-onset presentations include recurrent emesis, ataxia, liver dysfunction or apparent failure with coagulopathy, postpartum psychosis, and other psychiatric symptoms such as aggression, agitation, mania, and personality changes (Serrano et al., 2010).

Without rapid treatment severe UCDs are almost always fatal or result in severe and irreversible brain damage. The primary goal of treatment is to remove excess ammonia, which is neurotoxic. Its effects include alterations in amino acid pathways, neurotransmitters, energy production, nitric oxide synthesis, axonal and dendritic growth, and signal transduction in the developing brain. Additionally, excess glutamine may cause cerebral swelling and edema in the mature brain (Braissant et al., 2013). Acute hyperammonemic episodes may be associated with transaminase elevation and synthetic liver dysfunction, and it is important to assess these.



• **Fig. 22.3** Overview of urea cycle metabolism (blue) and nitrogen scavenger therapies (green). Stars indicate supplementation with citrulline or arginine. Plus sign indicates allosteric activator of carbamyl phosphate synthetase I. Dashed line indicates accumulation of orotic acid in ornithine transcarbamylase deficiency. Purple indicates transport between the mitochondrion and the cytosol. ARG, arginase; ASL, argininosuccinate lyase; ASS, argininosuccinate synthetase; ATP, adenosine triphosphate; CPSI, carbamyl phosphate synthetase I; CO_2 , carbon dioxide; NAGS, N-acetylglutamate synthase; NH_3 , ammonia; OTCD, ornithine transcarbamylase.

Individuals affected by proximal UCDs have been reported to present earlier in life, to have a higher peak ammonia level, and to have a longer average length of stay compared with patients with distal UCDs. Reports identify the age at first admission for hyperammonemia as less than or equal to 2 days in 55% of OTCD and CPSI patients and at less than 7 days in 84% of these patients (Ah Mew et al., 2013). For OTCD patients, nearly half of the males but only 4% of females will present between 0 to 30 days of age (Summar et al., 2008).

The critical laboratory abnormality in a UCD is elevated plasma ammonia. While artifactual elevations may occur due to problems with sample collection and processing, hyperammonemia in a newborn is a medical emergency, and, if elevated, the test should be repeated and additional evaluation and management initiated immediately. Normal plasma ammonia levels in newborns are as high as 110 $\mu\text{mol/L}$ (although care should be taken to confirm units as some laboratories report mg/dL). A level of greater than 150 $\mu\text{mol/L}$ (255 mg/dL) should prompt suspicion of, and evaluation for, an inborn error of metabolism in neonates. In older infants, children, and adults the reference range for ammonia is less than 35 $\mu\text{mol/L}$ (60 mg/dL). In primary or secondary disorders of the urea cycle that present in the newborn period, ammonia levels may be in the thousands. The differential diagnosis of hyperammonemia in the newborn period includes urea cycle defects, organic acidemias, fatty acid oxidation disorders (FAODs), and transient hyperammonemia of the newborn (THAN). In UCDs the hyperammonemia is often associated with a respiratory alkalosis caused by the effect of ammonia on the respiratory control centers in the brainstem. A primary respiratory alkalosis in a

newborn should prompt a physician to order an ammonia level. Early involvement of a geneticist with experience in the evaluation and management of inborn errors of metabolism is critical. Specialized biochemical laboratory testing should include plasma amino acids, total and free plasma carnitine, plasma acylcarnitine profile, total plasma homocysteine, plasma B12 level, urine organic acids, urine amino acids, and a quantitative urine orotic acid.

In proximal UCDs, there is decreased citrulline on plasma amino acid analysis, while patients with distal UCDs have either elevated citrulline (in CIT1 and ASA deficiency), elevated argininosuccinic acid (in ASA deficiency), or elevated arginine (in arginase deficiency). In OTCD, increased urinary orotic acid is present and may be identified on urine organic acid analysis, but a quantitative value is recommended because of variability in detection. NBS for CIT1, ASA, and arginase deficiency is performed through measurements of citrulline (CIT1, ASA) and arginine (arginase deficiency) in blood spots as these are elevated in these conditions. Proximal UCDs are not well identified on NBS because of the poor sensitivity of low citrulline levels, and most states do not have a low citrulline cutoff. When suspected clinically or through NBS, further evaluation should include metabolite testing of blood and urine, as previously described, and confirmation of a diagnosis is generally performed through DNA testing. For OTCD this should include analysis for gene deletions, as sequencing alone detects just over 80% of patients (Yamaguchi et al., 2006; Shchelochkov et al., 2009). In some cases, liver biopsy and enzyme analysis of liver tissue are required.

Hemodialysis is the primary method for rapid removal of ammonia. No clear guidelines have been established for initiation

of hemodialysis, but common indications include a rapidly increasing ammonia level, the presence of neurologic symptoms, or an ammonia level of greater than 400 $\mu\text{mol/L}$. Neurologic outcomes were improved when peak ammonia concentration was less than 180 $\mu\text{mol/L}$ and poorer when greater than 360 $\mu\text{mol/L}$ (Kido et al., 2012). Continuous arteriovenous hemofiltration (CAVH) provides a lower clearance rate but has the added benefit of continuous use and a lesser likelihood of major swings in intravascular volume that can exacerbate an already fragile state and cerebral edema. Ammonia clearance with peritoneal dialysis is approximately one-tenth that of CAVH and is not recommended for UCD therapy in the newborn period. Ammonia is not cleared effectively by exchange transfusion.

Alternative pathway or nitrogen-scavenging therapies are effective in helping to control ammonia levels and are critical in acute and chronic management (Fig. 22.3). When hyperammonemia is recognized and a UCD suspected, alternative pathway therapy can be rapidly implemented before hemodialysis, continued throughout, and maintained afterward and transitioned from intravenous (IV) to oral therapy for chronic management. The only approved IV therapy for treatment of hyperammonemia is sodium benzoate plus sodium phenylacetate (Ammonul). Arginine becomes an essential amino acid in severe early urea cycle defects; it stimulates the CPSI enzyme and is required for urea cycle function as it is one of the amino acids that comprises the urea cycle. It should be provided IV for suspected neonatal OTCD or CPSI and is especially effective in patients with CIT1 and ASA. It should not be given in known or suspected arginase 1 deficiency. Ammonul is rarely stocked except in pharmacies of tertiary care metabolic centers, and 10% arginine HCl is commonly available.

Nitrogen-scavenging therapies can effectively help control mild-to-moderate hyperammonemia in combination with other therapies and are critical to the acute management of marked hyperammonemia. A prospective study of IV sodium benzoate and sodium phenylacetate in neonatal hyperammonemia demonstrated the ability of these medications to lower plasma ammonia levels and improve survival (Enns et al., 2007). In acute management of hyperammonemia Ammonul is given as a loading dose of 250 mg/kg (of sodium benzoate) over 90 minutes followed by a 250 mg/kg dose over 24 hours by continuous IV (Summar, 2001). Arginine hydrochloride should be provided in known or suspected cases of NAGS, CPSI, or OTCD and is given as a 250 mg/kg loading dose over 90 minutes followed by a 250 mg/kg 24-hour maintenance dose. The dose is 600 mg/kg for known or suspected CIT1 or ASA (Summar, 2001). Also critical is reversal of catabolism, and age appropriate and higher calories must be provided by IV glucose at high concentration and IV intralipid (once a disorder of fat metabolism has been excluded). Parenteral nutrition, including provision of catabolism-sparing essential amino acids, should be initiated when ammonia has been controlled, ideally within 24 to 36 hours of the initiation of treatment. This treatment should be performed in collaboration with a clinical biochemical geneticist with experience in the treatment of UCDs.

Chronic management of infants with UCDs consists of providing adequate dietary protein, which will require a combination of natural (whole) protein from a regular infant formula as well as a special metabolic formula consisting of only essential amino acids to decrease the nitrogen burden; oral/enteral ammonia-scavenging medications; and arginine or citrulline, depending on the defect and severity. Also critical is the prevention of protein catabolism during times of illness or other physiologic stress, and an emergency sick-day diet and emergency letter should be provided at hospital

discharge. Children must be monitored frequently and medications and diet adjusted to prevent hyperammonemia and to allow adequate growth without overrestriction of protein resulting in poor growth and provoking catabolism. Long-term management requires a multidisciplinary team of a clinical biochemical geneticist, biochemical nutritionist, and genetic counselors and, in some cases nurses, a neurodevelopmental pediatrician, a neurologist, and rehabilitative medicine specialist, depending on the presence and severity of early brain injury.

Historically, the outcome of individuals with severe neonatal-onset CPSI or OTCD has been poor. A 2005 review estimated a poor outcome with a mortality of 84% in neonatal-onset cases and 28% in late-onset cases, before the use of nitrogen-scavenging therapies in Europe (Nassogne et al., 2005). When reviewing survival according to age, diagnosis, and first or recurrent episodes, the lowest survival is seen in male OTCD neonates at first episode. In an open label trial, newborns less than 30 days old had survival rates of 73% compared with 94% in infants greater than 30 days of age (Enns et al., 2007). In a 2009 study, in neonatal-onset cases intellectual disability was reported at almost 50%, compared with historical estimates of 60%–80% (Krivitzky et al., 2009). Currently, early liver transplantation in the first year of life, especially if initial rescue therapy has limited the peak and duration of hyperammonemia, is recommended (Campeau et al., 2010). Early recognition of hyperammonemia and rapid lowering of ammonia is critical to decreasing the extent and severity of early brain injury (Bireley et al., 2012).

Transient Hyperammonemia of the Newborn

THAN is most common in preterm newborns less than 36 weeks' gestational age that have a birth weight of less than 2.5 kg. Typically, it occurs following respiratory distress syndrome in the first 24 hours of life, and coma develops within the first 48 hours of life (Ballard et al., 1978). Serum ammonia levels can surpass 1500 $\mu\text{mol/L}$, and infants may require hemodialysis and protein restriction (Stojanovic et al., 2010).

The cause of this disease is unknown, although in some cases it may be due to a patent ductus venosus. Doppler ultrasound of the portal vein should be performed to evaluate for a clot in the portal vein. Plasma amino acid levels may have elevations of citrulline and arginine. The glutamine-to-ammonia ratio may distinguish this from a urea cycle defect (glutamine to ammonia ratio <1.6 in THAN, ensuring units are the same). There may be no respiratory alkalosis (Stojanovic et al., 2010). The mortality rate in THAN appears to be linked to the duration of coma; outcome can be good in surviving infants, and long-term treatment and protein restriction may not be necessary.

Amino Acid Metabolism Disorders

Maple Syrup Urine Disease

Maple syrup urine disease (MSUD) is a rare autosomal recessive inborn error of amino acid metabolism caused by branched-chain α -ketoacid dehydrogenase (BCKAD) complex deficiency. This enzyme is involved in the metabolism of the three branched-chain amino acids (BCAAs), leucine, isoleucine, and valine, at the step of conversion of each of their respective α -ketoacid derivatives into their decarboxylated coenzyme A (CoA) metabolites in the mitochondria (Fig. 22.4). The enzyme complex is composed of three components, E1, E2, and E3. E1 has two subunits, E1 α

long-term mental health (Muelly et al., 2013). Liver transplantation appears to result in similar outcomes when compared with those who have not had transplantation, but liver transplant may prevent further neurocognitive impairment from prevention of injury during recurrent acute events (Mazariegos et al., 2012).

Long-term MSUD treatment focuses on a BCAA-free formula balanced with the provision of sufficient BCAAs to maintain normal growth and development. The goal is that plasma leucine, isoleucine, and valine levels are in the normal range, though this may be difficult to achieve outside of infancy. Affected individuals must be closely monitored, and careful management by a biochemical genetic nutritionist is critical. Care must be given to ensure adequate supplementation with isoleucine and valine as BCAA-free formulas may lead to overrestriction of these. Overrestriction of isoleucine can result in anemia and a severe exfoliative rash similar to acrodermatitis enteropathica. A rare thiamine-responsive variant of MSUD may show improved BCAA levels and a decreased need for protein restriction with thiamine supplementation.

Tyrosinemia Type 1

Tyrosinemia type 1 (TYR1), or *hepatorenal tyrosinemia*, is an autosomal recessive disorder caused by a deficiency of the enzyme fumarylacetoacetate hydrolase as a result of mutations in the *FAH* gene. This enzymatic reaction is the last in the catabolism of phenylalanine and tyrosine to fumaric acid and acetoacetate, and the accumulation of tyrosine is due to other accumulating metabolites. The primary metabolites that accumulate are maleylacetoacetic acid and fumarylacetoacetic acid, and these both result in the elevation of succinylacetone. This compound is pathognomonic for this disease and is the primary confirmatory metabolite identified on urine organic acid analysis. It is a more sensitive and specific marker than tyrosine in NBS but is not available in all NBS programs. The estimated incidence is 1 in 100,000 to 1 in 120,000 in the general population. The incidence is higher in specific populations with estimates of 1 in 60,000 to 1 in 74,000 in Norway and Finland, 1 in 16,000 in Quebec, and 1 in 1846 in the Saguenay-Lac Saint-Jean region of Quebec because of common founder mutations in these areas (Sniderman King et al., 1993).

The phenotype is variable. One presentation is of an acute, early-onset, severe liver disease at less than 2 months of age; there is also an infantile-onset presentation and a chronic presentation after 1 year of age. The acute early-infantile presentation may be fatal with hepatomegaly, jaundice, elevated transaminases, and profound prolongations of prothrombin time and partial thromboplastin time. Affected individuals develop a renal Fanconi syndrome with generalized aminoaciduria, glycosuria, hypophosphatemia, hypouricemia, proteinuria, and an unusual urine odor of “boiled cabbage.” Children with the chronic phenotype exhibit liver disease, hypophosphatemic rickets as a result of the renal Fanconi syndrome, cardiomyopathy (in 20%–30%), and porphyria-like neurologic crises with abdominal pain, peripheral neuropathy, and respiratory failure (Sniderman King et al., 1993).

Plasma amino acid analysis will demonstrate elevated tyrosine levels, but this is not diagnostic as elevations of tyrosine are nonspecific and may be found, along with hypermethioninemia, in any disease causing liver dysfunction. Serum alpha fetoprotein levels are abnormally high. The identification of succinylacetone on urine organic acid analysis is diagnostic, and this may be detected within the first 12 hours of life (Schlump et al., 2010). As for most metabolic disorders, the characteristic metabolite abnormalities may not be present or detectable at all times.

NBS has allowed early treatment with 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC), and this has improved clinical outcome (Mayorandan et al., 2014; McKiernan et al., 2015). Treatment for TYR1 includes NTBC, which inhibits *p*-hydroxyphenylpyruvate dioxygenase, a proximal enzyme in the pathway, reducing the accumulation of succinylacetone but resulting in increased tyrosine levels. NTBC improves liver and renal disease but requires the implementation of a low-tyrosine, low-phenylalanine diet for improved neurologic outcome (Bendadi et al., 2014). Early treatment, ideally within the first month of life, results in a significant reduction in the development of acute liver disease, hepatomegaly, cirrhosis, hepatocellular carcinoma, renal dysfunction, rickets, and the need for liver transplantation (Mayorandan et al., 2014). Before the use of NTBC, most infants with the early-onset form of TYR1 died in early to late infancy. Unfortunately, patients treated with NTBC have shown impaired cognitive outcomes including a lower intelligence quotient, sub-optimal executive functioning (working memory and cognitive flexibility), and social cognition (face recognition and the identification of facial emotion) when treated with a natural protein-restricted diet (Bendadi et al., 2014; van Ginkel et al., 2016). Dietary overrestriction leading to hypophenylalaninemia may be the cause of these neurocognitive deficits, poor growth, cortical myoclonus, and eczema, although these have been seen to improve or resolve following phenylalanine supplementation (van Vliet et al., 2015).

One of the most severe complications of TYR1 is the development of hepatocellular carcinoma, typically occurring in later presentations in older children. Monitoring through serial liver ultrasounds and alpha fetoprotein levels is necessary. Treatment with NTBC will improve the biochemical markers and liver dysfunction, but liver transplantation may still be necessary in those who are NTBC-resistant or who have chronic liver disease or poor quality of life (Mayorandan et al., 2014).

Other forms of tyrosinemia may be detected with elevated tyrosine levels on NBS or plasma amino acid analysis. Tyrosinemia type 2, or oculocutaneous tyrosinemia, presents with corneal tyrosine crystals causing photophobia and hyperkeratotic plaques on the hands and soles of the feet. Tyrosinemia type 3 is extremely rare and has a variable phenotype including ataxia and mild mental retardation (Scott, 2006).

Transient tyrosinemia of the newborn is common in premature infants and is probably the most common disturbance of amino acid metabolism identified on NBS. It is due to delayed maturation of *p*-hydroxyphenylpyruvate dioxygenase or liver immaturity.

Nonketotic Hyperglycinemia

Glycine encephalopathy, also termed *nonketotic hyperglycinemia* (NKH), is an autosomal recessive disorder of the catabolism of glycine to carbon dioxide and ammonia (Van Hove et al., 1993). The incidence is about 1 in 60,000. The glycine cleavage system is composed of four proteins, glycine decarboxylase (GLDC), amino-methyltransferase (AMT), the glycine cleavage H protein, and lipoamide dehydrogenase. These are also called the *P* (pyridoxal-phosphate), *T* (tetrahydrofolate), *H* (hydrogen), and *L* (lipoamide) proteins, respectively, for the cofactor each utilizes. GLDC removes carbon dioxide, the AMT protein removes ammonia, the H protein removes the hydrogen, and the L protein regenerates the reduced form of the protein (Van Hove et al., 1993). The majority of affected individuals have mutations in the *GLDC* or *AMT* genes (Swanson et al., 2015). The pathophysiology is likely related to glycine's role in the CNS as both an inhibitory and an excitatory

neurotransmitter. The most common form of the disorder manifests in the 1st week of life as apnea and treatment refractory seizures associated with a burst-suppression pattern on electroencephalogram (EEG). This neonatal-onset form is associated with a very poor prognosis, even with early diagnosis and treatment (Swanson et al., 2015; Bjoraker et al., 2016). There are milder forms of the disorder that manifest in the first months of life or later.

The diagnosis of NKH is based on both the absolute value of glycine in cerebrospinal fluid (CSF) and on the ratio of CSF glycine to plasma glycine. CSF and plasma amino acids must be obtained concurrently. However, the presence of blood in the CSF invalidates the results as CSF amino acid values will not be accurate (Applegarth and Toone, 2001). CSF glycine is generally greater than 40 $\mu\text{mol/L}$ in affected individuals, and a CSF-to-plasma glycine ratio of 0.08 or greater is considered diagnostic of NKH (Swanson et al., 2015). This disorder is not identified through NBS because of high false-positive rates of blood spot glycine levels.

One goal of treatment is to lower CSF glycine through high-dose sodium benzoate therapy. Benzoate is conjugated with glycine to form hippuric acid, which is excreted. In addition, dextromethorphan, an *N*-methyl-D-aspartate (NMDA) receptor antagonist, is prescribed to counteract the activation of the NMDA receptor by glycine. Restriction of dietary protein to restrict the amino acid glycine is not an effective therapy. Other care is supportive as the majority of affected individuals have profound intellectual disability and develop spastic quadriplegic cerebral palsy. Mildly affected individuals with a later-onset presentation with autism have been described. Withdrawal of support in the newborn period has been performed because of the poor prognosis, but the existence of a “mimic” of the disorder associated with identical, but transient, biochemical and clinical features—and with a good outcome—may complicate this decision (Boneh et al., 2008).

Recently, two new classes of disorders that have a secondary effect on the glycine cleavage system have been described. One class was identified through candidate gene sequencing of individuals with defects in the glycine cleavage system that lacked mutations in the known causative genes (Baker et al., 2014). The two classes are defects in lipoate synthesis, as lipoate is a cofactor of the L protein, and defects in iron–sulfur cluster biogenesis, as lipoate synthase is an iron–sulfur-containing protein. Affected individuals with defects in genes in both pathways have been described. The disorders have unique clinical and biochemical features, and all have deficient glycine cleavage (Mayr et al., 2014). It is important to consider these variant disorders in a neonate with apparent NKH. DNA testing should be performed to confirm the correct diagnosis, as the results are necessary for accurate treatment, prognosis, and genetic counseling.

Hyperhomocystinemias and Remethylation Disorders

In humans, as the essential amino acid methionine is converted to homocysteine, a methyl group is provided for many different methylation reactions. Homocysteine may then either be metabolized to cysteine (the transsulfuration pathway) via cystathionine β -synthase or be remethylated back to methionine; defects in these enzymes may lead to homocystinemia (or homocystinuria).

Classic homocystinuria is caused by an autosomal recessive deficiency of the cystathionine β -synthase enzyme as a result of mutations in the *CBS* gene and rarely presents in early infancy. The population frequency is estimated to be between 1 in 1800 and 1 in 900,000. NBS detects elevated methionine levels, but this will not detect all affected infants, particularly those with the

pyridoxine-responsive form of the disease. Early treatment improves clinical outcomes, including ectopia lentis, myopia, mental impairment, a marfanoid body habitus, osteoporosis, thromboembolic events, and behavioral problems (Morris et al., 2017). Infants may present with thrombosis, and homocysteine should be assayed for any thrombotic event, regardless of the NBS result.

Defective methionine remethylation may be due to methionine synthetase deficiency, methionine synthetase reductase deficiency, 5-methylenetetrahydrofolate reductase (MTHFR) deficiency, and other defects in cobalamin metabolism or transport (a cofactor for methionine synthase). Newborns may come to clinical attention with encephalopathy with lethargy, feeding difficulties, hypotonia, seizures, pancytopenia with megaloblastic anemia, cardiomyopathy, and optic atrophy and retinopathy (Morris et al., 2017). Previously thought to be rare, these disorders are now identified more frequently as a result of NBS, and false positives for these may be due to maternal nutritional cobalamin deficiency. The disorders of cobalamin intracellular processing or transport are identified by decreased methionine, elevated homocysteine, and/or elevated methylmalonic acid and C3-(methylmalonyl)-acylcarnitine levels (see *Methylmalonic Acidemia*). Infants may quickly develop postnatal failure to thrive, and appropriate treatment, which may include hydroxocobalamin, betaine, folinic acid, carnitine, and methionine supplementation, is now resulting in improvements in clinical outcome (Morris et al., 2017).

Patients with MTHFR deficiency may present as neonates with decreased consciousness, hydrocephalus, hypotonia, and feeding difficulties. There may be severe cortical atrophy and brain lesions caused by thromboses of the arteries or veins. This may present as an acute disorder of intoxication in the newborn period. Treatment with high-dose betaine, which enhances the remethylation of homocysteine to methionine through an alternate betaine–homocysteine methyltransferase pathway, is reported to improve or normalize development (Morris et al., 2017).

Early detection of disorders of remethylation and of classic homocystinuria is possible through NBS, which utilizes a high methionine level to detect classic homocystinuria and a low methionine level to detect remethylation disorders. NBS is not fully sensitive or specific for these disorders. One cause of false positives is heterozygous mutations in the *MAT1A* gene causing hypermethioninemia, which may be benign. The sensitivity of NBS for cystathionine β -synthase deficiency is improved by using elevated methionine levels together with methionine:phenylalanine and methionine:total homocysteine ratios, although measurement of total homocysteine is not available universally (Huemer et al., 2015). NBS for some remethylation disorders is possible by detection of low methionine levels with elevated C3-acylcarnitine levels, depending on the individual state programs (Huemer et al., 2015). Confirmatory DNA testing is available for all of the disorders.

Phenylketonuria

Phenylketonuria (PKU) is an autosomal recessive disorder resulting from deficiency of phenylalanine hydroxylase (PAH). It is the most common inborn error of amino acid metabolism, affecting about 1 in 10,000 northern European or east Asians, 1 in 2600 individuals in Turkey, and 1 in 4500 in Ireland but only 1 in 143,000 in Japan and 1 in 200,000 both in Finland and among those of Ashkenazi Jewish ancestry (Regier and Greene, 1993). The enzyme defect results in decreased levels of tyrosine. PAH requires the cofactor tetrahydrobiopterin (BH_4), therefore PAH enzyme deficiency may also result from a deficiency in the synthesis or recycling of BH_4 . Disorders of BH_4 metabolism may result in elevated

phenylalanine levels and should be tested for when performing follow-up NBS testing, as treatment and outcomes differ (Camp et al., 2014). BH_4 is also a cofactor for tyrosine hydroxylase, tryptophan hydroxylase, and nitric oxide synthase, and so BH_4 deficiency will also lead to impaired levels of L-dopa, dopamine, norepinephrine, melanin, serotonin, citrulline, and nitric oxide. PKU does not present with symptoms in the newborn period. It is a cause of intellectual disability, and there are no systemic manifestations. Treatment, however, should be instituted in the first several weeks of life for optimal outcome, and it is important that physicians caring for newborns are familiar with the evaluation and management of newborns with an abnormal NBS for PKU.

PKU was the first disorder for which NBS was implemented in the 1960s. NBS for PKU is a model for preventative and personalized medicine, as well as for public health screening and intervention. The blood spot phenylalanine level and the phenylalanine:tyrosine ratio will be elevated and detected by NBS. The blood spot should be collected after 24 hours of age following breastfeeding or formula feeding. Elevated urine phenyl ketones and phenylacetic acid result in a “mousey” urine odor. Diagnosis should be confirmed through plasma amino acid analysis and testing for BH_4 metabolism disorders in blood and urine. DNA sequencing of the *PAH* gene may provide helpful information for genetic counseling and treatment.

Untreated PKU patients will develop developmental and intellectual disabilities, eczema, hypopigmented skin and hair, and epilepsy in infancy and at older ages. Dietary phenylalanine restriction with strict compliance is necessary to prevent intellectual disability and other features. Dietary compliance may be difficult for teenagers and adults, but lifelong dietary treatment is necessary to avoid neuropsychiatric symptoms such as inattention, hyperactivity, depression, and anxiety (Bilder et al., 2016). Mothers who are poorly compliant during pregnancy and have phenylalanine levels above the recommended treatment range may have children with microcephaly, fetal growth restriction, congenital heart defects, and other malformations, known as *maternal PKU syndrome*. Attaining plasma phenylalanine levels within treatment range before the 8th week of pregnancy is essential and ideally should be achieved before pregnancy. Phenylalanine levels should be maintained between 120 and 360 $\mu\text{mol/L}$, the typical treatment range in the United States, throughout the pregnancy (Camp et al., 2014).

Dietary therapy for PKU is effective and involves a low natural-protein diet supplemented with phenylalanine-free medical formula to ensure adequate total protein and micronutrients. An adequate amount of phenylalanine from whole protein in formulas and foods is necessary for normal growth and protein synthesis, with a goal of maintaining plasma phenylalanine levels less than 360 $\mu\text{mol/L}$ (Camp et al., 2014). Sapropterin (a synthetic form of the BH_4 cofactor) may be beneficial in lowering phenylalanine levels in the 25%–50% of patients who are sapropterin responsive. Newer therapies being developed include using large neutral amino acids that compete for the same gut and brain transporter as phenylalanine in older children and adults, to lower brain phenylalanine levels. There is a current clinical trial of polyethylene glycol-conjugated phenylalanine ammonia lyase that converts phenylalanine to transcinnamic acid and is given subcutaneously to reduce plasma phenylalanine levels in patients on normal diets (Camp et al., 2014).

Organic Acidemias

The classical organic acidemias (or acidurias, the terms are interchangeable) are characterized by systemic illness and presentation

in the newborn period in the severe forms and include methylmalonic acidemia (MMA), propionic acidemia, and isovaleric acidemia. These are due to defects in pathways that affect the catabolism of one or more of the BCAAs (leucine, isoleucine, and valine) as well as other amino acids (Fig. 22.4). An organic acid is any organic compound that contains a carboxy functional group but no α -amino group. They are intermediates in multiple pathways, including those of amino acid, fatty acid, cholesterol, and neurotransmitter metabolism. Disorders of fatty acid oxidation, ketone body metabolism, and lactic acid metabolism may also be detected through organic acid analysis.

Before NBS, these disorders were diagnosed only after patients became symptomatic, unless there was a known affected family member. While early presentation does occur before NBS results are available, with widespread NBS it is more common to be confronted with an asymptomatic or early symptomatic patient and a differential diagnosis that is based on the NBS results. Presenting symptoms of the classic organic acidemias in the neonate result from the progressive hyperammonemia, keto lactic acidosis, and hypoglycemia.

Methylmalonic Acidemia

Multiple genetic defects can lead to the elevation of methylmalonic acid. Isolated MMA is caused by methylmalonyl-CoA mutase enzyme deficiency as a result of mutations in the *MUT* gene. Adenosylcobalamin is a required cofactor for the mutase enzyme, and cobalamin (vitamin B_{12}) disorders of processing or transport may present with elevations of methylmalonic acid alone or in combination with elevated homocysteine, as homocysteine is also metabolized by a cobalamin-dependent enzyme, cystathionine β -synthase (see [Hyperhomocystinemias and Remethylation Disorders](#) previously). Cobalamin is acquired through dietary sources and must be appropriately transported and then undergoes a series of intracellular modifications. Thus impaired cellular cobalamin metabolism and other inherited defects in cobalamin transport or modification result in MMA (e.g., the deficient activity of cobalamin adenosyltransferase causes cobalamin B disease). Other inherited defects of cobalamin transport or modification may cause an isolated or a combined defect with homocystinuria (Carrillo et al., 1993; Manoli et al., 1993). Methylmalonic acid is detected in the blood, CSF, and urine of affected individuals. The disorder is typically identified through urine organic acid analysis after an abnormal NBS or in a clinically presenting neonate. The precursors of methylmalonyl-CoA are primarily isoleucine and valine but additionally include methionine, threonine, thymine, and odd-chain fatty acids.

There is a spectrum of severity of isolated MMA that ranges from severe, catastrophic newborn-onset disease to benign forms (Manoli et al., 1993). Patients presenting in the newborn period have a severe phenotype but may have a vitamin responsive form of the disorder (e.g., cobalamin A deficiency). Levels of homocysteine and vitamin B_{12} should always be assessed when elevated methylmalonic acid has been identified, and cobalamin should be provided empirically. The neonate appears well at birth but may rapidly progress as early as the 2nd or 3rd day of life to having problems with feeding and then develop vomiting, lethargy, and perhaps seizures. There may be tachypnea to compensate for metabolic acidosis. Crucial laboratory findings include metabolic acidosis with an increased anion gap, elevated lactate, elevated ketones, and elevated ammonia. The elevation of ammonia may be as high as that found in neonates presenting with early-onset UCDs, and

specialized biochemical genetic laboratory testing is required for diagnosis and should include a urine organic acid analysis and a plasma amino acid analysis, a plasma acylcarnitine profile, and a quantitative orotic acid level. Ketonuria is uncommon in newborns, and the physician must always consider an organic acidemia in an acutely ill newborn with ketosis. Other laboratory findings are thrombocytopenia, leukopenia, and anemia caused by effects of the metabolite on hematopoietic elements in bone marrow. Plasma amino acid analysis may reveal elevations of glycine and alanine. Elevations of C3-(methylmalonyl)-acylcarnitines may result in a secondary free carnitine deficiency caused by conjugation of accumulating metabolites, and so carnitine supplementation should be initiated (Baumgartner et al., 2014). Methylmalonic acid and other metabolites are detected in urine by organic acid analysis. The specific genetic diagnosis should be identified by DNA testing. Next-generation DNA sequencing panels are available, although results often take weeks to return. Most are inherited as autosomal recessive conditions, except for cobalamin X deficiency caused by a defect in *HCFC1*, a transcription factor that regulates the cobalamin C gene (Yu et al., 2013).

Rapid institution of empiric therapy may improve outcome, even in a patient who has presented clinically. Acute management protocols are available and should be administered in conjunction with a biochemical geneticist (Baumgartner et al., 2014). The treatment of acute metabolic decompensation includes cessation (for no more than 12–24 hours) of protein intake, empiric therapy with cobalamin, provision of high calories through protein-free enteral formula, and/or IV glucose at high concentration with IV intralipids, insulin, and carnitine. Some patients are responsive to pharmacologic doses of cobalamin, and 1 mg/day intramuscular hydroxocobalamin (preferred formulation) should be given as empiric therapy to a newborn presenting with hyperammonemia and acidosis (Baumgartner et al., 2014). Sodium bicarbonate should be used if indicated to correct acidosis. Dialysis is indicated for refractory hyperammonemia or acidosis. Additional therapies may include ammonia scavenger medications and carglumic acid, a stable analogue of the coactivator of the urea cycle that is depleted in MMA, causing the secondary hyperammonemia (Baumgartner et al., 2014). Chronic treatment includes a low natural-protein diet, an amino acid supplement lacking the MMA precursors, carnitine, and appropriate calories and fluid. Cobalamin is used chronically only when a specific and reproducible response is noted.

Most patients are now identified by NBS, although there are missed cases. False-positive NBS may result from maternal cobalamin deficiency. If the NBS and follow-up results are rapidly available, the outlook for the neonatal period may be improved. Episodes of decompensation will still occur. Patients who are particularly brittle or who develop renal failure receive either liver, kidney, or combined liver–kidney transplants. Long-term complications include basal ganglia stroke (which may occur in the newborn period), renal disease and failure, and pancreatitis (Baumgartner et al., 2014).

Propionic Acidemia

Propionic acidemia (PA) is due to a deficiency of propionyl-CoA carboxylase and is an autosomal recessive disorder. It was originally referred to as “ketotic hyperglycinemia,” because patients may have elevations of glycine as well as ketosis. Propionyl-CoA carboxylase is the enzymatic reaction just upstream of methylmalonyl-CoA mutase, and the precursors are identical (Fig. 22.4). The clinical

presentation and therapy of PA in the newborn period are similar to that of MMA. A biochemical geneticist with experience in the management of inborn errors should guide care and help guide diagnosis and the differentiation of MMA and PA from other possible disorders. Empiric therapy should be administered as described for MMA, with the provision of calories, IV glucose at high concentration with IV intralipid (once a disorder of fat metabolism has been ruled out), insulin (if needed), carnitine, cobalamin (for possible cobalamin-responsive MMA), and temporary cessation (no more than 12–24 hours from last intake) of protein. Hyperammonemia may be marked, and dialysis may be indicated. Ammonia-scavenging medications and carglumic acid may also be used, as for MMA (Chapman et al., 2012).

Elevated ammonia levels may be associated with CNS injury. Metabolic stroke, because of cell death in the absence of thrombosis or ischemia, may be seen in both MMA and PA and may result in damage to the basal ganglia. In MMA the globus pallidus is classically affected, while in PA the caudate and putamen are typically affected, resulting in severe choreoathetosis (Schreiber et al., 2012; Baumgartner et al., 2014). Chronic therapy consists of a low natural-protein diet, supplementation with metabolic formula lacking the MMA/PA precursors, carnitine, and adequate calories and micronutrients (Chapman et al., 2012). Biotin is a cofactor for the enzyme, but no case of biotin-responsive PA has been described; an empiric trial can be considered. Intestinal bacteria (*Propionibacterium* species) contribute to propionate production. Antimicrobial therapy with metronidazole has been used to lower propionate metabolites in acute illness and may be given as intermittent therapy to lower metabolites as part of chronic therapy. While not curative of the disorder, liver transplantation is being performed in individuals with early-onset PA with the goal of ameliorating long-term complications and enhancing quality of life (Chapman et al., 2012). The efficacy of liver transplant is unclear.

Outside of the newborn period, or in late-presenting individuals, episodes of metabolic decompensation are characterized by hyperammonemia, acidosis, and ketosis precipitated by excessive protein intake or more commonly by infection resulting in poor oral intake and protein catabolism. Families may use home urine ketone strips for monitoring so that early decompensation can be recognized and aggressive measures instituted to avoid further decompensation, as episodes can result in permanent neurologic damage. Affected individuals may exhibit developmental delay, seizures, cerebral atrophy, and EEG abnormalities (Schreiber et al., 2012). Other complications include optic atrophy, cardiomyopathy, and pancreatitis (Schreiber et al., 2012). Late-onset presentations include those with neurologic features only (Schreiber et al., 2012).

Diagnosis is made through urine organic acid analysis and confirmed by enzymatic and/or DNA testing. The acylcarnitine profile, on NBS or in plasma, will have elevated C3-(propionyl)-acylcarnitine, and urine organic acid analysis is required to distinguish between MMA and PA. In PA the urine has elevated 3-hydroxypropionic, propionyl glycine, and methylcitric acid but not methylmalonic acid. Both chronically and during acute decompensation, plasma ammonia may be mild to moderately elevated. The enzyme activity of propionyl-CoA carboxylase can be assayed in white blood cells or cultured skin fibroblasts. DNA testing may identify the numerous mutations that have been described in the two genes (*PCCA* and *PCCB*) that encode the subunits of this multimeric enzyme. Most patients are now identified by NBS, and the severe neonatal decompensation may be ameliorated if the results are rapidly available.

Isovaleric Acidemia

Isovaleric acidemia (IVA) is an autosomal recessive disorder caused by deficiency of the enzyme isovaleryl-CoA dehydrogenase. This is a defect in catabolism of the amino acid leucine. There are two major phenotypes, distinguished by the degree of enzyme deficiency and differing mutations: the acute form manifests as catastrophic disease in the newborn period, and the late-onset type is characterized by chronic, intermittent episodes of metabolic decompensation (Vockley and Ensenauer, 2006). In the acute form the infants become ill in the 1st week of life, similar to MMA and PA. Marked acidosis and ketosis suggest an organic acidemia, and urine organic acid analysis is required for diagnosis and shows elevations of isovalerylglycine and 3-hydroxyisovaleric acid. An elevated C5-(isovaleryl)-acylcarnitine value on plasma acylcarnitine analysis or on NBS is strongly suggestive of this diagnosis, although urine organic acid analysis is still required. DNA mutation analysis of the *IVD* gene is available. Another diagnostic clue is the characteristic “sweaty-feet, rancid cheese, or dirty socks” odor caused by isovaleric acid, which is detectable in blood and urine, especially during acidosis. Dialysis may be necessary if there is marked hyperammonemia. As for other organic acid disorders, acute treatment consists of protein restriction (for 12–24 hours) with the provision of calories, IV glucose at high concentration with IV intralipids, insulin (if necessary), carnitine, and sodium bicarbonate if indicated, followed by a protein-free formula to provide additional calories via nasogastric tube if enteral nutrition is tolerated and oral glycine (150 to 250 mg/kg per day) for known or suspected IVA and oral or IV carnitine supplementation (Vockley and Ensenauer, 2006). The excretion of isovaleric acid as the glycine conjugate is highly efficient, and improvement can occur rapidly with provision of glycine and other therapy.

In the chronic, intermittent form of IVA, patients have repeated episodes of metabolic decompensation precipitated by infection, other catabolic stress such as surgery, or excessive protein intake. These episodes may mimic Reye syndrome. The same therapeutic principles are applied as for the acute treatment of the neonatal disorder. Chronic therapy consists of a diet with low natural protein (limiting leucine to the amount required for growth), a leucine-free amino acid supplement to provide sufficient protein for growth, and glycine, which enhances isovalerylglycine production and reduces free isovaleric acid levels in body fluids (Vockley and Ensenauer, 2006). In addition, carnitine administration can augment the excretion of isovalerylcarnitine (Vockley and Ensenauer, 2006). Some patients who remain largely asymptomatic are ascertained through NBS. This form of IVA is associated with a specific mutation (p.A282V) in the *IVD* gene (Ensenauer et al., 2004). NBS and early institution of treatment can alter the prognosis of affected individuals (Grunert et al., 2012).

Multiple Carboxylase Deficiency

Two enzymatic defects, holocarboxylase synthetase deficiency and biotinidase deficiency, lead to deficiency of the four carboxylase enzymes, propionyl-CoA carboxylase, pyruvate carboxylase (PC), 3-methylcrotonyl-CoA carboxylase, and acetyl-CoA carboxylase, that require covalent linkage of the cofactor biotin to a lysine residue of the enzyme for normal activity (Wolf, 1993). Holocarboxylase synthase is the enzyme that catalyzes this covalent linkage, and a severe defect of this enzyme causes early-onset multiple carboxylase deficiency. The biotinidase enzyme regenerates biotin from the amide biocytin, formed by biotin and lysine. Biotinidase

deficiency causes late-onset multiple carboxylase deficiency because of the failure to recycle biotin. This condition typically does not manifest in the newborn period. High-dose biotin therapy is extremely efficacious in both conditions.

Infants with severe deficiency of holocarboxylase synthase become ill in the newborn period. They develop a marked metabolic acidosis with severe lactic acidosis and become encephalopathic. Administration of enteral biotin is life-saving, and empiric therapy should be administered if there is a concern for this disorder. In an affected neonate, administration of this vitamin results in a dramatic improvement in clinical and laboratory findings. As described for MMA, PA, and IVA, empiric management should include initial protein restriction, high calories, correction of acidosis, and empiric administration of cofactors and carnitine. After an initial period of empiric management, specific management should be based on the results of specialized biochemical testing. The goal is to have these results within 24 hours in order to provide the correct therapy. Evaluation and management should be guided by a biochemical geneticist familiar with the management of inborn errors.

The characteristic plasma acylcarnitine profile exhibits elevations of C3-(propionyl)-acylcarnitine and C5-OH-(3-hydroxyisovaleryl)-acylcarnitine and may be detected on NBS. Urine organic acid analysis identifies marked elevations of metabolites because of the deficiencies of pyruvate, propionyl-CoA, and 3-methylcrotonyl carboxylases. These include lactate, 3-hydroxypropionic acid and propionylglycine, and 3-methylcrotonylglycine, respectively. In most patients the defect is associated with decreased affinity of the holocarboxylase synthetase enzyme for biotin, and effective treatment requires between 10 mg and 100 mg of oral biotin per day. Long-term treatment may include dietary protein restriction and carnitine supplementation. The enzyme defect can be confirmed in cultured skin fibroblasts, or the diagnosis can be confirmed by DNA mutation analysis of the *BTD* or *HCLS* gene. Assay of individual carboxylases in white blood cells will show deficiency of all three carboxylases assayed, but this is not specific. The disorder is inherited as an autosomal recessive trait.

NBS also detects individuals with partial biotinidase deficiency (30% enzyme activity) who do not develop the severe symptoms of the profound deficiency. Enzyme analysis and mutation analysis distinguish these (Cowan et al., 2010; Wolf, 2010).

Late-onset multiple carboxylase deficiency caused by profound biotinidase deficiency may present as seizures and lactic acidosis and episodic decompensation. Long-term sequelae include hearing loss and optic atrophy. This disorder is effectively treated with biotin (10–20 mg/day), which prevents these long-term complications (Wolf, 1993). Effective NBS requires a specific biotinidase enzyme assay as it is not reliably detected by tandem mass spectrometry.

Glutaric Aciduria Type 1

Glutaric aciduria type 1 (GA1) is due to a deficiency of glutaryl-CoA dehydrogenase, an enzyme in the catabolic pathway of the amino acids lysine, hydroxylysine, and tryptophan (Boy et al., 2017). Unlike the organic acid disorders described previously that have systemic manifestations including hyperammonemia, lactic acidosis, ketosis, and bone marrow suppression, GA1 is termed a “cerebral” organic aciduria. In most instances the systemic manifestations of decompensation are not present, and GA1 does not have a catastrophic presentation in the newborn period that includes hyperammonemia and acidosis. The classic presentation is that of a child with normal growth and development in the first 6–18

months of life who experiences a metabolic stroke of the basal ganglia during illness, surgery, or another event that provokes catabolism (Kolker et al., 2006). Clinically affected children sustain irreversible bilateral damage to the caudate and putamen and, more rarely, the globus pallidus, structures of the basal ganglia that influence the control of voluntary movement, resulting in incapacitating dystonia and decreased life expectancy. Other affected individuals appear to have a slowly progressive course with hypotonia, dystonia, and dyskinesia in the first several years of life; these may have sustained early injury without a recognized encephalopathic illness. Remarkably, there is a vulnerable period for neurologic injury, and affected individuals who do not have injury in the first 6 years of life may have normal growth and development. This disorder is identified on NBS with elevations of C5DC-(glutaryl)-acylcarnitine, though some individuals termed “low excretors” may be missed (Boy et al., 2017). Chronic therapy includes a low natural-protein diet balanced with supplementation of a lysine-free, tryptophan-reduced, and arginine-containing formula, carnitine, and riboflavin (the enzyme cofactor that may show biochemical improvements in some cases). Acute emergency therapy includes protein restriction (for 12–24 hours) with the provision of calories as IV glucose at high concentrations with IV intralipids, insulin (if necessary), and carnitine, with supportive care from neurology or neurosurgery. These are critical to decreasing the incidence of devastating neurologic injury in affected individuals (Heringer et al., 2010; Boy et al., 2017).

GA1 may mimic nonaccidental trauma, as some individuals have subdural hematomata and retinal hemorrhages, independent of basal ganglia injury. Subdural hematomas may be identified in the investigation of crossing of percentiles for head circumference, a finding in GA1. Another finding that may prompt investigation for GA1 is increased fluid in the perisylvian fissures observed on brain computerized tomography or magnetic resonance imaging (MRI) (Boy et al., 2017). Manifestations in the newborn period include nonfamilial macrocephaly, increased fluid in the perisylvian fissures, and an abnormal NBS. Initial testing should include a plasma acylcarnitine profile, measurement of total and free carnitine, and a urine organic acid analysis.

Urine organic acid analysis will identify elevated glutaric acid and 3-hydroxyglutaric acid, though the latter compound, considered diagnostic of GA1, may be normal or near normal in a nondecompensated state in some individuals. Critically, these individuals, termed low excretors, are at no less risk of severe neurologic injury. The diagnosis may be confirmed by enzyme assay in fibroblasts or by DNA mutation analysis of the *GCDH* gene. NBS has resulted in a decrease in the incidence of neurologic injury in affected individuals (Heringer et al., 2010; Boy et al., 2017).

Fatty Acid Oxidation Disorders

FAODs are inborn errors of metabolism resulting in failure of β -oxidation within, or transport of fatty acids into, the mitochondria. There are at least 31 enzymes described. While short- and medium-chain fatty acids enter the mitochondria directly, carnitine is required for transport of long-chain fatty acids. Within the mitochondria the β -oxidation cycle will form acetyl-CoA and generate nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH₂), enabling electron transfer to the respiratory chain for adenosine triphosphate (ATP) generation. Acetyl-CoA is used as a substrate in the Krebs cycle to produce reducing equivalents for the electron transport chain (ETC) and ketone synthesis. FAODs lead to a deficit of energy production

and produce a wide range of clinical presentations from mild hypotonia in adults to sudden death (Rinaldo et al., 2002).

FAODs present at all ages and spare the CNS when in good metabolic control. Life-threatening phenotypes present in the first few days of life (typically after 24 to 48 hours of life) because of the severe catabolic state and enhanced breakdown of free fatty acids from adipose stores, often before NBS results being received. Signs include hypoglycemia, liver disease and liver failure, cardiac and skeletal myopathy, rhabdomyolysis, and retinal degeneration (Roe and Ding, 2001). The accumulation of long-chain acylcarnitine species has been suggested to be dysrhythmogenic and may be associated with cardiac dysfunction (Bonnet et al., 1999).

Currently, many countries diagnose most FAODs through NBS, but there is significant variability. The diagnosis is confirmed with a plasma acylcarnitine profile, total and free carnitine levels, and DNA testing. All disorders are autosomal recessive. Urine organic acid analysis and measurement of urine acylglycines should also be performed. If variants of uncertain significance are identified on DNA sequencing, leukocyte, fibroblast, or liver enzyme assays may be implemented to determine whether an individual is affected. False-positive NBSs are commonly due to carrier status. Many infants are now being identified with milder, and perhaps persistent, elevations of specific acylcarnitines but are not thought to be at significant risk for clinical disease, although long-term follow-up studies are needed to determine what, if any, risk is present for later-onset forms of these disorders, such as very long chain acyl-CoA dehydrogenase deficiency (VLCADD).

Treatment of FAOD shares many common features, and unique aspects will be reviewed in each section respectively. Treatment primarily is targeted at prolonged fasting avoidance to maintain a constant energy supply and prevent the use of fat for energy, together with a higher-carbohydrate, low-fat diet. Safe fasting periods for different age groups have been proposed: neonates until about 3 months of age should fast no longer than 3–4 hours, with this time gradually increasing through infancy (Spiekerkoetter et al., 2009b). IV fluids with 10% dextrose at 150% maintenance rate should be given when the patient is fasting for surgery.

For long-chain FAOD, supplementation with medium-chain triglyceride (MCT) oil is used to provide a substrate for β -oxidation and should be prescribed to provide 20%–25% of total calories (Spiekerkoetter et al., 2009b). Avoiding essential fatty acid deficiency requires supplementation with additional oils. MCT supplementation has been associated with a reversal of cardiomyopathy in carnitine acylcarnitine translocase deficiency (CACT) and VLCADD, although compliant VLCADD patients still had significant muscle weakness, muscle pain, or myoglobinuria (Pierre et al., 2007; Spiekerkoetter et al., 2009a; Pervaiz et al., 2011; Sharef et al., 2013).

Carnitine supplementation is controversial although secondary carnitine deficiency is common. Long-chain hydroxyl-acylcarnitines may exert toxicity by inducing arrhythmias, although mouse studies have been inconclusive (DaTorre et al., 1991).

Medium-Chain Acyl-CoA Dehydrogenase Deficiency

Medium-chain acyl-CoA dehydrogenase deficiency (MCADD) is the most common FAOD with a frequency of approximately 1 in 20,000 births in northern European populations. Classic presentations occur in older infants during an infection, with poor oral intake, vomiting, dehydration, lethargy, hypoglycemia, seizures, and a presentation similar to Reye syndrome, leading to death from brain edema and hyperammonemia. Severe lethal presentations

will develop during the first days of life, and MCADD is a recognized cause of sudden infant death syndrome.

The initial presenting episode historically has a high mortality rate, but this has improved with NBS (Wilcken et al., 2007; Arnold et al., 2010). Plasma acylcarnitine profiles demonstrate increased levels of C6, C8, C10, and C10:1 values. Urine acylglycine analysis shows elevations of suberylglycine and hexanoylglycine. Sequence analysis of *ACADM* confirms the diagnosis with a common mutation, c.985A>G, seen frequently in those of northern European ancestry. Treatment focuses on avoiding prolonged fasting, especially during intercurrent illness, and carnitine supplementation if necessary.

Very Long Chain Acyl-CoA Dehydrogenase Deficiency

VLCADD has been estimated to affect between 1 in 100,000 to 1 in 120,000 individuals, but prevalence may be as high as 1 in 42,500 (Zytkovicz et al., 2001; Chace et al., 2002; Spiekerkoetter et al., 2003; Wilcken et al., 2003). VLCADD presents with variable phenotypes ranging from severe cardiomyopathy that may result in death in the first few days of life to recurrent hypoketotic hypoglycemia or to later-onset presentations with myopathy and/or rhabdomyolysis in adolescence or adulthood (Bertrand et al., 1993; Bonnet et al., 1998; de Lonlay-Debeney et al., 1998; Bonnet et al., 1999; Kluge et al., 2003; Hoffman et al., 2006). Cardiomyopathy and arrhythmias have been reported in a high proportion of cases presenting at less than 6 years in a country without newborn screening (Baruteau et al., 2014).

Most patients with VLCADD are detected through NBS but present a significant challenge in that a large number of individuals appear to have mild or perhaps benign DNA variants and normal follow-up plasma acylcarnitine profiles. Many of these cases will have a single heterozygous mutation and so appear to be unaffected carriers. Others may have two compound heterozygous mutations but have a reduced clinical risk of symptoms. Plasma acylcarnitines show a pattern of elevations of C14:1-, C14-, C16:1-, and C16-acylcarnitines levels with low secondary free carnitine levels in some infants. With an acute metabolic decompensation, urine organic acid analysis may demonstrate dicarboxylic aciduria. Confirmation by sequencing and deletion/duplication analysis of *ACADVL* is recommended. Functional enzyme assay or fibroblast acylcarnitine probe analysis may be helpful to determine treatment when a single heterozygous mutation is found, novel uncharacterized variants are found, and/or there are persistent elevations of acylcarnitines inconsistent with the genotype (Pena et al., 2016).

Treatment follows the general principles for long-chain FAOD treatment, consisting of fasting avoidance, dietary fat restriction, MCT supplementation, and carnitine supplementation if necessary. Infants should discontinue breastfeeding because of the high fat content in breast milk, with implementation of an MCT-containing formula (Spiekerkoetter et al., 2009b). Treatment of milder forms of VLCADD may include supplementation of breastfeeding with an MCT-containing formula.

Short-Chain Acyl-CoA Dehydrogenase Deficiency

Short-chain acyl-CoA dehydrogenase deficiency (SCADD) is diagnosed through the detection of elevations of C4-acylcarnitine, urinary ethylmalonic acid, and butyrylglycine. Prior reports found decreased SCAD enzyme activity to be associated with failure to thrive, poor feeding, hypotonia, and seizures. Subsequently, up to 14% of the

normal population has been found compound heterozygous or homozygous for two common polymorphisms (c.511C>T and c.625G>A) with the biochemical abnormalities of SCADD (Pedersen et al., 2008). Currently, most infants with SCADD are detected on NBS and remain clinically asymptomatic leading many to consider this a benign condition (Gallant et al., 2012). The need for treatment, carnitine or riboflavin supplementation, or management during illness is unclear (Wolfe et al., 1993).

Long-Chain 3-Hydroxy Acyl-CoA Dehydrogenase Deficiency and Trifunctional Protein Deficiency

The mitochondrial trifunctional protein complex of four alpha and four beta subunits comprises three enzymes: long-chain enoyl-CoA hydratase, long-chain 3-hydroxy acyl-CoA dehydrogenase, and 3-ketoacyl-CoA thiolase. Long-chain 3-hydroxy acyl-CoA dehydrogenase deficiency (LCHADD) occurs when there is only reduced dehydrogenase activity because of mutations in the *HADHA* gene, while trifunctional protein deficiency (TFP) results from mutations in either the *HADHA* or *HADHB* gene and in deficient activity of all three enzymes. The prevalence of LCHADD is approximately 1 in 110,000 while TFP is much rarer (Das et al., 2006). The most severe forms of LCHADD or TFP present with a rapidly progressive neonatal cardiomyopathy (Spiekerkoetter et al., 2009a; Sperk et al., 2010). Infants may later develop recurrent hypoketotic hypoglycemia with acute catabolic illness resulting in liver dysfunction (a Reye-like syndrome), cardiomyopathy, myopathy, and rhabdomyolysis. Sixty-five percent of surviving individuals with LCHADD or TFP experience skeletal myopathy, 21% develop a slowly progressing peripheral neuropathy, and 43% have pigmentary retinopathy (Spiekerkoetter et al., 2009a). Some patients may have severe liver disease with fibrosis in addition to necrosis and steatosis. Older children, adolescents, and adults may develop recurrent rhabdomyolysis during illness.

Heterozygous mothers may rarely develop either acute fatty liver of pregnancy or hemolysis, elevated liver enzymes, and low platelets (HELLP syndrome) when carrying a child with LCHADD (Ibdah et al., 1999).

Diagnosis is made through the demonstration of elevations of C16:1-OH-, C16-OH-, C18:1-OH-, and C18-OH-acylcarnitines levels and the demonstration of longer-chain 3-hydroxydicarboxylic acids on urine organic acid analysis. Enzymatic diagnosis can be made in lymphocytes or in skin fibroblasts, but a combination of clinical biochemical abnormalities and *HADHA* or *HADHB* mutation analysis is often sufficient. The majority of moderate-to-severe cases are diagnosed by NBS. Follow-up of cases diagnosed on NBS demonstrates improved growth and development, but NBS does not completely prevent morbidity and mortality, especially in TFP, which has poorer survival (Sander et al., 2005; Spiekerkoetter et al., 2009a; Sperk et al., 2010; Wilcken, 2010).

Treatment of LCHADD and TFP involves avoidance of prolonged fasting, dietary fat restriction, and MCT supplementation. A low-fat diet and MCT supplementation decrease plasma hydroxyl acylcarnitine levels, and most LCHADD patients remain healthy without metabolic decompensation but still require supplementation with essential fatty acids and fat-soluble vitamins (Gillingham et al., 2003).

Primary Carnitine Transporter Deficiency

Carnitine is essential for long-chain fatty acid transport across the mitochondrial inner membrane. This is dependent upon a

sodium-dependent carnitine transporter, two transferases that covalently link and then remove carnitine to the long-chain fatty acid, and a translocase.

Carnitine transporter deficiency (CTD, primary carnitine deficiency, carnitine uptake defect) is characterized by hypoketotic hypoglycemia, hyperammonemia, liver dysfunction, cardiomyopathy, and skeletal hypotonia. Neonatal presentations are not common. In some older patients, cardiomyopathy may be the presenting sign. Profoundly low plasma total and free carnitine levels (typically $<10 \mu\text{mol/L}$ in plasma) are found. False-positive NBS for low free carnitine levels may be due to neonatal nutritional deficiency or secondary to low maternal plasma carnitine levels from either a dietary restriction (e.g., vegan diet) or previously unrecognized maternal primary carnitine deficiency. Measurement of maternal total and free carnitine levels is often necessary when performing follow-up testing of an abnormal NBS. Women with true maternal primary carnitine deficiency may be apparently asymptomatic at diagnosis. Diagnostic confirmation is through DNA sequencing of *SLC22A5* or analysis of fibroblast carnitine uptake (El-Hattab, 1993).

Treatment of primary carnitine deficiency involves supplementation of carnitine at 100 to 200 mg/kg per day to maintain normal carnitine levels. Treatment is successful in preventing or reversing symptoms but is dependent upon compliance.

Carnitine Palmitoyltransferase Type I Deficiency

Carnitine palmitoyltransferase type I (CPTI) covalently links long-chain fatty acids to carnitine. CPTI deficiency will present in early childhood with hypoketotic hypoglycemia and liver dysfunction, less commonly as an adult-onset skeletal myopathy, and only with rare presentations of neonatal hypoglycemia (Bennett and Santani, 1993). Cardiomyopathy is typically not seen, although renal tubular acidosis has been reported (Bonnefont et al., 2004). Diagnostic laboratory findings include elevated total and free plasma carnitine levels, while NBS additionally uses a C0/(C16+C18) ratio to improve specificity. Confirmatory testing will demonstrate mutations in the *CPT1A* gene or through enzyme activity in skin fibroblasts. A common *CPT1A* variant, c.1436C>T (p.P479L), has been identified in the Arctic populations of the Inuit, Alaskan Native, Canadian First Nation, and Hutterite and has been associated with higher infant mortality and impaired fasting intolerance in these populations (Collins et al., 2010; Gillingham et al., 2011; Clemente et al., 2014). Treatment involves fasting avoidance, low-fat diet, and MCT supplementation and results in a normal outcome, although some suffer neurologic impairment from repeated episodes of metabolic decompensation (Bonnefont et al., 2004; Longo et al., 2006).

Carnitine Acylcarnitine Translocase Deficiency

CACT is one of the more severe FAODs, and the most common presentation is ventricular dysrhythmia and sudden neonatal death (Yang et al., 2001; Wilcken, 2010). Symptoms include hypoglycemia, vomiting, gastroesophageal reflux, and mild chronic hyperammonemia, as well as severe skeletal myopathy and mild hypertrophic cardiomyopathy. Early diagnosis and treatment can be beneficial, although significant morbidity includes profound developmental delay, seizures, and other complications despite NBS (Pierre et al., 2007; Al-Sannaa and Cheriyan, 2010; Spiekerkoetter, 2010; Wilcken, 2010). Milder disease is associated with higher residual enzyme activity (Rubio-Gozalbo et al., 2004).

Individuals will have elevated C16-, C16:1-, C18-, and C18:1-acylcarnitine levels with low free carnitine levels on diagnostic testing and NBS. DNA sequencing of *SLC25A20* will confirm disease. Treatment includes fasting avoidance with a low-fat, high-carbohydrate formula, MCT supplementation, and carnitine.

Carnitine Palmitoyltransferase Type II Deficiency

Carnitine palmitoyltransferase type II deficiency (CPTII) results in elevations of C16- and C18:1-acylcarnitines in NBS as found with CACT deficiency; DNA testing is required for diagnosis; treatment depends on severity. Children with the severe form of CPTII deficiency may have congenital anomalies including renal cysts, dysmorphic facies, and brain malformations and may present with hypotonia, cardiomyopathy, arrhythmias, and seizures within the newborn period (Albers et al., 2001). The later-onset form of CPTII deficiency is much more common and presents in the second or third decade of life with exercise intolerance or rhabdomyolysis (Longo et al., 2006). Confirmatory sequencing of the gene *CPT2* will reveal mutations (Wieser et al., 2003).

Multiple Acyl-CoA Dehydrogenase Deficiency

Multiple acyl-CoA dehydrogenase deficiency (MADD; also called *glutaric acidemia type 2*) is the result of a defect of electron transfer from multiple acyl-CoA dehydrogenases to the mitochondrial ETC. Each acyl-CoA dehydrogenase enzyme binds electron transfer flavoprotein (ETF). ETF accepts electrons from the FADH_2 cofactor in the oxidative dehydrogenation reactions and is made up of three subunits. Mutations in the genes for the three subunits, *ETFA*, *ETFB*, and *ETFDH*, will interfere with electron transfer from ETF to coenzyme Q10 within the mitochondria. Riboflavin (an FADH_2 component) deficiency or deficient riboflavin transport may show a similar presentation—a riboflavin-responsive form of MADD has been described. Because of the multiple dehydrogenase enzymes involved there will be elevations of metabolites from short-, medium-, and long-chain fatty acids and amino acid metabolism.

MADD may present in three major ways. The first two present in the newborn period, with or without congenital anomalies, and the third is a later-onset type. Neonatal MADD presents with metabolic acidosis, hypoketotic hypoglycemia, and often hypertrophic cardiomyopathy. Those with congenital malformations may have enlarged polycystic kidneys, rocker-bottom feet, defects of the inferior abdominal musculature, and hypospadias and chordee. Hypotonia, cerebral cortical dysplasia, and gliosis have been reported, and dysmorphic facies may include telecanthus, malformed ears, macrocephaly, large anterior fontanel, high forehead, and a flat nasal bridge (Wilson et al., 1989). Older patients with the later-onset MADD do not have congenital malformations but have a lifelong risk of acute intermittent episodes with vomiting, dehydration, hypoketotic hypoglycemia, and acidosis. In some there may be hepatomegaly and muscle disease.

Many infants do not survive beyond the first few weeks or months of life because of rapidly progressing cardiomyopathy. In individuals identified through NBS and in whom treatment is initiated early, acute, life-threatening events or sudden death may still occur (Angle and Burton, 2008; Singla et al., 2008).

Diagnosis of MADD is suspected due to the combination of increased anion gap lactic acidosis, hypoketotic hypoglycemia, and hyperammonemia. They may have an odor of isovaleric acid. Increased serum transaminases and prolongations of prothrombin

time and partial thromboplastin time suggest liver dysfunction. Diagnostic testing should include plasma acylcarnitines with elevations of C4 and C5 acylcarnitines (which are the primary analytes on NBS), but medium- and long-chain acylcarnitines may also be present (i.e., C8-, C10:1-, C12-, C14-, C14:1-, C16-, C16:1-, C18-, C18:1-, C16-OH-, C16:1-OH-, C18-OH-, and C18:1-OH-acylcarnitines). Urine organic acid analysis will show elevations of ethylmalonic acid, glutaric acid, and 3-hydroxyisovalerate in addition to lactic acid, medium- and long-chain dicarboxylic acids, the glycine conjugates isovalerylglycine, isobutyrylglycine, and 2-methylbutyrylglycine. Ketone bodies, including acetoacetic acid and 3-hydroxybutyric acids, are minimal or undetectable. Generalized aminoaciduria will reflect impaired renal tubular function. DNA sequencing of *ETFA*, *ETFB*, and *ETFDH* will confirm the diagnosis.

Treatment of MADD includes a low-protein and low-fat diet, fasting avoidance, and supplementation with carnitine, riboflavin, and glycine. Individualized metabolic formulas often have to be designed to meet nutritional goals. Individuals at least heterozygous for common *ETFDH* mutations confer a milder riboflavin-responsive phenotype with some cases of complete correction of clinical and biochemical parameters after riboflavin treatment (Olsen et al., 2007). Acute decompensation should be treated with IV glucose and carnitine to restore anabolism with close monitoring of cardiac, hepatic, and renal function.

Ketone Metabolism Disorders

Disorders of ketone metabolism result from the inability to use ketone bodies, 3-hydroxybutyric acid and acetoacetic acid, for energy generation. Each cycle of the fatty acid β -oxidation generates a single acetyl-CoA. This acetyl-CoA has to be converted to acetoacetic acid by mitochondrial acetoacetyl-CoA thiolase, 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) synthase, and then HMG-CoA lyase. Acetoacetic acid and 3-hydroxybutyric acid are transported out of liver mitochondria and hepatocytes into blood to be used by other tissues, especially the brain and heart.

Patients with mitochondrial acetoacetyl-CoA thiolase deficiency (β -ketothiolase or 3-oxothiolase deficiency) have a metabolic acidosis associated with excess ketosis. Most commonly presenting in children around 15 months of age, cases are reported as early as 3 and 4 days of age with lethargy, metabolic ketoacidosis, and an elevated anion gap (Fukao et al., 2001; Cubillo Serna et al., 2007). The clinical presentation varies from severe, acute metabolic decompensation in infants to asymptomatic adults. The episodes are intermittent, are typically associated with a catabolic stressor or a high dietary protein intake, and have been associated with mild hyperketotic hypoglycemia and liver dysfunction, without hyperammonemia. Cardiomyopathy is rare. Plasma acylcarnitines and NBS will demonstrate elevations of C5-OH- and C5:1-acylcarnitines. Urine organic acid analysis demonstrates significant elevations of lactate and ketone bodies and a specific pattern of elevations of 2-methylacetoacetate, 2-methyl-3-hydroxybutyrate, and tiglylglycine. Older children and adults have ketoacidosis while others have remained asymptomatic. Diagnosis is confirmed by DNA sequence analysis of the *ACAT1* gene looking for mutations in this autosomal recessive disorder. Acute treatment involves IV glucose and bicarbonate to correct metabolic acidosis, which is often severe, while long-term therapy includes mild protein restriction, avoidance of fasting, and prompt attention to any intercurrent illness or catabolic stressor, as treatment and avoidance of severe ketoacidosis may lead to normal development.

The most severe disorder of ketone metabolism is the last step in synthesis of acetoacetate from HMG-CoA via the HMG-CoA lyase enzyme. Neonates present as early as 3 days of life with an often-catastrophic illness characterized by vomiting, lethargy, hypoketotic hypoglycemia, metabolic acidosis, hyperammonemia, elevated transaminases, hepatomegaly, seizures, and coma. Urine organic acid analysis reveals 3-hydroxy-3-methylglutaric acid, 3-methylglutaconic acid, and 3-hydroxyisovaleric acid in a specific diagnostic pattern with elevations of C5-OH- and C6-DC-acylcarnitines on plasma acylcarnitine analysis and NBS. Levels of acetoacetic acid and 3-hydroxybutyric acid may be unexpectedly low. Lactate values may be elevated during the acute metabolic decompensation.

An autosomal recessive disorder, the HMG-CoA lyase deficiency is confirmed by mutation analysis of the *HMGCL* gene. Acute treatment of the episode consists of administration of IV rehydration and administration of glucose and bicarbonate, to correct metabolic acidosis. Long-term treatment consists of avoiding prolonged fasting, a low-protein and high-carbohydrate diet, and carnitine supplementation (Gibson et al., 1988).

Succinyl-CoA 3-ketoacid-CoA transferase deficiency results from the failure of extrahepatic tissues to convert acetoacetate back to acetoacetyl-CoA. This is required for hydrolysis to acetyl-CoA for final metabolism in the TCA cycle. Affected patients have a persistent ketosis with intermittent ketoacidosis that does not resolve, even postprandially. Affected newborns often present in the 1st week of life with severe ketosis, lactic acidosis, hypoglycemia, and coma, and many do not survive (Fukao et al., 2014). Elevated acetoacetate and 3-hydroxybutyrate levels are almost always present in blood and urine. Therapy focuses on fasting avoidance, which can cause profound acidosis and ketosis, and IV fluids, glucose, and sodium bicarbonate during crisis. Enzyme analysis in fibroblasts is available, but DNA sequencing of the *OXCT1* gene will confirm the diagnosis in this autosomal recessive disorder. Milder forms do exist, and patients may have nonketotic periods.

Primary Lactic Acidosis

Congenital lactic acidosis (CLA) is due to a severe disorder of energy metabolism. This can be caused by one of multiple diseases, including those in which lactate and pyruvate metabolism are impaired because of a primary defect in the mitochondrial ETC or the tricarboxylic acid (TCA) cycle. These disorders affect pyruvate metabolism, which, in turn, affects lactate. The majority of neonates presenting with CLA have defects of the mitochondrial ETC or the PDH complex or PC deficiency. Inborn errors of the TCA cycle are much rarer but should be considered in the differential diagnosis. A recently described autosomal recessive disorder of metabolism of the amino acid valine, short-chain enoyl-CoA hydratase deficiency because of mutations in the *ECHS1* gene, appears to cause a secondary inhibition of PDH and can present with refractory CLA with a Leigh disease-like presentation, neurodegeneration, and neonatal death (Ferdinandusse et al., 2015).

Some patients with CLA present with overwhelming lactic acidosis in the neonatal period. In others, lactate may be elevated only in CSF. This “cerebral” lactic acidosis may present more indolently. When blood lactate is elevated, the ratio of blood lactate to pyruvate can help narrow the differential diagnosis. The ratio is low to normal (10 to 20) in PDH deficiency, may be modestly elevated in PC type B deficiency (see later), but may be greatly elevated (>25) in an ETC defect. This ratio reflects the oxidation–reduction state of the mitochondria. When calculating this

it is important to ensure the units of the two compounds are the same. These conditions are not identified through NBS, and patients will present symptomatically unless there has been a prior affected family member such as an older affected sibling.

Pyruvate Dehydrogenase Complex Deficiency

The PDH complex converts pyruvate, which is derived from the catabolism of glucose, to acetyl-CoA, which enters the TCA cycle at citrate synthase. Severe PDH deficiency may manifest in the neonatal period with profound lactic acidosis and a low to normal lactate-to-pyruvate ratio. Patients may have moderately elevated plasma ammonia and congenital brain anomalies including an absent or underdeveloped corpus callosum and heterotopias. They are hypotonic and may require mechanical ventilation. The prognosis is poor, even with early recognition and intervention. Importantly, the high concentration dextrose-containing IV and enteral empiric therapy that may be life-saving for a child with a possible organic aciduria or urea cycle defect exacerbates the lactic acidosis in the neonate with PDH deficiency or other mitochondrial energy metabolism disorders. This worsening may suggest a primary energy metabolism disorder in the neonate. A possible diagnosis of PDH deficiency is inferred from the patient presentation, the clinical course, and the results of routine and specialized biochemical laboratory testing. Alanine will likely be elevated on plasma amino acid analysis, and TCA cycle intermediates may be elevated in the urine organic acid analysis. There is no specific diagnostic compound that identifies this disorder. The diagnosis is made through abnormal enzyme analysis in skin fibroblasts or white blood cells and/or by DNA testing. The majority of individuals have a mutation in the *PDHA1* gene, which encodes the E1- α subunit of the PDH enzyme complex. This is an X-linked dominant condition, and both males and females are affected (Patel et al., 2012).

PDH is a large, multisubunit complex. It contains three enzymatic subunits and several regulatory subunits, including a phosphatase and a kinase. The first enzymatic step is a decarboxylation reaction catalyzed by a heterodimeric system consisting of the E1- α subunit and E1- β . All other subunits, including E1- β , result in autosomal recessive inheritance. Defects in all the known genes have been reported, and DNA sequencing is available, but mutations in *PDHA1* account for 80% of cases of PDH deficiency.

The majority of patients are indolent on clinical presentation with developmental delay and an MRI indicative of Leigh disease. This group of patients often responds well biochemically to a high-fat and low-carbohydrate, or ketogenic, diet. Fat, as acetyl-CoA, enters the energy pathway after the block, whereas glucose must traverse the defective PDH enzymatic reaction to provide energy.

Defects of the PDH complex because of defects in the two other enzyme subunits, the activating and deactivating enzymes and a structural protein that binds one subunit (E3 binding protein), are rarer. These usually result in chronic psychomotor retardation syndrome in late infancy and childhood. Deficiency of the E3 subunit of the PDH complex has pleiotropic biochemical effects, because the subunit is a component of two other dehydrogenase complexes: the BCKAD complex and the α -ketoglutarate dehydrogenase complex of the TCA cycle. Therefore these patients have elevated BCAAs as in MSUD, which is due to BCKAD deficiency, as well as elevated TCA cycle metabolites because of α -ketoglutarate dehydrogenase complex deficiency. Most patients with E3 deficiency present after the newborn period and have

progressive neurodegenerative disease. The key laboratory findings are the elevation of lactic acid in blood with elevated pyruvate and a low lactate-to-pyruvate ratio, elevated BCAAs on plasma amino acid analysis, and detection of α -ketoglutarate and BCAA metabolites in urine organic acid analysis. PDH phosphatase deficiency is a rare cause of CLA. Apart from E3 deficiency, defects in the PDH complex are responsive to the ketogenic diet.

In addition to E3 deficiency there is another class of defects that affects PDH and other enzyme complexes, including BCKAD and α -ketoglutarate dehydrogenase complex, because of the requirement for lipoate in these and the complex synthesis of lipoate. Defects in eight genes encoding proteins required for lipoate synthesis have been reported, and these may present as CLA (Tort et al., 2016). Symptoms are variable and may be characteristic of the specific genetic defect. In an early-onset presentation, symptoms can include seizures, encephalopathy, and cardiomyopathy (Tort et al., 2016).

Pyruvate Carboxylase Deficiency

PC is involved in gluconeogenesis and adds bicarbonate to pyruvate to form oxaloacetate, a compound also involved in replenishing intermediates of the TCA cycle. PC is one of the four carboxylases that are biotin-requiring enzymes. There are three main types of PC deficiency. Type A is characterized by lactic acidosis in the newborn period and delayed development, and the disease has a chronic course. The catastrophic form of the disorder is type B where the neonate is acutely ill in the first week of life, is encephalopathic, and develops severe metabolic acidosis with lactic acidosis and hyperammonemia. The mortality rate in this form of PC deficiency is high. Type C is considered intermittent and benign (Wang and De Vivo, 1993).

Most patients with the type B form of PC deficiency have been of French or English background. The blood lactate-to-pyruvate ratio is normal in type A as both lactate and pyruvate are comparably elevated, while patients with type B often have an elevated lactate-to-pyruvate ratio. Because oxaloacetate produces aspartate, which then combines with citrulline to create argininosuccinate through the urea cycle, PC deficiency leads to elevations of plasma citrulline and plasma ammonia. Although PC is an important enzyme in gluconeogenesis, hypoglycemia is not common. The liver may be enlarged. The prognosis is poor. Enzyme testing may be performed in white blood cells or fibroblasts. DNA mutational analysis may also be performed for diagnosis.

Electron Transport Chain Defects

Oxidative phosphorylation results in the generation of ATP and is the central process performed by mitochondria. Genetic defects affecting the tightly coupled and regulated process of ATP generation may have profound effects on one or more organ systems. Derivatives of nutrients such as pyruvate and fatty acids are converted to carbon dioxide in mitochondria. The energy derived from this is harnessed by allowing the reducing equivalents (as NADH or FADH₂, which are derived from such metabolism) to combine with oxygen to form water and, in the process the synthesis of ATP, is coupled to the flow of electrons down the ETC.

The important components in the mitochondrial respiratory chain are complex I (NADH dehydrogenase), complex II (ETF dehydrogenase), complex III (cytochromes *b*, *c*₁), and the terminal complex in this chain, complex IV (cytochrome *c* oxidase). In addition, there is a complex V (ATP synthetase) and an adenine

nucleotide translocase that permit transport of adenosine diphosphate into, and ATP out of, the mitochondria. Complex II is involved primarily in fatty acid oxidation and oxidation of succinate derived from the TCA cycle, because the reducing equivalents extracted from fatty acids, glutaric acid, and succinate flow from ETF into complex II. Early understanding of the molecular mechanisms that produce ETC disturbances concerned those attributed to mutations of mitochondrial DNA (mtDNA—a small circular DNA molecule located within the mitochondria) genes, although in recent years multiple new disorders that effect the ETC caused by nuclear (chromosomal) encoded genes have been identified through DNA sequencing. mtDNA is important in production of the subunits of each respiratory chain complex as at least one subunit is encoded by the mtDNA except for complex II. CLA caused by defects in ETC components can be due to either nuclear or mtDNA encoded subunits, which is an important factor in genetic counseling of families of affected children. In addition to the structural components of a respiratory chain complex, defects in genes encoding proteins responsible for the assembly of the protein subunits into functional complexes can also cause lactic acidosis. Nuclear DNA encodes these assembly proteins, and defects in more than 1000 genes can result in ETC deficiency (Parikh et al., 2015).

The relationship between phenotype and a specific mtDNA mutation is not straightforward, due, at least in part, to heteroplasmy (the proportion of mutant and nonmutant mtDNA molecules within each cell, each of which contain many mitochondria that also each contain multiple copies of mtDNA). Mitochondria and their mtDNA are inherited solely from the mother. Random segregation of mitochondria having greater or fewer mtDNA mutations as oocytes are formed leads to a “bottleneck effect” in which the fetus has a higher concentration of cells with mutant mtDNA than the mother, who may have little or no mutant mtDNA detectable in blood. Often there is a different proportion of defective mitochondria in different cells and different tissues, and, crucially, there is a tissue-specific “threshold effect”; that is, a certain proportion of mutant mtDNA molecules must be present to have clinical consequences in a given tissue (Wallace et al., 1988; Parikh et al., 2015).

The disorders caused by nuclear DNA gene mutations are most often autosomal recessive, and they comprise the majority of ETC disease presenting in neonates and infants, with a minority resulting from mtDNA gene mutations with a maternal inheritance pattern. With the exception of neurogenic muscle weakness, ataxia, and retinitis pigmentosa (NARP, caused by a mutation at position 8993 of the mtDNA), only a minority of diseases caused by mtDNA mutations manifest in the newborn period (Wong, 2007). Other examples of syndromes caused by mtDNA mutations are MELAS (mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes, mtDNA position 3243) and MERRF (myoclonic epilepsy with ragged-red fibers, position 8344) syndromes, Leber hereditary optic neuropathy (Wallace et al., 1988), and sporadic deletion–duplication syndromes such as Pearson marrow–pancreas syndrome (Di Donato, 2009). Important mitochondrial energy metabolism disorders that affect infants include CLA, the most dramatic form, with presentation often in the first few days of life during which there is marked lactic acidosis (Carrozzo et al., 2007; Gibson et al., 2008); subacute necrotizing encephalomyopathy or Leigh disease that classically presents at later than 3 months of age but can present earlier; Alpers disease presenting with seizures and liver disease; benign infantile mitochondrial myopathy, cardiomyopathy, or both; lethal infantile mitochondrial disease; lethal infantile

cardiomyopathy; and Pearson syndrome. The hallmarks of mitochondrial disease are often multisystem involvement and lactic acidosis, but involvement of only one organ system and the absence of lactic acidosis do not exclude it.

Important classes of mitochondrial disorders include nuclear encoded mtDNA depletion syndromes resulting from defects in mtDNA replication component proteins or in the availability of nucleotides for DNA replication, which may include fatal hepatopathy (El-Hattab and Scaglia, 2013; Mayr et al., 2015). Another class is the defects in lipoate synthesis, including those of iron–sulfur protein synthesis (Tort et al., 2016). A potentially treatable class is that of coenzyme Q10 biosynthesis diagnosed by DNA testing of nuclear genes or by coenzyme Q10 levels in muscle. This includes a severe infantile multisystem disease, and it is important to identify these defects as they are one of the few classes of mitochondrial disorders in which administration of coenzyme Q10 may be effective (Quinzii and Hirano, 2011).

The diagnostic tools include measurement of plasma lactate and pyruvate and analysis of urine organic and plasma amino acids. CSF lactate may be measured by lumbar puncture and by magnetic resonance spectroscopy during MRI. The current availability of mtDNA sequencing panels and whole exome sequencing with full sequencing of the mitochondrial genome has decreased the use of muscle biopsy. However, histologic analysis of muscle tissue by light and electron microscopy and mitochondrial respiratory chain complex assay on either fresh (preferred, but infrequently available) or flash-frozen tissue may be of aid in diagnosis. In many cases however, DNA sequencing has replaced muscle biopsy as a first-line test for suspected mitochondrial disease (Parikh et al., 2015). In fatal cases, rapid (metabolic) autopsy and proper preservation of tissue specimens are essential if functional assays are to be performed. DNA testing can also be performed on tissues.

Benign Infantile Mitochondrial Myopathy, Cardiomyopathy, or Both

Benign infantile mitochondrial myopathy is associated with congenital hypotonia and weakness at birth, feeding difficulties, respiratory difficulties, and lactic acidosis. In this poorly understood disorder, only skeletal muscle appears to be affected, and histochemical analyses show a cytochrome *c* oxidase deficiency that returns to normal levels after 1 to 3 years of age. A nuclear DNA mutation in a gene-encoding fetal isoform of an ETC polypeptide specific for muscle oxidative phosphorylation was hypothesized to be the cause of this. A developmental switch from the defective fetal gene to the adult form may be responsible for the gradual improvement. It was thought to be the only example of a developmental defect in oxidative phosphorylation that is probably nuclear encoded and in which the treatment is supportive during the early newborn period to prevent death from respiratory disease. A study suggested the etiology may be due to a maternally inherited homoplasmic m.14674T>C or T>G mitochondrial tRNA^{Glu} mutation or may be due to mutations in the nuclear gene *TMRU* (Horvath et al., 2009; Uusimaa et al., 2011).

The form also associated with cardiomyopathy may be a variant of the benign isolated myopathy and involves striated muscle in both skeletal and cardiac muscle. It manifests in the newborn period with lactic acidosis and a cardiomyopathy that improves during the first year of life. The exact gene defect is unknown. More attention must be paid to these two disease entities because with early optimal medical care affected infants may have an excellent prognosis.

Lethal Infantile Mitochondrial Disease

Infants with lethal infantile mitochondrial disease are severely ill in the first few days or weeks of life or in the extended newborn period. They exhibit hypotonia, muscle weakness, failure to thrive, and severe lactic acidosis. Death often occurs by 6 months of age and almost always is associated with overwhelming lactic acidosis. Skeletal muscle shows lipid and glycogen accumulation and abnormally shaped mitochondria on electron microscopic examination. Hepatic dysfunction may be a prominent finding in these patients. Generalized proximal renal tubular dysfunction may occur, leading to renal Fanconi syndrome. The ETC defects reported in these patients include defects in complexes I, III, and IV. Genes responsible for combined ETC defects continue to be identified (Mayr et al., 2015).

Leigh Disease: Subacute Necrotizing Encephalomyelopathy

Leigh disease is a progressive neurodegenerative disorder with severe hypotonia, seizures, extrapyramidal movement disorder, optic atrophy, and defects in automatic ventilation or respiratory control (Baertling et al., 2014). Generally, disease onset is outside of the neonatal period, but symptoms may be evident in the first months of life. There are more than 70 genetic defects known to be associated with Leigh disease (Gerards et al., 2016). These include defects in the PDH complex, ETC structural proteins, assembly factors of individual ETC complexes, coenzyme Q10 biosynthesis, biotinidase, and others. MRI characteristically shows bilateral symmetrical T2-weighted hyperintense lesions in the basal ganglia. One disorder with features of Leigh disease is biotin–thiamine-responsive basal ganglia disease caused by *SLC19A3* deficiency, and empiric biotin and thiamine should be trialed (Baertling et al., 2014).

Clinical findings in infants with Leigh disease include optic atrophy, ophthalmoplegia, nystagmus, respiratory abnormalities, ataxia, hypotonia, spasticity, seizures, developmental delay, psychomotor retardation, myopathy, and renal tubular dysfunction. Some patients may manifest hypertrophic cardiomyopathy, liver dysfunction, and microcephaly. The neuropathologic lesions include demyelination, gliosis, necrosis, relative neuronal sparing, and capillary proliferation in specific brain lesions.

Pearson Syndrome

One class of mitochondrial disorders that is genetic but not familial is caused by spontaneous mtDNA deletions or duplications. These include Kearns–Sayre syndrome and chronic progressive external ophthalmoplegia, which manifest in older individuals. The manifestation in early infancy is Pearson syndrome. This disorder manifests with anemia, ringed sideroblasts, and exocrine pancreatic dysfunction. This disease of the bone marrow can lead to death in infancy. However, patients able to recover or who benefit from aggressive therapy may demonstrate other signs of this systemic disorder in late infancy or childhood, such as poor growth, pancreas dysfunction, mitochondrial myopathy, lactic acidosis, and progressive neurologic damage, and develop Kearns–Sayre syndrome (DiMauro and Hirano, 1993).

Barth Syndrome

Barth syndrome is an X-linked disorder associated with cardiomyopathy, skeletal muscle disease, and neutropenia (Ferreira et al.,

1993). Skeletal muscle shows abnormal mitochondrial morphology and deficiencies of respiratory chain complexes III and IV. The *TAZ* gene encodes tafazzin, a cardiolipin acyltransferase, which leads to disruption of the inner mitochondrial membrane and disruption of ETC function. The accumulation of NADH and FADH₂ inhibit the TCA cycle, driving lactic acidosis, the development of muscle weakness, and cardiomyopathy. Urine organic acid analysis will show an increased urinary excretion of 3-methylglutaconic acid. Barth syndrome is also known as *3-methylglutaconic aciduria type II* but is genetically distinct from primary 3-methylglutaconic aciduria type I, caused by mutations in *AUH* involved in leucine metabolism, and other forms of 3-methylglutaconic aciduria (Gaspard and McMaster, 2015). Patients must be supported from birth to early infancy as acute neonatal presentations may have lactic acidosis, hypoglycemia, hyperammonemia, and liver dysfunction. Fetal loss and stillbirth have also been reported. Treatment includes standard support for heart failure and neutropenia as well as nutrition support and physical therapy (Ferreira et al., 1993).

Early Lethal Lactic Acidosis

In some patients with primary disturbances of mitochondrial ETC, massive lactic acidosis develops within 24 to 72 hours of birth. Commonly the condition is untreatable, because it is relentless and unresponsive to buffer therapy (Danhauser et al., 2015). Dialysis may be helpful but is not a cure and is not feasible in all patients. Often, affected infants have no obvious organ damage early in the course or evidence of malformations. In addition, acidemia per se can easily cause the coma or impaired cardiac contractility that may be encountered. Some infants have survived with aggressive therapy, and specific treatments can include thiamine, biotin, riboflavin, coenzyme Q10, or carnitine (Danhauser et al., 2015). Overall prognosis is likely poor, and the care of neonates with severe lactic acidosis is difficult as the prognosis is unclear. Decisions regarding management must be individualized, because the mitochondrial dysfunction and resultant pathophysiology can vary among infants, and expedited nuclear and mitochondrial DNA testing, to facilitate diagnosis and therefore guide prognosis, should be considered.

Suggested Readings

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23

Lysosomal Storage, Peroxisomal, and Glycosylation Disorders and Smith–Lemli–Opitz Syndrome Presenting in the Neonate

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KEY POINTS

- Lysosomal storage diseases (LSDs) may present in the extended neonatal period. Findings may include nonimmune hydrops fetalis, brain disease and seizures, cherry-red spot, dysmorphic facial features, dysostosis multiplex, and/or hepatomegaly. An LSD may be diagnosed via an enzyme assay and/or DNA sequencing; newborn screening (NBS) for LSDs has generated ethical and moral concerns, as well as controversy.
- Many of the LSDs may be treated with enzyme replacement therapy (ERT).
- The phenotypic spectrum of glycosylation disorders is broad and ranges from mild to severe and from single organ system to multisystem disease; glycosylation defects should be considered in any unexplained clinical condition but especially in multiorgan disease with neurologic involvement.
- Most glycosylation disorders have been diagnosed molecularly since the advent of clinical next-generation sequencing testing.
- Treatment of glycosylation defects is mainly supportive, with the exceptions of more targeted therapies being available for MPI-CDG, SLC35C1-CDG, PIGM-CDG, and PGM1-CDG.
- Peroxisomal disorders are a broad and heterogeneous group of inherited diseases that result from the absence or dysfunction of one or more peroxisomal enzymes.
- Features are typically evident in the newborn and are most often multisystem features, including craniofacial dysmorphism, neurologic dysfunction, including hearing and vision dysfunction, hepatodigestive dysfunction, renal cysts, and skeletal abnormalities.
- Diagnosis of peroxisomal disorders is best made by next-generation sequencing techniques following abnormal biochemical screening test findings.
- Treatment of individuals with peroxisomal disorders is largely symptomatic and supportive.
- Smith–Lemli–Opitz syndrome (SLOS) is a multisystemic, developmental, and dysmorphic disorder with a wide clinical spectrum caused by a defect in cholesterol biosynthesis.
- Diagnosis of SLOS is based on elevated 7-dehydrocholesterol and 8-dehydrocholesterol levels in the blood.
- Treatment of SLOS mainly involves supportive management and exogenous cholesterol supplementation.

CONTROVERSY BOX

Newborn Screening for Lysosomal Storage Diseases

Certain physicians and ethicists consider it premature to mandate state newborn screening for lysosomal storage diseases such as Krabbe disease, as the natural history of each disease with its different genotypes and phenotypes is not known and improvement in outcome with early intervention is not proven. In other disorders the presentation is later in life and not in the newborn period (e.g., Fabry disease) and thus is not consistent with the original paradigm of newborn screening. In addition, many states do not have the human resources or financial resources to ensure an accurate diagnosis and proper long-term medical care. An opposing view is that it is our moral duty to treat these patients and alleviate suffering as soon and as best as possible and to reduce or eliminate the diagnostic odyssey that many individuals suffer before diagnosis.

Suggested Readings

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Lysosomal storage disorders (LSDs), peroxisomal disorders, congenital disorders of glycosylation (CDGs), and Smith–Lemli–Opitz syndrome (SLOS) are single-gene disorders, most of which demonstrate autosomal recessive inheritance. The combined incidence of LSDs has been reported to be 1 in 1500 to 1 in 8000 live births in the United States, Europe, and Australia (Stone and Sidransky, 1999; Winchester et al., 2000; Wenger et al., 2003; Fletcher, 2006; Meikle et al., 2006; Staretz-Chacham et al., 2009). The incidence of peroxisomal disorders is estimated to be more than 1 in 20,000. The most current estimate for SLOS

Case Study 1

C.J. was a 2200 g girl born to a 24-year-old mother (third pregnancy, second viable child) after a 32-week gestation by cesarean delivery performed for fetal distress. The pregnancy was complicated by the finding on ultrasonography of fetal hydrops and ascites and possible hepatosplenomegaly at 24 weeks' gestation. Fetal blood sampling showed a hematocrit of 31% and elevations of γ -glutamyltransferase and aspartate transaminase values. The results of viral studies were negative and chromosomes were normal. At delivery, the newborn was limp and blue, with a heart rate of 60 beats per minute. Physical examination and chest radiograph showed marked abdominal distention, hepatosplenomegaly, multiple petechiae and bruises, a bell-shaped thorax, generalized hypotonia, talipes equinovarus, contractures at the knees, a large heart, and hazy lung fields with low volumes. Disseminated intravascular coagulopathy and evidence of liver disease developed rapidly, with elevated aspartate transaminase and γ -glutamyltransferase levels and increasing hyperbilirubinemia. The patient received mechanical ventilatory support and antibiotics for possible sepsis.

The results of evaluations for bacterial and viral agents were negative. Metabolic studies, including studies of ammonia, lactate, very long chain fatty acids, and urine amino and organic acids, yielded unremarkable measurements. The white blood cells were noted to have marked toxic granularity consistent with overwhelming bacterial sepsis or metabolic storage disease.

The patient experienced continued cardiorespiratory deterioration, had bilateral pneumothoraces and pneumopericardium, and died on the third day of life. Consent for autopsy was obtained from the family. A standard autopsy was performed and showed the presence of large, membrane-bound vacuoles within hepatocytes, endothelial cells, pericytes, and bone marrow stromal cells, which are typical of a metabolic storage disorder. Similar cells were also found within the placenta. There was no evidence of an infectious cause. Unfortunately, because a lysosomal storage disorder was not considered as a possible cause at the time of death, no frozen tissue or cultured fibroblasts were available to pursue the diagnosis. As a result of efforts by a research laboratory and the recurrence of disease in the couple's subsequent pregnancy, a diagnosis of β -glucuronidase deficiency, or mucopolysaccharidosis type VII, was confirmed.

is 1 in 20,000, and a similar frequency of 1 in 20,000 is estimated for the CDGs.

These four categories of metabolic diseases involve molecules important in cell membranes and share overlapping clinical presentations. The clinical presentations are heterogeneous, with a broad range of age at presentation and severity of symptoms. All are chronic and progressive. The age of onset ranges from prenatal to adulthood, and severity can range from severe disability and early death to nearly normal lifestyle and life span. For each condition, interfamilial variability is greater than intrafamilial variability. The genetic and clinical characteristics of conditions in these categories that can manifest themselves in the neonatal period (except Pompe disease, which is addressed in Chapter 22) are summarized in [Tables 23.1–23.2](#).

Important presentations that should lead the neonatologist to consider these disorders in the differential diagnosis are as follows:

1. In utero infection—hepatosplenomegaly and hepatopathy, possibly with extramedullary hematopoiesis
2. Nonimmune hydrops fetalis, ichthyotic or collodion skin, or both
3. Neurologic only—early and often difficult to control seizures, hypertonia, or hypotonia, with or without altered head size and with or without eye findings

Case Study 2

M.E. was born by normal spontaneous vaginal delivery, at term according to dates based on early ultrasonography, with a weight of 2200 g, a length of 45 cm, and a head circumference of 31.5 cm. On the basis of physical examination, the gestational age was assessed as 36 weeks. A heart murmur was noted, and investigation showed the presence of a small ventricular septal defect with no hemodynamic significance. Submucous cleft palate was noted. Examination for dysmorphic features showed simple, posteriorly rotated ears, mild epicanthic folds, micrognathia, and unilateral simian crease. Tone was moderately decreased. Irritability and severe feeding problems were noted, and gavage feeding was required; growth was poor despite adequate intake of calories. The results of a karyotype analysis were normal, and the results of studies for velocardiofacial syndrome were negative. Vomiting developed, and further evaluation showed no acidosis, hypoglycemia, or hyperammonemia. Liver-associated values and cholesterol level were normal, as were results of studies of amino acids, organic acids, and acylcarnitine profile. Vomiting became severe and did not respond to elemental formula, and pyloric stenosis was detected. Feeding problems persisted after successful surgical correction. Delivery of more than 140 kcal/kg by gavage was poorly tolerated but resulted in weight gain; however, length and head growth remained poor.

Smith–Lemli–Opitz syndrome was suggested despite the normal cholesterol value obtained on analysis in the hospital laboratory. Studies performed in a specialized laboratory showed the 7-dehydrocholesterol and 8-dehydrocholesterol values to be elevated and the cholesterol value to be decreased. Cholesterol supplementation led to some improvement in behavior and feeding. A decrease to 110 kcal/kg per day was tolerated without worsening of growth, and weight for height gradually returned to normal. A review of records confirmed that the pregnancy had been accurately dated by ultrasonography at 10 weeks' gestation, confirming that M.E. was small for gestational age and microcephalic at birth, with subsequent growth typical of Smith–Lemli–Opitz syndrome. The incorrect assessment of gestational age as 36 weeks on examination was found to result from a failure to appreciate the effect of hypotonia on the findings for gestational age. The family was counseled about autosomal recessive inheritance, including the availability of prenatal diagnosis.

Case Study 3

H.K. was born at term to healthy parents by cesarean delivery performed for breech presentation after an otherwise uncomplicated pregnancy. Hypotonia and dysmorphic features were noted in the delivery room, including inner epicanthic folds, flat occiput, large fontanelles, shallow orbital ridges, low nasal bridge, micrognathia, redundant skin folds at the neck, and unilateral simian crease. Brushfield spots were present. Investigation of a heart murmur revealed patent ductus arteriosus and a small atrial septal defect. There was mild hepatomegaly but normal liver function, no acidosis, and no hypoglycemia. Suck was poor, and gavage feeding was required.

The results of a karyotype analysis were normal, and there was no evidence of trisomy 21 in blood in 50 interphase cells examined. The option of skin biopsy to search further for evidence of mosaicism for trisomy 21 was considered. Thyroid function values were normal. Urine amino and organic acid values were normal, as was the acylcarnitine profile. Plasma very long chain fatty acid analysis showed elevation consistent with a diagnosis of Zellweger syndrome, along with a typical increase in pipecolic acid value and impaired capacity for fibroblast synthesis of plasmalogens. The baby died at 3 months of age, and autopsy showed polymicrogyria and small hepatic and renal cysts. The family was counseled about autosomal recessive inheritance, including the availability of prenatal diagnosis.

Case Study 4

M.J. had hypotonia at birth after an uncomplicated pregnancy. Minor dysmorphic features were noted, including high nasal bridge, large ears, and inverted nipples. Feeding difficulties were significant, and growth was poor. The findings on head ultrasonography were unremarkable, as were those of head magnetic resonance imaging, although the radiologist questioned whether the cerebellum might be slightly small. The results of a karyotype analysis were normal. There was no acidosis or hypoglycemia, and liver enzyme values were normal; the results of amino and organic acid analyses and the acylcarnitine profile were normal.

The baby was discharged on a diet providing 130 kcal/kg per day. On follow-up, growth remained poor, and development was severely delayed. At 6 months of age, she was admitted to the hospital for an episode of dehydration and irritability after gastroenteritis. Mild acidosis, borderline elevations of lactate and ammonia levels, and significant elevation of liver enzyme levels were noted over the course of the hospital stay. The liver enzyme levels remained elevated after discharge. Cardiac ultrasonography showed a small pericardial effusion, which resolved. Amino and organic acid values were normal, as was the acylcarnitine profile. Urine oligosaccharide levels showed an unusual pattern, and urine mucopolysaccharide values were normal.

At 2 years of age, developmental delay remained marked, and hypotonia persisted with reflexes absent. The creatinine phosphokinase level was normal, but liver function values remained abnormal. Because mitochondrial disease was suspected, the patient was scheduled for liver biopsy, but clotting values were abnormal. A congenital disorder of glycosylation was suspected, and a transferrin assay confirmed the diagnosis. A review of neonatal records revealed a comment from a neurology consultant about the unusual distribution of fat on the buttocks and thighs of M.J. as a neonate. The family was counseled about autosomal recessive inheritance, including availability of prenatal diagnosis.

4. Coarse facial features with bone changes, dysostosis multiplex, or osteoporosis
 5. Dysmorphic facial features with or without major malformations
 6. Rarely, known family history or positive prenatal diagnosis
- Only for the last three presentations are these conditions likely to be considered early in the differential diagnosis. Most babies with these conditions are born to healthy, nonconsanguineous couples with normal family histories, and these disorders are usually considered late, if at all, as in Case Study 1.

Lysosomal Storage Disorders

Lysosomes are single membrane-bound intracellular organelles that contain enzymes called *hydrolases*. These lysosomal enzymes are responsible for splitting large molecules into simple, low-molecular-weight compounds, which can be recycled. The materials digested by lysosomes and derived from endocytosis and phagocytosis are separated from other intracellular materials by the process of autophagy, which is the main mechanism whereby endogenous molecules are delivered to lysosomes. The common element of all compounds digested by lysosomal enzymes is that they contain a carbohydrate portion attached to a protein or lipid. These glycoconjugates include glycoproteins, glycosaminoglycans (GAGs), and glycolipids.

Glycolipids are large molecules with carbohydrates attached to a lipid moiety. Sphingolipids, globosides, gangliosides, cerebroside, and lipid sulfates all are glycolipids. The different classes of glycolipids are distinguished from one another primarily by different polar groups at C-1. Sphingolipids are complex membrane lipids

composed of one molecule of each of the amino alcohol sphingosine, a long-chain fatty acid, and various polar head groups attached by a β -glycosidic linkage. Sphingolipids occur in the blood and nearly all tissues of the body, the highest concentration being found in white matter of the central nervous system (CNS). In addition, various sphingolipids are components of the plasma membrane of practically all cells. The core structure of natural sphingolipids is ceramide, a long-chain fatty acid amide derivative of sphingosine. Free ceramide, an intermediate in the biosynthesis and catabolism of glycosphingolipids and sphingomyelin, composes 16%–20% of normal lipid content of stratum corneum of the skin. Sphingomyelin, a ceramide phosphocholine, is one of the principal structural lipids of membranes of nervous tissue.

Cerebrosides are a group of ceramide monohexosides with a single sugar, either glucose or galactose, and an additional sulfate group on galactose. The two most common cerebroside are galactocerebroside and glucocerebroside. The largest concentration of galactocerebroside is found in the brain. Glucocerebroside is an intermediate in the synthesis and degradation of more complex glycosphingolipids. Gangliosides, the most complex class of glycolipids, contain several sugar units and one or more sialic acid residues. Gangliosides are normal components of cell membranes and are found in high concentrations in ganglion cells of the CNS, particularly in nerve endings and dendrites. GM1 is the major ganglioside in the brain of vertebrates.

Gangliosides function as receptors for toxic agents, hormones, and certain viruses, are involved in cell differentiation, and can also have a role in cell–cell interaction by providing specific recognition determinants on the surface of cells. Ceramide oligosaccharides (i.e., globosides) are a family of cerebroside that contain two or more sugar residues, usually galactose, glucose, or *N*-acetylgalactosamine. GAGs and oligosaccharides are essential constituents of connective tissue, parenchymal organs, cartilage, and the nervous system.

GAGs, also called *mucopolysaccharides*, are complex heterosaccharides consisting of long sugar chains rich in sulfate groups. The polymeric chains are bound to specific proteins (core proteins). Glycoproteins contain oligosaccharide chains (long sugar molecules) attached covalently to a peptide core. Glycosylation occurs in the endoplasmic reticulum and Golgi apparatus. Most glycoproteins are secreted from cells and include transport proteins, glycoprotein hormones, complement factors, enzymes, and enzyme inhibitors. There is extensive diversity in the composition and structure of oligosaccharides.

The degradation of glycolipids, GAGs, and glycoproteins occurs especially within lysosomes of phagocytic cells, related to histiocytes and macrophages, in any tissue or organ. A series of hydrolytic enzymes cleaves specific bonds, resulting in sequential, stepwise removal of constituents such as sugars and sulfate and degrading complex glycoconjugates to the level of their basic building blocks. LSDs most commonly result when an inherited defect causes significantly decreased activity in one of these hydrolases. Other causes are failure of transport of an enzyme, substrate, or product. Whatever the specific cause, incompletely metabolized molecules accumulate, especially within the tissue responsible for catabolism of the glycoconjugate. Additional excess storage material may be excreted in urine. The mechanisms of cellular dysfunction and damage in most LSDs remain unknown. Various hypotheses have been offered, such as a pivotal disturbance in the normal process of autophagy (Kiselyov et al., 2007; Ballabio and Gieselmann, 2009). In this pathophysiologic construct, endoplasmic reticulum membrane engulfment of cellular components, such as mitochondrial derivatives targeted for destruction, is perturbed. As a consequence,

TABLE
23.1

Lysosomal Storage Disorders in the Newborn Period: Genetic and Clinical Characteristics of Neonatal Presentation

| Disorder | Onset | Facies | Neurologic Findings | Distinctive Features | Eye Findings |
|---------------------------------------|-----------------------------|---------------------|--|--|---|
| Niemann–Pick A disease | Early infancy | Frontal bossing | Difficulty feeding, apathy, deafness, blindness, hypotonia | Brownish-yellow skin, xanthomas | Cherry-red spot (50%) |
| Niemann–Pick C disease | Birth–3 months | Normal | Developmental delay, vertical gaze paralysis, hypotonia, later spasticity | – | – |
| Gaucher disease type 2 | In utero–6 months | Normal | Poor suck and swallow, weak cry, squint, trismus, strabismus, opsochonus, hypertonic, later flaccidity | Congenital ichthyosis, collodion skin | – |
| Krabbe disease | 3–6 months | Normal | Irritability, tonic spasms with light or noise stimulation, seizures, hypertonia, later flaccidity | Increased CSF protein level | Optic atrophy |
| GM1 gangliosidosis | Birth | Coarse | Poor suck, weak cry, lethargy, exaggerated startle, blindness, hypotonia, later spasticity | Gingival hypertrophy, edema, rashes | Cherry-red spot (50%) |
| Farber disease type I | 2 weeks–4 months | Normal | Progressive psychomotor impairment, seizures, decreased reflexes, hypotonia | Joint swelling with nodules, hoarseness, lung disease, contractures, fever, granulomas, dysphagia, vomiting, increased CSF protein level | Grayish opacification surrounding retina in some patients, subtle cherry-red spot |
| Farber disease types II and III | Birth–9 months (≤20 months) | Normal | | Joint swelling with nodules, hoarseness | Normal macula, corneal opacities |
| Farber disease type IV (neonatal) | Birth | Normal | Nodules not consistent findings | Corneal opacities (1/3) | – |
| Congenital sialidosis | In utero–birth | Coarse, edema | Mental retardation, hypotonia | Neonatal ascites, inguinal hernias, renal disease | Corneal clouding |
| Galactosialidosis | In utero–birth | Coarse | Mental retardation, occasional deafness, hypotonia | Ascites, edema, inguinal hernias, renal disease, telangiectasias | Cherry-red spot, corneal clouding |
| Wolman disease | First weeks of life | Normal | Mental deterioration | Vomiting, diarrhea, steatorrhea, abdominal distention, failure to thrive, anemia, adrenal calcifications | – |
| Infantile sialic acid storage disease | In utero–birth | Coarse, dysmorphic | Mental retardation, hypotonia | Ascites, anemia, diarrhea, failure to thrive | – |
| I-cell disease | In utero–birth | Coarse | Mental retardation, deafness | Gingival hyperplasia, restricted joint mobility, hernias | Corneal clouding |
| Mucopolipidosis type IV | Birth–3 months | Normal | Mental retardation, hypotonia | – | Severe corneal clouding, retinal degeneration, blindness |
| Mucopolysaccharidosis type VII | In utero–childhood | Variable coarseness | Mild to severe mental retardation | Hernias | Variable corneal clouding |

–, Not seen; +, typically present, usually not severe; ++, usually present and moderately severe; +++, always present, usually severe; CSF, cerebrospinal fluid, HSM, hepatosplenomegaly.

| Cardiovascular Findings | Dysostosis Multiplex | Hepatomegaly/Splenomegaly | Defect | Gene Location/Molecular Findings | Ethnic Predilection |
|---|----------------------|---|--|--|---|
| — | — | ++/++ | Sphingomyelinase deficiency | <i>SMPD1</i> gene at 11p15.4; three of 18 mutations account for approximately 92% of mutant alleles in the Ashkenazi population | 1 : 40,000 in Ashkenazi Jews with carrier frequency of 1 : 60 |
| — | — | + / ++ | Abnormal cholesterol esterification | <i>NPC1</i> gene at 18q11 accounts for >95% of cases; <i>HE1</i> gene mutations may account for remaining cases | Increased in French Canadians of Nova Scotia and Spanish Americans in the southwest United States |
| — | — | + / ++ | Glucocerebrosidase deficiency | 1q21; large number of mutations known; five mutations account for approximately 97% of mutant alleles in the Ashkenazi population but approximately 75% in the non-Jewish population | Panethnic |
| — | — | — / — | Galactocerebrosidase deficiency | 14q 24.3-q32.1; >60 mutations with some common mutations in specific populations | Increased in Scandinavian countries and in a large Druze kindred in Israel |
| — | + | + / + | β-Galactosidase deficiency | 3pter-3p21; heterogeneous mutations; common mutations in specific populations | Panethnic |
| Occasional | — | Hepatomegaly in 50%, splenomegaly less common | Lysosomal acid ceramidase | 8p21.3-22; nine disease-causing mutations identified | Panethnic |
| — | — | HSM less common than in type I | | 8p21.3-p22 | Panethnic |
| — | | ++ / ++ | | Unknown | Panethnic |
| — | + | + / + | Neuraminidase deficiency | <i>NEU 1</i> gene (sialidase) at 6p21 | Panethnic |
| Cardiomegaly progressing to failure | + | + / + | Absence of a protective protein that safeguards neuraminidase and β-galactosidase from premature degradation | 20q13.1 | Panethnic |
| — | — | + / + | Lysosomal acid lipase deficiency | 10q23.2-q23.3; variety of mutations identified | Increased in Iranian Jews and in non-Jewish and Arab populations of Galilee |
| Congestive heart failure | + | + / + | Defective transport of sialic acid out of the lysosome | <i>SLC17A5</i> gene at 6q | Panethnic |
| Valvular disease, congestive heart failure, cor pulmonale | ++ | +++ / +++ | Lysosomal enzymes lack mannose 6-phosphate recognition marker and fail to enter the lysosome (phosphotransferase deficiency, 3-subunit complex [α 2 β 2 γ 2]) | Enzyme encoded by two genes; α and β subunits encoded by gene at 12p; γ subunit encoded by gene at 16p | Panethnic |
| — | — | — / — | Unknown; some patients with partial deficiency of ganglioside sialidase | <i>MCOLN1</i> gene at 19p13.2-13.3 encoding mucopolipin 1; two founder mutations accounting for 95% of mutant alleles in the Ashkenazi population | Increased in Ashkenazi Jews |
| Variable | ++ | Variable | β-Glucuronidase deficiency | <i>GUSB</i> gene at 7q21.2-q22; heterogeneous mutations | Panethnic |

TABLE 23.2**Common Clinical Features of Congenital Disorders of Glycosylation by Pathway**

| Pathway | Example Disorders | Neurologic | Ophthalmologic | Cardiologic | Gastroenterologic |
|------------------------|--|--|--|---|--|
| N-linked glycosylation | PMM2-CDG, MPI-CDG, ALGx-CDG, MOGS-CDG | ID (except MPI), DD, seizures (50%), hypotonia, ataxia, dysmetria, dysarthria, peripheral neuropathy, cerebral and cerebellar atrophy, myasthenic syndrome | Strabismus, nystagmus, optic hypoplasia, retinal pigmentary changes, alacrima | Pericardial effusion, cardiomyopathy, fetal hydrops | Protein-losing enteropathy, diarrhea, failure to thrive, gastroesophageal reflux, hepatopathy with elevated AST and ALT levels, edema and hypoalbuminemia, low cholesterol level |
| O-linked glycosylation | GALNT3-CDG, B3GLCT-CDG, POMK-CDG, EXT1-CDG, CHST-CDG | ID (not universal), DD, congenital and later-onset muscular dystrophy, hypotonia, polymicrogyria, lissencephaly | Peters plus syndrome, other structural eye abnormalities, glaucoma, isolated macular corneal dystrophy, corneal opacity, cataracts | | Failure to thrive |
| Mixed glycosylation | COGx-CDG, TMEMx-CDG | Seizures, ID (not universal), DD, microcephaly, hypotonia, cortical and cerebellar atrophy | All findings seen in N-linked pathway possible | Cardiomyopathy, congenital structural heart defects | All findings seen in N-linked pathway possible, isolated polycystic liver disease, high cholesterol level, cholestatic liver disease, prenatal growth retardation |
| GPI anchor disorder | PIGx-CDG, PGAPx-CDG | Seizures, ID, DD, macrocephaly, hypotonia | | Congenital heart defects, cardiomyopathy | |
| Lipid glycosylation | ST3GAL5-CDG (Amish infantile epilepsy syndrome) | Seizures, ID, DD, hypotonia, diffuse brain atrophy, irritability, microcephaly | Optic atrophy, cortical visual impairment | | Failure to thrive |

ALT, Alanine transaminase; AST, aspartate transaminase; DD, developmental disability; GPI, glycosylphosphatidylinositol; ID, intellectual disability; IGF1, insulin-like growth factor 1.

deleterious pathways become activated, leading to unwanted ubiquitination of targeted molecules and apoptosis.

LSDs are classified according to the stored compound. The clinical phenotype depends partially on the type and amount of storage substance. There are more than 50 different LSDs, and a significant fraction, approximately 20 LSDs, may have manifestations in the newborn (Staretz-Chacham et al., 2009). The disorders selected for discussion in this chapter are all known to manifest themselves in the neonatal period.

Clinical Presentations

Table 23.1 summarizes the clinical characteristics of the neonatal presentations of LSDs.

Niemann–Pick A Disease (Acute, Sphingomyelinase Deficient)

Etiology

Niemann–Pick A disease is caused by a deficiency of sphingomyelinase. Sphingomyelinase catalyzes the breakdown of sphingomyelin

to ceramide and phosphocholine, and its deficiency results in sphingomyelin storage within lysosomes. Cholesterol is also stored, suggesting that its metabolism is tied to that of sphingomyelin. Sphingomyelin normally composes 5%–20% of phospholipids in the liver, spleen, and brain, but in these disorders it can compose up to 70% of phospholipids. Individuals with Niemann–Pick A disease usually have enzyme activity less than 5% of normal.

Clinical Features

Clinical features of this disorder may appear in utero or up to 1 year of age. Affected infants usually have massive hepatosplenomegaly (hepatomegaly greater than splenomegaly), constipation, feeding difficulties, and vomiting, with consequent failure to thrive. Patients eventually appear strikingly emaciated with a protuberant abdomen and thin extremities. Neurologic disease is evident by 6 months of age, with hypotonia, decrease or absence of deep tendon reflexes, and weakness. Loss of motor skills, spasticity, rigidity, and loss of vision and hearing occur later. Seizures are rare. A retinal cherry-red spot is present in about half of cases, and the electroretinographic findings are abnormal. Respiratory infections are

KEY FEATURES BY SYSTEM

| Hematologic | Renal | Endocrine | Dermatologic | Musculoskeletal/Other | Diagnostic Screen |
|---|---|---|--|--|---|
| Factors II, V, VII, VIII, IX, X, and XI, antithrombin III, protein C, protein S deficiency, increased bleeding tendency, thrombotic events, hypogammopathy, coagulopathy and thrombosis | Hyperechoic kidneys, microcystic changes, proteinuria | Abnormal thyroid function test findings, short stature, IGF1 deficiency, hypogonadotropic hypogonadism, hyperinsulinemic hypoglycemia | Lipodystrophy, hypohidrosis | Osteopenia, kyphoscoliosis, dysmorphic features, skeletal dysplasia | Transferrin profiling, urine oligosaccharide analysis |
| | | Tumoral calcinosis with phosphatemia | Loose skin, Dowling–Degos disease | Skeletal dysplasia, short stature, Ehlers–Danlos syndrome, hypermobility, exostoses, elevated creatine kinase, dysmorphic facies | |
| Isolated leukocyte adhesion deficiency, isolated congenital dyserythropoietic anemia type II, immunodeficiency | Obstructive uropathy, micropenis, hypospadias | | Ichthyosis, cutis laxa, hypohidrosis | Skeletal dysplasia, dysmorphic features, elevated creatine kinase level | Transferrin profiling with apolipoprotein CIII profiling |
| | | Accelerated linear growth, advanced bone age, with or without hyperphosphatasia | | Dysmorphic features, multiple congenital anomalies | Flow cytometry studies using cell surface markers such as FLAER and CD59 on granulocytes, lymphocytes, etc. |
| | | | Dyspigmentation, “salt and pepper” pattern on skin macules | | |

common. The skin may have an ochre or brownish-yellow color, and xanthomas have been observed. Radiographic findings consist of widening of medullary cavities, cortical thinning of long bones, and osteoporosis. In the brain and spinal cord, neuronal storage is widespread, leading to cytoplasmic swelling together with atrophy of cerebellum. Bone marrow and tissue biopsy samples may show foam cells or sea-blue histiocytes, which represent lipid-laden cells of the monocyte–macrophage system. Similarly, vacuolated lymphocytes or monocytes may be present in peripheral blood. Tissue cholesterol levels may be threefold to tenfold that of normal, and patients may have a microcytic anemia and thrombocytopenia. Death occurs by 2–3 years of age.

Niemann–Pick C Disease

Etiology

Niemann–Pick C disease is caused by an error in the intracellular transport of exogenous low-density lipoprotein (LDL)-derived cholesterol, which leads to impaired esterification of cholesterol and trapping of unesterified cholesterol in lysosomes. The incidence may be higher than 1 in 150,000 births (Wraith et al., 2009).

Cell lines from patients can be divided into two complementation groups, Niemann–Pick C (NPC)1 and NPC2, corresponding to different genes (Millat et al., 2001). In each group the primary defect is abnormal cholesterol esterification, but the enzyme responsible for cholesterol esterification—acetyl coenzyme A (CoA) acetyltransferase (ACAT)—is not deficient. The storage of sphingomyelin is secondary. It has been suggested that the defect is in transport of cholesterol out of the lysosome, making cholesterol unavailable to ACAT (Natowicz et al., 1995). Sphingomyelinase activity appears normal or elevated in most tissues but is partially deficient (60%–70%) in fibroblasts from most patients with this disorder. Storage of sphingomyelin in tissues is much less than in Niemann–Pick A or Niemann–Pick B disease and is accompanied by additional storage of unesterified cholesterol, phospholipids, and glycolipids in the liver and spleen. Only glycolipids levels are increased in the brain.

Clinical Features

The age of onset, clinical features, and natural history of Niemann–Pick C disease are highly variable. Onset can occur from birth to

18 years of age. Fifty percent of children with onset in the neonatal period have conjugated hyperbilirubinemia, which usually resolves spontaneously but is followed by neurologic symptoms later in childhood. In the severe infantile form, hepatosplenomegaly is common, accompanied by hypotonia and delayed motor development. Further mental regression is usually evident by the age of 1–1.5 years, in association with behavior problems, vertical supranuclear ophthalmoplegia, progressive ataxia, dystonia, spasticity, dementia, drooling, dysphagia, and dysarthria. Seizures are rare. Foam cells and sea-blue histiocytes may be found in many tissues. Neuronal storage with cytoplasmic ballooning, inclusions, meganeurites, and axonal spheroids are also seen. Death may occur in infancy or as late as the third decade of life. Niemann–Pick C disease can also manifest itself as fatal neonatal liver disease, often misdiagnosed as fetal hepatitis. Patients with mutations in the *NPC2* gene (also known as *HE1*) may have remarkable features consisting of pronounced pulmonary involvement leading to early death caused by respiratory failure (Millat et al., 2001).

Gaucher Disease Type 2 (Acute Neuropathic)

Etiology

Three types of Gaucher disease have been defined. Type 1, the nonneuropathic form, is the most common and is distinguished from types 2 and 3 by the lack of CNS involvement. Type 1 disease most commonly manifests itself in early childhood but may do so in adulthood. Type 2 disease, the acute neuropathic form, is characterized by infantile onset of severe CNS involvement. Type 3 disease, the subacute neuropathic form, is also late in onset, with slow neurologic progression. Almost all types of Gaucher disease are caused by a deficiency of lysosomal glucocerebrosidase and result in storage of glucocerebroside in visceral organs; the brain is affected in types 2 and 3. Although there is significant variability in clinical presentation among individuals with the same mutations, there is a clear correlation between certain mutations and clinical symptoms involving the CNS (Beutler and Grabowski, 2001). Glucocerebrosidase splits glucose from cerebroside, yielding ceramide and glucose. A few patients with Gaucher disease type 2 have a deficiency of saposin C, a cohydrolase required by glucocerebrosidase.

Clinical Features

Typically, the age of onset of Gaucher disease type 2 is approximately 3 months, consisting of hepatosplenomegaly (splenomegaly predominates) with subsequent neurologic deterioration. Hydrops fetalis, congenital ichthyosis, and collodion skin, however, are well-described presentations (Lipson et al., 1991; Sidransky et al., 1992; Sherer et al., 1993; Fujimoto et al., 1995; Ince et al., 1995; Liu et al., 1988). In a review of 18 cases of Gaucher disease manifesting itself in the newborn period, Sidransky et al. (1992) found that eight patients had associated dermatologic findings and six patients had hydrops. The cause of the association of such findings in Gaucher disease is unclear, although the enzyme deficiency appears to be directly responsible (Sidransky et al., 1992). Ceramides have been shown to be major components of intracellular bilayers in epidermal stratum corneum, and they have an important role in skin homeostasis (Fujimoto et al., 1995). Therefore Gaucher disease should be considered in the differential diagnosis for infants with hydrops fetalis and congenital ichthyosis. For the subset of patients in the prenatal period or at birth, death frequently occurs within hours to days or at least within 2–3 months.

Krabbe Disease (Globoid Cell Leukodystrophy)

Etiology

The synonym for Krabbe disease, globoid cell leukodystrophy, is derived from the finding of large numbers of multinuclear macrophages in cerebral white matter that contain undigested galactocerebroside. Disease is caused by a deficiency of lysosomal galactocerebroside β -galactosidase, which normally degrades galactocerebroside to ceramide and galactose. Deficiency of the enzyme results in storage of galactocerebroside. Galactocerebroside is present almost exclusively in myelin sheaths. Accumulation of the toxic metabolite psychosine, also a substrate for the enzyme, has been postulated to lead to early destruction of oligodendroglia. Impaired catabolism of galactosylceramide is also important in the pathogenesis of the disease.

Clinical Features

The age of onset ranges from the first weeks of life to adulthood. The typical age of onset of infantile Krabbe disease is between 3 and 6 months, but there are cases of early onset in which neurologic symptoms are evident within weeks after birth. Symptoms and signs are confined to the nervous system; no visceral involvement is present. The clinical course has been divided into three stages. In stage I, patients who appeared relatively normal after birth exhibit hyperirritability, vomiting, episodic fevers, hyperesthesia, tonic spasms with light or noise stimulation, stiffness, and seizures. Peripheral neuropathy is present, but reflexes are increased. Stage II is marked by CNS deterioration and hypertonia that progresses to hypotonia and flaccidity. Deep tendon reflexes are eventually lost. Patients with stage III disease are decerebrate, deaf, and blind with hyperpyrexia, hypersalivation, and frequent seizures. Routine laboratory findings are unremarkable except for an elevation of the level of cerebrospinal fluid protein. Cerebral atrophy and demyelination become evident in the CNS, and segmental demyelination, axonal degeneration, fibrosis, and macrophage infiltration are common in the peripheral nervous system. The segmental demyelination of peripheral nerves is demonstrated by the finding of decreased motor nerve conduction. The white matter is severely depleted of all lipids, especially glycolipids, and nerve and brain biopsies show globoid cells. Death from hyperpyrexia, respiratory complications, or aspiration occurs at a median age of 13 months.

GM1 Gangliosidosis

Etiology

Infantile GM1 gangliosidosis is caused by a deficiency in lysosomal β -galactosidase. The enzyme cleaves the terminal galactose in a β linkage from oligosaccharides, keratan sulfate, and GM1 ganglioside. Deficiency of the enzyme results in storage of GM1 ganglioside and oligosaccharides. Clinical severity correlates with the extent of substrate storage and residual enzyme activity. The same enzyme is deficient in Morquio disease type B.

Clinical Features

The age of onset ranges from prenatal to adult. Infantile or type 1 GM1 gangliosidosis may be evident at birth as coarse and thick skin, hirsutism on the forehead and neck, and coarse facial features consisting of a puffy face, frontal bossing, depressed nasal bridge, maxillary hyperplasia, large and low-set ears, wide upper lip, moderate macroglossia, and gingival hypertrophy. These dysmorphic features, however, are not always obvious in the neonate. A retinal cherry-red spot is seen in 50% of patients, and corneal clouding is often observed. Shortly after birth, or by 3–6 months of age,

failure to thrive and hepatosplenomegaly become evident, as does neurologic involvement with poor development, hyperreflexia, hypotonia, and seizures. Cranial imaging shows diffuse atrophy of the brain, enlargement of the ventricular system, and evidence of myelin loss in white matter.

The neurologic deterioration is progressive, resulting in generalized rigidity and spasticity and sensorimotor and psychointellectual dysfunction. By 6 months of age, skeletal features are present, including kyphoscoliosis and stiff joints with generalized contractures, and striking bone changes are seen—vertebral beaking in the thoracolumbar region, broadening of shafts of the long bones with distal and proximal tapering, and widening of the metacarpal shafts with proximal pinching of four lateral metacarpals. Tissue biopsy samples demonstrate neurons filled with membranous cytoplasmic bodies and various types of inclusions as well as foam cells in the bone marrow. Death generally occurs before 2 years of age. A severe neonatal-onset type of GM1 gangliosidosis with cardiomyopathy has also been described (Kohlschütter et al., 1982).

Farber Lipogranulomatosis

Etiology

Farber lipogranulomatosis results from a deficiency of lysosomal acid ceramidase. Ceramidase catalyzes the degradation of ceramide to its long-chain base, sphingosine, and a fatty acid. Clinical disease is a consequence of storage of ceramide in various organs and body fluids.

Clinical Features

Four types of Farber lipogranulomatosis can manifest themselves in the neonatal period. Type I, classic disease, is a unique disorder with onset from approximately 2 weeks to 4 months of age. Patients exhibit hoarseness progressing to aphonia, feeding and respiratory difficulties, poor weight gain, and intermittent fever caused by granuloma formation and swelling of the epiglottis and larynx. Palpable nodules appear over joints and pressure points, and joints become painful and swollen. Later, joint contractures and pulmonary disease appear. Liver and cardiac involvement can occur, and patients can have a subtle retinal cherry-red spot. Severe and progressive psychomotor impairment can occur, as can seizures, decreased deep tendon reflexes, hypotonia, and muscle atrophy. Affected patients die in early infancy, usually of pulmonary disease.

Type 2, or intermediate, Farber lipogranulomatosis manifests itself from birth to 9 months of age as joint and laryngeal involvement and nodules. Death occurs in early childhood. Type 3 disease (mild) manifests itself slightly later, from approximately 2 months to 20 months of age, with survival into the third decade. Clinically types 2 and 3 are both dominated by subcutaneous nodules, joint deformity, and laryngeal involvement. Liver and pulmonary involvement may be absent. Two-thirds of patients have a normal intelligence quotient. Type 4, or neonatal visceral, Farber lipogranulomatosis manifests itself at birth as hepatosplenomegaly caused by massive histiocyte infiltration of the liver and spleen, with infiltration also in the lungs, thymus, and lymphocytes. Subcutaneous nodules and laryngeal involvement may be subtle. Death occurs by 6 months of age.

In all types of Farber lipogranulomatosis, tissue biopsy samples show granulomatous infiltration, foam cells, and lysosomes with comma-shaped, curvilinear tubular structures called *Farber bodies*. Cerebrospinal fluid protein level may be elevated in patients with type 1 disease.

Sialidosis

Etiology

Sialidosis is caused by a deficiency of neuraminidase, which is responsible for the cleavage of terminal sialyl linkages of several oligosaccharides and glycopeptides. The defect results in multisystem lysosomal accumulation of sugars rich in sialic acid.

Clinical Features

Type I sialidosis is characterized by retinal cherry-red spots and generalized myoclonus with onset generally in the second decade of life. Type II is distinguished from type I by the early onset of a progressive, severe phenotype with somatic features. Type II is often subdivided into juvenile, infantile, and congenital forms. Congenital sialidosis begins in utero and manifests itself at birth as coarse features, facial edema, hepatosplenomegaly, ascites, hernias, and hypotonia and occasionally frank hydrops fetalis. Radiographs demonstrate dysostosis multiplex and epiphyseal stippling. Delayed mental development is quickly apparent. The patient may have recurrent infections. Severely dilated coronary arteries, excessive retinal vascular tortuosity, and an erythematous macular rash may also be features of this disease (Buchholz et al., 2001). Most patients are stillborn or die before 1 year of age. The age of onset for the infantile form of sialidosis ranges from birth to 12 months. The clinical features include coarse facial features, organomegaly, dysostosis multiplex, retinal cherry-red spot, and mental retardation. Death occurs by the second or third decade. In both types of sialidosis, vacuolated cells can be seen in almost all tissues, and bone marrow foam cells are present.

Galactosialidosis

Etiology

Galactosialidosis results from a deficiency of two lysosomal enzymes, neuraminidase and β -galactosidase. The primary defect in galactosialidosis is a defect in the protective protein cathepsin A, an intralysosomal protein that protects the two enzymes from premature proteolytic processing. The protective protein has catalytic and protective functions, and the two functions appear to be distinct. Deficiency of the enzymes results in the accumulation of sialyloligosaccharides in tissue lysosomes and in excreted body fluids.

Clinical Features

Galactosialidosis has been divided into three phenotypic subtypes on the basis of age at onset and severity of clinical manifestations. Most cases occur in adolescence and adulthood, but early infantile and late infantile presentations occur. Patients develop early infantile galactosialidosis between birth and 3 months of age, with ascites, edema, coarse facial features, inguinal hernias, proteinuria, hypotonia, and telangiectasias, and, occasionally, frank hydrops fetalis. Patients subsequently demonstrate organomegaly, including cardiomegaly progressing to cardiac failure, psychomotor delay, and skeletal changes, particularly in the spine. Ocular abnormalities can occur, including corneal clouding and retinal cherry-red spots. Death occurs at an average age of 8 months, usually from cardiac and renal failure. Galactosialidosis can be a cause of recurrent fetal loss or recurrent hydrops fetalis.

Late infantile galactosialidosis manifests itself in the first months of life as coarse facial features, hepatosplenomegaly, and skeletal changes consistent with dysostosis multiplex. Cherry-red spots and corneal clouding may also be present. Neurologic involvement may be absent or mild. Valvular heart disease is a common feature,

as is growth retardation, partially because of spinal involvement and often in association with muscular atrophy. Early death is not a feature of the late infantile form. Vacuolated cells in blood smears and foam cells in bone marrow are present in all forms of galactosialidosis.

Wolman Disease

Etiology

Wolman disease is caused by a deficiency of lysosomal acid lipase, which is an enzyme involved in cellular cholesterol homeostasis and responsible for hydrolysis of cholesterol esters and triglycerides. The result of enzyme deficiency is defective release of free cholesterol from lysosomes, which leads to upregulation of LDL receptors and 3-hydroxy-3-methylglutaryl-CoA reductase activity. De novo synthesis of cholesterol and activation of receptor-mediated endocytosis of LDL then occur, leading to further deposition of lipid in lysosomes. The result is the accumulation of cholesterol esters and triglycerides in most tissues of the body, including the liver, spleen, lymph nodes, heart, blood vessels, and brain. An extreme level of lipid storage occurs in cells of the small intestine, particularly in the mucosa. In addition, neurons of the myenteric plexus demonstrate a high level of storage, with evidence of neuronal cell death, which may account for the prominence of gastrointestinal (GI) symptoms (Wolman, 1995).

Clinical Features

Clinical presentation of Wolman disease is within weeks of birth, with evidence of malnutrition and malabsorption, including symptoms of vomiting, diarrhea, steatorrhea, failure to thrive, abdominal distention, and hepatosplenomegaly. Adrenal calcifications may be seen on radiographs, and adrenal insufficiency appears. The presence of adrenal calcifications in association with hepatosplenomegaly and GI symptoms is strongly suggestive of Wolman disease. Later, mental deterioration becomes apparent. Laboratory findings include anemia secondary to foam cell infiltration of the bone marrow and evidence of adrenal insufficiency. The serum cholesterol level is normal. Death usually occurs before 1 year of age.

Infantile Sialic Acid Storage Disease

Etiology

Infantile sialic acid storage disease is caused by a defective lysosomal sialic acid transporter that is responsible for efflux of sialic acid and other acidic monosaccharides from the lysosomal compartment. The defective transporter results in greater storage of free sialic acid and glucuronic acid within lysosomes and increased sialic acid excretion.

Clinical Features

Infantile sialic acid storage disease often manifests itself at birth as mildly coarse features, hepatosplenomegaly, ascites, hypopigmentation, and generalized hypotonia. Mild dysostosis multiplex may be seen on radiographs. Failure to thrive and severe mental and motor retardation soon appear. Cardiomegaly may be present. Corneas are clear, but albinoid fundi have been reported (Lemyre et al., 1999). Vacuolated cells are seen in a tissue biopsy sample, and electron microscopy demonstrates swollen lysosomes filled with finely granular material. CNS changes include myelin loss, axonal spheroids, gliosis, and neuronal storage. Death occurs in early childhood. Infantile sialic acid storage disease can also manifest itself as fetal ascites, nonimmune fetal hydrops, or infantile nephrotic syndrome (Lemyre et al., 1999).

I-Cell Disease (Mucopolidosis Type II)

Etiology

In normal cells, targeting of enzymes to lysosomes is mediated by receptors that bind a mannose 6-phosphate recognition marker on the enzyme. The recognition marker is synthesized in a two-step reaction in the Golgi complex. It is the enzyme that catalyzes the first step of this process, uridine diphosphate-*N*-acetylglucosamine:lysosomal enzyme *N*-acetylglucosaminyl-1-phosphotransferase, that is defective in I-cell disease. As a result, the enzymes lack the mannose 6-phosphate recognition signal, and the newly synthesized lysosomal enzymes are secreted into the extracellular matrix instead of being targeted to the lysosome. Consequently, multiple lysosomal enzymes are found in plasma at 10–20 times their normal concentrations. Affected cells, especially fibroblasts, show dense inclusions of storage material that probably consists of oligosaccharides, GAGs, and lipids; these are the inclusion bodies from which the disease name is derived. This disorder is found more frequently in Ashkenazi Jews, because of a putative founder effect.

Clinical Features

I-cell disease can manifest itself at birth as coarse features, corneal clouding, organomegaly, hypotonia, and gingival hyperplasia. Birthweight and length are often below normal. Kyphoscoliosis, lumbar gibbus, and restricted joint movement are often present, and there may be hip dislocation, fractures, hernias, or bilateral talipes equinovarus. Dysostosis multiplex may be seen on radiographs. Severe psychomotor retardation, evident by 6 months of age, and progressive failure to thrive occur. The facial features become progressively coarser, with a high forehead, puffy eyelids, epicanthal folds, flat nasal bridge, anteverted nares, and macroglossia. Linear growth slows during the first year of life and halts completely thereafter. The skeletal involvement is also progressive, with development of increasing joint immobility and claw-hand deformities. Respiratory infections, otitis media, and cardiac involvement are common complications. Death usually occurs in the first decade of life because of cardiorespiratory complications.

Mucopolidosis Type IV

Etiology

Although mucopolidosis type IV is associated with a partial deficiency of the lysosomal enzyme ganglioside sialidase, a deficiency of mucopolipin 1, a member of the transient receptor potential mucopolipin subfamily of channel proteins, is the cause of the disorder (Bargal et al., 2000; Sun et al., 2000). Mutations in the *MCOLN1* gene result in lysosomal storage of lipids such as gangliosides, plus water-soluble materials such as GAGs and glycoproteins in cells from almost all tissues.

Clinical Features

The age of onset for mucopolidosis type IV ranges from infancy to 5 years. Presenting features include corneal clouding (may be congenital), retinal degeneration, blindness, hypotonia, and mental retardation. Survival of affected patients into the fourth decade of life has been reported (Chitayat et al., 1991). Cytoplasmic inclusions are noted in many cells, including those in conjunctiva, liver, and spleen, as well as fibroblasts.

Mucopolysaccharidosis Type VII (Sly Disease)

Etiology

Sly disease is a member of a group of LSDs that are caused by a deficiency of enzymes catalyzing the stepwise degradation of GAGs. Skeletal and neurologic involvement are variable. There is a wide

spectrum of clinical severity among the mucopolysaccharidoses and even within a single enzyme deficiency. Most of these disorders manifest themselves in childhood, but type VII is included in this chapter because of its well-recognized neonatal and infantile presentations. Sly disease is caused by β -glucuronidase deficiency and results in lysosomal accumulation of GAGs, including dermatan sulfate, heparan sulfate, and chondroitin sulfate, causing cell, tissue, and organ dysfunction.

Clinical Features

Sly disease can manifest itself as a wide spectrum of severity. Patients with the early-onset or neonatal form may have coarse features, hepatosplenomegaly, moderate dysostosis multiplex, hernias, and nonprogressive mental retardation. Corneal clouding is variably present. Frequent episodes of pneumonia during the first year of life are common. Short stature becomes evident. Granulocytes have coarse metachromatic granules. A severe neonatal form associated with hydrops fetalis and early death has been recognized frequently. Milder forms of the disease with later onset are also known.

Diagnosis, Management, and Prognosis

Growing recognition of LSDs in the neonate has led to expansion of the spectrum of possible clinical presentations in the newborn period. Diagnostic tools and options for treatment also continue to advance. For example, newborn screening (NBS) for mucopolysaccharidoses has begun in several states, with the goal to offer treatment with enzyme infusion or hematopoietic stem cell transplantation (HSCT) for affected babies (Vogler et al., 1999; Hopkins et al., 2015). The state of New York has implemented NBS for Krabbe disease that uses dried blood spots. The test uses a tandem mass spectrometry–based enzyme analysis (Li et al., 2004a). This test has resulted in a fairly large number of positive newborn screens for Krabbe disease, most of which appear to be false positives, including enzyme perturbations that are not linked with clinical disease (Duffner et al., 2009). As a consequence, an expert advising panel, the Krabbe Consortium of New York State, has been generated to establish standardized clinical evaluation guidelines (Wasserstein et al., 2016). The goal is to help physicians determine which infant with a positive newborn screen may express disease and require treatment, such as HSCT in early infancy. The neonatologist is urged to work closely with appropriate experts to explore diagnostic and treatment protocols on an individual basis. Larger panels of multiplex testing for various other LSDs are in the testing stages (Li et al., 2004b), and some states in the United States are poised to begin implementing LSD NBS. Currently a federal advisory committee actively reviews and makes recommendations to the US Secretary of Health and Human Services about the introduction of new NBS tests in the United States, with the aim of vetting proposed tests for need, cost-effectiveness, and availability of effective and timely therapy. The Recommended Universal Screening Panel has added mucopolysaccharidosis type I to the list of diseases that ought to be screened in every state. Ross (2012) performed an ethical and policy analysis using the Wilson and Jungner criteria for public health screening and concluded that the data do not support the incorporation of screens for LSDs into NBS programs. Instead, they should entail institutional review board–approved research protocols that require parental consent.

Recognizing LSDs in the newborn period can be difficult, because they often mimic more common causes of illness in newborns, such as respiratory distress, nonimmune hydrops fetalis, liver disease,

and sepsis. The initial step in the diagnosis of these disorders is to consider them in the differential diagnosis of a sick or unusual-appearing newborn. At times the phenotype may suggest a specific diagnosis, such as respiratory distress and painful, swollen joints in Farber lipogranulomatosis or GI symptoms, hepatosplenomegaly, and adrenal calcifications in Wolman disease. Subtle dysmorphic features, coarsening of features, and radiographic evidence of dysostosis multiplex are also strong indications that LSDs should be considered. Routine laboratory findings are often normal or nonspecific. Affected infants do not have episodes of acute metabolic decompensation. Anemia and thrombocytopenia may be seen because of bone marrow involvement. Vacuolated cells may be found in peripheral blood, but the absence of this finding does not exclude LSD. Elevated cerebrospinal fluid protein level is seen in Krabbe disease and Farber lipogranulomatosis type I.

Nonimmune hydrops fetalis deserves special mention. The physician must consider LSDs as the cause of nonimmune hydrops fetalis or unexplained ascites in the affected newborn. The following LSDs are potential causes: sialidosis type II, mucopolysaccharidosis types VII and IV, infantile sialic acid storage disease, Salla disease, galactosialidosis, Gaucher disease type 2, GM1 gangliosidosis, I-cell disease, Niemann–Pick disease types A and C, Wolman disease, and Farber disease (Staretz-Chacham et al., 2009). The mechanisms of edema are unclear. Furthermore, not all of the 13 LSDs routinely appear in the neonatal period.

Directed analysis of urine is helpful for conditions in which characteristic metabolites are excreted in urine. One- or two-dimensional electrophoresis or thin-layer chromatography can detect excess excretion of urine GAGs, oligosaccharides, or free sialic acid, but all urinary tests for the diagnosis of LSDs can have false-negative results. Examination of bone marrow or other tissues may demonstrate storage macrophages in Gaucher disease and in Niemann–Pick disease types A and C. Small skin or conjunctival biopsy specimens may demonstrate storage within lysosomes in most of these disorders.

Definitive diagnosis for all LSDs, except for Niemann–Pick C disease, is confirmed by enzymatic assays in serum, leukocytes, fibroblasts, or a combination of these. The diagnosis of Niemann–Pick C disease requires measurement of cellular cholesterol esterification and documentation of a characteristic pattern of filipin–cholesterol staining in cultured fibroblasts during LDL uptake. Analysis of DNA mutations may be helpful for the diagnosis of Niemann–Pick C disease, Gaucher disease, and some other conditions, and it will become increasingly available for other conditions. An imperfect genotype–phenotype correlation impedes the use of mutation analysis as a prognostic tool. In addition, prenatal diagnosis is available for most LSDs through the use of enzyme assays performed on amniocytes or chorionic villus cells or measurements of levels of stored substrate in cultured cells or amniotic fluid. As mutation analysis becomes more prevalent, it will increasingly substitute for biochemical and enzymatic methods.

These conditions must also be considered in the dying infant, and the neonatologist must be prepared to request the appropriate samples for diagnosis at the time of death. In surviving patients, treatment and management must be considered. All the LSDs are chronic and progressive conditions for which there is no curative treatment. Gene transfer therapy holds promise but is not currently available for LSDs. With few exceptions, current standard medical management is supportive and palliative. Patients must be continually reassessed for evidence of disease progression and associated complications. These complications manifest themselves at variable

ages and can include hydrocephalus, valvular heart disease, joint limitation, and obstructive airway disease.

For several disorders, particularly neonatal Gaucher disease and Niemann–Pick C disease, splenectomy may be indicated to relieve severe anemia and thrombocytopenia. This procedure enhances the risk of serious infections, and it can accelerate the progression of disease at other sites. Patients with Krabbe disease may have significant pain of radiculopathy and spasms, and alleviation of that pain is important for the patient's comfort. The administration of a glutamic acid transaminase inhibitor, vigabatrin, has been used in a small number of patients with Krabbe disease, because part of the disease may involve a secondary deficiency of γ -aminobutyric acid (Barth, 1995). Low-dose morphine has also been reported to reduce the irritability associated with this disorder (Stewart et al., 2001).

Enzyme replacement therapy (ERT) with imiglucerase (Cerezyme®, Sanofi Genzyme), a recombinant enzyme, is available for Gaucher disease. Although ERT has successfully reversed many of the systemic manifestations of the disease, it has been suggested that ERT should not be given to patients with Gaucher disease type 2 who already have severe neurologic signs, because no substantial relief has been demonstrated to occur in the neurologic symptoms of patients treated (Erikson et al., 1993; McCabe et al., 1996). ERT should be discussed with families, and in some instances it may be appropriate to provide ERT until it has been established that the patient does not have the less severe form of Gaucher disease type 3 (Weiss et al., 2015).

HSCT has been tried for a variety of LSDs. The rationale for the procedure is that circulating blood cells derived from the transplanted marrow become a source of the missing enzyme. Results of HSCT in disorders of GAGs show that after successful engraftment, leukocyte and liver tissue enzyme activity normalizes, organomegaly decreases, and joint mobility increases. Skeletal abnormalities stabilize but do not abate. Whether brain function can be improved in patients with CNS disease remains questionable. Some patients maintain their learning capability or intelligence quotient, but others continue to deteriorate. Clinical experience and studies in animal models indicate that HSCT before the onset of neurologic symptoms can prevent or delay the occurrence of symptoms, whereas there is no clear benefit if transplantation is performed when symptoms are already present (Hoogerbrugge et al., 1995). HSCT in patients with nonneuropathic Gaucher disease can result in complete disappearance of all symptoms; however, the procedure is associated with significant risks (Hoogerbrugge et al., 1995) that must be balanced against lifelong ERT. Currently it is unclear to what extent patients with Gaucher disease type 2 would benefit from transplantation (Weiss et al., 2015); therefore it is generally not recommended.

HSCT has also been attempted in a small number of patients with infantile Krabbe disease, Farber lipogranulomatosis, and Niemann–Pick A disease. The outcome after transplantation for these few patients has been poor, with continued disease progression and death. Success may depend on treatments started very early in life before the onset of neurologic signs of diseases (Escobar et al., 2005, 2006). Krivit et al. (2000) reported successful long-term bone marrow engraftment in a patient with Wolman disease that resulted in normalization of peripheral leukocyte lysosomal acid lipase enzyme activity. The patient's diarrhea resolved; cholesterol, triglyceride, and liver function values normalized, and the patient attained developmental milestones. LSDs are not all equally amenable to HSCT, and the use of HSCT as a treatment modality for most LSDs remains uncertain. In a small number of cases,

HSCT has been performed in utero after prenatal diagnosis showing an affected fetus, and experimental protocols are available for families who wish to pursue this option.

The preferred treatment to reduce the accumulation of storage material in intestine and phagocytes in lysosomal acid lipase deficiency is ERT (Burton et al., 2015; Rader 2015), and there is now a trial using sebelipase alfa to treat Wolman disease (www.ClinicalTrials.gov identifier NCT01757184).

Congenital Disorders of Glycosylation

Etiology

CDGs are a group of more than 100 genetic diseases that involve various defects in the process of modifying proteins, lipids, or other biomolecules with glycans (sugar molecules or chains) (Freeze et al., 2014). Glycosylation, the addition of glycans to biomolecules, is essential to many biologic processes, such as aiding with correct folding, protecting against premature destruction, directing intracellular localization and transport, and modifying the biologic function of these biomolecules.

The first CDG discovered (PMM2-CDG) was described by Jaak Jaeken in 1980 and was initially termed *carbohydrate-deficient glycoprotein syndrome* because of abnormalities seen in multiple serum glycoproteins in the affected individuals (Jaeken et al., 1980; Jaeken et al., 1984). When several more human glycosylation disorders were identified, this group of disorders was renamed *congenital disorders of glycosylation*. The decision was made to designate the types of CDG into either a group I or a group II disorder on the basis of the transferrin pattern obtained by isoelectric focusing, with specific diagnoses alphabetized consecutively as they were identified (i.e., CDG Ia, Ib, Ic, IIa, IIb, etc.) (Aebi et al., 1999). Improved molecular diagnostics expanded the definition of CDGs to include genetic diseases that primarily disrupt the process of formation of any glycoconjugate (i.e., glycoproteins, glycolipids, glycosaminoglycan, etc.), resulting in an exponential growth of the number of pathways and individual disorders (Jaeken, 2010). In 2009 the nomenclature was updated, and currently specific CDG types are named starting with the affected gene symbol (not in italics) followed by CDG (e.g., CDG Ia is now PMM2-CDG) (Jaeken et al., 2009).

It is estimated that approximately 2% (~400) of our genes encode proteins involved with the glycosylation process, which occurs in a variety of locations within the cell, including the cytosol, endoplasmic reticulum, and Golgi apparatus. The underlying mechanism for the clinical manifestations of most of these disorders is still unclear. Given the complexity of glycosylation, there are multiple methods to subdivide these disorders. One classification schema sorts CDGs into protein N-linked glycosylation defects, protein O-linked glycosylation defects, glycosylphosphatidylinositol (GPI) anchor glycosylation defects, lipid glycosylation defects, and defects in multiple glycosylation and other pathways (Jaeken, 2011). In this chapter we use this classification method to help organize our discussion of the CDGs that manifest themselves in the neonatal period.

Clinical Presentations

Because so many biologic functions are dependent on the correct glycosylation, the phenotypic spectrum of CDG defects is extremely broad and ranges from mild to severe disease and from a single-organ system to multisystem disease. Clinical features alone are insufficient

to define the CDG type. A CDG should be considered in any unexplained clinical condition, but especially in multiorgan disease with neurologic involvement (Table 23.2).

N-Linked Protein Glycosylation Defects

Etiology

N-linked protein glycosylation, the process involved with attaching glycans to the asparagine residue of target proteins, was the first discovered and is the best understood glycosylation pathway in humans. Classically, these disorders were divided into two categories: type I, which results in defects in *N*-glycan assembly, and type II, which results from defects in *N*-glycan processing. The initial assembly steps of *N*-glycosylation occur on the endoplasmic reticulum membrane, where sugars are attached in a stepwise manner to dolichol phosphate to form a lipid-linked oligosaccharide. Sugars are donated by an activated nucleotide sugar (uridine diphosphate-*N*-acetylglucosamine and guanosine diphosphate-mannose), with the attached nucleotide providing the necessary energy for the transfer of the sugar to the lipid-linked oligosaccharide. This oligosaccharide is then transferred to the nascent protein cotranslationally. Once the oligosaccharide chain has been transferred to the protein, further processing occurs. The oligosaccharide is then transported to the Golgi apparatus, where further processing occurs. Different types of CDGs have been found in affected individuals who have defective enzymes in individual steps of this complex pathway, including the enzymes that form the dolichol backbone, transfer single sugars to the growing chain, interconvert activated monosaccharides, and transfer the oligosaccharide from dolichol to protein (Hennet, 2012; Jaeken, 2012).

Clinical Features

N-linked glycosylation defects encompass a large number of disorders. Taken together these several dozen disorders are typically multisystemic with significant neurologic involvement with the notable exception of MPI-CDG, in which development can be normal (Sparks and Krasnewich, 1993). The most common perinatal findings include hypotonia, nonspecific dysmorphic features (mostly without inverted nipples or abnormal fat pads), feeding problems with growth delay, hepatopathy with elevated levels of transaminases, and abnormal coagulation profiles. Discriminating findings include neonatal hemorrhages (including cerebral hemorrhage) and thrombotic events, strabismus, nystagmus and other ophthalmologic findings, neonatal seizures, and an abnormal thyroid function screening result (Funke et al., 2013). Transferrin glycosylation analysis previously performed by isoelectric profiling and now performed by mass spectrometry methods shows an abnormal glycosylation pattern in many, but not all, of these disorders.

Three disorders warrant special mention. PMM2-CDG (CDG Ia) is the classic and most common presentation, and many other N-linked CDGs mirror its presentation. Most affected infants appear normal at birth. In infancy, patients with PMM2-CDG can exhibit dysmorphic features, strabismus, nystagmus, and feeding difficulties; subsequently patients may exhibit growth failure, hypotonia, lipocutaneous abnormalities (including prominent fat pads on the buttocks), coagulopathy with thrombosis and bleeding, pericardial effusion, and mild to moderate hepatomegaly and hepatopathy. Approximately 20% of patients die during the first year of life after a course of severe fluid imbalance and sometimes anasarca in response to infection or their underlying glycosylation disorder (Grunewald, 2009). Having survived infancy, patients with PMM2-CDG can live into their seventh and eighth decades. Later manifestations include retinitis pigmentosa or retinal

degeneration, pericardial effusion, renal cysts, coagulopathy, stroke-like episodes, thrombotic disease, cerebral and olivopontocerebellar hypoplasia, ataxia, peripheral neuropathy followed by lower extremity atrophy, and hypogonadism. In general, patients have an extroverted disposition and happy appearance (Krasnewich et al., 2007). MPI-CDG (CDG Ib) stands out in this group of disorders because patients with MPI-CDG can have normal development, and mannose is a known targeted therapy. These individuals can experience vomiting, protein-losing enteropathy, and progressive liver fibrosis (Jaeken and Matthijs, 2007) but can also survive to adulthood. NGLY1-CDDG is the first described disorder of N-linked deglycosylation. It presents with hepatopathy, alacrima (lack of tears), intellectual disability, and movement disorder. In infancy many times the first symptom is poor feeding and motor delay with hyperkinesia (Need et al., 2012).

O-Linked Protein Glycosylation Defects

Etiology

O-glycosylation consists of attachment of a monosaccharide (mannose, fructose, or xylose) or the assembly of a glycan and its attachment to a serine or threonine residue of a target protein. O-glycosylation differs from N-glycosylation in that it does not occur at the same time as the protein is being translated but occurs posttranslationally, exclusively in the Golgi apparatus, without further processing (Jaeken and Matthijs, 2007). O-glycosylation can be classified according to the type of sugar that is attached to the serine or threonine. Examples of O-glycosylation include O-mannosylation, O-xylosylation, and O-fucosylation.

Clinical Features

The clinical features differ significantly depending on which type of O-glycosylation is defective. Deficiency of *O*-*N*-acetylglucosamine linkage can lead to familial tumoral calcinosis with phosphatemia and massive calcium deposits in the skin and subcutaneous tissues (Freeze and Schachter, 2009). A defect in O-fucosylation has been shown to lead to Peters plus syndrome characterized by anterior eye chamber defects, disproportionate short stature, developmental delay, and cleft lip and/or palate (Lesnik Oberstein et al., 2006). Defects in O-xylosylation will lead to defective anchoring of GAGs to proteins and thus impaired proteoglycan formation. Defective O-xylosylation can lead to progeroid-type Ehlers–Danlos syndrome characterized by failure to thrive, loose skin, skeletal abnormalities, hypotonia, and hypermobile joints. Defects in forming heparin sulfate, also attached to proteins via O-xylosylation, cause congenital exostosis, an autosomal dominant disorder where patients have bony outgrowths usually at the growth plate of the long bones. Defective cartilage proteoglycan sulfation leads to achondrogenesis, diastrophic dystrophy, and atelosteogenesis that manifest themselves as symptoms in cartilage and bone such as cleft palate and club feet and in the severest cases lead to perinatal death from respiratory insufficiency (Freeze and Schachter, 2009).

Additionally, there are more than a dozen different genetic disorders that lead to a defect in O-mannosylation (Endo, 2015). O-mannosylation defects lead to hypoglycosylation of α -dystroglycan, an important glycoprotein needed to link the intracellular cytoskeleton of muscle to the extracellular matrix. These disorders, collectively referred to as α -dystroglycanopathies, have a wide spectrum of clinical severity and encompass previously described disorders, ranging from Walker–Warburg syndrome, muscle–eye–brain disease, and Fukuyama congenital muscular dystrophy to limb–girdle muscular dystrophy (Mercuri et al., 2009; Topaloglu, 2009). In the neonate, clinical features involve the triad of muscle,

eye, and brain, and may include hypotonia; muscle weakness; microcornea; microphthalmia; pale, hypoplastic or absent optic nerves; colobomas; cataracts; iris hypoplasia; glaucoma; retinal dysplasia or detachment; and brain structural abnormalities, including hydrocephalus, brainstem hypoplasia, cerebellar cysts, cobblestone lissencephaly, polymicrogyria, cerebellar vermis and hemisphere atrophy, hypoplasia of the pyramidal tracts, and absence of the corpus callosum. There is no specific blood or urine biochemical marker available for this group of disorders. Elevated creatine kinase level is frequently noted. Muscle biopsy with specialized immunohistochemical staining may show deficient glycosylated α -dystroglycan and normal β -dystroglycan level. Molecular testing is needed to confirm the specific type (Sparks et al., 1993).

Combined Glycosylation Defects

Etiology

Combined N-glycosylation and O-glycosylation and other glycosylation defects are important because they appear to affect trafficking in the glycosylation machinery (Grünwald, 2007). Several of these disorders involve defects in channels involved in activated sugar-nucleotide transport (SLCx-CDG). Some affect vesicular transport (COGx-CDG) in general. Others affect the process of sugar activation (attaching nucleotides to monosaccharides so that they can be used for glycosylation). Yet others cause abnormalities in the Golgi apparatus structure (TMemx-CDG), which needs to be intact for glycosylation to proceed (Ungar et al., 2002).

Clinical Features

In the neonate the most frequent presenting symptoms include neonatal microcephaly; neonatal seizures; strabismus; hypotonia; dysmorphic features, especially cutis laxa; feeding problems with growth delay; and hepatic involvement (Funke et al., 2013). Again, encompassing a very large group of disorders, the presentations are very heterogeneous and include not only multisystemic diseases with the aforementioned symptoms but also single system disorders such as congenital dyserythropoietic anemia type II due to SEC23B-CDG.

Glycosylphosphatidylinositol Anchor Glycosylation Defects

Etiology

The biosynthesis and attachment of GPI anchors to proteins occur in the endoplasmic reticulum and Golgi apparatus and involve 11 steps and at least 27 genes (Kinoshita et al., 2008). To date, inherited loss-of-function mutations in more than a dozen of these genes have been implicated in human disease. GPI anchors are attached during posttranslational modification and allow these proteins to attach to the outer leaflet of the cell membrane and face the extracellular environment. This permits these proteins to participate in processes such as signal transduction and immune response (Paulick and Bertozzi, 2008; Ferguson et al., 2009).

Clinical Features

Typically, individuals affected with GPI anchor disorders have a severe phenotype and present in infancy with epilepsy, intellectual disability, and multiple congenital anomalies, including heart, skeletal (especially abnormalities in phalanges), endocrine, ophthalmologic, and facial anomalies, with possible abnormalities in alkaline phosphatase levels depending on the specific diagnosis (Jezela-Stanek et al., 2016). Although there is no standard blood or urine biomarker, flow cytometry markers show promise to be effective biomarkers in many of these disorders (Freeze et al., 2012).

Lipid Glycosylation Defects

To date, three disorders of lipid glycosylation have been described. SIAT9-CDG, also known as Amish infantile epilepsy, was the first identified and is caused by a defect of lactosylceramide α -2,3-sialyltransferase (GM₃ synthase) (Jaeken, 2006). This enzyme catalyzes the initial step in the biosynthesis of most complex gangliosides from lactosylceramide (Jaeken and Matthijs, 2007). The defect causes accumulation of lactosylceramide associated with decreased levels of gangliosides (Jaeken, 2006). Individuals with this disorder present with infantile-onset epilepsy with developmental stagnation, blindness, poor feeding, vomiting, failure to thrive, later-onset “salt and pepper” macules, and variable survival (Boccuto et al., 2014). ST3GAL-CDG is a cause of West syndrome (Freeze et al., 2015). B4GALNT1-CDG, also known as spastic paraplegia 26, is also a defect in ganglioside biosynthesis. However, onset of symptoms including gait abnormalities and central and peripheral nervous system involvement typically occurs after the neonatal period, in the first 2 decades of life (Boukhris et al., 2013).

Diagnosis

CDG should be considered in young infants with several of the following features:

- Neurologic signs, including hypotonia, hyporeflexia, or seizures
- Ophthalmic signs, including abnormal eye movements, cataracts, optic nerve atrophy, retinitis pigmentosa, or glaucoma
- Hepatic and GI signs, including ascites or hydrops, hepatomegaly, diarrhea, and protein-losing enteropathy
- Endocrinologic signs, including hyperinsulinemic hypoglycemia and hypothyroidism
- Hematologic signs, including thrombosis or coagulopathy with factor deficiency
- Signs of renal or cardiac disease
- Musculoskeletal signs, including congenital muscular dystrophy, and congenital joint contractures
- Dysmorphic features, microcephaly, or abnormal skin findings

Serum transferrin isoform analysis is the most available screening method, but it detects only N-glycosylation and some mixed glycosylation defects. Until about 2000, transferrin screening was achieved by isoelectric focusing of transferrin; failure to correctly synthesize the N-linked glycans alters the charge on serum transferrin and consequently its migration in an electrophoretic field. Since then, however, mass spectrometry methods, capable of identifying individual oligosaccharides and complete glycans by mass and charge, have replaced transferrin isoelectric focusing as the standard method for screening patients for CDGs (Sturiale et al., 2011). Transferrin and glycan analysis may yield false positive results in galactosemia, inborn errors of fructose metabolism, alcohol consumption, certain bacterial (neuraminidase-producing) infections, and in cases of mutations in transferrin itself. False negatives can occur in the first 3 weeks of life (Freeze et al., 2012). There are also reported cases where initially abnormal transferrin glycosylation normalizes without relief of symptoms. There are also N-linked defects known to not show transferrin isoform abnormalities (MOGS-CDG, TUSC3-CDG, SLC35A1-CDG, SLC35C1-CDG) (He et al., 2012).

Apolipoprotein CIII glycan analysis has been used in the screening of some mixed and O-glycosylation disorders. Urine oligosaccharide screening is useful in detecting MOGS-CDG. Many defects

in GPI synthesis can be identified by flow cytometry of GPI-anchored proteins, such as FLAER or CD59 on leukocytes. Not all subtypes of CDGs have convenient biochemical markers; for example, screening for congenital muscular dystrophies caused by defective O-mannosylation requires a muscle biopsy with the use of monoclonal antibodies directed against the glycan (He et al., 2012). There are also no simple markers for defects in GAG biosynthesis. Since the advent of next-generation sequencing and exome analysis, most CDGs have been diagnosed molecularly (Timal et al., 2012). Once variants are identified in the specific gene, if novel, the functional consequence of the mutation can be confirmed by enzymatic assays in peripheral blood leukocytes or cultured fibroblasts for PMM2-CDG and MPI-CDG and on a research basis for other types. Prenatal diagnosis is possible in all types of CDG for which the molecular defect is known (Grunewald, 2007). The vast majority of CDGs are autosomal recessive disorders; POGlut1-CDG and POFUT1-CDG (Dowling–Degos disease), EXT1&2-CDG (hereditary multiple exostoses syndrome), and SEC63-CDG and PRKCSH-CDG (polycystic liver disease) are autosomal dominant; C1GALT1C1-CDG, PIGA-CDG, SSR4-CDG, SLC35A2-CDG, ALG13-CDG, and MAGT1-CDG are X-linked.

Treatment, Management, and Prognosis

A specific treatment is available for only a minority of CDGs. MPI-CDG can be treated with orally administered mannose (Thiel and Korner, 2013), which can help significantly with the protein-losing enteropathy but does not necessarily halt the progression of the liver disease. Heparin has also been used for protein-losing enteropathy in MPI-CDG (de Lonlay and Seta, 2009). In SLC35C1-CDG, some patients respond to oral fucose supplementation; this treatment is effective only with regard to the typical recurrent infections with hyperleukocytosis and does not correct the neurodevelopmental aspects (Marquardt et al., 1999). In PIGM-CDG, butyrate has been shown to control the seizures in some cases (Almeida et al., 2007). There are ongoing trials to assess the efficacy of galactose in PGM1-CDG, and preliminary findings show promise that this therapy may alleviate the hypoglycemia, coagulopathy, and endocrinopathy seen in this disorder (Morava, 2014). The treatment and management of other types of CDGs are primarily supportive and palliative. In infancy, evidence of multisystem involvement and the resulting complications must be treated promptly. There is substantial mortality in the first years of life because of severe infection or vital organ failure (Jaeken, 2006; Grunewald, 2007).

Peroxisomal Disorders

Peroxisomes are small, evolutionarily conserved, single membrane-bound cellular organelles that contain no internal structure or DNA and are characterized by an electron-dense core and a homogeneous matrix. Peroxisomes are found in all cells and tissues except mature erythrocytes and are in highest concentration in the liver and kidneys. They are formed predominantly by growth and division of preexisting peroxisomes, but they can also arise de novo from peroxisomal vesicles that originate from specialized compartments of the endoplasmic reticulum (Waterham and Ebberink, 2012; Braverman et al., 2016; Waterham et al., 2016). Their half-life is 1.5–2 days before they are randomly destroyed by autophagy. All peroxisomal proteins are encoded by nuclear genes, synthesized in cytosol, and imported posttranslationally

into the peroxisome (Waterham and Ebberink, 2012). The import of proteins into the peroxisome is mediated by specific targeting sequences known as peroxisomal targeting sequences (Waterham et al., 2016).

Peroxisomes contain enzymes that use oxygen to oxidize a variety of substrates, thereby forming peroxide. The peroxide is decomposed within the organelle by the enzyme catalase to water. This process protects the cell against peroxide damage through compartmentalization of peroxide metabolism within the organelle. Peroxisomes can also function to dispose of excess reducing equivalents and may contribute to thermogenesis, producing heat from cellular respiration (Gould et al., 2001).

More than 70 enzymes have been found within peroxisomes (Braverman et al., 2016). The proteins have multiple functions, both synthetic and degradative (Braverman et al., 2016; Waterham et al., 2016). The primary synthetic functions are plasmalogen synthesis and bile acid and docosahexanoic acid formation. Plasmalogens constitute 5%–20% of phospholipids in cell membranes and 80%–90% of phospholipids in myelin. They are involved in platelet activation and may also protect cells against oxidative stress. Degradative functions include (1) β -oxidation of very long chain fatty acids (VLCFAs) ($\geq C_{23}$), fatty acids (down to C_8 to C_6), long-chain dicarboxylic acids, prostaglandins, and polyunsaturated fatty acids; (2) oxidation of bile acid intermediates, pipercolic acid and glutaric acid (intermediates in lysine metabolism), and phytanic acid; (3) deamination of D-amino acids and L-amino acids; (4) metabolism of glycolate to glyoxylate; (5) polyamine degradation (spermine and spermidine); and (6) ethanol clearance. At least 16 conditions caused by peroxisomal enzyme deficiencies have been confirmed (Klouwier et al., 2015; Braverman et al., 2016; Waterham et al., 2016).

Peroxisomal disorders constitute a clinically and biochemically heterogeneous group of inherited diseases that result from the absence or dysfunction of one or more peroxisomal enzymes. Disorders in which more than one enzyme is affected are collectively termed *peroxisomal biogenesis disorders* (PBDs). Disorders in which only one enzyme is affected encompass the remaining known disorders. All but one are inherited in an autosomal recessive manner. The pathophysiologic features apparently involve either deficiency of necessary products of peroxisomal metabolism or excess of unmetabolized substrates. Disorders with similar biochemical defects may have markedly different clinical features, and disorders with similar clinical features may be associated with different biochemical findings. General features of peroxisomal disorders, each of which can be evident in the newborn period, are as follows:

- Dysmorphic craniofacial features
- Neurologic dysfunction, primarily consisting of severe hypotonia, possibly associated with hypertonia of extremities, seizures, and abnormalities in neuronal migration
- Hepatodigestive dysfunction, including hepatomegaly, cholestasis, prolonged hyperbilirubinemia, and feeding difficulties
- Rhizomelic shortening of the limbs, stippled calcifications of epiphyses, and renal cysts

In this section we discuss the peroxisomal disorders that can manifest themselves in the newborn period.

Disorders of Peroxisomal Biogenesis

Conditions in which multiple peroxisomal enzymes are affected can result from a disturbance of biogenesis of the organelle. Peroxisomal assembly includes matrix protein import, synthesis of new organelles, and fusion of existing organelles. The coordinated

activity of 16 PEX proteins, or peroxins, encoded by their corresponding genes is required for this process (Braverman et al., 2013). The PEX genes responsible for disease in most human patients are known, with more than 60% of patients with PBD having mutations in *PEX1*; the second most commonly involved gene is *PEX6* (Waterham et al., 2012; Braverman et al., 2013; Braverman et al., 2016). The overall incidence of PBD is estimated to be approximately 1 in 50,000 newborns (Klouwer et al., 2015; Braverman et al., 2016).

Zellweger syndrome is the prototype of neonatal peroxisomal disease. It is a disorder of peroxisome biogenesis caused by failure to import newly synthesized peroxisomal proteins into the peroxisome. The proteins remain in the cytosol, where they are rapidly degraded. In this condition, peroxisomes are absent from liver hepatocytes or exist as “ghosts.” Neonatal adrenoleukodystrophy and infantile Refsum disease are also disorders of peroxisome biogenesis in which, as in Zellweger syndrome, disruption of function of more than one peroxisomal enzyme is demonstrable. A few residual peroxisomes, however, may be seen in the liver. These disorders represent a continuum of clinical severity, and the term *Zellweger spectrum disorders* (ZSDs) is now suggested (Braverman et al., 2013; Braverman et al., 2016). Features common across the spectrum include liver disease, variable neurologic dysfunction, developmental delay, retinopathy, neurosensory hearing loss, and adrenocortical dysfunction (Poll-The and Gärtner, 2012; Klouwer et al., 2015). Rhizomelic chondrodysplasia punctata, types 1 and 5, are caused by a defect in a subset of peroxisomal enzymes resulting from mutations in the *PEX7* gene and the *PEX5L* isoform respectively. In these disorders, liver peroxisomes are demonstrable and normal in number, but their distribution and structure are abnormal. A new category of disorders, referred to as *peroxisomal fission defects*, has also been recognized. Peroxisomal fission defects are disorders caused by defects in proteins known to be involved with the proliferation and division of peroxisomes (Mff, Fisl, PEX11, DLP1) (Waterham et al., 2007; Schrader et al., 2012; Waterham and Ebberink, 2012; Waterham et al., 2016). Finally, Heimler syndrome, a rare recessive disorder, typically presenting in young childhood with sensorineural hearing loss, amelogenesis imperfecta, nail abnormalities, and retinal pigmentation, was recognized as a mild PBD disorder involving mutations in *PEX1* and *PEX6* (Ratbi et al., 2015).

Zellweger Syndrome

Zellweger syndrome is most often evident at birth, with affected newborns having dysmorphic facial features including large fontanels, high forehead, flat occiput, epicanthus, hypertelorism, upward-slanting palpebral fissures, hypoplastic supraorbital ridges, abnormal ears, severe weakness and hypotonia, hepatomegaly, multicystic kidneys, and congenital heart disease. Seizures, feeding difficulties, and postnatal growth failure soon manifest themselves. Ophthalmologic examination may detect cataracts, corneal clouding, glaucoma, optic atrophy, retinitis pigmentosa, and Brushfield spots. Somatic sensory evoked responses and electroretinograms are abnormal. Hearing assessment often shows an abnormal brainstem auditory evoked response consistent with sensorineural hearing loss. Skeletal radiographs demonstrate epiphyseal stippling, and cranial imaging shows leukodystrophy and neuronal migration abnormalities. Hepatic cirrhosis and severe psychomotor retardation occur later. Laboratory analysis may demonstrate abnormal liver function values, hyperbilirubinemia, or hypoprothrombinemia. Death usually occurs within the first year of life, the average life span being 12.5 weeks.

Neonatal Adrenoleukodystrophy

Clinically, neonatal adrenoleukodystrophy is similar to, but less severe than, Zellweger syndrome. Differences include less dysmorphism, absence of chondrodysplasia punctata and renal cysts, and fewer neuronal and gray matter changes. Patients with neonatal adrenoleukodystrophy may have striking white matter disease, however, and often show degenerative changes in adrenal glands. They also have slow psychomotor development followed by neurodegeneration that usually begins before the end of the first year of life. Disease progression is slower than that observed in Zellweger syndrome, and longer survival is usual, to an average of approximately 15 months of age or into the teen years (Waterham et al., 2016).

Infantile Refsum Disease

Patients with infantile Refsum disease also have relatively mild dysmorphic features, such as epicanthic folds, midface hypoplasia with low-set ears, and mild hypotonia. Early neurodevelopment is normal, possibly up to 6 months of age, but then slow deterioration begins. Later, sensorineural hearing loss (100%), anosmia, retinitis pigmentosa, hepatomegaly with impaired function, and severe cognitive impairment are evident. Patients learn to walk, although their gait may be ataxic and broad based. Diarrhea and failure to thrive may also be seen. Chondrodysplasia punctata and renal cysts are absent. Neuronal migration defects are minor and adrenal hypoplasia occurs. The life span of patients with infantile Refsum disease ranges from 3 to 11 years or into adulthood.

Rhizomelic Chondrodysplasia Punctata

Patients with defects in the biosynthesis of ether phospholipids present with rhizomelic chondrodysplasia punctata. Five genetically distinct, but clinically indistinguishable, groups exist, three of which are single enzyme defects (types 2, 3, and 4) and the other two of which are peroxisomal biogenesis defects (types 1 and 5) (Waterham et al., 2016). Patients with rhizomelic chondrodysplasia punctata at birth have facial dysmorphism, microcephaly, cataracts, rhizomelic shortening of extremities with prominent stippling, and coronal clefting of vertebral bodies. The chondrodysplasia punctata is more widespread than in Zellweger syndrome and may involve extraskel-etal tissues. Infants with this disorder have severe psychomotor retardation from birth onward and severe failure to thrive. In addition, patients may have joint contractures, and 25% experience ichthyosis. Neuronal migration is normal. The life span is usually less than 1 year.

Peroxisomal Fission Defects

The first described patient with a peroxisomal fission defect was a severely affected female patient with mitochondrial encephalopathy who died at 1 month of age (Waterham et al., 2007). She was noted to have microcephaly, mild dysmorphic features, truncal hypotonia, absent deep tendon reflexes, optic atrophy, failure to thrive, abnormal brain development, and severe developmental delay. She had elevated peripheral and central lactic acid and alanine levels, mildly elevated VLCFA levels, and abnormal-appearing peroxisomes and mitochondria in fibroblasts but normal oxidative phosphorylation values in fibroblasts and skeletal muscle specimens (Waterham et al., 2007). Evaluation revealed a peroxisomal and mitochondrial fission defect with a heterozygous, dominant-negative mutation in the dynamin-like protein 1 gene (*DLP1*) (Waterham et al., 2007). Additional patients with *DLP1* mutations have been described (Vanstone et al., 2016; Chao et al., 2016; Sheffer et al.,

2016). Patients may not show significant peroxisomal or biochemical abnormalities.

Single Peroxisomal Enzyme Defects

Of patients with suspected ZSD and elevated VLCFA levels, approximately 10%–15% will have a single enzyme defect (Braverman et al., 2013; Braverman et al., 2016). To date, three childhood disorders of peroxisomal fatty acid β -oxidation have been defined: D-bifunctional protein deficiency, acyl-CoA oxidase deficiency, and 2-methylacyl-CoA racemase deficiency (Waterham et al., 2016). The clinical presentation resembles that of biogenesis disorders. Previously, an isolated case of a fourth disorder, peroxisomal thiolase deficiency, was described (Goldfischer et al., 1986). On reinvestigation, however, this case was identified as D-bifunctional protein deficiency (Ferdinandusse et al., 2002).

D-Bifunctional Protein Deficiency

D-bifunctional protein deficiency is a rare single peroxisomal enzyme defect that results in a phenotype similar to Zellweger syndrome. It is caused by mutations in the *HSD17B4* gene encoding 17 β -estradiol dehydrogenase, an enzyme involved in β -oxidation of VLCFAs and branched-chain fatty acids, including pristanic acid and bile acid intermediates, resulting in accumulation of VLCFAs, pristanic acid, and dihydroxycholestanoic acid and trihydroxycholestanoic acid (Shimozawa et al., 2011; Waterham et al., 2016). In general, children have severe CNS involvement consisting of profound hypotonia, uncontrolled seizures, and failure to acquire any significant developmental milestones. Children are usually born at term without evidence of intrauterine growth restriction. Dysmorphic features, similar to those seen in Zellweger syndrome, are notable in most children. In most cases, neuronal migration is disturbed, with areas of polymicrogyria and heterotopic neurons in the cerebrum and cerebellum. Death generally occurs before 1 year of age, but survival to at least 3 years of age is possible.

Acyl Coenzyme A Oxidase Deficiency

Acyl-CoA oxidase deficiency, also called *pseudoneonatal adrenoleukodystrophy*, is a rare, neuroinflammatory, neurodegenerative disorder (El Hajj et al., 2012; Wang et al., 2015). It is caused by mutations in *ACOX1* exclusively involved in the β -oxidation of straight-chain fatty acids resulting in the accumulation of VLCFAs (El Hajj et al., 2012; Waterham et al., 2016). Patients exhibit global hypotonia, deafness, and delayed developmental milestones with or without facial dysmorphic features. Patients may demonstrate early developmental skills but then show regression of skills typically between 24 and 48 months of age (Wang et al., 2015). Retinopathy with extinguished electroretinograms, nystagmus, optic atrophy, failure to thrive, hepatomegaly, areflexia, seizures, and white matter demyelination have also been reported (Poll-The et al., 1988; Carrozzo et al., 2008; El Hajj et al., 2012).

2-Methylacyl Coenzyme A Racemase Deficiency

2-Methylacyl-CoA racemase (AMACR) deficiency is a rare disorder caused by mutations in the *AMACR* gene encoding the enzyme 2-methylacyl-CoA racemase. The enzyme catalyzes the isomerization of fatty acids with a methyl group in the *R* configuration to the corresponding *S* configuration, an obligatory reaction in the steps leading to peroxisomal β -oxidation. This results in impaired bile acid synthesis and pristanic acid metabolism and subsequent accumulation of pristanic acid, (25*R*)-trihydroxycholestanoic acid, and (25*R*)-dihydroxycholestanoic acid (Setchell et al., 2003). Most

patients present with an adult-onset ataxia and sensory neuropathy; however, an infantile presentation with cholestatic liver disease, coagulopathy, and fat-soluble vitamin deficiency has been reported (Setchell et al., 2003; Waterham et al., 2016).

X-Linked Adrenoleukodystrophy

X-linked adrenoleukodystrophy (X-ALD) is the most common peroxisomal disorder, with an estimated incidence of 1 in 17,000 (Waterham et al., 2016). It is caused by the altered function of the membrane-bound protein ABCD1, which predominantly catalyzes the import of straight-chain VLCFAs into peroxisomes (Waterham et al., 2016). It does not usually present in the newborn period; however, contiguous *ABCD1 DXS1357E* deletion syndrome, caused by a contiguous gene deletion of *ABCD1* and its upstream gene *DXS1357E*, may. Four male patients have been reported with profound neonatal hypotonia, severe growth and developmental retardation, cholestatic liver disease, accumulation of VLCFAs, and death within the first year of life (Corzo et al., 2002; Shimozawa et al., 2011; Iwasa et al., 2013).

Diagnosis, Management, and Prognosis

The key to diagnosing peroxisomal disorders is a high index of suspicion. Peroxisomal disorders should be considered in newborns with dysmorphic facial features, skeletal abnormalities, shortened proximal limbs, neurologic abnormalities (including hypotonia or hypertonia), ocular abnormalities, and hepatic and renal abnormalities. Babies with abnormal vision, hearing, or somatosensory evoked potentials should also be considered for these diagnoses.

Peroxisomal disorders are not associated with acute metabolic derangements or abnormal routine laboratory test findings. Measurements of the levels of VLCFAs, phytanic acid, pristanic acid, pipecolic acid, bile acid intermediates, and plasmalogens are required for diagnosis. Zellweger syndrome is associated with elevations of the levels of VLCFAs, phytanic acid, pipecolic acid, and bile acid intermediates and a decrease in plasmalogen synthesis. Neonatal adrenoleukodystrophy and infantile Refsum disease have similar biochemical findings; however, the defect in plasmalogen synthesis and the degree of VLCFA accumulation are less severe. Laboratory findings in rhizomelic chondrodysplasia punctata include elevations of the levels of phytanic and pipecolic acids, a decrease in the levels of plasmalogens, and normal levels of VLCFAs and bile acid intermediates. Therefore screening that uses only levels of VLCFAs fails to detect rhizomelic chondrodysplasia punctata. Also, a small number of patients with mutations in PEX genes have been identified with mild or absent elevations in VLCFA levels (Braverman et al., 2016). D-bifunctional protein deficiency is associated with deficient oxidation of C23:0 and pristanic acid, leading to elevations of the levels of pristanic acid and, to a lesser extent, phytanic acid. This deficiency results in an elevated pristanic acid to phytanic acid ratio, which is generally not elevated in PBD. Abnormal VLCFA levels and elevations of the levels of varanic acid, an intermediate metabolite in β -oxidation, are also seen. Accumulation of bile acid intermediates is a variable finding.

Abnormalities in the levels of phytanic acid and plasmalogens are age dependent. The elevation of the levels of phytanic and pristanic acids might not be demonstrable in newborns not consuming dairy products or other dietary sources of these fatty acids, and reduction in red blood cell plasmalogen levels may not be evident in children older than 20 weeks (Gould et al., 2001; Lee and Raymond, 2013; Braverman et al., 2016). Pipecolic acid levels are more likely to be abnormal in the urine of newborns and more

abnormal in plasma at later ages (Braverman et al., 2016). A ketogenic diet may elevate VLCFA levels (Lee and Raymond, 2013; Braverman et al., 2016). A liver biopsy may be a useful adjunct diagnostic tool to assess the presence or absence and structure of peroxisomes. Definitive diagnosis for all types may require cultured skin fibroblasts for measurement of the levels of VLCFAs and their β -oxidation and, as needed, assay of the peroxisomal steps of plasmalogen synthesis, phytanic acid oxidation, subcellular localization of catalase, enzyme assays, and immunocytochemistry studies. More recently, however, next-generation sequencing panels for the PEX genes are being used for confirmatory diagnostic testing (Braverman et al., 2016). DNA study for deletions also has a role in diagnostic evaluation in some cases as demonstrated by the neonatal presentation of cases with deletion of the *ABCD1* gene on the X chromosome (Corzo et al., 2002; Shimozawa et al., 2011; Iwasa et al., 2013). Diagnostic flow diagrams have been published by Shimozawa et al. (2011) and Klouwer et al. (2015).

Prenatal diagnosis with a variety of methods is available. It can be accomplished in the first or second trimester by biochemical or genetic testing in chorionic villi cells or cultured amniocytes (Waterham and Ebberink, 2012; Klouwer et al., 2015; Braverman et al., 2016). Preimplantation genetic diagnosis can be performed when the PEX mutations are known. Carriers cannot be identified by biochemical testing (Waterham and Ebberink, 2012).

One of the more interesting recent developments in peroxisomal disease is consideration of NBS. The combination of liquid chromatography and tandem mass spectrometry to detect elevated levels of VLCFAs (C26:0-lysophosphatidylcholine) in newborn dried blood spots has been validated as a diagnostic approach for X-ALD (Braverman et al., 2016). Legislation for X-ALD screening has passed in several states. Screening for X-ALD has recently been approved for addition to the Recommended Uniform Screening Panel. NBS for X-ALD should also detect the majority of ZSDs, permitting early diagnosis and intervention (Klouwer et al., 2015; Braverman et al., 2016).

The prognosis for patients with a neonatal-onset peroxisomal disease remains poor, and patients frequently die within the first year of life (Klouwer et al., 2015). Patients with later presentation have a better prognosis but still have progressive disease. Plasma levels of metabolites do not correlate well with disease severity (Klouwer et al., 2015). There is, however, a generally good correlation between the defective PEX gene, the type of mutation, and the impact on peroxisomal assembly and function and the clinical severity (Waterham and Ebberink, 2012).

Treatment for all peroxisomal disorders in the newborn period remains symptomatic and supportive. These disorders are chronic, progressive diseases with no currently available curative therapy. In patients with severe disease, seizure control, feeding, and respiratory support are the main focus of management (Braverman et al., 2016). Feeding difficulties, including malabsorption, are prominent and may require the use of elemental formulas and/or gastrostomy tube placement. Dietary reduction in VLCFAs has not been shown to reduce plasma VLCFA levels as most VLCFAs are produced endogenously (Braverman et al., 2016). In patients with X-ALD, dietary reduction of VLCFAs in combination with supplementation with Lorenzo's oil (a 4:1 mixture of glyceryl trioleate and glyceryl trierucate) can reduce plasma VLCFA levels but does not affect progression of already present leukodystrophy (Braverman et al., 2016). Use of Lorenzo's oil has not been studied in ZSDs but may be contraindicated because of the presence of increased levels of dietary monounsaturated fatty acids in patients who already accumulate large amounts of C26:1 (Klouwer et al., 2015;

Braverman et al., 2016). Because of impaired synthesis of docosahexanoic acid, supplementation with docosahexanoic acid was previously recommended. A placebo-controlled study, however, showed no clinical benefit with supplementation (Parker et al., 2010). Also, because of defective bile acid synthesis, supplementation with the fat-soluble vitamins, A, D, E, and K, is recommended (Braverman et al., 2016). Studies evaluating the effectiveness of bile acid supplementation (cholic acid and chenodeoxycholic acid) are limited, but bile acid supplementation may improve liver function especially in AMACR deficiency (Setchell et al., 2003; Braverman et al., 2016).

Further supportive care includes use of antiepileptic medications for seizure control, oxygen supplementation as needed for respiratory difficulties, use of hearing aids or cochlear implants for hearing loss, use of glasses for vision difficulties, routine dental care, and routine immunizations. Screening for adrenal insufficiency should occur regularly, and replacement therapy should be started as indicated with stress doses when necessary. Citrate therapy may help prevent renal oxalate stones. Bone density and vitamin D status should be monitored. Comprehensive developmental services should be provided. Treatment guidelines have recently been proposed and published by Braverman et al. (2016).

More recently, betaine and arginine have been recognized to be molecular chaperones that can improve peroxisomal assembly and may have a future therapeutic role (Waterham and Ebberink, 2012). HSCT is the established therapy for the cerebral childhood form of X-ALD, but there are no reports describing HSCT in ZSDs (Klouwer et al., 2015). Use of HSCT was recently reported in a young child with acyl-CoA oxidase deficiency. It was considered as a possible disease-arresting therapeutic intervention following recognition that the neuropathologic features of acyl-CoA oxidase deficiency resemble those of X-ALD (Wang et al., 2015). Despite full engraftment, the child experienced neurodegeneration and died in childhood (Wang et al., 2015). Hepatocyte transplantation and orthotopic liver transplantation have been described in patients with infantile Refsum disease with improvement in biochemical parameters and clinical course (Sokal et al., 2003; Van Maldergem et al., 2005). Gene therapy may provide future hope.

Smith–Lemli–Opitz Syndrome

Etiology

SLOS is a well-recognized autosomal recessive malformation syndrome, with an estimated incidence ranging from 1 in 10,000 to 1 in 70,000 in various populations (Smith et al., 1964; Porter, 2008; Cross et al., 2015). In 1993 it was discovered that SLOS is caused by a defect in cholesterol biosynthesis that results in low levels of cholesterol and elevated levels of 7-dehydrocholesterol (7DHC) and its isomer 8-dehydrocholesterol (8DHC) (Irons et al., 1993; Tint et al., 1994). Patients have markedly reduced activity of 7DHC reductase (Honda et al., 1995), the enzyme responsible for conversion of 7DHC to cholesterol encoded by the gene *DHCR7* (Wassif et al., 1998). The cause of the clinical phenotype of SLOS may be related to deficient cholesterol, deficient total sterols, and toxic effects of either 7DHC or compounds derived from it, or a combination of these factors (Bianconi et al., 2015).

Cholesterol is a major lipid component of cellular membranes such as myelin, and it is an important structural component of lipid rafts, which play a major role in intracellular signaling. In animal and in vitro models of SLOS, altered ratios of cholesterol, its dehydrocholesterol precursors, and its derivatives have been

noted to alter membrane rigidity, alter electrostatic properties of biologic membranes that can change the activity of ion-dependent adenosine triphosphatases and channels, decrease the stability of lipid rafts leading to increases in degranulation of mast cells, and reduce ligand binding to receptors such as the serotonin 1A receptor. In addition, bile acids, steroid hormones, neuroactive steroids, and oxysterols are all synthesized from cholesterol, and dehydrocholesterols can also serve as precursors of related steroids, bile acids, and oxysterols that may be antagonists or agonists of the ones derived from cholesterol (Bianconi et al., 2015). Cholesterol is also involved in hedgehog signaling by acting as a cofactor and covalent adduct to hedgehog members (Cooper et al., 2003). Hedgehog is a family of signaling proteins that are critical to pattern formation through interactions with the homeobox genes during embryonic development, and altered hedgehog signaling could explain some malformations seen in SLOS, such as holoprosencephaly and postaxial polydactyly (Farese and Herz, 1998; Kelley and Hennekam, 2000; Cooper et al., 2003). Recently, attention has been drawn to the high sensitivity of 7DHC to oxidation, and thus increased free radical generation may be a possible contributor to certain aspects of the disease, such as retinal degeneration (Chang et al., 2014; Xu and Porter, 2015).

Clinical Features

Recognition of the biochemical defect in SLOS provided the diagnostic test required to recognize the mildest and severest cases, substantially expanding the clinical spectrum of the condition. Classic SLOS is often evident at or before birth; affected patients have prenatal and postnatal growth retardation, microcephaly, and facial dysmorphism, including bitemporal narrowing, ptosis, epicanthic folds, anteverted nares, broad nasal tip, prominent lateral palatine ridges, retromicrognathia, and low-set ears. Other features include two- or three-toe syndactyly (found in 95% of patients), small proximally placed thumbs, occasionally postaxial polydactyly, and cataracts. Males usually have hypospadias, cryptorchidism, and a hypoplastic scrotum but may have ambiguous or female genitalia. Females may have a bicornuate uterus and/or septate vagina. Pyloric stenosis, cleft palate, bifid uvula, pancreatic anomalies, constipation, Hirschsprung disease, renal anomalies, congenital heart defects, and lung segmentation defects have also been reported. Hypotonia progressing to hypertonia is also present. Feeding difficulties and vomiting are common problems in infancy. Irritable behavior and shrill screaming may also pose problems during infancy. Older children frequently have hyperactivity, self-injurious behavior, sleep difficulties, and autistic characteristics. Cranial imaging studies and autopsies show defects in brain morphogenesis, including holoprosencephaly, frontal lobes, cerebellum, and brainstem hypoplasia, irregular gyral patterns, and irregular neuronal organization (Nowaczyk, 1993; Bianconi et al., 2015).

Historically, approximately 20% of patients die within the first year of life, although others may survive for more than 30 years. The clinical severity in SLOS correlates best with either reduction in absolute cholesterol levels or the sum of 7DHC and 8DHC levels expressed as a fraction of total sterol levels (Waterham and Clayton, 2006). Life expectancy appears to correlate inversely with the number and severity of organ defects and with the kinds and numbers of limb, facial, and genital abnormalities (Tint et al., 1995). Developmental outcomes are also highly variable, ranging from severe mental retardation to normal intelligence. Growth is typically lower than in unaffected individuals, and specific growth charts have been developed (Lee et al., 2012). In adults, depression

and anxiety may manifest themselves, and there has at least been one mildly affected female who has undergone a pregnancy with a good outcome (Ellingson et al., 2014). Testing for SLOS has been suggested for all patients with idiopathic intellectual impairment, behavioral anomalies, or both when associated with nonfamilial two- and three-toe syndactyly and failure to thrive (Jezela-Stanek et al., 2008).

Diagnosis

The diagnosis of SLOS is based on findings of elevated levels of 7DHC and 8DHC. False-positive elevations of 7DHC levels occur in patients taking psychoactive medications such as aripiprazole, trazodone, and haloperidol and in patients with increased cholesterol synthesis because of bile acid loss after ileal resection (Bianconi et al., 2015). Plasma cholesterol levels are usually low, but cholesterol is a poor diagnostic marker since as many as 10% of patients at all ages have normal cholesterol levels. Also, in many laboratories, measured cholesterol levels include cholesterol as well as 7DHC and 8DHC (Kelley and Hennekam, 2000).

Confirmation of diagnosis through molecular analysis of *DHCR7* is possible and recommended in cases where the serum concentration of 7DHC is difficult to interpret or prenatal or preimplantation genetic diagnosis is desired. Patients with two null mutations or with mutations in putative loop 8 or 9 have a severer phenotype, and patients with two missense mutations seem to be more mildly affected. However, patients with the same genotype can have markedly different severity (Waterham and Hennekam, 2012). Modifier genes are likely present, and maternal *APOE* and *ABCA1* genotypes that alter maternoplacental cholesterol transfer appear to modify disease severity (Witsch-Baumgartner et al., 2004; Lanthaler et al., 2013). If the genotype is unknown but prenatal testing is desired, abnormal levels of 7DHC from amniotic fluid or tissue from chorionic villus samples can be used for prenatal diagnosis, although false negatives can occur in mild cases. Prenatal sonographic findings of intrauterine growth retardation, increased nuchal translucency, nonimmune hydrops, unusual facial features, cystic hygroma, or major malformations in brain, heart, kidneys, limbs, genitalia, and palate are consistent with SLOS but have low sensitivity and specificity. Maternal serum screening showing low levels of unconjugated estriol, human chorionic gonadotropin, and alpha fetoprotein is also consistent with SLOS (Nowaczyk, 1993).

Treatment

Because of the underlying biochemical defect in SLOS, targeted treatment strategies to date have mainly focused on supplying exogenous cholesterol with the goal of raising cholesterol levels and secondarily lowering 7DHC and 8DHC levels by downregulating the patient's endogenous cholesterol synthesis. Cholesterol is typically given as a dietary modification (egg yolk, breast milk in infants), as a crystalline cholesterol suspension, or as a microencapsulated cholesterol powder with dosing dependent on the formulation ranging from 20 to 300 mg/kg (Irons et al., 1997; Kelley and Hennekam, 2000; Lin et al., 2005). Unfortunately dietary studies on cholesterol supplementation have not been conducted in a randomized fashion except for one short-term study that found no difference in short-term behavior in patients treated with cholesterol supplementation (Tierney et al., 2010). Case series have reported that cholesterol supplementation in SLOS has improved growth, development, and behavior, increased

nerve conduction velocity, and decreased skin photosensitivity, susceptibility to infection, and cholestatic liver disease of infancy when used with and without bile acid replacement. Cholesterol supplementation in SLOS has minimal side effects (Nowaczyk, 1993; Bianconi et al., 2015). However, given that dietary cholesterol does not cross the blood–brain barrier and that SLOS cells have impaired intracellular cholesterol transport, the efficacy of cholesterol supplementation is likely limited (Wassif et al., 2002; Dietschy, 2009).

Other targeted therapies have also been attempted, but none have been validated by controlled studies. Bile acid replacement has been used with cholestatic liver disease in infancy. Fresh frozen plasma, which contains high levels of cholesterol-rich lipoproteins such as LDL, has been used in acutely ill or severely stressed patients and in the setting of fetal intravenous and intraperitoneal transfusion. Stress steroid dosing has been used when there is evidence of adrenal insufficiency. A 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitor (simvastatin) has been used to improve cholesterol profiles, but its use had to be stopped in one individual who experienced liver dysfunction (Bianconi et al., 2015). Additionally, there may be a role for antioxidants in SLOS since 7DHC is highly reactive and gives rise to biologically active oxysterols (Korade et al., 2014). Direct delivery of cholesterol to the CNS by low-pressure catheter infusions has been proposed but not tested (Yu and Patel, 2005). Gene therapy, the use of neuroactive steroids, and inhibition of glycosphingolipids are also being investigated as possible therapeutic options in SLOS (Merkens et al., 2009).

Even without proven targeted treatments, appropriate supportive management is important. Following the initial diagnosis, to establish the extent of disease and the needs of the individual, recommended evaluations include a developmental assessment, an ophthalmologic evaluation, ECG, echocardiogram, a musculoskeletal evaluation especially for the need for orthotics, a genital urinary examination, nutritional assessment, renal ultrasonography, brain magnetic resonance imaging, hearing evaluation, GI evaluation with special effort to evaluate the patient for pyloric stenosis, gastroesophageal reflux, and Hirschsprung disease if indicated, laboratory evaluation to evaluate the patient for adrenal insufficiency and cholestatic liver disease, and a medical genetics consultation. Referral to early intervention and physical, occupational, and speech therapies is needed in many cases. Surgical interventions, such as gastrectomy tube insertion, surgical repair of cataracts, ptosis, or strabismus, pyloromyotomy, surgical repair of syndactyly or polydactyly, tendon release surgery in cases with significant hypertonia, and tympanostomy may be required in individuals with SLOS. Anesthetic complications of malignant hyperthermia have been reported. Treatment with medications with high affinity for the 7DHC reductase substrate may worsen the biochemical abnormalities so when medications such as haloperidol, trazodone, or aripiprazole are being used, potential benefits need to be weighed against the theoretical risk of worsening the underlying disease. Some infants with severe feeding problems benefit from use of

hypoallergenic, elemental formulas. Patients also need to avoid extended periods of sun exposure and use appropriate sun protection measures given the issue with photosensitivity (Nowaczyk, 1993).

Suggested Readings

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24

Newborn Resuscitation

ANUP KATHERIA AND NEIL N. FINER

KEY POINTS

- Adequate preparation for newborn resuscitation ensures that care can be provided in a timely and competent manner.
- Avoiding early umbilical cord clamping following delivery may have a significant impact on newborn outcomes.
- The use of additional monitoring (such as electrocardiography, carbon dioxide detection, and respiratory function) can be helpful during resuscitation.

The transition from fetal to neonatal life is a dramatic and complex process involving extensive physiologic changes that are most obvious at the time of birth. Individuals who care for newborns must monitor the progress of this transition and be prepared to intervene when necessary. In most births this transition occurs without a requirement for any significant assistance. However, when the need for intervention arises, the presence of providers skilled in neonatal resuscitation can be lifesaving. Each year approximately 4 million children are born in the United States ([Martin et al., 2008](#)), and more than 30 times as many are born worldwide. It is estimated that approximately 5%–10% of all births will require some form of resuscitation beyond basic care, making neonatal resuscitation the most frequently practiced form of resuscitation in medical care. Throughout the world approximately 1 million newborn deaths are associated with birth asphyxia ([Lawn et al., 2005](#)). While early effective newborn resuscitation will not eliminate all early neonatal deaths, such intervention will save many lives and significantly reduce subsequent morbidities.

Attempts to revive nonbreathing newborns immediately after birth have been made throughout recorded time, with references in the literature, religion, and early medicine. Although the organization and sophistication have changed, the basic principle and goal of initiating breathing has remained constant throughout time. It has been just in the last 30 years that more attention has been focused on the process of neonatal resuscitation.

Resuscitation programs in other areas of medicine were initiated in the 1970s in an effort to improve knowledge of effective resuscitation and provide an action plan for early responders. The first such program (1974) was focused on adult cardiopulmonary resuscitation. These programs then began increasing in complexity and becoming

more specific to different types of resuscitation needs. With the collaboration of the American Heart Association and the American Academy of Pediatrics, the Neonatal Resuscitation Program (NRP) was initiated in 1987—designed to address the specific needs of the newborn. Recent editions of the NRP textbook ([Kattwinkel 2006](#)) contained several revisions, including specific recommendations for the preterm newborn. Various groups throughout the world also provide resuscitation recommendations that may be more specific to the practices in certain regions. An international group of scientists, the International Liaison Committee on Resuscitation (ILCOR), meets on a regular basis to review available resuscitation evidence for all the different areas of resuscitation and puts forth a summary of its review ([Chamberlain, 2005](#)). The most recent recommendation by ILCOR ([Perlmán et al., 2015](#)) and the recommendations in the seventh edition of the NRP textbook (2016) are outlined in this review.

The overall goal of the NRP is similar to that of other resuscitation programs in that it intends to teach large groups of individuals of varied backgrounds the principles of newborn resuscitation and to provide an action plan for providers. Similarly, a satisfactory end result of resuscitation would be common to all forms of resuscitation: namely, to provide adequate tissue oxygenation to prevent tissue injury and restore spontaneous cardiopulmonary function. However, when one is comparing neonatal resuscitation with other forms of resuscitation, there are two distinctions. First, the birth of a child is a more predictable occurrence than most events requiring resuscitation in an adult such as an arrhythmia or a myocardial infarction. While not every birth will require “resuscitation,” it is more reasonable to expect that skilled individuals can be present when the need for neonatal resuscitation arises. It is possible to anticipate with some accuracy which newborns will more likely require resuscitation on the basis of perinatal factors and thus allow time for preparation.

The second distinction of neonatal resuscitation compared with other forms of resuscitation involves the unique physiology involved in the normal transition from fetal to neonatal life. The fetus exists in the protected environment of the uterus, where temperature is closely controlled, the lungs are filled with fluid, continuous fetal breathing is not essential, and the gas exchange organ is the placenta. The transition that occurs at birth requires the newborn to increase heat production, initiate continuous breathing, replace the lung fluid with air/oxygen, and significantly increase pulmonary blood

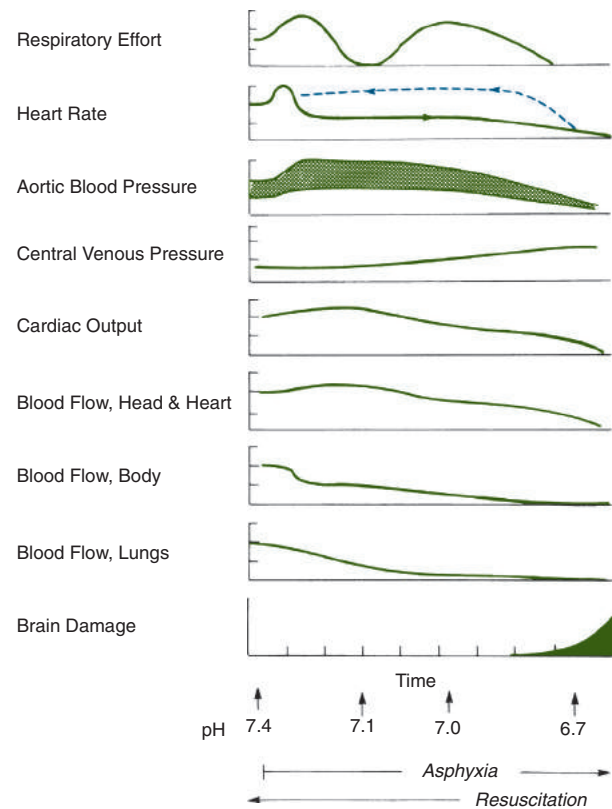
flow so that gas exchange can occur in the lungs. The expectations for this transitional process and knowledge of how to effectively assist the process help guide the current practice of newborn resuscitation.

Transition From Fetal to Extrauterine Life

While the complete transition from fetal to extrauterine life is complex and much more intricate than can be discussed in a few short paragraphs, basic knowledge of these processes will contribute to the understanding of the rationale for resuscitation practices. The key elements necessary for a successful transition to extrauterine life involve changes in thermoregulation, respiration, and circulation. In utero, the fetal core temperature is approximately 0.5°C greater than the mother's temperature (Gunn and Gluckman, 1983). Heat is produced by metabolic processes and is lost over this small temperature gradient through the placenta and skin (Gilbert et al., 1985). After birth the temperature gradient between the newborn and the environment becomes much greater, and heat is lost through the skin by radiation, convection, conduction, and evaporation. The newborn must begin producing heat through other mechanisms, such as lipolysis of brown adipose tissue (Dawkins and Scopes, 1965). If heat is lost at a pace greater than it is produced, the newborn will become hypothermic. Preterm newborns are at particular risk because of increased heat loss through immature skin, a greater surface area to body weight ratio, and decreased brown adipose tissue stores.

The fetus lives in a fluid-filled environment, and the developing alveolar spaces are filled with lung fluid. Lung fluid production decreases in the days before delivery (Kitterman et al., 1979), and the remainder of lung fluid is reabsorbed into the pulmonary interstitial spaces after delivery (Bland, 1988). As the newborn takes its first breaths after birth, a negative intrathoracic pressure of approximately $50\text{ cmH}_2\text{O}$ is generated (Vyas et al., 1986). The alveoli become filled with air, and with the help of pulmonary surfactant, the lungs retain a small amount of air persisting at the end of exhalation that is known as the functional residual capacity (FRC). Although the fetus makes breathing movements in utero, these efforts are intermittent and are not required for fetal gas exchange. Continuous spontaneous breathing is maintained after birth by several mechanisms, including the activation of chemoreceptors, the decrease in the levels of hormones that inhibit respirations, and the presence of natural environmental stimulation.

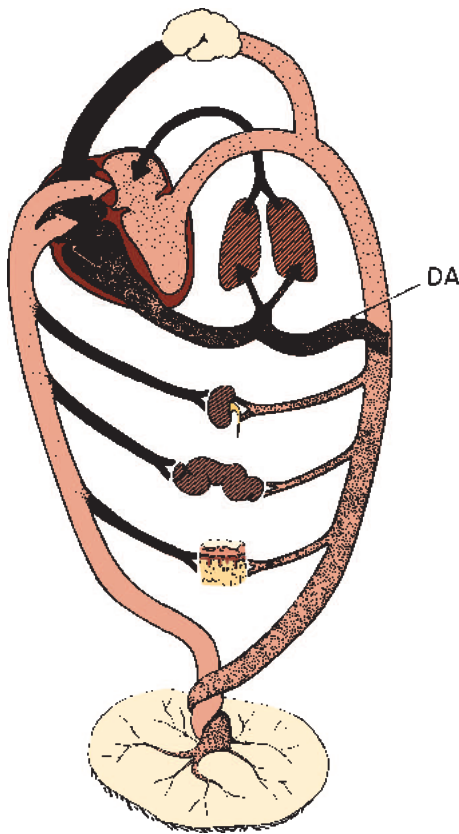
Spontaneous breathing can be suppressed at birth for several reasons, most critical of which is the presence of acidosis secondary to compromised fetal circulation. The natural history of the physiologic responses to asphyxia and acidosis has been described by researchers evaluating animal models. Dawes (1968) described the breathing response to acidosis in different animal species. He noted that when the pH is decreased, animals typically have a relatively short period of apnea followed by gasping. The gasping pattern then increases in rate until breathing ceases again for a second period of apnea. The physiologic effects that occur with worsening acidosis are noted in Fig. 24.1. Dawes also noted that the first period, or primary apnea, could be reversed with stimulation, while the second period, secondary or terminal apnea, required assisted ventilation to ultimately establish spontaneous breathing. The first sign of improvement was noted to be an increase in heart rate. Further recovery was noted when the newborn begins gasping again. The secondary period of apnea differs in duration depending on the duration of asphyxia and the degree of acidosis. In the clinical situation the exact timing of the onset of acidosis is generally



• **Fig. 24.1** The Sequence of Cardiopulmonary Changes With Asphyxia and Resuscitation. Time is on the horizontal axis. Asphyxia progresses from left to right; resuscitation proceeds from right to left. Units of time are not given. If there is complete interruption of respiratory gas exchange, the entire process of asphyxia from extreme left to right could occur in approximately 10 minutes. It could take much longer with an asphyxiating process that only partly interrupts gas exchange or does so completely but only for repeated brief periods. With resuscitation, the process reverses, beginning at the point to which asphyxia has proceeded. The blue dotted line is the reversal of asphyxia with resuscitation. (Modified from Dawes G. *Foetal and Neonatal Physiology*. Chicago: Year Book; 1968; and Avery GN. *Neonatology*. Philadelphia: JB Lippincott; 1987.)

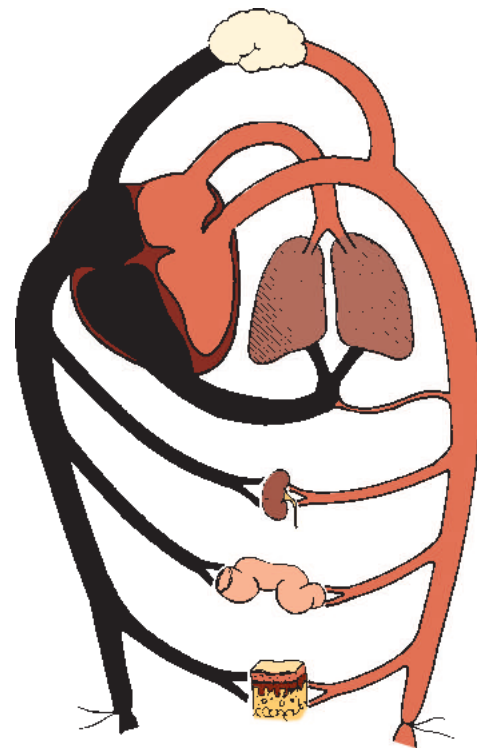
unknown, and therefore any observed apnea may be either primary or secondary. This is the basis of the resuscitation recommendation that stimulation may be attempted in the presence of apnea, but if it is not quickly successful, assisted ventilation should be initiated promptly. Without the presence of acidosis a newborn may also develop apnea because of recent exposure to respiratory-suppressing medications such as narcotics, anesthetics, and magnesium. These medications when given to the mother cross the placenta and depending on the time of administration and dose may depress the newborn's respiratory drive.

Fetal circulation is unique because gas exchange occurs in the placenta. In the fetal heart, oxygenated blood returning via the umbilical vein is mixed with deoxygenated blood from the superior vena cava and inferior vena cava and is differentially distributed throughout the body. The most oxygenated blood is directed toward the brain, while the most deoxygenated blood is directed toward the placenta. Thus blood returning from the placenta to the right atrium is preferentially streamed via the foramen ovale to the left atrium and left ventricle and then to the ascending aorta, providing the brain with the most oxygenated blood. Fetal channels, including the ductus arteriosus and foramen ovale, allow most blood flow to bypass the lungs with their intrinsically high vascular resistance,



• **Fig. 24.2** Fetal Circulation. Oxygenated blood leaves the placenta by way of the umbilical vein (vessel without stippling). The blood flows into the portal sinus in the liver (not shown), and a variable portion of it perfuses the liver. The remainder passes from the portal sinus through the ductus venosus into the inferior vena cava, where it joins blood from the viscera (represented by the kidney, gut, and skin). Approximately half of the inferior vena cava flow passes through the foramen ovale to the left atrium, where it mixes with a small amount of pulmonary venous blood. This relatively well oxygenated blood (light stippling) supplies the heart and brain by way of the ascending aorta. The other half of the inferior vena cava stream mixes with superior vena cava blood and enters the right ventricle (blood in the right atrium and ventricle has little oxygen, which is denoted by heavy stippling). Because the pulmonary arterioles are constricted, most of the blood in the main pulmonary artery flows through the ductus arteriosus (DA), so the descending aorta's blood has less oxygen (heavy stippling) than blood in the ascending aorta (light stippling). (From Avery GN. *Neonatology*. Philadelphia: JB Lippincott; 1987.)

and as a result pulmonary blood flow is approximately 8% of the total cardiac output. In the mature postnatal circulation the lungs must receive 100% of the cardiac output. When the low-resistance placental circulation is removed after birth, the newborn's systemic vascular resistance increases, while the pulmonary vascular resistance begins to fall as a result of pulmonary expansion, increased arterial and alveolar oxygen tension, and local vasodilators. These changes result in a dramatic increase in pulmonary blood flow. The average fetal oxyhemoglobin saturation as measured in fetal lambs is approximately 50% (Nijland et al., 1995) but ranges in different sites within the fetal circulation between 20%–80% (Teitel, 1988). The oxyhemoglobin saturation rises gradually over the first 5–15 minutes of life to 90% or greater as the air spaces are cleared of fluid. Diagrams of the blood flow patterns in the fetus and normally transitioning newborn are shown in Figs. 24.2–24.3. In the face of poor transition secondary to asphyxia, meconium aspiration,



• **Fig. 24.3** Circulation in the Normal Newborn. After expansion of the lungs and ligation of the umbilical cord, pulmonary blood flow increases and left atrial and systemic arterial pressures increase, while pulmonary arterial and right-sided heart pressures decrease. When the left atrial pressure exceeds the right atrial pressure, the foramen ovale closes so that all of the inferior and superior vena cava blood leave the right atrium, enter the right ventricle, and are pumped through the pulmonary artery toward the lung. With the increase in systemic arterial pressure and decrease in pulmonary arterial pressure, flow through the ductus arteriosus becomes left-to-right, and the ductus arteriosus constricts and closes. The course of the circulation is the same as in the adult. (From Avery GN. *Neonatology*. Philadelphia: JB Lippincott; 1987.)

pneumonia, or extreme prematurity, the lungs may not be able to develop efficient gas exchange, and thus the oxygen saturation may not increase as expected. In addition, in some situations the normal reduction in pulmonary vascular resistance may not fully occur, resulting in persistent pulmonary hypertension and decreased effective pulmonary blood flow with continued right to left shunting through the aforementioned fetal channels. This will lead to persistent hypoxemia and potentially to significant newborn illness requiring intensive care until the circulatory pattern adjusts to extrauterine life. The circulatory pattern associated with poor transition is noted in Fig. 24.4.

Environment and Preparation

The environment in which the newborn is born should facilitate the transition to neonatal life as much as possible and should be able to readily accommodate the needs of a resuscitation team when necessary. Hospitals differ in their approach to the details of how to prepare for resuscitation. For example, some hospitals have a separate room designated for resuscitation where the newborn will be taken after birth, while others have the delivery room adjacent to the neonatal intensive care unit (NICU), and the newborn is resuscitated in the NICU if necessary. Hospitals may



• **Fig. 24.4** Circulation in an Asphyxiated Newborn With Incomplete Expansion of the Lungs. Pulmonary vascular resistance is high, pulmonary blood flow is low (normal number of pulmonary veins), and flow through the ductus arteriosus is high. With little pulmonary arterial flow, left atrial pressure decreases below right atrial pressure, the foramen ovale opens, and vena cava blood flows through the foramen into the left atrium. Partially venous blood goes to the brain via the ascending aorta. The blood of the descending aorta that goes to the viscera has less oxygen than that of the ascending aorta (*heavy stippling*) because of the reverse flow through the ductus arteriosus. Therefore the circulation is the same as in the fetus, except that there is less well-oxygenated blood in the inferior vena cava and umbilical vein. (From Avery GN. *Neonatology*. Philadelphia: JB Lippincott; 1987.)

bring all the necessary equipment into the delivery room when resuscitation is expected or may have every delivery room already equipped for any resuscitation. Wherever the resuscitation will happen, a few key elements must be considered. The room should be warm enough to prevent excessive newborn heat loss, bright enough for assessment of the newborn's clinical status, and large enough to accommodate the necessary personnel and equipment to care for the baby.

When no added risks to the newborn are identified, term births frequently occur without the attendance of a specific neonatal resuscitation team. However, it is recommended that one individual be present who is responsible only for the newborn and can quickly alert a neonatal resuscitation team if necessary. Even the best neonatal resuscitation triage systems will not anticipate the need for resuscitation in all cases. A review found that when a risk-based determination of neonatal resuscitation team attendance at deliveries was used, 22% of newborns at attended deliveries required at least assisted ventilation (Aziz et al., 2008). These investigators found that the most significant risk factors were preterm birth, emergency cesarean delivery, and meconium-stained amniotic fluid. Other significant risk factors for the need for resuscitation are listed in

TABLE 24.1 Risk Factors for Neonatal Resuscitation

| Maternal Factors | Fetal Factors | Intrapartum Factors |
|-------------------------------|---------------------|---|
| Maternal hypertension | Preterm delivery | Opiates in labor |
| Maternal infection | Breech presentation | Rupture of membranes >18 h |
| Multiple gestations (preterm) | Shoulder dystocia | Meconium-stained amniotic fluid |
| | | Nonreassuring fetal heart rate patterns |
| | | Emergency cesarean delivery |
| | | Prolapsed cord |

Data from Aziz K, Chadwick M, Baker M, Andrews E. Ante- and intra-partum factors that predict increased need for neonatal resuscitation. *Resuscitation*. 2008;79(3):444–452.

Table 24.1. Antenatal determination of risk allows the resuscitation team to be present for the delivery and to be more thoroughly prepared for the situation.

The composition of the neonatal resuscitation team will differ tremendously among institutions. Probably the most important factor in how well a team functions is how the team has prepared for the delivery. Preparation involves both the immediate tasks of readying equipment and personnel for an individual situation and the more broad institutional preparation of training team members and providing appropriate space and equipment. We believe that when there is a strong suspicion that the newborn will be born in a compromised state, a minimum of three team members should be present, including one member with significant experience in leading neonatal resuscitations.

Each team member has assigned tasks that are performed on a regular basis. The leader is expected to ensure that the appropriate interventions are performed and that they are performed well. All team members are encouraged and expected to speak up if a problem is noticed or if they believe an alternative course would be beneficial. It seems logical that teams that regularly work together and divide tasks in a routine manner will have a better chance of functioning smoothly during a critical situation.

Institutions can facilitate team readiness with regular review of practices and mock codes or simulator training to practice uncommon scenarios. In our institution, Sharp Mary Birch Hospital for Women & Newborns, we review recorded resuscitations monthly with representatives from all disciplines involved in the resuscitation team. This is done as a quality assurance procedure and allows ongoing identification of areas needing improvement (Carbine et al., 2000). Additionally, this practice provides an opportunity for education and discussion about potential solutions to repetitive problems of newborn resuscitation. We have also instituted a supplemental training program for our pediatric trainees to obtain experience in a preclinical situation (Garey, 2009). These training sessions allow adequate time to review scenarios in detail, and trainees are given the opportunity to prepare and operate the equipment and practice procedures on an individualized basis. Others have used simulators to provide additional resuscitation training (Halamek, 2008). All of these training elements help prepare teams for future resuscitations.

The process of neonatal resuscitation requires that the medical team make rapid medical decisions to effectively transition a newborn from fetal to neonatal life. If the possible need for resuscitation is anticipated, the use of checklists can help the care team prepare for the specific circumstances of the particular delivery, familiarize themselves with other team members and the team leader, reinforce appropriate communication, ensure that the necessary equipment is available for prompt initiation of support critical to a successful neonatal resuscitation, and encourage a debrief to determine if further improvements are necessary for this process. The use of checklists in neonatal resuscitation would therefore seem logical. Checklists have been used in the aviation industry for many years to reduce errors and improve safety of passengers. In the last several years, these tools have begun to be embraced by the medical community to improve patient safety and patient care. They have been found to be useful in helping teams function more effectively, both in simulated environments and in clinical environments. While their use has been shown to yield low compliance when first introduced (Finer and Rich, 2010; Vats et al., 2010), the use of checklists has become a required standard for high-risk interventions such as emergency room traumas and surgical procedures and has been shown to reduce operative mortality (Haynes et al., 2009). Although the initial World Health Organization safe surgery checklist showed a reduction in surgical mortality, the implementation in routine practice in one study failed to show a benefit (Urbach et al., 2014). Some have suspected this is because the implementation of checklists requires some training and a culture of quality as necessary accompanying factors.

The most recent American Academy of Pediatrics NRP guidelines recommend the use of checklists for mock codes and related equipment but fall short of recommending them for use in actual resuscitations (NRP, 2016). The use of checklists with a debrief for actual resuscitations provides a mechanism for improving communication and recognizing and resolving problems and should be an essential component of neonatal resuscitation. The use of checklists during neonatal resuscitation is helpful in improving overall communication and allows rapid identification of issues that need to be addressed by institutional leaders (Katheria et al., 2013). We have shown that the introduction of such checklists for neonatal resuscitation has led to improved communication and better overall team function. An example of our institutional checklist is shown in Fig. 24.5. While there needs to be further evaluation of the utility and benefit of checklists for neonatal resuscitation, we encourage the use of institution-specific checklists for neonatal resuscitation teams. The ILCOR recommendations state: “A standardized checklist to ensure that all necessary supplies and equipment are present and functioning may be helpful...” When perinatal risk factors are identified, a team should be mobilized and a team leader should conduct a pre-resuscitation briefing, identify interventions that may be required, and assign roles and responsibilities to the team members. During resuscitation, it is vital that the team demonstrates effective communication and teamwork skills to help ensure quality and patient safety... It is still suggested that briefing and debriefing techniques be used whenever possible for neonatal resuscitation” (Perlman et al., 2015).

Transition and Resuscitation

After birth, blood flow in the umbilical arteries and vein usually continues for a few minutes. The additional blood volume transferred to the baby during this time is known as a placental transfusion. During the first 30 seconds of delayed cord clamping (DCC),

blood volume in the newborn increases by at least 12 mL/kg (Aladangady et al., 2006; Meyer and Mildenhall, 2012; Sommers et al., 2012; Takami et al., 2012; Katheria et al., 2014). The timing of umbilical cord clamping influences the amount of placental transfusion and subsequent plasma and red blood cell volume of the newborn (Yao et al., 1969; Yao and Lind, 1977). Early clamping may deprive newborns of blood that has an important role in opening the lungs (Jaykka, 1958), increasing cardiac output (Katheria et al., 2015), enhancing lung fluid clearance, and improving oxygen delivery to the newborn's tissues (Isobe et al., 2000; Jaiswal et al., 2015).

Recommendations and Evidence for Delayed Cord Clamping

The established practice of clamping the umbilical cord immediately after the delivery of the newborn was a result of the practice of limiting postpartum hemorrhage, which included immediate cord clamping. However, it was subsequently realized that immediate cord clamping was not required to reduce such hemorrhages. In fact there is no high-level trial-based evidence supporting the use of immediate cord clamping, and such immediate cord clamping has never been subjected to any controlled trial apart from its use as the control group in recent trials of other approaches to allow an adequate placental transfusion. The American College of Obstetricians and Gynecologists recommends a 30–60-second delay before the umbilical cord is clamped in all preterm deliveries, when feasible, to ensure that at-risk newborns receive an adequate placental transfusion (Raju, 2012). The timing for clamping of the umbilical cord after birth is a critical part of the resuscitation of preterm newborns and may have important benefits for perinatal outcomes. Some potential risks that have been raised with DCC, largely derived from term newborn studies, include increased rates of hyperbilirubinemia, polycythemia, and transient tachypnea in the newborn and increased risks of postpartum hemorrhage in the mother. However, several metaanalyses have demonstrated that there were no increases in these morbidities in preterm newborns (Rabe et al., 2012; Backes et al., 2014), and further, DCC does not increase maternal hemorrhage or blood loss (Eichenbaum-Pikser and Zasloff, 2009). In addition, these metaanalyses demonstrated that providing additional placental blood by either DCC or cord milking was associated with less need for transfusion, better circulatory stability, less intraventricular hemorrhage (all grades), decreased mortality, and lower risk of necrotizing enterocolitis (Rabe et al., 2012; Backes et al., 2014). The evidence to date shows that DCC substantively increases hemoglobin and iron stores in early infancy (Andersson et al., 2011). Inadequate iron stores in infancy may have an irreversible impact on the developing brain despite oral iron supplementation. Iron deficiency in infancy can lead to neurologic issues in older children, including poor school performance, decreased cognitive abilities, and behavioral problems. (Mercer and Erickson-Owens, 2012). Andersson et al. (2015) demonstrated that DCC increased scores in the fine-motor and social domains at 4 years of age, particularly in boys.

Cord Milking

Cord milking is an alternative to DCC that is used when the cord must be cut immediately for medical reasons, often because the newborn is in need of immediate resuscitation as judged by the clinician overseeing the resuscitation. Cord milking consists of encircling the umbilical cord with the thumb and forefingers,

| DR Resuscitation Check list | | Patient Label Here |
|---|--|--------------------|
| Pre-Resuscitation Briefing | | |
| Leader | _____ | |
| MD(s) | _____ | |
| RN(s) | _____ | |
| RT(s) | _____ | |
| <input type="checkbox"/> | Introductions/Roles | |
| <input type="checkbox"/> | Discuss Plan, communication expectations | |
| | o Special considerations? | |
| | o Additional personnel/equipment? | |
| | o "If any team member sees any developing problem or concern, I want to have it brought to my attention as soon as possible." | |
| | o Please call back all orders from Leader (e.g. "PIP is now 40") | |
| Pre-Resuscitation Checklist | | |
| Lead Resuscitator | | |
| <input checked="" type="checkbox"/> | Need urgent assistance, call x10770 | |
| <input type="checkbox"/> | Ensure briefing completed and introductions done | |
| <input type="checkbox"/> | Ensure RT checklist done | |
| <input type="checkbox"/> | Ensure RN checklist done | |
| <input type="checkbox"/> | Check status with resident receiving infant | |
| Respiratory Therapy | | |
| <input type="checkbox"/> | Brings RT bag (bring surfactant for < 28 weeks) | |
| <input type="checkbox"/> | Sets up Neopuff (30/5 and FiO ₂ .40, flow 8-10), appropriate masks | |
| <input type="checkbox"/> | Pedicap | |
| <input type="checkbox"/> | Sets up hand bag, checked (black bag if expecting difficult resus.) | |
| <input type="checkbox"/> | Intubation equipment checked, appropriate sized tubes | |
| <input type="checkbox"/> | Suction set at 80-100 mmHg, catheters, meconium aspirator if needed | |
| <input type="checkbox"/> | Pulse ox on and probe out | |
| <input type="checkbox"/> | NICO ET CO ₂ sensor | |
| <input type="checkbox"/> | Turn on video recorder | |
| Nursing | | |
| <input type="checkbox"/> | If crash C/section (call 2 nd RN/MD) ensure line is set up, Epi drawn up. | |
| <input type="checkbox"/> | Barney bag | |
| <input type="checkbox"/> | Radiant warmer on MANUAL at 100%, probe and cover available, hat | |
| <input type="checkbox"/> | Stethoscope | |
| <input type="checkbox"/> | Plastic wrap for < 28 weeks, Chemical mattress for <25 weeks | |
| <input type="checkbox"/> | ECG Leads | |
| Debrief | | |
| Did we have all the information we need to admit this patient? Y/N | | |
| What did we do well? (Resident, Nurse, RT, Fellow, Attending in that order) | | |
| _____ | | |
| What can we improve upon? _____ | | |
| Do we need follow-up on any items? _____ | | |

• **Fig. 24.5** Delivery Room Resuscitation Checklist. DR, Delivery room; ET, endotracheal; FiO₂, oxygen concentration; PIP, peak inspiratory pressure; MD, medical doctor; NICO ET, NICO Monitor (Philips-Respironics, Inc.; Wallingford, CT); RN, registered nurse; RT, respiratory therapist.

gently squeezing a short segment of the cord, and slowly pushing the blood through the cord to the newborn's abdomen three to four times.

For newborns born by cesarean there has been a concern that DCC may not provide an adequate placental transfusion. Aladangady et al. (2006) reported lower circulating red cell volume with DCC in newborns born by cesarean compared with vaginal delivery. They also found the duration of delay, up to 90 seconds, increased blood volume in neonates born by vaginal delivery but not cesarean delivery. For neonates born by cesarean, cord milking appears to offer benefits over DCC for 45–60 seconds. Compared with DCC,

cord milking results in greater blood flow to and from the heart, higher hemoglobin levels, and higher blood pressure in neonates born by cesarean (Katheria et al., 2015). Among a smaller number of vaginal births, there was no difference in blood volume between newborns undergoing cord milking and those undergoing DCC.

Breathing During Delayed Cord Clamping

Animal studies and one epidemiologic study suggest cord clamping should not occur until the newborn is breathing (Bhatt et al., 2013; Ersdal et al., 2014). One clinical study suggested that DCC

results in an inadequate transfusion in depressed newborns who are not breathing during the delay (Nevill and Meyer, 2015). It compared nonbreathing newborns with breathing newborns who underwent DCC and found that nonbreathing newborns had a lower 1-minute Apgar score, were more likely to be intubated, and were at greater risk of chronic lung disease or severe intraventricular hemorrhage (Nevill and Meyer, 2015). However, it is unclear in preterm newborns whether a few gasping breaths or positive pressure ventilation (PPV) is required. Most newborns will tolerate 60 seconds of delay without obvious deterioration. We need more observational data to characterize the need for resuscitation during this critical interval. While hypotonia and pallor may be relevant, most newborns will improve during DCC, and few if any studies have used oximetry during DCC to determine actionable levels. Our own study (Neonatal Resuscitation with Intact Cord, $n = 150$), where newborns were randomized to undergo DCC alone or receive ventilation during DCC (ClinicalTrials.gov identifier NCT02231411; Katheria et al., 2017), found that ventilation during DCC was feasible but did not lead to any improvements immediately after delivery or reduce neonatal morbidity when compared with simple stimulation during DCC. Our study suggests that more than 90% of preterm newborns will initiate spontaneous breathing during DCC if given some form of stimulation. Thus stimulation to encourage breathing may be as effective as attempting to establish ventilation during DCC in premature newborns. While organizations such as the World Health Organization (2012) have suggested that interventions such as PPV can be started during DCC, our study did not show a measurable benefit from starting interventions when compared with stimulation alone, and larger trials are needed in this area.

Clinical trials have demonstrated that both DCC and cord milking in preterm newborns increase cardiac output (Katheria et al., 2015), measures of systemic blood flow (Sommers et al., 2012), and brain oxygen extraction (Takami et al., 2012). Importantly, there is no high-level trial-based evidence supporting the use of immediate cord clamping, which has never been subjected to any controlled trial apart from its use as the control group in recent trials.

Providers currently need to evaluate the large body of evidence supporting early placental transfusions (from DCC or cord milking) and compare it with the lack of evidence supporting immediate cord clamping. Providing only warmth and stimulation during DCC may be as good as initiation of respiratory support during DCC, but more studies are needed. Further studies are also needed to determine the optimal type of placental transfusion, the optimal duration of delay, and the use of supportive respiratory interventions; many such studies are ongoing. In the interim, the best available evidence to date along with policy statements from national organizations suggests that DCC should be the standard of care for preterm newborns who do not require resuscitation, and cord milking should be reserved for cases when DCC cannot be performed (Perlman et al., 2015).

Delivery Room Monitoring

Assessment

Immediately after birth the newborn's condition is evaluated by general observation as well as measurement of specific parameters. Typically a healthy newborn will cry vigorously and maintain adequate respirations. The color will transition from blue to pink in the first 2–5 minutes, the heart rate will remain in the region

of 140–160 beats per minute (bpm), and the newborn will demonstrate adequate muscle tone with some flexion of the extremities. The overall assessment of a newborn who is having difficulty with the transition to extrauterine life will often reveal apnea, bradycardia, hypotonia, and cyanosis or pallor.

Following the initial steps of resuscitation, interventions are based mainly on the evaluation of respiratory effort and heart rate, so both must be continually assessed throughout the resuscitation.

Heart Rate

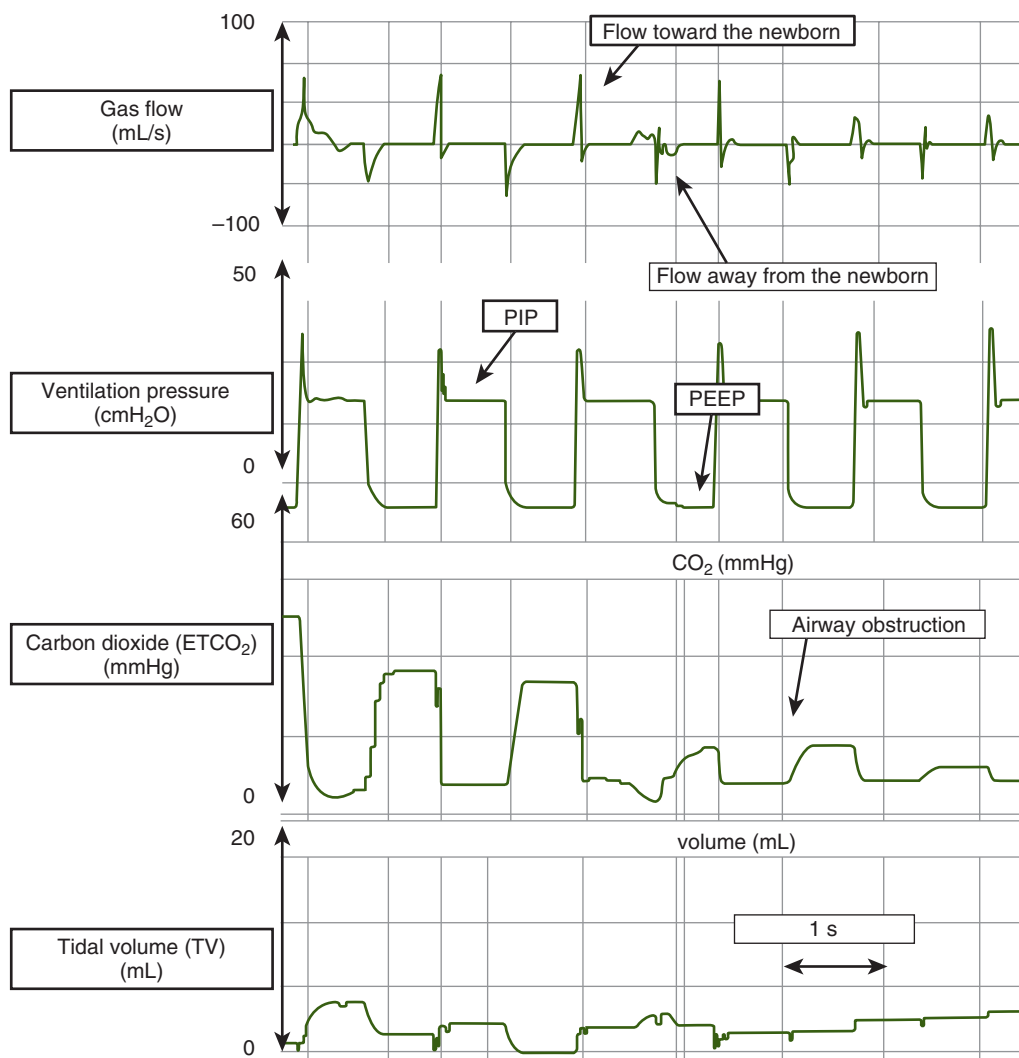
Previous NRP recommendations only required a snapshot of the heart rate every 30 seconds to determine whether it fell between two critical cut points (60 and 100 bpm) as defined in the guidelines. Even if the heart rate is being auscultated and manually tapped out by hand, it can be difficult for the leader of the resuscitation to recognize changes quickly. With the inclusion of pulse oximetry for high-risk deliveries, all resuscitation teams can now monitor the heart rate continuously as long as the oximeter is functioning (Kattwinkel et al., 2010). However, the pulse oximeter, while helpful, does not provide a reliable heart rate in the first few minutes of life. Importantly, this is a critical period when decisions, such as the need to begin PPV, must be made. Our group demonstrated that the median time to obtain the heart rate of very low birth weight newborns (<1500 g), by means of oximetry, was 67 seconds (interquartile range 50–93 seconds) (Gandhi et al., 2013).

ECG, which derives from the electrical activity of the heart, is not dependent on the circulation and so is less affected by the transitional state of the newborn. In trials comparing oximetry with ECG, early application of ECG electrodes during newborn resuscitation can provide the resuscitation team with a continuous reliable audible heart rate earlier, and its use may improve the timeliness of appropriate critical interventions when compared with pulse oximetry alone (Katheria et al., 2012; Mizumoto et al., 2012). In addition, heart rate measured by ECG has been found to be higher than that measured by oximetry particularly early in resuscitation when low signal messages occur (Narayan et al., 2015). The seventh edition of the NRP textbook suggests the use of ECG to provide a rapid and accurate estimation of heart rate (Perlman et al., 2015; NRP, 2016).

Airway Pressure

Managing ventilation in the transitioning newborn may be one of the most difficult procedures faced by the resuscitation team. Positive end-expiratory pressure (PEEP) is used during resuscitation of preterm newborns to assist in establishing and maintaining FRC in the surfactant-deficient lung. It is difficult to recognize subtle changes in PEEP with a manometer. Real-time tracings of airway pressure allow the recognition of patterns, such as slow rises or falls in PEEP. When a T-piece resuscitation device is used, the airway pressure plot also provides the team with a visual cue of mask leak during ventilation, as the inspired airway pressure changes from consistent and flat to variable and curvy.

Mechanical ventilation in preterm newborns has been moving in the direction of “gentler ventilation” for years. This means low mean airway pressures and low ventilation rates. In the delivery room, where devices that regulate respiratory support are not generally present, ventilation rates and mean airway pressures can quickly be increased to levels reserved for much sicker newborns in the NICU. The visual display of the airway pressure waveform, along with digital readouts of mean airway pressure and respiratory



• **Fig. 24.6** Complete Airway Occlusion During Mask Positive Pressure Ventilation in an Extremely Low Birth Weight Newborn. Adequate positive pressure ventilation is provided, and suddenly the inflation and expiratory flow curves both display fewer flow movements and no tidal volume, and reducing expiratory CO_2 is displayed. The peak inspiratory pressure and positive end-expiratory pressure are maintained during positive pressure ventilation. ETCO_2 , end-tidal carbon dioxide; PEEP , positive end-expiratory pressure; PPV , positive pressure ventilation. (Courtesy of G. Schmolzer.)

rate, can quickly alert the team with feedback that excessive pressure is being provided to the newborn. However, the main issue with airway pressure monitoring is that achievement of a target pressure does not ensure that the newborn has received an adequate breath because of possible airway obstruction or leak (Finer et al., 2009). Therefore additional tools to measure tidal volumes, determine airway patency, and detect carbon dioxide (CO_2) are required.

Tidal volume can now be monitored with respiratory function monitors (RFMs) that are placed in the respiratory circuit (Schmolzer et al., 2010). Monitoring of tidal volume is standard on any current neonatal ventilator, but measuring spontaneous tidal volume, either in the NICU or in the delivery room, can be difficult. RFMs, which record airflow, airway pressure, and tidal volume, have been shown to be an effective tool in providing feedback to users in the delivery room (Schmolzer et al., 2012). In addition, it has been suggested that respiratory function monitoring should be used to measure tidal volumes to avoid harming the lungs (Schmolzer et al., 2008). The use of RFMs has been shown to

reduce mask leak, increase mask adjustments, and lower the rate of excessive tidal volumes. RFMs can display airway obstruction by reduction in both the expiratory gas flow and the inspiratory gas flow and no tidal volume (Fig. 24.6). Trials are needed to determine whether the RFM in the delivery room reduces neonatal morbidity.

The use of a colorimetric CO_2 detector during hand ventilation provides confirmation that gas exchange is occurring by the observed color change of the device, alerting the operator to an obstructed airway with lack of such color change (Leone et al., 2006). Airway obstruction is very common in the preterm newborn during PPV immediately after birth (Finer et al., 2009; Schmolzer et al., 2011). The site of obstruction may be laryngeal or supralaryngeal. It is important to remember that these CO_2 detectors will not change color in the absence of pulmonary blood flow, as occurs with inadequate cardiac output. One should be aware, however, that such colorimetric detectors can increase the overall resistance of commonly used resuscitation devices, including T-pieces, bags,

and masks. At times, multiple maneuvers are required to achieve an open airway, such as readjusting the head and mask positions, choosing a mask of more appropriate size, and further suctioning of the pharynx.

Our recommendation for most neonatal centers includes the use of ECG for early heart rate assessment, now referred to in the 2016 NRP recommendations, early use of pulse oximetry, and the use of a colorimetric CO₂ detector (also discussed in the 2016 NRP guidelines), which would provide adequate delivery room monitoring for resuscitation of newborns. RFMs providing real-time tidal volume and airway leak are ideally suited for academic centers focusing on neonatal resuscitation research.

Initial Steps: Temperature Management and Maintaining the Airway

In the first few seconds after birth all newborns are evaluated for signs of life, and a determination of the need for further assistance is made. This is done both formally as described in the NRP guidelines and informally as the initial care providers observe the newborn in the first few moments after birth. When the determination that further assistance and formal resuscitation are necessary, the newborn is placed on a radiant warmer and positioned appropriately for resuscitation to proceed. Appropriate positioning includes placing the newborn supine on the warmer in such a way that care providers have easy access. In addition, the head should be in a neutral or “sniffing” position to facilitate maintenance of an open airway. Frequently the oropharynx contains fluid that can be removed by suctioning with a standard bulb syringe.

A neonate born through meconium-stained amniotic fluid is at risk of aspirating meconium and developing significant pulmonary disease, known as meconium aspiration syndrome, which may also be accompanied by persistent pulmonary hypertension. For many years routine management of all newborns with meconium-stained amniotic fluid included suctioning of the mouth and pharynx once the head had been delivered but before the shoulders were delivered and endotracheal intubation and tracheal suctioning in an attempt to remove any meconium from the trachea and prevent the development of meconium aspiration syndrome. Routine suctioning of the mouth and nose before delivery of the shoulders has since been shown to be of no benefit in decreasing the incidence of meconium aspiration syndrome, the need for assisted ventilation, or mortality (Vain et al., 2004). Recognizing that intubation may not be necessary and may be associated with complications (Wiswell et al., 2000), the NRP recently stated that there was insufficient evidence to recommend routine suctioning of meconium in *nonvigorous* neonates born through meconium-stained amniotic fluid (NRP, 2016). The concern for routine suctioning of nonvigorous newborns is that it is more likely to result in delays in initiating ventilation, especially during difficult intubations or multiple attempts. The emphasis should be on initiating ventilation within the first minute after delivery in nonbreathing or ineffectively breathing newborns.

While temperature control is important for all newborns, it is particularly important for the extremely preterm newborn. Preterm newborns are commonly admitted to the NICU with core temperatures well below 37°C, and in a population-based analysis of all newborns of less than 26 weeks' gestation, more than one-third of them had an admission temperature below 35°C (Costeloe et al., 2000). More disturbing is the fact that these newborns with hypothermia on admission survived less often than those with

admission temperatures above 35°C. Admission temperatures can be increased in preterm newborns by immediate covering of the newborn's body with polyethylene wrap before the newborn is dried (Vohra et al., 2004). With this approach the newborn's head is left out of the wrap and is dried, but the body is not dried before wrap application. Other measures for maintaining newborn temperatures include performing resuscitations in a room that is kept at an ambient temperature of approximately 27°C–30°C, the use of radiant warmers with servo-controlled temperature probes placed on the newborn within minutes of delivery, and the use of an accessory prewarmed mattress or heating pads for the tiniest newborns. While hats are routinely used as a method of decreasing heat loss, they have not been shown to be consistently effective (McCall et al., 2008). It is important to note that as a required safety feature, radiant warmers substantially decrease their power output after 15 minutes of continuous operation in full-power mode. If this decrease in power is unrecognized, the newborn will be exposed to much less radiant heat. By application of the temperature probe and use of the warmer in servo-controlled mode, the temperature output will adjust as needed, and the power will not automatically decrease. The NRP has recently recommended that the temperature of newly born nonasphyxiated neonates be maintained between 36.5°C and 37.5°C after birth through admission and stabilization (NRP, 2016).

Assisting Ventilation

As the newborn begins breathing and replaces the lung fluid with air, the lung becomes inflated and an FRC is developed and maintained. With inadequate establishment of FRC, the newborn will not be adequately oxygenated and if this is prolonged will develop bradycardia. The provision of assisted ventilation when the newborn's spontaneous breathing is inadequate is probably the most important step in newborn resuscitation. The indications for providing assisted ventilation (PPV) include apnea or bradycardia (heart rate less than 100 bpm). PPV can be delivered noninvasively with a pressure delivery device and a face mask or a laryngeal mask airway (LMA) or delivered invasively with the same pressure delivery device and an endotracheal tube. Pressure delivery devices can include self-inflating bags, flow-inflating or anesthesia bags, and T-piece resuscitators, each with its advantages and disadvantages. The self-inflating bag is easy to use for inexperienced personnel and will work in the absence of a gas source. However, the self-inflating bag requires a reservoir to provide nearly 100% oxygen and does not consistently provide adequate PEEP. These devices may deliver very high pressure if not used carefully. Although they have pressure blow-off valves, these valves do not always open at the target blow-off pressures (Finer et al., 1986). An anesthesia bag or flow-inflating bag requires a gas source for use and allows the operator to vary delivery pressures continuously on the basis of the compliance felt but requires significant practice to develop expertise. However, using a test lung and intermittent airway occlusion, even experienced anesthesiologists were unable to recognize the increased resistance from an airway obstruction using only their hands (Spears et al., 1991). A T-piece resuscitator is easy to use, requires a gas source, and delivers the most consistent levels of pressure but requires intentional effort to vary pressures (Hoskyns et al., 1987). The flow-inflating bag and T-piece resuscitator allow the operator to deliver continuous positive airway pressure (CPAP) or PEEP relatively easily (Finer et al., 2001; Bennett et al., 2005).

A level of experience is required to perform assisted ventilation with a face mask and resuscitation device, and this is especially

true for an extremely low birth weight newborn. It is important to maintain an open airway for pressure to be transmitted to the lungs. The procedure for obtaining and maintaining an open airway includes, at minimum, clearing of fluid with a suction device, holding the head in a neutral position, and sometimes lifting the jaw slightly anteriorly. The face mask must make an adequate seal with the face for air to pass to the lungs effectively. No device will adequately inflate the lungs if there is a large leak between the mask and the face. Wood et al. (2008) measured face mask leaks of more than 55% when participants were evaluated providing positive pressure to manikins without instruction. The amount of leakage was decreased to approximately 30% with specific instruction. Until recently there were no masks that were small enough to provide an adequate seal over the mouth and nose for the tiniest newborns. Such masks are now readily available and facilitate bag-and-mask resuscitation of very small newborns. Signs that the airway is open and air is being delivered to the lungs include visual inspection of chest rise with each breath and, more importantly, improvement in the clinical condition, including heart rate and color.

We recommend that the resuscitation team attempt to achieve the target inspiratory pressures as the first goal in providing bag-and-mask resuscitation. Failure to achieve such pressures usually indicates a significant leak, which should be addressed by the repositioning of the mask and jaw or selection of a better-fitting mask. Once the target pressure has been achieved, the colorimetric detector is useful in determining whether there is airway obstruction, and failure to achieve an adequate color change in the face of delivering targeted pressures requires further attention to the airway, including repositioning, suctioning, and use of higher airway pressures. The seventh edition of the NRP textbook states that a colorimetric CO₂ detector can be used during ventilation corrective steps (NRP, 2016). Alternative methods of providing an open airway include the use of a nasopharyngeal tube (Lindner et al., 1999) or an LMA device (Grein and Weiner, 2005). Failure of these interventions requires immediate intubation. Prolonged attempts at ventilation against an unrecognized obstructed airway could result in further, more aggressive and hazardous procedures, such as chest compressions and/or medication administration.

The amount of pressure provided with each breath during assisted ventilation is critical to the establishment of lung inflation and therefore adequate oxygenation. Although it is important to provide adequate pressure for ventilation, excessive pressure can contribute to lung injury. Achieving the correct balance of these goals is not simple and is an area of resuscitation that requires more study. A single specific level of inspiratory pressure will never be appropriate for every baby. Initial inflation pressures of 25–30 cmH₂O are probably adequate for most term babies. The current NRP textbook recommends initial pressures of 20–25 cmH₂O for preterm newborns. The first few breaths may require increased pressure if lung fluid has not been cleared, as occurs when the newborn does not initiate spontaneous breathing. Newborns with specific pulmonary disorders such as pneumonia or pulmonary hypoplasia also frequently require increased inspiratory pressure. It has been shown that use of enough pressure to produce a visible chest rise is associated with hypocarbia on admission blood gas evaluation (Tracy et al., 2004), and excessive pressure may decrease the effectiveness of surfactant therapy (Bjorklund et al., 1997). It may be possible to establish FRC without increasing peak inspiratory pressures by providing a few prolonged inflations (inspiration for 3–5 seconds) although the use of prolonged inflations has not been associated with better outcomes than conventional breaths

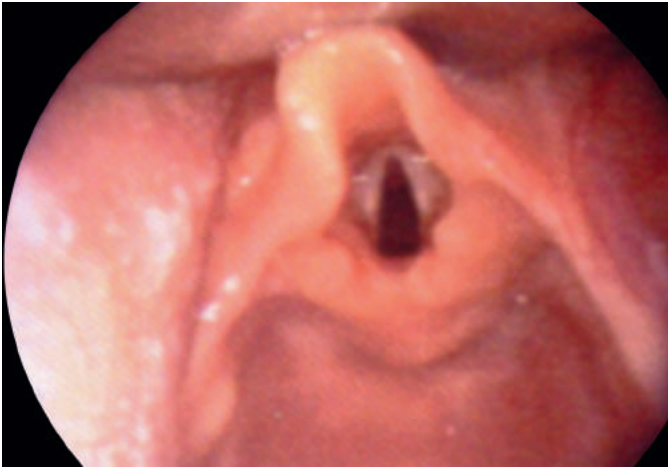
during resuscitation (Lindner et al., 2005). Choosing the actual initial inspiratory pressure is less important than continuously assessing the progress of the intervention. A manometer in the circuit during assisted ventilation provides the clinician with an indication of the administered pressure, although if the airway is blocked this pressure is not delivered to the lungs. The volume of air delivered to the lungs seems to be more important than the absolute pressure delivered in the development of lung injury.

The most critical component of continued assessment is evaluation of the newborn's response to the intervention. If after ventilation has been initiated the condition of the newborn does not improve, then the ventilation is most likely inadequate. In our experience the two most likely reasons for inadequate ventilation are a blocked airway and insufficient inspiratory pressure. The occluded airway can be noted with use of a colorimetric CO₂ device as described earlier and frequently can be corrected with changes in position or suctioning, while inadequate pressure is corrected by adjustment of the ventilating device.

Use of continuous pressure throughout the breathing cycle seems to be beneficial for the establishment of FRC and improvement in surfactant function (Michna et al., 1999; Hartog et al., 2000; Siew et al., 2009). This is accomplished during assisted ventilation with the use of PEEP or with CPAP when additional inspiratory pressure is not needed. In the absence of PEEP, a lung that has been inflated with assisted inspiratory pressure will lose on expiration most of the volume that had been delivered on inspiration. This pattern of repeated inflation and deflation is frequently thought to be associated with lung injury. In preterm newborns a general approach of using CPAP as a primary mode of respiratory support in NICUs has been associated with a low incidence of chronic lung disease (Avery et al., 1987; Van Marter et al., 2000; Ammari et al., 2005; Vanpee et al., 2007). te Pas and Walther (2007) evaluated a ventilation strategy that included use of a T-piece resuscitator and a nasopharyngeal tube to deliver a prolonged breath followed by CPAP, compared with use of a self-inflating bag to deliver PPV when needed without any CPAP provided, until newborns reached the NICU. Newborns treated with the prolonged breath and CPAP required less endotracheal intubation and had lower rates of bronchopulmonary dysplasia. More recently, this same group demonstrated that probably because of airway obstruction at or above the larynx, they could not deliver a sustained inflation to premature newborns who were apneic (van Vonderen et al., 2014).

The use of CPAP compared with endotracheal intubation and mechanical ventilation as the initial mode of respiratory support was evaluated in the Continuous Positive Airway Pressure or Intubation at Birth (COIN) trial (gestational age [GA] 25–28 6/7 weeks) (Morley et al., 2008), but the intervention did not begin until 5 minutes after delivery and can therefore not inform the decision about use of these therapies as immediate resuscitative interventions. The Surfactant, Positive Pressure, and Oxygenation Randomized Trial (SUPPORT) (GA 24–27 6/7 weeks) demonstrated that the use of early CPAP can be an effective alternative to intubation and surfactant as the primary mode of respiratory support in preterm newborns (Finer et al., 2010). While these trials did not evaluate these therapies specifically in the resuscitation period, the respiratory support provided shortly after birth should probably mimic the support provided in the NICU following delivery.

If assisted ventilation is necessary for a prolonged period or if other resuscitative measures have been unsuccessful, ventilation must be provided by a securer device such as an endotracheal tube.



• **Fig. 24.7** View of the Glottis and Vocal Cords as the Laryngoscope Is Gently Lifted. (From Kattwinkel J, ed. *Neonatal Resuscitation Textbook*. 5th ed. Elk Grove Village: American Heart Association and American Academy of Pediatrics; 2006.)

If it has been difficult to maintain an open airway while ventilation has been given via a face mask, the appropriately placed endotracheal tube will provide a stable airway. This will allow more consistent delivery of gas to the lungs and therefore provide the ability to establish and maintain FRC. Currently in the United States, intubation with an endotracheal tube is required for administration of surfactant and may be used to administer other medications, such as epinephrine, if necessary for resuscitation. The intubation procedure is often critical for successful resuscitation, requires a significant amount of skill and experience to perform reliably, and may be associated with serious complications. The placement of a laryngoscope in the pharynx often produces vagal nerve stimulation that leads to bradycardia. A photograph of the desired view of the larynx is presented in Fig. 24.7. Assisted ventilation must be paused for the procedure, which if prolonged can lead to hypoxemia and bradycardia. Intubation has been shown to increase blood pressure and intracranial pressure (Kelly and Finer, 1984). Trauma to the mouth, to the pharynx, to the vocal cords, and to the trachea is a possible complication of intubation. If the intubation procedure is performed when the newborn already has bradycardia and hypoxemia, this can lead to a further decline in heart rate and oxygenation (O'Donnell et al., 2006). Additionally, hypoxia and bradycardia are more likely when intubation attempts are prolonged beyond 30 seconds. Therefore it is most appropriate to attempt to stabilize the newborn with noninvasive ventilation before the procedure is performed and to limit each attempt to 30 seconds or less (Lane et al., 2004) and allow time for the newborn to recover with noninvasive ventilation between attempts. If misplacement of the endotracheal tube in the esophagus goes unrecognized, the newborn may experience further clinical deterioration. Clinical signs that the endotracheal tube has been correctly placed in the trachea include auscultation of breath sounds over the anterolateral aspects of the lungs (near the axilla), mist visible in the endotracheal tube, chest rise, and clinical increase in heart rate and improvement in color or increase in oxygen saturation. The use of a colorimetric CO₂ detector to confirm intubation significantly decreases the amount of time necessary to determine correct placement of the endotracheal tube from approximately 40 seconds to less than 10 seconds (Aziz et al., 1999; Repetto et al., 2001). We consider this to be the primary method of determining endotracheal tube placement.

Successful placement of the endotracheal tube is not always easy and is sometimes not possible. Airway anomalies may make alternative methods of airway management necessary. An LMA is one such alternative that has been described for use in patients with Pierre Robin sequence or other airway anomalies (Yao et al., 2004). Some practitioners have reported using the LMA for all positive pressure delivery after birth, with success noted in newborns as small as 1000 g (Gandini and Brimacombe, 1999). Administration of medications, including surfactant and epinephrine, through this device has undergone preliminary investigation (Trevisanuto et al., 2005; Chen et al., 2008). The most recent comparison of the use of an LMA versus mask resuscitation demonstrated better outcomes for newborns weighing 1500 g or more at birth with the LMA, and we advise that a small LMA should always be available in the resuscitation area.

Transitional Oxygenation and Oxygen Use

It is critical to remember that fetal arterial oxygen levels are much lower than newborn arterial oxygen levels. The transition from fetal to newborn levels does not happen instantaneously. Using pulse oximetry in the immediate newborn period, several investigators have established that this transition with an ultimate oxygen saturation level of more than 90% takes 5–15 minutes to occur in newborns who do not otherwise require resuscitation. For term and preterm nonresuscitated newborns the median oxygen saturation at 3 minutes was 76% (interquartile range 64%–87%) and at 5 minutes was 90% (interquartile range 79%–91%) (Kamlin et al., 2006). The expected oxygen saturation levels are slightly lower for preterm neonates compared with term neonates and for neonates delivered via cesarean compared with those delivered vaginally (Rabi et al., 2006). Oxygen saturation levels measured in preductal sites are 5%–10% higher than those measured from postductal sites for approximately 15 minutes of life (Mariani et al., 2007). A great deal of variability occurs in the saturation values among different well individuals during the first 5 minutes of life, but a resuscitation team can expect there to be a steady, albeit slow, increase in levels over several minutes. If the values are below a threshold at different time points or not progressively increasing, intervention should be considered.

The use of pure oxygen for ventilation became routine practice in resuscitation simply because it seemed logical that oxygen would be beneficial. However, the recognition that oxygen could also be toxic led some investigators to question this previously well-accepted practice. The toxicity of oxygen is anticipated when the cellular antioxidant capacity is impaired as occurs during the reperfusion phase following an hypoxic–ischemic insult. After animal studies showed the potential harmful effects of oxygen (Rootwelt et al., 1992; Poulsen et al., 1993), clinical trials were conducted to evaluate the effects of supplemental oxygen use during resuscitation of depressed newborns. Several worldwide trials have compared the use of pure (100%) oxygen with room (ambient) air (21% oxygen) as the initial ventilating gas for primarily term asphyxiated newborns. These trials found that air was as successful as oxygen in achieving resuscitation, and newborns resuscitated with air had a shorter time to initiate spontaneous breathing and less evidence of oxidative stress (Saugstad et al., 1998; Vento et al., 2001; Ramji et al., 2003; Vento et al., 2003). Metaanalyses of trials demonstrated that newborns resuscitated with air had a lower risk of death than those resuscitated with pure oxygen (Tan et al., 2005; Rabi et al., 2007). These trials have been criticized because they were not all strictly randomized, and some sites were in developing countries. However,

metaanalysis of the strictly randomized trials that were mostly carried out in European centers demonstrates significant benefit for survival with the use of air as compared with pure oxygen (Saugstad et al., 2008).

The preterm newborn may be more susceptible to any harmful effects of excessive oxygen exposure because of decreased antioxidant enzyme capacity. Some of the newborns in the previous oxygen trials were preterm but a few weighed less than 1000 g. Review of the outcomes for preterm newborns (<37 weeks) demonstrated an even greater reduction in death for those initially resuscitated with room air compared with oxygen (Saugstad et al., 2005). NICUs generally attempt to reduce oxygen toxicity by limiting the amount of oxygen administered to newborns using an upper limit for oxygen saturation and adjusting supplemented oxygen levels to maintain saturation levels within that limit. The unlimited use of oxygen during resuscitation exposes the preterm newborn to higher oxygen saturation levels than would routinely be accepted in the NICU. Several small trials of oxygen use during resuscitation of preterm newborns have been performed in the last 3 years. Among 42 preterm neonates born at less than 32 weeks' gestation treated with either pure oxygen for 5 minutes or a targeted oxygen strategy beginning with 21% oxygen (Wang et al., 2008), those newborns initially given pure oxygen had higher peripheral arterial oxygen saturation (SpO_2) levels during the transition but did not have any differences in heart rate, need for intubation, or survival. The newborns provided with air from the start of resuscitation all required an increase in the amount of inspired oxygen to obtain the prespecified oxygen saturation targets. Using 30% versus 90% oxygen at the start of resuscitation and adjusting the concentration on the basis of the clinical status of the newborn, Escrig et al. (2008) found that newborns initially given 30% oxygen had lower overall exposure to oxygen without any adverse effects. The group initially given 30% oxygen received increased oxygen concentrations up to approximately 55% at 5 minutes, but both groups received similar oxygen concentrations after 4 minutes of life, with a level of approximately 35% by 15 minutes after delivery. In another study, these investigators reported a decrease in the incidence of bronchopulmonary dysplasia in newborns initially given 30% oxygen compared with 90% oxygen (Vento et al., 2009). The Room-Air Versus Oxygen Administration for Resuscitation of Preterm Infants (ROAR) study was a blinded, randomized controlled trial in 106 neonates born at 32 weeks' gestation or less comparing three oxygen strategies: one group received 100% oxygen throughout, one received 100% oxygen initially, and the third received 21% oxygen initially; in the last two groups the oxygen concentration was titrated to keep SpO_2 at 85%–92%. SpO_2 levels were below the prespecified target range 61% of the time in the group that received 21% oxygen initially and were above the target range 49% of the time in the group that received 100% oxygen throughout ($P < .001$) (Rabi et al., 2011). A recent retrospective study from the Canadian Neonatal Network demonstrated an increased risk of severe neurologic injury or death with resuscitation using room air (or room air with titrated increases to 40% oxygen) compared with 100% oxygen (Rabi et al., 2015).

The most recent large trial involving preterm newborns compared room air with 100% oxygen, the Targeted Oxygenation in the Resuscitation of Premature Infants and their Developmental Outcome (TO2RPIDO) study (ACTRN12610001059055), was stopped because of poor enrollment but also found increased mortality (<28 days) in neonates born at less than 28 weeks' gestation who were treated with low or titrated oxygen compared with

those who received 100% at the initiation of resuscitation (Vento et al., 2016).

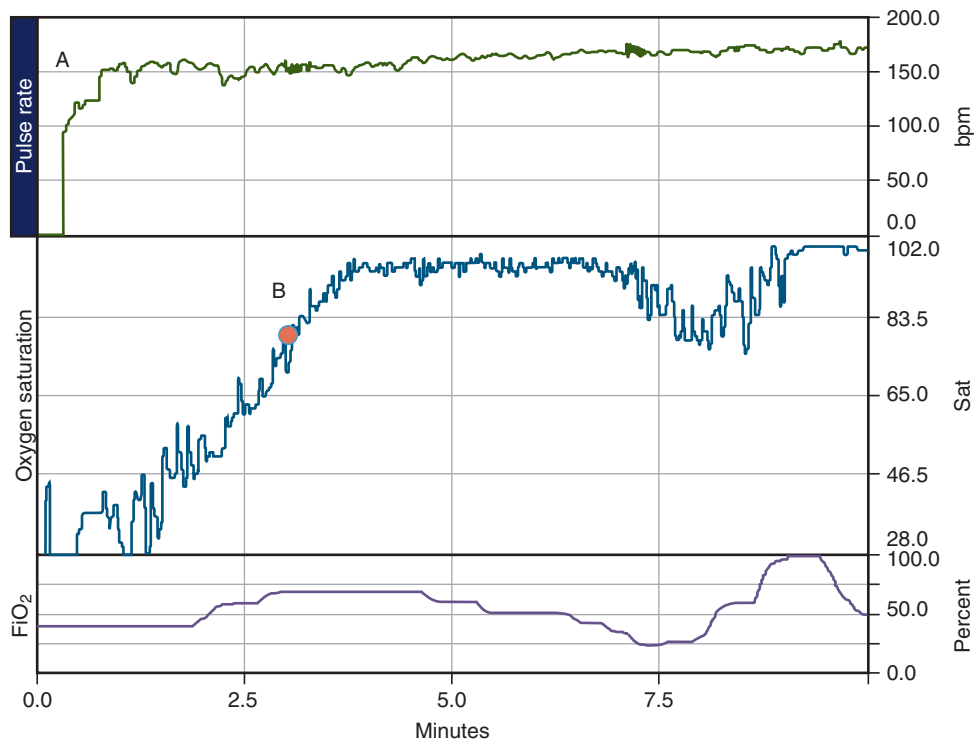
An updated metaanalysis including these trials comparing initiation of resuscitation with high (>65%) or low (21%–30%) oxygen concentration no longer demonstrated any differences in survival (Oei et al., 2017) (Fig. 24.8). Larger trials such as the ongoing Premature Infants Resuscitated with Oxygen or Air (PRESOX) study (NCT01773746) and the planned TO2RPIDO trial involving preterm newborns treated with different oxygen strategies are necessary to determine the long-term effects of resuscitation with different oxygen concentrations. Oxygen use in the first few minutes of life could impact survival and common neonatal morbidities associated with prematurity and free-radical disease such as neurodevelopmental impairment, retinopathy of prematurity, bronchopulmonary dysplasia, and necrotizing enterocolitis.

The use of oxygen concentrations between 21% and 100% requires compressed air and a blender. Different organizations throughout the world have provided differing recommendations on the use of oxygen for newborn resuscitation. The NRP has recommended initiating resuscitation for term newborns with air when ventilation is needed (Perlman et al., 2010). For the preterm neonate born at less than 35 weeks' gestation, ILCOR recommended initiating resuscitation with a low oxygen concentration (21%–30%) and adjustment based on target oxygen saturations (1 minute 60%–65%, 2 minutes 65%–70%, 3 minutes 70%–75%, 4 minutes 75%–80%, 5 minutes 80%–85%, 10 minutes 85%–95%) (Perlman et al., 2015). Our approach has been to begin with 30% oxygen and adjust the concentration slowly, attempting to mimic the gradual transition in these SpO_2 values. Fig. 24.9 demonstrates continuous physiologic data during the first 10 minutes of life for a newborn resuscitated with this targeted oxygen strategy.

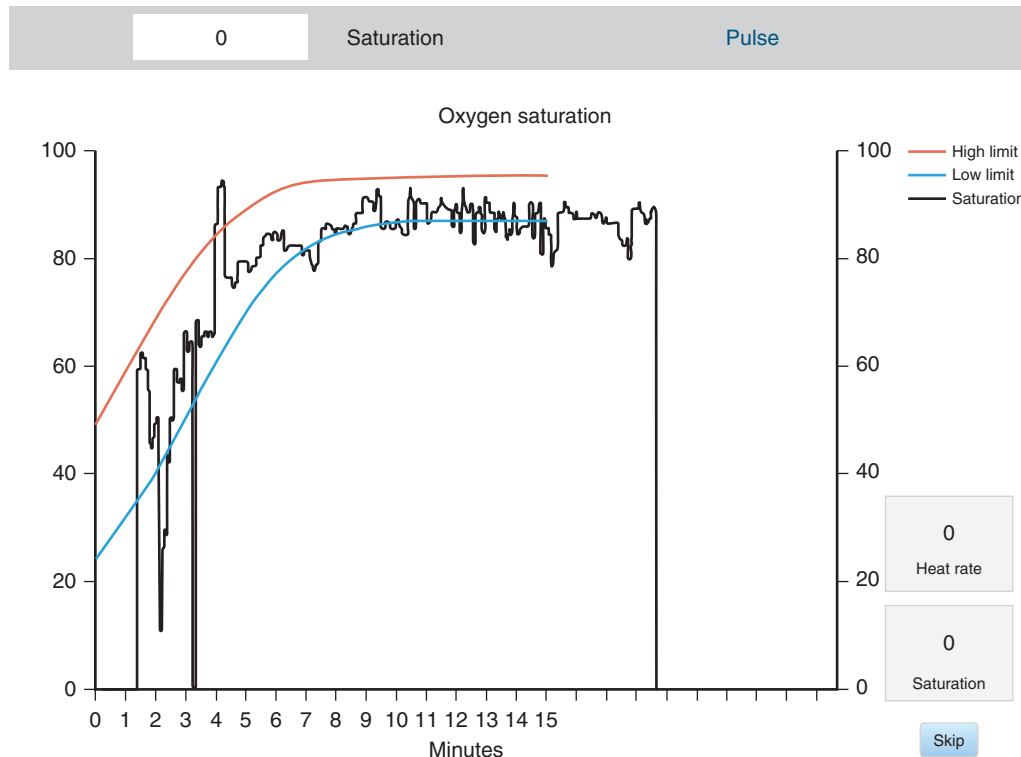
Assisting Circulation

In newborns the need for resuscitative measures beyond assisted ventilation is extremely rare. Additional circulatory assistance can include chest compressions, administration of epinephrine, and volume infusion. In a cohort study of neonates born at less than 32 weeks' gestation in Canadian NICUs, 5.2% of all neonates weighing less than 1000 g received chest compressions and/or epinephrine compared with 3.4% of neonates weighing more than 1000 g. (Soraisham et al., 2014). Ventilation remains the most critical priority in neonatal resuscitation. However, if adequate ventilation is provided for 30 seconds and bradycardia with a heart rate of less than 60 bpm persists, chest compressions are initiated. Further attention to ventilation with the use of increased pressures and/or intubation may be required. Chest compressions are preferably provided with two thumbs on the sternum while both hands encircle the chest (Menegazzi et al., 1993). The chest is then compressed in a 3:1 ratio coordinated with ventilation breaths.

Further circulatory support may be necessary if adequate chest compressions do not result in an increase in heart rate after 30 seconds. Epinephrine is then indicated as a vasoactive substance that increases blood pressure by α -receptor agonism, coronary perfusion pressure, and heart rate by β -receptor agonism. Intravenous (IV) administration of epinephrine is more likely to be effective than endotracheal administration. The IV dose of 0.01–0.03 mg/kg (0.1–0.3 mL/kg of a 1:10,000 solution) is currently recommended. Early placement of an umbilical venous catheter is critical to deliver epinephrine by IV quickly enough to be effective. To place an umbilical catheter as quickly as possible, it is necessary to have the equipment readily available and to begin



• **Fig. 24.8** Tracing of Physiologic Data During Transition. The pulse rate, oxygen saturation, and oxygen concentration measured within 30 seconds of birth are displayed here. The pulse oximeter is placed on the newborn's right hand as soon as possible. Point A shows when the pulse oximeter begins working. Point B shows the oxygen saturation (SpO_2) at 3 minutes (approximately 80%). This newborn was initially treated with 40% oxygen, and the concentration was adjusted to achieve SpO_2 of 85% to 90% by 5 minutes of life. When the oxygen is reduced to 21% at approximately 7.5 minutes of life, the SpO_2 decreases, and the oxygen concentration is increased again. *Bpm*, Beats per minute; *FiO₂*, oxygen concentration.



• **Fig. 24.9** Tracing of a Newborn Resuscitated With a Targeted Oxygen Saturation Strategy.

the procedure as soon as possible. This could be accomplished by the lead resuscitator assigning the task of placing the catheter as soon as chest compressions are initiated. If there is any prenatal indication that substantial resuscitation will be required, the necessary equipment for umbilical venous catheter placement should be prepared before delivery as completely as possible. Epinephrine may be given by an endotracheal tube but the efficacy of this delivery method is not as certain, and therefore an increased dose (0.03–0.1 mg/kg) is currently recommended. Epinephrine doses may be repeated every 3 minutes if heart rate does not increase. Excessive epinephrine may result in hypertension, which in preterm newborns may be a factor in the development of intraventricular hemorrhage. However, the risks are balanced by the benefit of successful resuscitation in a newborn who might not otherwise survive.

If the newborn has not responded to any of the prior measures, a trial of increasing intravascular volume should be considered by the administration of crystalloid or blood. Situations associated with fetal blood loss are also frequently associated with the need for resuscitation. These include placental abruption, cord prolapse, and fetal maternal transfusion. Some of these clinical circumstances will have an obvious history associated with blood loss, whereas others may not be readily evident at the time of birth. Signs of hypovolemia in the newborn are nonspecific but include pallor and weak pulses. Volume replacement requires IV access, for which emergent placement of an umbilical venous catheter is essential. Any newborn who has signs of hypovolemia and has not responded quickly to other resuscitative measures should have an umbilical venous line placed and a volume infusion administered. The most common (and currently recommended) fluid for volume replacement is isotonic saline. A trial volume of 10 mL/kg is given initially and repeated if necessary. If a substantial blood loss has occurred, the newborn may require infusion of red blood cells to provide adequate oxygen-carrying capacity. This can be accomplished emergently with noncrossmatched O-negative blood, with blood collected from the placenta, or with blood drawn from the mother, who will have an antibody profile compatible with her newborn at the time of birth. Because not all blood loss is obvious and resuscitation algorithms usually discuss volume replacement as the last resort of a difficult resuscitation, the clinician needs to keep a high index of suspicion for significant hypovolemia so that action may be taken to correct the problem as promptly as possible. Therefore in situations where the possibility of hypovolemia is known before birth, it would be wise to provide a placental transfusion, prepare an umbilical catheter, prepare an initial syringe of isotonic saline, and discuss with the blood bank and the delivering physicians the possibility that noncrossmatched blood may be required.

Apgar Score

The overall assessment of a newborn was quantified by the anesthesiologist Virginia Apgar in the 1950s with the Apgar score (Apgar, 1953). The score describes the newborn's condition at the time it is assigned and consists of a 10-point scale with a maximum of 2 points assigned for each of the following categories: respirations, heart rate, color, tone, and reflex irritability. Table 24.2 shows the detailed components of the score. While the components of the score include items that are assessed for determination of interventions, the score itself is not used to determine the need for interventions. The score was initially intended to provide a uniform, objective assessment of the newborn's condition and was used as a tool to compare different practices, especially obstetric anesthesia. Despite

TABLE 24.2 The Apgar Score

| Feature Evaluated | 0 Points | 1 Point | 2 Points |
|------------------------|------------|---|---------------------|
| Heart rate (beats/min) | 0 | <100 | >100 |
| Respiratory effort | Apnea | Irregular, shallow, or gasping respirations | Vigorous and crying |
| Color | Pale, blue | Pale or blue extremities | Pink |
| Muscle tone | Absent | Weak, passive tone | Active movement |
| Reflex irritability | Absent | Grimace | Active avoidance |

the intent of objectivity, there is often disagreement in score assignment among various practitioners (O'Donnell et al., 2006). Low scores are associated with increased risk of neonatal death (Casey et al., 2001) but have not been predictive of neurodevelopmental outcome (Nelson and Ellenberg, 1981). Interpreting the score when interventions are being provided may be difficult, and current recommendations suggest that clinicians should document the interventions used at the time the score is assigned (American Academy of Pediatrics, 2006).

Specific Problems Encountered During Resuscitation

Neonatal Response to Maternal Anesthesia/Analgesia

Medications administered to the mother during labor can affect the fetus by transfer across the placenta and direct action on the fetus or by adversely affecting the mother's condition and therefore altering uteroplacental circulation and fetal oxygen delivery. The most commonly discussed complication of intrapartum medication exposure is perinatal respiratory depression after maternal opiate administration. The fetus can develop respiratory depression from the direct effect of the drug. Naloxone has been used during neonatal resuscitation as an opiate receptor antagonist to reverse the effects of fetal opiate exposure. Newborns of mothers who have long-term opiate exposure can potentially have a sudden withdrawal syndrome, including seizures, if they receive a narcotic antagonist. It is also critical that assisted ventilation be provided as long as spontaneous respirations are inadequate. ILCOR does not recommend naloxone as part of the initial resuscitation for newborns with respiratory depression in the delivery room (Perleman et al., 2010). The administration of a narcotic antagonist is never an acutely required intervention during neonatal resuscitation as such newborns can and should be treated with assisted ventilation.

The Use of Early Caffeine

Respiratory distress syndrome can be successfully treated in very preterm newborns with nasal CPAP beginning at birth. However, CPAP treatment will fail in many of these newborns, and they will require endotracheal intubation and surfactant administration

to achieve adequate gas exchange (Morley et al., 2008; Finer et al., 2010). Caffeine therapy given during the first 10 days after birth reduced the need for positive airway pressure by 1 week compared with placebo (Schmidt et al., 2006). Very early use of caffeine (immediately after birth) as a respiratory stimulant represents a potential adjunctive therapy with CPAP to prevent intubation. Recently, some European centers have adopted a technique to administer surfactant via a thin flexible catheter during spontaneous breathing while nasal CPAP is being received (minimally invasive surfactant therapy) (Kribs et al., 2008). As part of this protocol, newborns are given IV caffeine in the first hour after delivery. Caffeine's favorable efficacy and safety record in the NICU for the treatment of apnea of prematurity, and for preventing failed extubation, is well known (Erenberg et al., 2000; Schmidt et al., 2006; Schmidt et al., 2007; Henderson-Smart and Davis, 2010). A potential new direction for caffeine therapy targets early administration for preterm neonates at risk of respiratory failure and cardiovascular insufficiency (Lodha et al., 2015). Such an intervention should be feasible in most settings as IV access is routinely achieved in these patients for provision of IV fluids and antibiotics. Newborns treated initially with CPAP alone may take time to develop respiratory insufficiency and apnea. Waiting to give caffeine when these symptoms develop may not prevent the need for intubation. In a feasibility study of very early caffeine use, we found that very preterm newborns receiving caffeine in the first 2 hours of life had increased systemic blood flow and blood pressure compared with newborns who received caffeine at 12 hours after delivery (Katheria et al., 2015). Larger prospective studies are needed to determine the effects of early caffeine use on the need for intubation, intraventricular hemorrhage, and related long-term outcomes such as chronic lung disease and neurodevelopmental impairment.

Conditions Complicating Resuscitation

When resuscitation has proceeded through the steps described without improvement in the newborn's clinical condition, other problems should be considered. Some of these problems may be modifiable with interventions that could improve the course of the resuscitation. For example, an unrecognized pneumothorax could prevent adequate pulmonary inflation and if under tension could impair cardiac function. If the pneumothorax is recognized and drained, both gas exchange and circulation can be improved. Some congenital anomalies that were not diagnosed antenatally make resuscitation more difficult. Congenital diaphragmatic hernia is one such anomaly that is difficult to recognize on initial inspection of the newborn but can cause significant problems with resuscitation. The abdominal organs are displaced into one hemithorax, and the lungs are unable to develop normally. This may cause ventilation to be quite difficult. If the intestines are displaced into the thorax and mask ventilation is provided, the intestines will become inflated, making ventilation even more difficult. If the congenital diaphragmatic hernia is known before delivery or a presumptive diagnosis is made in the delivery room, the baby should be intubated early to prevent intestinal inflation. An orogastric suction tube should also be placed to decompress the inflated intestines.

Many other congenital anomalies that can lead to a difficult resuscitation will be more visibly obvious when the baby is born. For example, hydrops fetalis occurring for any reason can be associated with very difficult resuscitation. Although most cases are diagnosed on fetal ultrasonography before delivery, severe hydrops would be clearly visible on examination because of

significant skin edema and abdominal distention. Frequently peritoneal and/or pleural fluid will need to be drained to achieve adequate ventilation. Abdominal wall defects such as gastroschisis require special attention in the delivery room to ensure that the exposed bowel is covered in plastic to prevent excessive fluid loss and is protected from twisting or trauma. An orogastric suction tube is also important to decompress the stomach and limit chances of further vomiting. If assisted ventilation is necessary, early intubation as opposed to mask ventilation should be considered to prevent gastric distention. Newborns with neural tube defects should also have any exposed tissues protected with a sterile covering. Additionally, all efforts should be made to avoid pressure on the defect.

A situation that may create a particularly difficult resuscitation is a fetal congenital high airway obstruction. If a significant airway obstruction is diagnosed antenatally, an ex utero intrapartum treatment or EXIT procedure can be planned. This allows the establishment of a stable airway before the clamping of the umbilical cord that maintains placental function until the airway is secure. An airway obstruction may not necessarily be diagnosed before delivery and may create a very difficult resuscitation. The therapy will differ depending on the cause of the obstruction. An alternative airway (oral or nasopharyngeal) can be helpful if endotracheal intubation is not possible as can occur with severe micrognathia. Tracheal suctioning can be attempted if a tracheal plug is suspected. In extreme situations of airway obstruction, an emergency cricothyroidotomy may be attempted.

Birth trauma of any kind has been documented to occur in approximate 2.6% of all deliveries in the United States (Moczygemba et al., 2010). The most serious injuries are the variety of head injuries that can occur, including subgaleal hemorrhage and intracranial hemorrhage. Subgaleal hemorrhage is more often associated with vacuum-assisted delivery and is important to recognize because of the rapid blood loss that can occur into this soft tissue space. Intracranial hemorrhages such as subdural hematomas can occur, though many such injuries are mild and can be found incidentally after uncomplicated vaginal delivery. Spinal cord injuries after birth are extremely rare but can be quite severe with long-lasting functional limitations. Brachial plexus and other peripheral nerve injuries may be noticed in the delivery room and evaluated shortly after birth but should not interfere with the resuscitation efforts. Pressure injuries from forceps-assisted deliveries can be noted on examination and evaluated as necessary in accordance with the location of the injury. Fractures and lacerations may occur and need to be evaluated after the newborn has adequately transitioned.

Limits of Viability

Neonatal intensive care has increased survival at lower GAs, resulting in changes in the definition of viability over time. A variety of opinions regarding the lower limit of GA when intensive care should be offered exist among practitioners (Backes et al., 2015; Janvier and Lantos, 2016). The most recent edition of the NRP textbook states that if there is no chance for survival, such as birth at a confirmed GA of less than 22 weeks or severe congenital anomalies, resuscitation should not be offered (NRP, 2016). When the outcome is most uncertain, parents of the newborn are frequently included in the decision-making process. Making an informed decision about the provision of intensive care should be done with the best available information. One of the resources that is used to determine the likelihood of survival without severe disabilities has been provided by Tyson et al. (2008) using the Eunice Kennedy

Shriver National Institute of Child Health and Human Development Neonatal Research Network data. Using information regarding the GA, birth weight, sex, number of fetuses, and antenatal steroid exposure, one can calculate the risk of death and neurodevelopmental impairment. These predictive data, which were compiled from the participating centers of the Neonatal Research Network, a group of mostly academic centers, may currently be the best available national data for predicting outcome but do not necessarily represent the outcome at any single unit or of any individual baby. For mothers who present to a small inexperienced center with the possibility of delivery at a very preterm GA, every effort should be made to transport the mother to an experienced tertiary inborn center when possible.

Care After Resuscitation

Newborns who survive a significant resuscitation require special attention in the hours and days that follow. Frequent complications immediately following resuscitation include hypoglycemia, hypotension, and persistent metabolic acidosis. In addition term and near-term newborns with evidence of hypoxic–ischemic encephalopathy benefit from mild therapeutic hypothermia (Shankaran et al., 2005; Jacobs et al., 2007; Azzopardi et al., 2009). Mild therapeutic hypothermia in which the core body temperature is kept at 33.5°C has now been extensively evaluated and has been effective at reducing death or impairment in newborns with moderate to severe hypoxic–ischemic encephalopathy. The decreased temperature is thought to decrease the secondary injury that occurs after an hypoxic–ischemic insult. Hypothermia can be accomplished with both whole-body and head cooling, though clinical trials of whole-body cooling more effectively achieved a reduction in adverse outcome. The therapy is most beneficial when initiated as quickly as possible after an insult, with beneficial effects noted when treatment was initiated within 6 hours of birth. The timing of the insult in relation to the time of birth is not always obvious, making it difficult to know the actual timing of initiation of therapy after the insult. Mild hypothermia therapy should be considered when a newborn has required a significant resuscitation after birth, Apgar scores are low (especially 5-minute score <5), fetal or neonatal acidosis is documented on cord or newborn blood gases, and signs of encephalopathy are apparent. In addition, a history of a significant event likely to cause a hypoxic–ischemic insult should trigger a thorough evaluation of the newborn to determine whether hypothermia therapy is indicated. Hypothermia therapy is not currently available at all institutions. The seventh edition of the NRP textbook recommends therapeutic hypothermia should be considered for neonates born at term or near term with evolving moderate to severe hypoxic–ischemic encephalopathy, with protocol and follow-up coordinated through a regional perinatal system (NRP, 2016). However, all delivery services must be able to recognize the indications for therapy so that, if necessary, a transfer can be initiated as quickly as possible.

In neonates born without a heart rate or any respiratory effort, if resuscitation is performed to the full extent without any response, discontinuation is recommended after 10 minutes. From a review of 13 years of a data including 81,603 deliveries, Haddad et al. (2000) found that survival with an Apgar score of 0 at 1 minute occurred in 1.26 in 1000 delivered newborns without major malformations. Of 33 newborns with an Apgar score of 0 at both 1 and 5 minutes, 67% died before hospital discharge. A review of the available literature for newborns with an Apgar score of 0 at 10 minutes found that 94% of them either died or were severely

handicapped, while 3% were mild or moderately handicapped (Harrington et al., 2007).

The transition from fetal to neonatal life is a critical time in an individual's life and is an opportunity for care providers to have significant impact on the outcome of those newborns who need assistance. The need for newborn resuscitation, even when no signs of encephalopathy are recognized, increases the risk that children will have lower scores on IQ tests at school age (Odd et al., 2009). This is most likely because resuscitation is a marker for a prior insult. However, a well-performed resuscitation could be critical for a successful recovery. Neonatal care providers have an obligation to ensure that this process is performed as well as possible and that the resuscitation techniques are evaluated in an objective manner to promote continued improvement.

Suggested Readings

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25

Newborn Evaluation

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KEY POINTS

- Detection of significant newborn problems in a timely fashion helps to prevent serious morbidities.
- A systematic approach to obtaining the prenatal and newborn history is essential.
- The initial newborn evaluation greatly informs the predischARGE evaluation and management plans.
- An appropriate level of vigilance is needed to detect the abnormal newborn “hiding” amid the healthy ones.
- An ongoing challenge of the newborn examination is the need to be efficient, thorough, and highly vigilant while simultaneously projecting an attitude of calming reassurance.
- The repetition of evaluating normal newborns and practicing calming reassurance should never promote complacency.

This chapter focuses on the medical evaluation of the apparently well newborn in the first few days after birth. As such, the emphasis will be on common problems, variations of normal and subtle abnormalities. Some clinical findings that suggest serious illness are also described but their inclusion is not intended to provide a complete differential diagnosis or guide to management. Physical findings pertinent to specific diseases and organ systems, evaluation of the dysmorphic newborn, and laboratory studies helpful in routine newborn care are described in greater detail in other chapters in this textbook.

Initial Newborn Evaluation: Key Elements

1. Obtain the history (prenatal, birth, family and social).
2. Perform a physical examination.
3. Evaluate and consider the impact of significant risk factors, history, and physical examination findings.
4. Determine the need for additional work-up and/or follow-up as indicated.
5. Provide education and counseling to parents/legal guardians. To complete these tasks requires skill and experience. For brevity, we refer to the person evaluating the newborn as a *pediatrician*. In some institutions the routine evaluation is performed by a general pediatrician, family practitioner, or pediatric nurse practitioner, and the neonatologist enters the well-baby nursery only as a consultant. In other institutions the neonatologist has direct responsibility for patients in the newborn nursery as well as in the neonatal intensive care unit (NICU). Regardless of which healthcare

professional is primarily responsible for the evaluation of newborns, this person should develop and maintain proficiency in the evaluation of the normal newborn, a task that requires its own particular set of skills.

In the normal nursery, the primary goal is to identify the small minority of babies with significant problems that may cause serious morbidity if not detected in a timely fashion. This includes the recognition of and consideration of the presence of psychosocial problems (e.g., maternal, family, and/or community) that may adversely impact both “normal” and “at risk” newborns. This goal must be accomplished with the understanding that the vast majority of babies encountered in the newborn nursery are completely healthy, despite wide variations in presentation and findings.

History

When obtaining the newborn’s history, the pediatrician may easily view himself/herself as an investigative reporter. Multiple sources of information are needed to complete the initial “fact gathering,” including:

1. The mother’s medical and obstetric history
2. The current pregnancy course
3. The social and family history (maternal and paternal)
4. Additional information sources:
 - a. Records of prenatal outpatient visits and laboratory studies
 - b. Records of the mother’s current and prior hospitalizations
 - c. Labor and delivery record
 - d. Newborn records created by nurses and other personnel
 - e. Direct communications from the obstetrician, midwife, and nurses
 - f. Interviews with the mother and other family members

In the era of transition from written to electronic health records, one must be cautious about “lost information” or misinformation inadvertently “carried over” between documents. Ideally, systems should be in place to ensure that the pediatrician responsible for the newborn is directly informed about high-risk conditions in a timely manner. However, the pediatrician has an independent responsibility to utilize information available in the maternal record to assess for potential high-risk issues. An outline of expected relevant prenatal and newborn history is presented in [Table 25.1](#).

It is essential to have a systematic approach to the collection and recording of the history so that important information is never presumed known when it is actually missing and/or when information becomes overlooked and thus neglected. This is particularly important when information gathering is interrupted and/or

TABLE 25.1 Components of an Emergency Prenatal and Newborn History

| Category | Components |
|--------------------------|--|
| Maternal identification | Age, gravida, parity, weeks in gestation |
| Maternal medical history | Significant illness, medications |
| Current pregnancy | Singleton or multiple fetuses |
| | Pertinent results of laboratory tests and studies |
| | Fetal growth (IUGR, LGA; hydrops) |
| Labor | Rupture of membrane (duration) |
| | Amniotic fluid (oligohydramnios/polyhydramnios; bloody; purulent; meconium; foul smelling) |
| | Signs of infection (maternal fever, elevated WBC count, tachycardia, uterine tenderness) |
| | Fetal tracing (tachycardia, decelerations, etc.) |
| Delivery | Indication for emergency (abruption; preeclampsia, fetal distress/intolerance to labor) |
| | Route of delivery |
| | Method of anesthesia (general vs. local) |
| | Medications administered |

IUGR, Intrauterine growth restriction; LGA, large for gestational age; WBC, white blood cell.

disjointed because of the need for immediate intervention to stabilize a newborn. For example, the pediatrician paged to attend the emergency delivery of a fetus in distress must focus information gathering, in just those few available minutes, on what is directly relevant to prepare for resuscitation.

For both the stabilized newborn sent to room with the mother and the critically ill newborn admitted to the NICU, one must quickly return to the systematic approach for collection and recording of the history. For example, if the mother does not have a documented negative test result for hepatitis B infection, the pediatrician may need to ascertain the hepatitis B status of the mother so that the hepatitis B vaccine can be administered within the recommended 12 hours after birth. Otherwise, for the healthy newborn, history gathering at the time of the initial encounter after birth will emphasize the prenatal history (including maternal and family history), the delivery and neonatal transition, the initiation of feeding, and any symptoms or parental concerns that have manifested themselves since birth.

During the initial evaluation, the pediatrician is expected to identify the following:

1. Risk factors for medical problems that may develop in the first few days of life (e.g., early-onset neonatal sepsis, severe hyperbilirubinemia)
2. Risk factors for psychosocial problems that may preclude safe discharge home

Although gathering the history and performing the physical examination are distinct activities and described in separate sections

of a pediatrician's documentation, they are not performed in isolation. Knowledge of specific concerns, events, and risk factors in the history should prompt a more focused or detailed examination of the relevant body region or organ system.

Clinical example: A newborn of a diabetic mother delivered with use of forceps and noted to have shoulder dystocia.

Pediatrician action: More detailed head, clavicular, and neurologic examinations.

Conversely, specific questioning prompted by physical examination findings will often elicit information that was not volunteered in the earlier routine history gathering, such as a family history of a specific anomaly.

Clinical example: An abnormal rhythm auscultated on examination with follow-up ECG findings suspicious of a prolonged corrected QT interval.

Pediatrician action: Query the parents regarding family history of sudden death, syncope, and/or early neonatal/infant death presumed to be due to sudden infant death syndrome.

Because of the potential of early-onset neonatal sepsis for morbidity and death, the presence or absence of risk factors for it should be assessed as part of the initial evaluation for every newborn.

The risk factors for early-onset sepsis include:

- Prematurity (<37 weeks' gestation) or low birthweight (<2500 g)
- Prolonged rupture of membranes (≥18 hours)
- Maternal body temperature of 38°C or higher, uterine tenderness, foul-smelling or purulent amniotic fluid, an elevated maternal white blood cell count or left shift, and fetal or maternal tachycardia
- Maternal vaginal colonization with group B streptococci unless adequate intrapartum prophylaxis was administered or the fetal membranes were intact until birth by cesarean delivery (CDC, 2010)
- Maternal urinary tract infection due to group B streptococci (*Escherichia coli* urinary tract infection is also a potential risk factor.)
- Poor fetal tolerance of labor
- Unexplained need for resuscitation at birth
- Delayed/"slow" extrauterine transition (noted by persistence of abnormal vital signs after birth)

After completion of the initial evaluation, it is important to document the history, physical examination findings, and laboratory study findings obtained. This information is the data used for crafting the newborn's assessment and plan of care. Once documented, the assessment and plan also become part of the ongoing newborn history. It is also important to know that the initial evaluation greatly informs the predischARGE evaluation, particularly if it is performed by two pediatricians. For the predischARGE evaluation, the prenatal and perinatal history will have already been documented and reviewed, so the pediatrician can direct his/her attention to the interval history (mostly collected from the nursing records and the parents). Knowledge of this information is critical in determining if the newborn can be discharged home safely.

Physical Examination Considerations

No matter how experienced the pediatrician is, every examination of a newborn adds to or refines one's appreciation of the wide ranges of variation in common and benign conditions observed. It also enhances the comfort level of the pediatrician for maintaining an appropriate level of vigilance to detect the abnormal newborn "hiding" amid the healthy ones. Repeated physical examinations

continually refine and merge the pediatrician's efficiency and thoroughness.

The examination of the healthy newborn entails a complete physical examination in the sense that all parts of the body are examined. However, it is impractical to exhaustively explore and explicitly document all possible findings. Fortunately, there are multiple opportunities to perform an examination and note pertinent findings, including:

1. Birth
2. Admission to the newborn nursery
3. Targeted/problem-directed evaluation
4. Daily follow-up encounter
5. Discharge from hospital or on follow-up home or office visits

Examination at Birth and Delivery Room Disposition

At the time of birth, attention focuses on the initiation of appropriate respiratory and cardiovascular stability. Ascertaining successful adaptation versus the need for ongoing resuscitation is described in more detail in Chapter 24. The newborn whose condition remains unstable or who has major anomalies that are apparent on initial inspection will usually be transferred to a NICU for further evaluation and management. However, it is reasonable for the pediatrician in attendance at the delivery to attempt the following brief assessment as feasible:

1. Presence of gross dysmorphic features
2. Auscultation for a potentially pathologic heart murmur
3. Patency of the anus

The newborn whose condition stabilizes after delivery, with or without intervention, is immediately evaluated to determine whether he/she can remain with the mother. This examination centers on determining adequacy of the cardiorespiratory transition and inspecting the newborn for congenital anomalies that may indicate a need for admission to a NICU or observation/stepdown unit. These latter inspections include the following:

1. Passage of a thin catheter through both nostrils to evaluate the newborn for potential choanal atresia
2. Passage of a thin catheter into the stomach to evaluate the newborn for potential esophageal atresia (with or without tracheoesophageal fistula)
3. Evaluation of the newborn for imperforate anus/anal patency

For routine deliveries of healthy newborns, this examination can also be performed by the obstetrician or nurse-midwife. If the pediatrician is present, an expanded examination can serve as the nursery admission examination. However, there is typically consideration for practices that encourage immediate bonding of the newborn and mother during the "golden hour" of transition after birth as well as initial breastfeeding attempts.

Nursery Admission Examination

For the healthy newborn this examination is done after the newborn completes transition and usually by 24 hours after birth. A complete physical examination at this time is designed to efficiently detect problems that were initially unapparent or are likely to soon develop. The pediatrician is again reminded that identification of historical risk factors or the detection of symptoms or abnormalities requires appropriate expansion of the basic examination (where indicated) to focus additional attention on the areas relevant to the developing differential diagnosis.

Targeted or Problem-Directed Evaluation

When the pediatrician is called to evaluate a newborn because of specific symptoms or concerns, the examination will naturally

focus on aspects relevant to those issues. Because of the notoriously nonspecific nature of many newborn symptoms, even the targeted examination will often need a wide focus to adequately inform the potentially wide differential diagnosis.

Daily Follow-Up Encounter

The daily follow-up examination for the newborn can be abbreviated but must always be guided by the newborn's overall condition. Areas of active attention should include the newborn's neurologic and cardiorespiratory status, hydration state, feeding and elimination patterns, and evaluation for jaundice.

Nursery Discharge Examination

Although similar in scope to the initial evaluations, the discharge examination has a slightly altered emphasis, based on the following:

1. Additional information provided during observation in the hospital
2. Determining whether the newborn is ready for routine care at home

The discharge examination is a complete physical examination. However, it is typically unnecessary to repeat a search for physical anomalies that do not change with time (e.g., examination of the oral cavity for cleft palate or the eyes for the red retinal reflexes) if they were not previously identified. If a different pediatrician performs the discharge examination, it is often more efficient to repeat the entire examination than to verify the completeness of previous examinations performed by different pediatricians. For hospital admissions of 24 hours or less, a combined admission–discharge examination is appropriate.

Performance of Examination

Approach to the Newborn Examination

An ongoing challenge of the newborn examination is the need to maintain efficiency, thoroughness, and a high level of vigilance while simultaneously projecting an attitude of calming reassurance. The air of appropriate reassurance reflects the reality that the overwhelming majority of newborns in the nursery are healthy. However, the repetition of evaluating normal newborns and practicing calming reassurance should never promote complacency.

Although an experienced examiner may detect many abnormalities by pattern recognition alone, even an expert can miss important findings by foregoing the disciplined and systematic approach advocated here. Still, the desire to ensure that no essential aspect of the examination is missed or slighted needs to be balanced with the newborn's tolerance for handling. It may seem easier to avoid omissions if the pediatrician always performs the examination in the same sequence. Unfortunately, a rigid approach that fails to adjust to the state and activity of the individual newborn may yield a suboptimal examination that is inefficient, inaccurate, and unnecessarily stressful for the newborn, parent, and examiner.

Environment

The environment in which the examination is performed can significantly affect the reliability of the results/findings via effects on the examiner and/or the baby. An optimal examination environment should be prioritized and maintained as much as possible. When this is not reasonably feasible, the examiner must be aware of the limitations produced by a suboptimal environment and adjust the approach to the examination to compensate, arrange

for the newborn to be moved, or defer selected parts of the examination when appropriate.

Important environmental considerations include:

1. Lighting
2. Temperature
3. Background noise
4. Other distractions (improper bed height/position, lack of appropriate equipment, parental and/or nursing queries, etc.)
5. Newborn's physiologic state (hungry, crying, etc.)

The healthy newborn is typically examined in a warmer bed in either the delivery room or the newborn nursery or in a bassinet in the mother's room. An open warmer bed provides the best access, allowing the newborn to be kept warm while completely undressed during the examination. However, the examination is often done with the newborn in a bassinet, starting with the newborn dressed in at least a shirt and hat, and wrapped in blankets.

An adequate well-baby examination can be performed under those basinet conditions, but the sequence of the examination should be modified to minimize the time the newborn is fully undressed. The examiner must take extra care to ensure that the examination is complete and the entire skin surface is visualized at some point during the examination.

Performing the examination in the presence of the parents allows the examiner to show how the newborn responds to handling and to demonstrate immediately any findings that require explanation or reassurance. Watching the examination may stimulate the parents to ask questions that might otherwise not occur to them until later, and it provides the physician with an immediate opportunity for further education. On the other hand, interruptions from parents and other family members can interfere with the examiner's train of thought, risking inadvertent distraction and omissions.

In large, busy nurseries, it may be more practical for the pediatrician to examine a series of newborns in the nursery and then report the results of the examination to each set of parents afterward. Specific findings are ideally demonstrated to the parents in real-time, if appropriate. Regardless of whether the parents are present for the examination, the results of the examination should be communicated promptly. Parents may be anxious about findings that the pediatrician believes are of little consequence and vice versa. Prompt and sensitive communication of the examination findings help to build parents' trust, whereas delayed or poor communication can undermine the parents' relationship with the physician and the hospital.

Evaluation of Gestational Age

If the obstetric estimate of the gestational age (GA) is uncertain or appears unreliable, the GA of the newborn can be estimated on the basis of physical examination criteria. This is important for determining highly relevant and pertinent information that will affect management and care. This includes an assessment of whether the patient is small for his/her GA or large for his/her GA. No individual feature is a reliable guide to the GA, but scoring systems that use multiple features of physical and neuromuscular maturity have been evaluated extensively (Amiel-Tison, 1968; Dubowitz et al., 1970). The Ballard score is probably the one most widely used in contemporary practice (Ballard et al., 1991). Detailed descriptions and a video demonstration of this examination are available at <http://www.ballardscore.com>. Pediatricians can also choose to use <http://www.medcalc.com/ballard.html> to assist them in calculating a Ballard score.

Determination of Key Metrics

The physical examination of the newborn includes measurements of the weight, length, and head circumference, which must be compared with standardized growth data (Fig. 25.1) to determine whether the newborn is small (<10th percentile), appropriately sized, or large (>90th percentile) for his/her GA (Battaglia and Lubchenco, 1967). For newborns born at less than 37 weeks' gestation, one should consider referencing the Fenton growth charts (Fig. 25.2).

The vital signs provided by nursing (body temperature, heart rate, and respiratory rate) may be reviewed, but the pediatrician should also note the heart rate and respiratory rate at the time of examination. The blood pressure is not routinely measured in healthy newborns, but the blood pressure should be checked in all four extremities if the history or examination suggests a circulation problem.

Of the standard physical newborn examination techniques, observation is often underappreciated and overlooked. Palpation and auscultation are also important, although percussion is of relatively limited use. The routine newborn examination does include specific physical maneuvers for examining the hips and for eliciting a variety of reflex responses.

A stethoscope, an ophthalmoscope, and a tape measure are the only pieces of equipment generally needed. A source of light for transillumination is helpful for specific purposes. Pulse oximetry can enhance early detection of critical congenital heart disease. In September 2011, US Secretary of Health and Human Services Kathleen Sebelius suggested that pulse oximetry screening for critical congenital heart defects be added to the Recommended Uniform Screening Panel for newborns before they are released from a hospital or birthing facility. (Martin et al., 2013). Several states have already passed laws to make this practice the standard of care in the initial newborn period. Screening is typically performed by appropriately trained staff 24–48 hours before discharge.

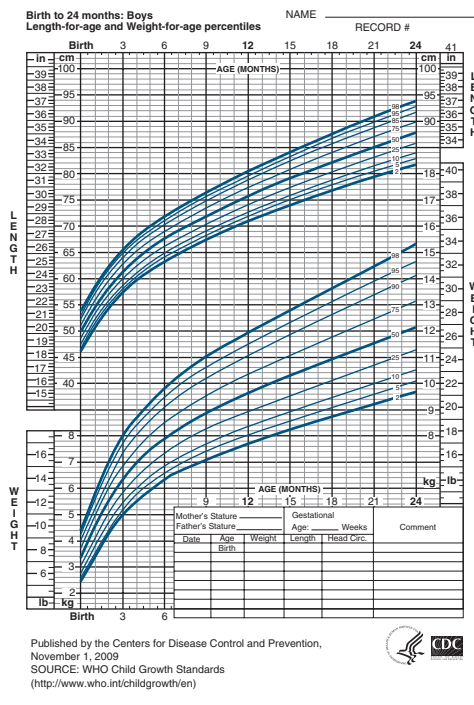
Initial Observation

Before approaching or touching the newborn, the pediatrician should stop and observe the newborn in an undisturbed state, making note of the following:

1. State of alertness or sleep
2. Color of visible portions of the skin and mucous membranes
3. Respiratory rate and any audible sounds or signs of increased work of breathing
4. Tone and posture
5. Spontaneous activity
6. Quality of cry and symmetry of facies when crying (if applicable)
7. General impression of whether the newborn is sick or healthy

These observations provide valuable information and should be repeated intermittently throughout the examination to compare and contrast them with the pediatrician's initial impressions. Attuning to the patient's state and responses can greatly facilitate the physical examination and make it less stressful for the newborn and less time-consuming for the examiner. The newborn generally responds more slowly to a stimulus than an older child or adult, and the response tends not to remain localized to the area of the stimulus.

Clinical example: Gentle touch of the face or extremity of a sleeping newborn, or merely loosening a blanket, typically results in some limited initial movement of the area touched,



Fenton preterm growth chart - girls

Girls

Centimeters

Weight (kilograms)

Gestational age (weeks)

Fenton preterm growth chart - boys

Boys

Centimeters

Weight (kilograms)

Gestational age (weeks)

Curves equal the WHO growth standard at 50 weeks.

Sources: Intrauterine section - Germany (Voight 2010), United States (Olsen 2010), Australia (Roberts 1999), Canada (Kramer 2001), Scotland (Bennell 2008), and Italy (Berlino 2010). Post term section - the World Health Organization Growth Standard, 2006.

www.ucalgary.ca/fenton

waking the newborn. With the newborn sleeping or in a quiet alert state, the examiner will usually begin by gently uncovering the chest. Depending on the type of clothing the newborn is wearing, it may be best at first to lift the shirt or gown just enough to slide the stethoscope underneath. Heart sounds and soft murmurs, if present, can easily be obscured by crying or the movement of fabric underneath the stethoscope. This may also be exacerbated by the relatively rapid respiratory rate for newborns as compared with adults and older children. Typically, the precordial area is auscultated first. If the newborn remains quiet, other locations on the chest can be auscultated for breath and heart sounds. The abdomen can also be assessed for the presence of bowel sounds. If the newborn is already crying or awakens and starts crying vigorously, auscultation can be deferred in favor of other parts of the examination. Once those have been completed, the newborn can be soothed and comforted back into a quiet resting state to complete auscultation.

In examining patients, one must remember that newborns are temperature sensitive. Ideally, the entire newborn should be exposed during an examination to inspect the newborn for skin abnormalities and dysmorphic features. However, there is a benefit from partially disrobing the newborn in sections as the examination progresses to facilitate maintenance of temperature and otherwise accomplish this goal.

Example Examination Sequence

1. Observation (is the baby sick or well?)
2. Auscultation of the anterior chest and abdomen (if the newborn is quiet and cooperative)
3. Inspect and palpate the head (the back of the head and neck will be inspected later).
4. Gently turn the head to each side (noting any restricted range of motion).
5. Inspect each ear when the head is turned.
6. Palpate the neck and clavicular areas (for masses and/or crepitus).
7. Determine overall features of facial shape and symmetry.
8. Confirm the presence or absence of abnormal findings involving skin, eyes, nose, mouth, and oral cavity.
9. Assess respiratory pattern.
10. Inspect and palpate the anterior chest and abdomen (including the umbilicus).
11. Open the diaper and palpate the femoral pulses.
12. Examine the genitalia and perineum.
13. Inspect the lower extremities for abnormal positioning to check alignment, plantar grasp, and the Babinski reflex (start by placing the thumbs on the soles of the feet with the fingers around the back of the ankles).
14. Perform the Barlow and Ortolani maneuvers.
15. Lift and abduct the legs into a frog-leg position to provide a full view of the perineum and anus. Evaluate the newborn for appropriate positioning of the anus and its patency.
16. Inspect the genitalia by gently retracting the labia majora in females or depressing the skin at the base of the penis in males. Inspect and palpate the scrotum and testes. Refasten the diaper.
17. Take an unobstructed observation of the overall shape, symmetry, and movements of the arms and hands (shirt/clothing removed).
18. Palpate each whole arm gently, starting with one of the examiner's hands on each of the baby's shoulders, and then slide down to the baby's hands, noting any swelling or discontinuities.

19. With the newborn supine, turn the head to elicit the asymmetric tonic neck reflex on each side.
20. Inspect the hands, fingers, nails, and palms. If the newborn's hand is tightly fistled, do not attempt to pry the fingers open. Instead, gently flex the wrist to 90 degrees, which will cause the fingers to relax naturally. Inspect the palms, and then elicit the palmar grasp reflex.
21. Without releasing the baby's hands, one can then perform the pull-to-sit maneuver. The pediatrician places his/her hand behind the newborn's head and neck to provide support as the newborn is gently lowered back toward the bed. When the newborn's head and shoulders are a few inches from the bed, the examiner drops his/her hand rapidly to elicit the Moro reflex. (Parents may be alerted to this portion of the examination to avoid unnecessary distress/anxiety.)
22. Next, place the hands on either side of the chest, under the arms at the shoulders, and raise the baby to an upright position. Note the strength and tone of the shoulder muscles.
23. Lower the newborn, still in an upright position, and try to elicit the supporting and stepping reflexes.
24. Turn the newborn to a prone position, suspended on the examiner's hand. Observe the newborn's posture and tone, and elicit the incurvation response. (Again, explanation in advance of these actions to parents who may be observing is helpful.)
25. Inspect the newborn's back, from the vertex of the head down to the sacrum (pulling the diaper down, if needed).
26. Gently place the newborn back in the crib, and fully redress the newborn. Attempt to successfully soothe newborn by swaddling if the newborn has cried during the more active portions of the examination.
27. Red reflex examination can be performed at this time or at any suitable time when the eyes are open spontaneously.

The previous sequence presumes a healthy newborn with findings within the range of normally accepted limits. With any abnormal and/or questionable findings, additional investigations are warranted, and the results should be documented. Occasionally, it may be preferable to defer portions of the examination. Any such decisions and/or limitations of the examination should be clearly documented, with the need for reexamination listed explicitly in the plan of care.

Detailed Physical Examination

Skin

To examine the skin of a newborn, the lighting must be adequate and consistent. Ambient natural light is best. Phototherapy lights must be turned off during the examination. Even if the skin examination is performed simultaneously with regional evaluations of the body, it is important to make a specific note of the skin findings as a separate documentation, particularly if abnormalities are seen. The size, shape, location, distribution, and time course of lesions are all important for differential diagnosis. Documentation should clearly state how measurements of skin findings/lesions were obtained to facilitate reproducibility by other examiners.

The entire skin surface should be inspected during the course of the examination, with attention to the following:

1. Color
2. Moisture
3. Temperature
4. Texture
5. Elasticity properties
6. Pattern and depth of skin creases
7. Presence and character of any lesion

Nails and hair (considered a skin appendage) are inspected along with the skin, with attention to similar findings:

For nails:

1. Color
2. Size
3. Shape
4. Presence of lesions

For hair:

1. Growth pattern
2. Color/discoloration
3. Texture/changes in texture
4. Distribution of scalp and body hair

What affects the color of newborn skin?

1. Newborn's basic skin pigmentation
2. Adequacy of tissue perfusion
3. Amounts of oxygenated and deoxygenated hemoglobin in the local circulation
4. (Optional): internal staining of skin by extravasated blood or pigmented molecules such as bilirubin; external staining by in utero exposure to meconium or postnatally by foreign substances applied to the skin

With polycythemia (excessive hemoglobin levels) a deep red or purple-red color (plethora) of the skin is seen. Paleness/pallor may be caused by anemia or poor perfusion. Central oxygenation is usually best evaluated by observing the color of the tongue and oral mucous membranes because lip color can be misleading. Visual detection of central cyanosis requires approximately 5 g of desaturated hemoglobin per 100 mL of blood and may not be apparent in the presence of significant anemia.

Acrocyanosis (cyanosis of the perioral area, hands, and feet) is common in the first 24 hours after birth or if the newborn is cold. It is due to relatively poor perfusion of those areas, resulting in increased O₂ extraction by the tissues and an increase in the concentration of deoxyhemoglobin. Because skin color is dependent on perfusion, it can be a sensitive indicator of systemic perfusion. However, the newborn's peripheral color can vary markedly and rapidly with activity and the local environmental temperature.

Cutis marmorata (transient mottling of skin of the extremities and trunk) is also a common skin finding in newborns in response to cold. However, if mottling does not resolve with warming, other conditions should be considered, such as hypothyroidism.

Harlequin color change is a striking but infrequently observed transient asymmetry of color and perfusion, in which one side of the body is vasodilated, with a clear line of demarcation at the midline.

Jaundice in newborns is characterized by a yellow-orange skin color caused by elevated unconjugated bilirubin concentrations. When present, jaundice typically becomes apparent in the face first and then progresses distally with rising bilirubin concentrations. However, visual inspection is not a reliable means to determine the severity of unconjugated hyperbilirubinemia nor can it be distinguished from pathologic conjugated hyperbilirubinemia. Furthermore, even expert examiners have difficulty visually assessing newborns for jaundice across ethnically diverse populations. For example, jaundice may be underestimated in African-American and Latino newborns and overestimated in Asian newborns because of variations in skin pigmentation.

Erythema neonatorum is typically viewed as a transient period of generalized erythema noted a few hours after birth. It is usually observed by the parents, often during a bath or with vigorous crying. It tends to resolve within minutes to an hour and appears to be associated with successful completion of neonatal transition

of the circulation. It rarely recurs with the same intensity after the first episode (Fletcher, 1998).

Skin creasing is affected by both development and movement:

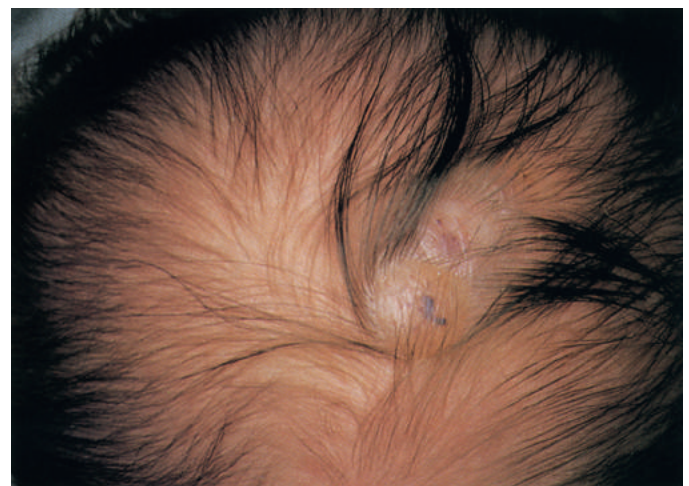
1. Increased creasing: constriction of fetal movement (e.g., fetus near term)
2. Absent creasing on the palms or soles of a term or near-term newborn: consider prolonged lack of movement secondary to a neuromuscular disorder.
3. Effect of edema: stretches the skin and obscures normal skin folds and creases; skin pits with gentle pressure; distribution of edema is affected by gravity and changes with patient positioning. Lymphedema (as in Turner syndrome) tends to *accentuate* skin creasing and is less affected by gravity (Fletcher, 1998).

Iatrogenic trauma and injuries (in utero or at delivery) are common. Cutaneous findings include scars from injury by an amniocentesis needle; minor lacerations and abscesses from fetal scalp electrodes or fetal blood gas sampling; scalpel lacerations during cesarean section; and ecchymoses and abrasions from application of forceps or vacuum.

Trauma can also occur from external pressure on the fetus during labor, particularly over bony prominences. This may lead to the development of subcutaneous fat necrosis, an uncommon condition hypothesized to be caused by hypoxic injury to fat and manifested by firm, subcutaneous nodules and plaques (Cohen, 2008). The lesions usually resolve spontaneously after several months but can become inflamed and fluctuant. On occasion, they are associated with later development of significant hypercalcemia.

Aplasia cutis congenita is a focal lesion characterized by congenital absence of some or all layers of skin (Fig. 25.3). It may occur sporadically, but it can be associated with chromosomal defects or other malformations (Kos and Drolet, 2008). It occurs most often on the scalp near the vertex and can be mistakenly presumed to be the result of a traumatic injury.

Some common and uncommon skin conditions observed in the newborn are outlined in Box 25.1. Fortunately, most of the skin findings encountered during a healthy newborn's examination are typically benign.



• **Fig. 25.3** Aplasia Cutis Congenita. Lesions are usually single. Two adjacent lesions near the vertex—one bullous and one membranous—can be appreciated. (From Rudolph AJ. *Atlas of the Newborn*. Vol 4. Hamilton, Ontario, Canada: BC Decker; 1997:30.)

• BOX 25.1 Common and Uncommon Causes of Skin Lesions in Neonates

Pustular, Vesiculopustular, and Vesiculobullous Lesions

- Common or benign: erythema toxicum neonatorum, transient neonatal pustular melanosis, miliaria crystallina, miliaria rubra, sucking blisters, neonatal acne (benign cephalic pustulosis)
- Infectious: herpes simplex, varicella, staphylococcal pustulosis, bullous impetigo, congenital candidiasis, syphilis, scabies
- Chronic or recurrent: epidermolysis bullosa, mastocytosis, epidermolytic hyperkeratosis, acropustulosis of infancy
- Positive Nikolsky sign: epidermolysis bullosa, staphylococcal scalded skin syndrome
- Other: incontinentia pigmenti

Nodules and Plaques

- Common or benign: milia, Epstein pearls, Bohn nodules, sebaceous hyperplasia
- Yellow: sebaceous nevus, juvenile xanthogranuloma
- Brown or black: congenital pigmented nevus, epidermal nevus
- Other: subcutaneous fat necrosis, dermoid cyst, fibroma, infantile myofibromatosis, hamartomas, malignant tumors, leukemia

Papulosquamous and Scaling Lesions

- Common or benign: physiologic desquamation
- Healthy newborn: atopic dermatitis, contact dermatitis, seborrheic dermatitis, local candida dermatitis, psoriasis
- Ill newborn: acrodermatitis enteropathica, Langerhans cell histiocytosis, syphilis
- Other: ichthyosis syndromes, collodion baby, harlequin baby

Erosions and Ulcerations

- Common or benign: sucking blisters, traumatic injury (e.g., scalp electrode, diaper erosions, reaction to adhesives)
- Other: aplasia cutis congenita, herpes simplex, epidermolysis bullosa, toxic epidermal necrolysis

Altered Pigmentation

- Common or benign: Mongolian spots, transient neonatal pustular melanosis, isolated café au lait macules
- Increased pigmentation: Mongolian spots, transient neonatal pustular melanosis, café au lait macules, lentigines, incontinentia pigmenti
- Decreased pigmentation: ash leaf macule, nevus depigmentosus, piebaldism, albinism
- Purpuric or erythematous: petechiae, dermal hematopoiesis (“blueberry muffin” lesions), neonatal lupus erythematosus

Vascular and Lymphatic Lesions

- Common or benign: nevus simplex or salmon patch, petechiae on the presenting part, small hemangioma
- Other vascular: complicated hemangioma, vascular malformation, port-wine stain
- Lymphatic: cystic hygroma, lymphangioma, lymphedema

Tutorial videos and demonstrations from the University of Utah (Paul Larsen, University of Nebraska, College of Medicine, and Susanne Stensaas, University of Utah School of Medicine).

If unusual or unfamiliar lesions are seen on the examination that fall outside the pediatrician's experience, consultation with an appropriate subspecialist should be arranged, and any appropriate additional studies should be initiated.

Erythema toxicum neonatorum (also known as erythema toxicum and “e. tox.”) is the most common rash in the newborn—observed in up to 70% of term newborns (Howard and Frieden, 2008; Lucky, 2008). The characteristic lesion is an isolated elevated



• **Fig. 25.4** Erythema toxicum neonatorum with erythematous macules, wheals, and pustules. Pustules predominate in this example. At times, patchy or confluent areas of erythema occur without pustules. (From Eichenfield LF, Frieden IJ, Esterly NB, eds. *Neonatal Dermatology*. 2nd ed. Philadelphia: Saunders; 2008:88.)

erythematous papule or pustule that is 1–2 mm in diameter and surrounded by an irregular area of erythema that is slightly larger at 1–3 cm in diameter (Fig. 25.4). They are occasionally present at birth but typically are first noted at 1–2 days of life. Although predominantly noted on the face and trunk, they can appear anywhere on the body *except* the palms and soles. New lesions may continue to appear for approximately 1 week while older lesions are simultaneously resolving. When extensive, the lesions can occur in clusters or become nearly confluent. Diagnosis can usually be made by appearance alone, but a scraping of the pustule will reveal an almost pure infiltrate of eosinophils if confirmation is needed for atypical cases.

Transient neonatal pustular melanosis is a benign and self-limited skin manifestation defined by the appearance and evolution of three distinct phases of skin lesions (Howard and Frieden, 2008; Lucky, 2008; Fig. 25.5). Initially, a superficial vesicopustule appears. It will then rupture and leave a fine rim of scale around the unroofed pustule. There is no associated erythema (unlike the erythema seen with erythema toxicum). The final stage is a hyperpigmented macule that gradually disappears and may be described as *freckles*. Different stages may be simultaneously seen on examination of the skin. The condition is most commonly seen in African-American newborns but is also recognized in other ethnically diverse newborns of color. In some cases, only the second or third stage is seen at birth, the initial stage presumably having occurred in utero.

Miliaria crystallina happens after superficial obstruction of the sweat ducts and produces small, crystal-clear vesicles that resemble water droplets (Fig. 25.6). It is classically seen where newborns live in warm climates or are febrile. The vesicles are fragile and can be easily removed by wiping of the skin with a soft damp cloth (Howard and Frieden, 2008; Lucky, 2008).

Miliaria rubra, also called *heat rash* or *prickly heat*, results from sweat duct obstruction deeper in the epidermal layer. This type of “rash” typically presents after the first week of life. It is mentioned in this context because it can occasionally appear as a pustular rash that mimics skin findings caused by cutaneous staphylococcal, candidal, or herpes simplex infection.

Sucking blisters and calluses result from vigorous sucking on a hand or forearm in utero. They can be visually quite impressive



• **Fig. 25.5** Transient Neonatal Pustular Melanosis. The first stage consists of small superficial pustules, without inflammation (A). Scales that rim the location of a pustule (comprising the second stage) may be seen at birth without evident pustules (B) or may develop postnatally after pustules have ruptured (C). Small hyperpigmented macules remain in the final stage (D), gradually fading over weeks or months. (From Eichenfield LF, Frieden IJ, Esterly NB, eds. *Neonatal Dermatology*. 2nd ed, Philadelphia: Saunders; 2008:89.)



• **Fig. 25.6** Miliaria Crystallina. The tiny, clear vesicles resemble water droplets, with no signs of inflammation. (From Rudolph AJ. *Atlas of the Newborn*. Vol 4. Hamilton, Ontario, Canada: BC Decker; 1997:13.)



• **Fig. 25.7** Neonatal Cephalic Pustulosis (Neonatal Acne). Small, red papules and pustules are seen on the cheeks and forehead, with some extension into the scalp. Comedones are absent. (From Eichenfield LF, Frieden IJ, Esterly NB, eds. *Neonatal Dermatology*. 2nd ed. Philadelphia: Saunders; 2008:90.)

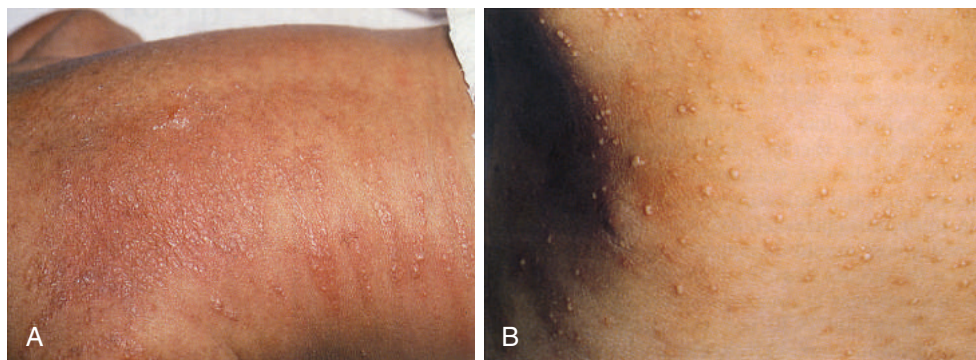
and cause a tense, fluid-filled blister. Once the blister ruptures, it forms an erosion or a callus. The lesion is most often solitary (occasionally bilateral) and is without inflammation. This is similar to the sucking pad or callus that can develop on the newborn's lips as a result of vigorous and frequent nursing.

Neonatal cephalic pustulosis ("neonatal acne") may occasionally be seen at birth. However, it has a mean onset of appearance at 2–3 weeks of life (Howard and Frieden, 2008; Lucky, 2008). It is characterized by inflammatory, erythematous papules and pustules located primarily on the cheeks with extension over the face and into the scalp (Fig. 25.7). Sometimes it is difficult to confidently

distinguish it from miliaria rubra. Fortunately, both are benign conditions. Therefore neither definitive diagnosis nor biopsies are warranted.

Infections presenting with skin lesions are relatively uncommon in the immediate newborn period but should be considered in the differential diagnosis, particularly for newborns who may be slightly premature:

1. Congenital candidiasis typically occurs immediately after birth (appearing on the first day). It is a papulovesicular eruption



• **Fig. 25.8** Congenital Candidiasis. The rash may be a diffuse, erythematous pustular eruption (A) or may have diffusely distributed but distinct pustules (B). In premature newborns, a diffuse scald-like erythematous dermatitis may be seen (not shown). (From Eichenfield LF, Frieden IJ, Esterly NB, eds. *Neonatal Dermatology*. 2nd ed. Philadelphia: Saunders; 2008:214.)

that progresses to pustules, followed by crusting and desquamation (Fig. 25.8). Lesions can be widespread and may appear on any part of the body, including the palms and soles (Darmstadt et al., 2000; Carder, 2008).

2. Thrush and candidal diaper dermatitis are distinct from congenital candidiasis. These infections are not seen immediately after birth but may present in the first week of life. Superficial skin infections of *Staphylococcus aureus* may also present during this time frame.
3. Neonatal herpes simplex is unlikely in the first few days after birth unless there is a history of severely prolonged rupture of membranes. However, it must be considered whenever vesicles are seen in the newborn. The skin lesions of herpes simplex start as small, 2–4-mm vesicles on an erythematous base. After 1–3 days the lesions become pustular and develop an eschar (Friedlander and Bradley, 2008; Fig. 25.9). The mucosal lesions are usually shallow ulcerations.
4. “Blueberry muffin” skin lesions of congenital rubella and cytomegalovirus (CMV) infection are caused by dermal hematopoiesis. When caused by infection, these lesions are unlikely found in isolation. Affected newborns typically have multiple stigmata of congenital infection, including growth retardation, microcephaly, and hepatosplenomegaly. However, one might separately include congenital histiocytosis in the differential diagnosis for this skin lesion.
5. Congenital syphilis skin findings are rarely seen, but they classically involve the palms, soles, perioral and anogenital areas (Dinulos and Pace, 2008; Howard and Frieden, 2008).

An additional difficulty is the *variable* presentation of the syphilitic rash. It may appear in papulosquamous, vesiculobullous, macular erythematous, annular, or polymorphous forms. However, desquamation limited to the palms and soles, with no rash or peeling elsewhere, is highly suggestive of congenital syphilis (Fig. 25.10). Given the resurgence of syphilis in some urban areas, the astute examiner should still maintain a watchful eye for this uncommon presentation of congenital syphilis.

Milia, Epstein pearls, and Bohn nodules are all forms of epidermal inclusion cysts (Lucky, 2008; Fig. 25.11):

1. Milia occur on the face and scalp in small numbers. They are smooth, firm, white papules with no associated erythema. They may be present at birth or appear somewhat later. They usually resolve within a few months.
2. Epstein pearls are larger inclusion cysts, which usually occur as distinct single lesions. Common locations include the central



• **Fig. 25.9** Herpes Simplex. (A) The first signs of herpes infection in this newborn were eroded vesicles at the corner of the mouth. (B) Herpetic vesicles on the face, scalp, and ear of a newborn with respiratory distress and hepatitis. (From Cohen B. *Pediatric Dermatology*. 3rd ed. Philadelphia: Mosby; 2005:36.)

hard palate, the foreskin, and the ventral surface of the penis and scrotum.

3. Bohn nodules are also larger inclusion cysts found on the alveolar ridge of the oral cavity.

Sebaceous hyperplasia occurs mostly on the face, especially the nose and upper lip. It is characterized by sheets of smooth, yellow-white papules with the regular spacing of involved follicles and no surrounding erythema (Fig. 25.12). Androgenic hormonal stimulation in utero causes this hypertrophy of the sebaceous glands. After birth it gradually resolves over several weeks.



• **Fig. 25.10** Desquamation on the palms (A) and soles (B) of a newborn with congenital syphilis. (From Rudolph AJ. *Atlas of the Newborn*. Vol 4. Hamilton, Ontario, Canada: BC Decker; 1997:108.)

Physiologic desquamation is frequently seen in term newborns at 1–2 days of age, particularly on the hands and feet. This is more pronounced in some postmature newborns (born later than 41 $\frac{1}{2}$ weeks). The dry, thickened skin cracks and peels extensively and then normalizes spontaneously without intervention in approximately 1 week. It is not typically confused with ichthyoses (rare inherited disorders of cornification) that manifest themselves in the neonatal period (Irvine and Paller, 2008).

Ichthyosis disorders include Harlequin ichthyosis (caused by mutations in the *ABCA12* gene) and “collodion babies” who appear encased in a thickened, shiny skin that resembles collodion, a phenotype associated with a number of different ichthyotic conditions.

Congenital dermal melanocytosis (“Mongolian spots”) are macular areas of a slate gray, blue-gray, blue-black, or deep brown color. The distinctive appearance is due to the presence of melanocytes located in the dermis, instead of their typical site at the dermal–epidermal junction. They most commonly occur on the lower back and buttocks but can occur on other extensor surfaces. The pigmentation tends to fade over several years but can persist without resolution (Gibbs and Makkar, 2008; Lucky, 2008). They are common in East Asian, East African, Native American, African-American, and Polynesian newborns and should not be confused with ecchymoses from nonaccidental trauma.

Dermal melanocytosis in the area of the first and second divisions of the trigeminal nerve is called the *nevus of Ota*. The *nevus of Ito* is a similar lesion occurring on the neck, upper back, and shoulders in the area of the posterior supraclavicular and lateral brachial cutaneous nerves. Unlike congenital dermal melanocytosis, these nevi do not become less pigmented with time. Rarely, malignant melanoma or malignant nevi can develop within them.

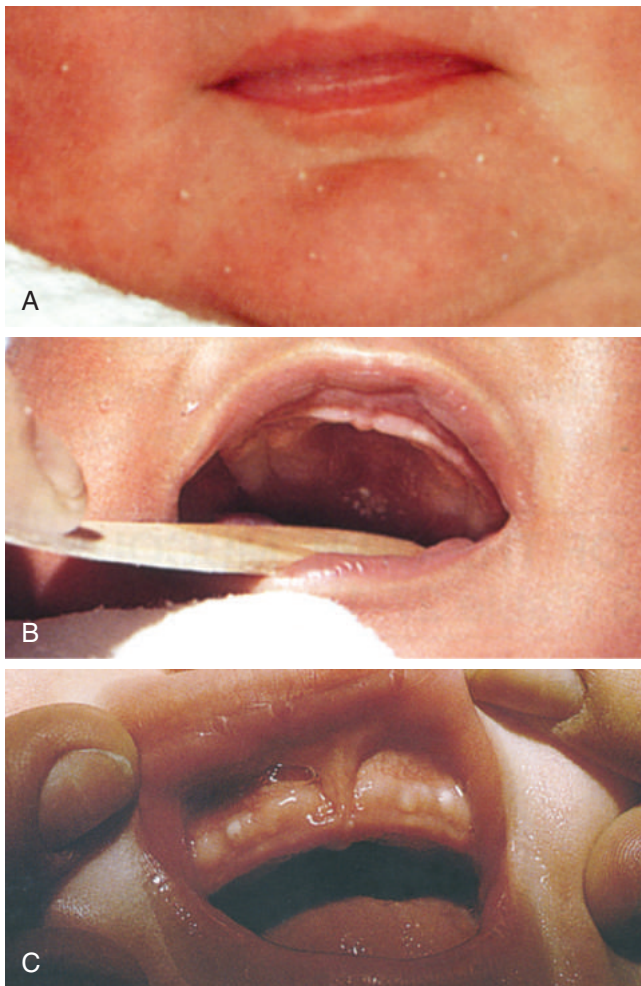
Café au lait macules are tan-brown macules that occur anywhere on the body. They are common as an isolated finding and generally benign. However, they can be markers of other conditions (Gibbs and Makkar, 2008). The presence of six or more café au lait macules

greater than 5 mm in diameter is considered presumptive evidence of neurofibromatosis type 1. Multiple large café au lait macules with irregular borders may be a manifestation of McCune–Albright syndrome.

Nevus simplex (“salmon patch”) is a benign vascular lesion consisting of erythematous macules or patches frequently seen at the nape of the neck (“stork bite”) or on the eyelids and glabella (“angel’s kisses”). Somewhat less frequently, it appears on the nose and upper lip (Lucky, 2008; Fig. 25.13). The lesions are caused by dilated capillaries in the upper dermis, with normal overlying skin. Facial lesions usually fade or resolve completely within 1–2 years, but 25%–50% of those on the neck persist throughout life (Lucky, 2008).

Petechiae after vaginal delivery of a healthy newborn on the presenting part (i.e., the part of the fetus closest to the pelvic inlet of the birth canal at the onset of labor) or on other areas of the body subjected to localized pressure during delivery are not unusual or concerning. A tight nuchal cord can cause extensive ecchymoses and petechiae on the entire head. Diffuse petechiae, however, are abnormal and suggest thrombocytopenia or platelet dysfunction.

Hemangiomas are soft, pink-red, compressible vascular tumors composed of proliferating endothelial cells. Small hemangiomas are recognized at or shortly after birth in 1%–3% of healthy term newborns and become apparent in 10% of all newborns by 1 month of age (Cohen, 2005). Hemangiomas are more common in female newborns (2:1) than in male newborns (9:1). The incidence is much higher (22%–30%) in preterm newborns weighing less than 1000 g but is only slightly higher (15%) in those weighing 1000–1500 g (Amir et al., 1986; Enjolras and Garzon, 2008). Infantile hemangiomas typically undergo a period of growth for 6–12 months, which is followed by spontaneous involution. Approximately 25% regress by 2 years of age, 40%–50% regress by 4 years of age, 60%–75% regress by 6 years of age, and 95% regress by adolescence (Cohen, 2005). Before the hemangioma



• **Fig. 25.11** Epidermal Inclusion Cysts. Milia are most commonly seen on the face (A) but can occur anywhere on the body. Epstein pearls occur on the midline of the hard palate, most commonly near the junction with the soft palate (B). Bohn nodules are found along the gum margins and on the lateral palate (C). Dental lamina cysts (not shown) are similar inclusions located on the crest of the alveolar ridge. (From Fletcher MA. *Physical Diagnosis in Neonatology*. Philadelphia: Lippincott-Raven; 1998:124 [A]; and Eichenfield LF, Frieden IJ, Esterly NB, eds. *Neonatal Dermatology*. 2nd ed. Philadelphia: Saunders; 2008:503–504 [B, C].)



• **Fig. 25.12** Sebaceous Hyperplasia. Sheets of tiny, white-yellow follicular papules, without inflammation, are seen on the nose. (From Eichenfield LF, Frieden IJ, Esterly NB, eds. *Neonatal Dermatology*. 2nd ed. Philadelphia: Saunders; 2008:87.)



• **Fig. 25.13** Salmon patches are commonly seen on the glabella, eyelids, nose, or upper lip, either singly or in all these locations (A) and on the nape of the neck (B). (From Eichenfield LF, Frieden IJ, Esterly NB, eds. *Neonatal Dermatology*. 2nd ed. Philadelphia: Saunders; 2008:95.)

becomes obvious, careful examination may reveal a precursor lesion manifesting itself as telangiectasias surrounded by an area of pallor, or it may be pale, erythematous, or ecchymotic-like macules and patches. In contrast, vascular malformations are nonproliferative lesions, usually present at birth.

Kasabach–Merritt phenomenon occurs in association with specific types of large vascular tumors that cause platelet trapping and severe thrombocytopenia. It is not associated with true hemangioma of infancy (Enjolras and Garzon, 2008).

Port-wine stains are capillary malformations evident at birth as pink or red patches that grow proportionately with the child and persist throughout life (Enjolras and Garzon, 2008). The initial pink-red color typically changes to a deeper red or purple hue with age. Approximately 10% of port-wine stains that involve the area supplied by the ophthalmic (V1) branch of the trigeminal nerve (Fig. 25.14) are associated with seizures, arterial brain malformations, and ocular abnormalities that are commonly referred to as Sturge–Weber syndrome (Enjolras et al., 1985).

Head

Scalp hair quantity in the newborn is highly variable, but abnormalities in the distribution, texture, and patterning of the hair are

potentially informative and should be documented. Hair usually forms a single whorl near the vertex, but double whorls occur in approximately 5% of newborns. Abnormal placement or the presence of more than two whorls may be a marker of abnormal brain development (Smith and Gong, 1974).

Caput succedaneum is a diffuse edematous swelling of the scalp caused by pressure during delivery that results in fluid accumulation external to the periosteum. The caput is boggy, has diffuse edges, is not limited by suture lines, and is most commonly located over the vertex. It is usually present at birth and resolves over several days.

Cephalohematoma is caused by hemorrhage *beneath* the periosteum. It forms a distinct, firm lateral mass that does not cross suture lines (Fig. 25.15). It may not be apparent until several hours after birth, and it often increases in size in the first 12–24 hours. It typically remains palpable for 2–3 weeks and may also develop a calcified rim.



• **Fig. 25.14** This newborn with Sturge–Weber syndrome has a port-wine stain in the distribution of the ophthalmic division of the facial nerve. (From Cohen B. *Pediatric Dermatology*. 3rd ed. Philadelphia: Mosby; 2005:49.)

Subgaleal hematoma is not confined by the periosteum and can involve massive loss of circulating blood volume due to the large potential space for hemorrhage to collect. Depending on the volume of blood that has accumulated, the hematoma can be palpated as a firm or fluctuant mass with poorly defined edges that may extend onto the neck or forehead. Large subgaleal hematomas are uncommon but are associated with high morbidity and mortality. Reasonable consideration for admission to the NICU for observation and evaluation should be considered.

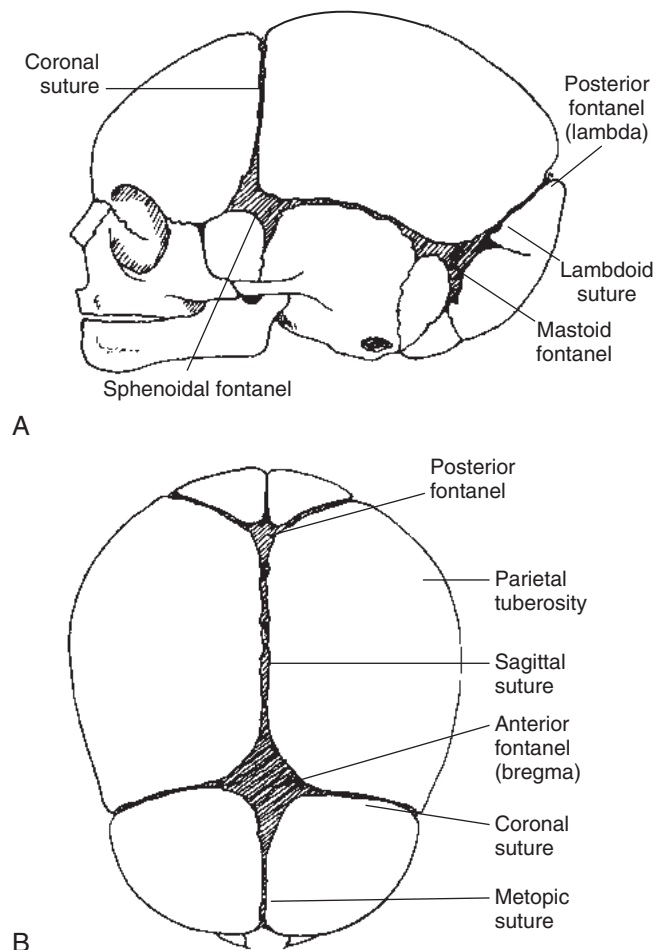
Skull inspection and palpation for overall size, shape, and features of the skull should be done. Head circumference (maximal occipital–frontal circumference) should always be measured and plotted on an appropriate growth chart (see Fig. 25.2). Head circumference measurement is subject to error and often significantly affected by molding of the skull during labor. The head circumference measurement should be repeated if the result appears discordant with the visual examination or the newborn's weight and length—after molding has resolved. The soft tissue of the scalp should be examined for swelling, ecchymoses, and other evidence of birth-related injury and for iatrogenic injuries (application of a vacuum device, placement of scalp electrodes, fetal blood sampling, and lacerations with a scalpel during cesarean section).

The newborn's skull is composed of several bony plates, separated by sutures and fontanels so that the skull appropriately deforms during labor (Fig. 25.16). The entire surface of the skull should be palpated to identify the location and size of the major fontanels and assess it for discontinuities. The soft tissue over a fontanel should normally be flat; a raised or bulging fontanel suggests that intracranial pressure is increased. It is common to find palpable discontinuity at a suture because of vertical displacement of a skull bone relative to the neighboring one (molding). In some cases, one bone can truly override the other, but this must still be distinguished from the step-off of a displaced skull fracture.

Craniosynostosis is premature fusion of one or more of the cranial sutures and causes a variety of abnormal skull shapes, depending on which sutures are involved (Fletcher, 1998; Volpe, 2008). Fusion of the sagittal suture produces a narrow skull elongated in the anterior–posterior dimension (scaphocephaly or dolichocephaly). Fusion of the coronal sutures causes a widened skull that is shortened in the anterior–posterior dimension (brachycephaly). Unilateral closure of either a coronal or a lambdoid suture causes an oblique deformity (frontal or occipital plagiocephaly respectively). Closure of a metopic suture produces a triangular skull with a prominent, narrow forehead (trigonocephaly). Depending on the timing of the fusion, the skull



• **Fig. 25.15** Anterior (A) and posterior (B) views of a large cephalohematoma under the periosteum of the right parietal bone. (From Fletcher MA. *Physical Diagnosis in Neonatology*. Philadelphia: Lippincott-Raven; 1998:185.)



• **Fig. 25.16** The Major Sutures and Fontanels of the Newborn Skull. (From Fletcher MA. *Physical Diagnosis in Neonatology*. Philadelphia: Lippincott-Raven; 1998:175.)

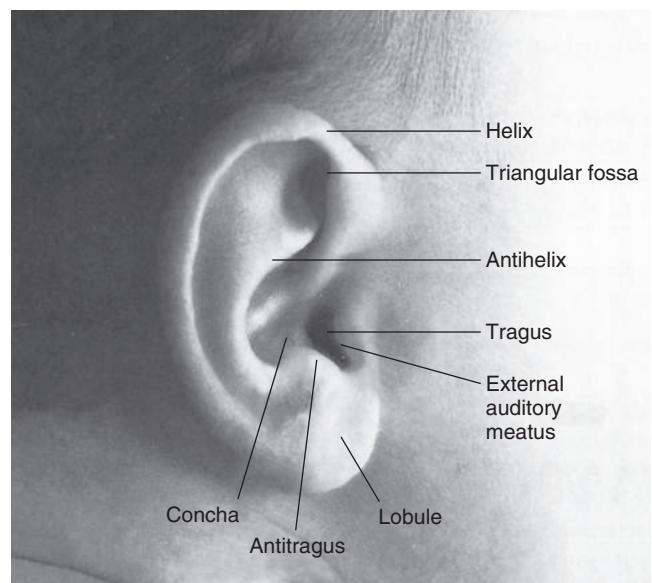
shape may be abnormal at birth or become visibly deformed later. The fused suture typically has a palpable elevation or ridge, which must be distinguished from displacement of a normal suture caused by molding. In the absence of craniosynostosis or overriding sutures, normal mobility at a suture can be verified by gentle application of alternating pressure on the bones on either side of the suture line.

Craniotabes (or craniomalacia) is a softening of the skull, most commonly involving the parietal bones near the vertex. Gentle pressure on the involved bone produces a sudden collapse, with recoil when the pressure is released, similar to the way a ping-pong ball collapses when squeezed. It has usually been attributed to localized bone resorption or interference with ossification caused by prolonged pressure on the fetal skull from the maternal pelvis, but it may be associated with maternal vitamin D deficiency in some populations (Yorifuji et al., 2008).

Encephaloceles may be small soft-tissue masses or bulges of brain and/or the membranes surrounding it at the occiput or in the midline of the forehead near the bridge of the nose. Large encephaloceles and major neural tube defects should otherwise be obvious if present.

Face

The face and facial expressions should be observed throughout the examination. It is helpful to view the face from the front, in



• **Fig. 25.17** Anatomy of the External Ear. (From Fletcher MA. *Physical Diagnosis in Neonatology*. Philadelphia: Lippincott-Raven; 1998:285.)

profile, and looking down from the top of the head. Important characteristics to note include the symmetry and relative sizes, spacing, and orientation of the features. Expressiveness of the face, both at rest and during crying, should be noted. Specific attention should be paid to the ears, eyes, eyebrows, eyelids, nose and nasal passages, lips, palate, and mouth. Suggested abnormalities should be described as precisely as possible. Measurements of the facial features are not part of the routine newborn examination, and comparison with reference nomograms for suspected dysmorphisms should ideally be done in consultation with a geneticist.

Ears

The external ear is a relatively common site of minor anomalies. The external ear develops from the first and second branchial arches. Most of the pinna derives from the second branchial arch. The third arch contributes the tragus, antitragus, and anterior wall of the canal (Fig. 25.17). The position and orientation of each ear should be noted, as should the size, shape, and structure of the helix, and any signs of trauma. The ear is palpated to assess the firmness and recoil of the cartilage and to detect any masses or abnormalities of texture. The skin near the pinna is examined for skin tags, pits, and sinus tracts. Minor variations in the shape of the ear and earlobe are common, and they usually have only cosmetic significance. A lop ear is characterized by downward folding of the superior helix caused by underdevelopment of the upper third of the pinna. However, this needs to be distinguished from folding caused by positioning in utero. A cup ear is a protruding ear with an excessively concave concha. Microtia implies a severely dysplastic and malformed ear, and it is often associated with abnormalities of the middle ear and other malformations.

More than 95% of all newborns born in the United States are screened for hearing loss shortly after birth by automated testing of hearing before hospital discharge (American Academy of Pediatrics, 2007; US Preventive Services Task Force, 2008). The screening test is effective and allows early identification of hearing loss. However, there is concern for ways to address the

newborns who are lost to follow-up. Additionally, ongoing studies are evaluating the role of CMV testing with universal hearing screening given that CMV infection (even in asymptomatic newborns) may account for 20%–30% of newborns in whom hearing loss is diagnosed.

Eyes

As part of the inspection of the face, the size, spacing, and orientation of the eyes, eyelids, and eyebrows should be assessed:

1. Microphthalmos: entire eye is small
2. Microcornea: possible isolated finding in otherwise normal eye
3. Hypertelorism: spacing between the bony orbits is excessively wide
4. Telecanthus: inner canthi are displaced laterally, giving a false impression of hypertelorism
5. Hypotelorism: often associated with holoprosencephaly, trisomy 13, and other genetic abnormalities
6. Downslanting palpebral fissures: medial canthi are higher than lateral canthi.
7. Upslanting palpebral fissures: lateral canthi are higher than medial canthi.
8. Ptosis: difficult to reliably detect in the newborn (unless severe or unilateral); may be congenital or it can be caused by trauma or inflammation
9. Transient episodes of gaze disjugation are common in otherwise healthy newborns, but persistent eye deviation requires follow-up.
10. Obstruction at both ends of the lacrimal sac produces a mucocoele (or dacryocoele) that appears as a bluish subcutaneous mass that can be mistaken for a hemangioma or an encephalocele. Mucocoeles can sometimes extend into the nasal passages and cause respiratory distress.

Routine examination of the sclera, cornea, and internal portions of the newborn eye is often limited by the significant eyelid edema present in the first few days after birth. If corneas and conjunctivae cannot be adequately visualized during spontaneous eye opening or with gentle lid retraction, it is normally preferable to defer further examination until the swelling recedes.

11. Coloboma: congenital defect in eye formation; can affect any or all of the external or internal structures of the eye. Colobomas of the iris (frequently located inferomedially and producing a keyhole-shaped pupil) are the most common type visible on external examination. With examination, the lower lid of each eye should be retracted so that the entire pupil is visible, if not the entire cornea. Ophthalmologic evaluation of internal structures of the eye is advisable when colobomas are seen.
12. Glaucoma: cloudy corneas represent glaucoma until proven otherwise and require prompt ophthalmologic evaluation even if obvious enlargement of the cornea and globe (buphthalmos) is not present.
13. Subconjunctival hemorrhages are common in newborns, particularly after vaginal delivery. They do not indicate trauma unless other findings are present, so it is helpful to document their presence.

A red reflex examination of the eyes should be performed in all newborns before discharge from the nursery. It is normal when the reflections of the two eyes viewed both individually and simultaneously are equivalent in color, intensity, and clarity, and there are no opacities or white spots within the area of either red

reflex or both red reflexes ([American Academy of Pediatrics, 2008](#)). Problems potentially detected by the red reflex examination include cataracts, aqueous and vitreous opacities, and retinal abnormalities, including tumors and chorioretinal colobomas. Dark spots in the red reflex, a markedly diminished reflex, the presence of a white reflex, or asymmetry of the reflexes is an indication for immediate referral to an ophthalmologist with pediatric expertise ([American Academy of Pediatrics, 2008](#)). The red reflex examination is vital for early detection of vision-threatening and potentially life-threatening abnormalities, including retinoblastoma. Unfortunately, the sensitivity of the routine red reflex examination for retinoblastoma detection is low, and most retinoblastomas are first detected by family members, despite routine screening ([Abramson et al., 2003](#)).

Nose

The nose should be evaluated for the size, shape, and symmetry of the nostrils, columella, alae nasi, and bridge, root, and tip and inspected for deformations. Asymmetries of the nose caused by in utero compression are common and need to be distinguished from more serious conditions such as septal deviation (prenatal or birth trauma), nasal fracture, or other deformations.

Passage of a catheter beyond the nasopharynx via each nare usually rules out choanal atresia but does not ensure that the nasal passages are adequately sized for normal breathing. One can evaluate the quality and volume of airflow through each nostril by listening with the bell of a stethoscope (or by observing the deflection of a wisp of cotton placed under the nostril). As obligate nose breathers, newborns should breathe comfortably via each nostril separately with the mouth closed. This can be assessed by the examiner briefly occluding each nostril with his/her fingertip. If unilateral atresia or stenosis is present, the newborn will exhibit signs of distress when the patent nostril is occluded. Persistent flaring of the alae nasi may occur as the sole or initial symptom of mild respiratory distress, or it may accompany grunting and retractions. Appropriate intervention is indicated.

Mouth and Oral Cavity

The lips, perioral area, and oral cavity should be inspected both at rest and during crying. The shape of the philtrum should be evaluated when the mouth is relaxed, because stretching of the upper lip during crying can give a false impression of the flat philtrum suggestive of fetal alcohol syndrome. Perioral cyanosis is common and benign in normal newborns in the immediate newborn period. However, cyanosis of the tongue and mucous membranes is always abnormal and requires immediate investigation. A well-hydrated newborn will have mucous membranes that are moist and shiny with saliva. Excessive oral secretions can be caused by esophageal atresia or impaired swallowing. An anteriorly displaced and/or excessively short frenulum that restricts protrusion and/or elevation of the tongue can be a cause of maternal pain with breastfeeding and subsequent breastfeeding difficulties.

No instruments are typically needed for the oral cavity examination. Epstein pearls, Bohn nodules, and dental lamina cysts (see [Fig. 25.11](#)) are common and benign findings. Tonsils are normally inconspicuous in newborns. With some adjustment of head position, the entire palate and much of the pharynx is seen, especially if the newborn cries. The soft palate elevates symmetrically during crying if cranial nerves (CNs) IX and X are intact. The uvula is short but highly mobile. A bifid uvula may be an isolated finding, but a submucosal cleft palate must be excluded.

After inspection the oral cavity should be palpated with a gloved finger to assess the shape and integrity of the palate and to feel for natal teeth and masses. Although clefting of the lip and anterior palate are easily seen, palpation decreases the chance that a pediatrician misses an isolated cleft of the posterior palate. Palpation may elicit the sucking reflex, and this can be used to gauge the strength and coordination of sucking. Elicitation of a gag reflex is not usually done in healthy newborns but should be done if the newborn is neurologically depressed or has difficulty swallowing.

Neck

It is usually convenient to combine palpation of the neck for lymphadenopathy and masses with palpation of the clavicles. The entire skin surface of the neck should be visualized and palpated while the head is being turned and the skin is being retracted to open the neck creases and folds. The range of motion of the head and neck is evaluated at the same time.

Congenital muscular torticollis at birth is commonly accompanied by a palpable fibrous tumor (fibromatosis colli) in the shortened sternocleidomastoid muscle. Nonmuscular causes of torticollis include tumors of the posterior fossa or cervical spine and cervical spine malformations.

Klippel-Feil syndrome is characterized by a short neck, low posterior hairline, and restricted mobility of the upper spine. Redundant skin or a webbed neck may be seen in trisomy 21 and in Turner and Noonan syndromes. Cystic hygromas are soft fluctuant masses (usually unilateral) that transilluminate. Branchial cleft cysts or sinuses are also found laterally, from the level of the mastoid to the center of the sternocleidomastoid muscle. Thyroglossal duct cysts are found at the midline and high in the neck or under the chin. Laryngeal or tracheal deviation from the midline or enlargement of the thyroid gland warrants a higher level of evaluation. Neuroblastoma may rarely occur with neck mass and signs consistent with Horner syndrome.

Chest Wall

The skin, soft tissue, and bony structures of the thorax should not be ignored when one is assessing cardiorespiratory status. The position of the nipples and the presence of any accessory nipples should be noted. Transient galactorrhea, a result of the lingering effect of maternal hormones, occurs in approximately 5% of term newborns (Madlon-Kay, 1986). Variations in the shape of the xiphoid process are common (a prominent or bifid xiphoid is a benign finding that is less pronounced over time). A mildly depressed sternum (pectus excavatum) or a protuberant one (pectus carinatum) may be of no clinical consequence but should be noted. A small, bell-shaped chest in a newborn with respiratory distress may reflect lung hypoplasia or a disorder of skeletal growth. An increase in the anterior-posterior diameter of the chest (barrel chest) may reflect an increase in the intrathoracic volume caused by air trapping from meconium aspiration or pneumothorax. Crepitus may be felt at the site of a fractured clavicle or rib. Crepitus can also be caused by dissection of air into the subcutaneous tissue from a pneumothorax or pneumomediastinum, but this is an unlikely occurrence in an asymptomatic newborn.

Lungs and Respiration

The respiratory examination begins with observation of the color of the skin and mucous membranes, respiratory rate, breathing pattern, and work of breathing. Respiratory problems are unlikely to be found in a newborn who is centrally pink and breathing comfortably at a normal rate.

In the normal newborn, the abdomen expands smoothly with each contraction of the diaphragm, while the chest moves inward slightly.

The respiratory rate of the newborn is highly variable when the newborn is awake and changes with activity such as feeding and crying. Tachypnea during sleep is more clearly associated with respiratory problems than tachypnea when awake. During an examination the respiratory rate should be determined if tachypnea or hypopnea is evident. Because short pauses and brief periods of rapid breathing are common in normal newborns, accurate measurement of the respiratory rate requires at least a 60-second assessment, preferably when the newborn is asleep or at least not crying. During crying the quality and vigor of vocalization are assessed, and the newborn is observed for changes in color and perfusion. Central cyanosis that *appears* during crying may indicate cardiac or respiratory disease and requires further evaluation. Cyanosis that *resolves* during crying may be due to choanal atresia/stenosis, apnea, or hypoventilation.

The classic signs of neonatal respiratory distress are nasal flaring, grunting, and retractions. Nasal flaring and mild grunting are common in the immediate postnatal period but should resolve within 15–20 minutes after birth. Increasing respiratory distress caused by decreasing lung compliance is typically characterized by a progression in severity of these classic symptoms. The respiratory rate generally decreases as the work or effort of breathing increases, as indicated by the development of grunting and increasing retractions. When respiratory distress is mild, intermittent grunting at a slower respiratory rate may alternate with periods of mild tachypnea. As grunting worsens, the expiratory phase becomes more prolonged. The length of the grunt, rather than its loudness, correlates with the severity of distress. Intermittent mild grunting can be misinterpreted by parents as crying or singing. The rhythm of grunting and its occurrence at the end of expiration are key features that help distinguish grunting from other vocalizations. Retractions require a forceful inspiratory effort and decreased lung compliance, and they may be absent or less prominent than expected in a newborn with neuromuscular depression.

Nasal congestion, airway obstruction, and airway secretions can produce sounds that are audible without a stethoscope. Noisy or congested nasal breathing and intermittent sneezing not associated with upper respiratory tract infection are common in the first few days after birth. A hoarse cry suggests an abnormality affecting the vocal cords. Because intubation of vigorous newborns born through meconium-stained amniotic fluid is no longer routine (Halliday and Sweet, 2001), hoarseness or stridor caused by vocal cord trauma in healthy term newborns has become less common. Inspiratory stridor is due to narrowing or partial obstruction of the upper airway. The presence and loudness of stridor depend on respiratory effort as well as the extent of airway narrowing, so stridor typically worsens with forceful inspiration during crying. Stridor during crying in a newborn with no respiratory distress when quiet is often due to tracheolaryngomalacia (usually benign). Stridor that is present during quiet breathing or present throughout inspiration and expiration suggests the presence of a more significant airway obstruction that requires further evaluation.

A brief chest auscultation of the newborn who is observed to be centrally pink and breathing comfortably in room air is usually sufficient to ensure that breath sounds are clear and that air entry is adequate and equal bilaterally. Breath sounds are not well localized in the newborn, and the newborn might not remain quiet for long, so delineating the quality of breath sounds is usually more helpful than attempting to compare multiple sites. Detection of

abnormal sounds such as crackles, wheezes, or rhonchi requires further assessment. If a more detailed examination is indicated, one should auscultate over the four major quadrants anteriorly, on both sides, and on the upper and lower back bilaterally. Diaphragmatic hernia presenting in the neonatal period usually causes significant respiratory distress. Rarely, a small diaphragmatic hernia is detected by the presence of bowel sounds in the chest of an asymptomatic newborn. Spontaneous cough is abnormal in newborns and is most commonly caused by infection or aspiration.

Although rarely done, percussion of the chest can be useful for estimating the position of the upper margin of the liver. Percussion can also be used to detect a large pleural effusion or lung consolidation, but newborns with these conditions will have other signs of respiratory distress, so the diagnosis will rely on imaging studies and not the physical examination. Transillumination can be useful for supporting a rapid diagnosis of pneumothorax in a distressed newborn, but it is not reliable for detecting a small pneumothorax that produces minimal symptoms.

Respiratory signs are sensitive but nonspecific indicators of illness in the newborn. Alterations in respiration (including apnea) can accompany illness of many different causes. Common causes of subtle or mild respiratory distress detected in routine evaluation include retained fetal lung fluid (transient tachypnea of the newborn), spontaneous pneumothorax, neonatal sepsis, pneumonia, meconium or amniotic fluid aspiration, and congenital heart disease. Any newborn with respiratory distress should be transferred to a NICU or observation nursery for further evaluation, monitoring, and treatment.

Cardiovascular System

The cardiovascular system evaluation has two major goals:

1. Assess the current status of the circulation.
2. Detect signs of congenital heart disease, particularly the critical, ductal-dependent forms that can produce rapid clinical deterioration in the newborn period.

Although detection of heart disease is important, abnormal circulatory findings in the newborn are more often secondary to other problems, including sepsis, hypovolemia, anemia, and hypoglycemia. The cardiovascular system undergoes marked changes after birth involving the transition to air breathing, the progressive decrease in pulmonary vascular resistance, and the closure of the ductus arteriosus. These changes affect the physical examination of healthy newborns and those with congenital heart disease. Timing after birth is always an important consideration in the interpretation of the examination.

The cardiovascular system examination also requires attention be paid to the newborn's general behavior and activity, respiratory signs, color of the oral mucosa, and temperature, color, and perfusion of all body regions. These features will usually be inspected at different times during the course of the examination, but they should be reevaluated with the heart and chest examination if cardiovascular abnormalities are suspected.

Pulse assessment is as follows:

1. Rate. The pulse rate of the well newborn at rest averages 120–130 beats per minute, with high variability. Transient sinus tachycardia during vigorous crying is common, but persistent tachycardia (≥ 160 –180 beats per minute) requires further investigation. A low resting heart rate during sleep (80–100 beats per minute) is common in healthy, hemodynamically stable term newborns.
2. Rhythm. Isolated premature beats occasionally occur in otherwise healthy newborns; these are typically benign.

3. Character. Pulses in a normal newborn can be challenging to palpate (particularly femoral) and are easily obliterated with pressure. “Normal pulses” receive a grade of 2 on the traditional 0–4 scale. If a decrease or delay of the femoral relative to the brachial pulses is detected, measurement of the blood pressure in all four extremities may reveal a gradient in the blood pressure caused by an aortic coarctation. If the ductus arteriosus remains patent, normal pulses and four-limb blood pressures do not rule out a coarctation. Bounding pulses may be palpated with a patent ductus arteriosus once pulmonary vascular resistance has dropped enough to allow significant left-to-right shunting. Uniformly weak pulses suggest a low output state, usually accompanied by signs of poor perfusion.

The precordium is examined by inspection, palpation, and auscultation. A precordial impulse can be visible in normal newborns, especially during activity, but visible prominence of the precordial area plus a palpably increased cardiac impulse suggests cardiomegaly or a hyperdynamic state. Displacement of the cardiac impulse to the right may tip the pediatrician to the presence of dextrocardia or a shift in the mediastinum. Thrills are rarely palpable in the newborn.

Most heart sounds and murmurs auscultated in the newborn are relatively high pitched, so the diaphragm of the stethoscope is usually used initially, and the bell can be used for further evaluation of low-pitched sounds, if needed:

1. S1 is produced by closure of the mitral and tricuspid valves, usually single in newborns, and best heard in the precordial area.
2. S2 is produced by closure of the aortic and pulmonary valves, usually best heard at the left upper sternal border. With focused attention, slight splitting of the second sound and its variation with respiration can be appreciated.
3. S3 and S4 are abnormal in the newborn.
4. Systolic ejection clicks may be heard in some normal newborns in the first several hours after birth, but ejection clicks are abnormal after this period (Johnson, 1990).

The timing, location, intensity, radiation, quality, and pitch are important characteristics of heart murmurs that can help distinguish physiologic from pathologic murmurs. With repeated examination, physiologic heart murmurs may be detected in as many as 60% of newborns during the first 48 hours (Johnson, 1990). These murmurs are most commonly systolic ejection murmurs that are transient and soft (grade I or II). They are usually attributed to flow through a closing ductus arteriosus or to increasing flow across the pulmonary valve as pulmonary vascular resistance drops. Vibratory systolic murmurs resembling Still murmur can be heard in some newborns. A murmur detected on a routine newborn examination that is not clearly physiologic needs further evaluation.

Detection of a suspicious murmur is the most common reason for further evaluation in an otherwise asymptomatic newborn. However, absence of a murmur does not rule out congenital heart disease (e.g., transposition of the great arteries and atrioventricular canal defect). A decrease or loss of a murmur can be an ominous sign that accompanies clinical deterioration associated with ductal closure in a newborn with ductal-dependent pulmonary or systemic blood flow.

Pathologic murmurs detected in the first few hours after birth are usually caused by obstruction of ventricular outflow such as aortic stenosis or pulmonic stenosis. They are crescendo-decrescendo murmurs (grade II or III). This type of murmur may also be caused by subaortic stenosis associated with hypertrophic cardiomyopathy in a macrosomic newborn of a diabetic mother. Murmurs associated with defects that produce left-to-right shunting usually appear

after a few days, when the pulmonary vascular resistance has dropped sufficiently. Pansystolic murmurs (which include and obscure the first heart sound) that are heard soon after birth are most commonly caused by atrioventricular valve insufficiency, whereas left-to-right shunting through a ventricular septal defect produces a pansystolic murmur that typically appears only after 1–2 days. Diastolic murmurs are rare in newborns. A continuous murmur in a newborn usually represents an aortopulmonary communication or an arteriovenous fistula. The location of an extrathoracic arteriovenous fistula may be revealed by auscultation of a bruit, most commonly in the head or liver.

Abdomen

The abdominal examination begins with observation of the configuration, fullness, and movement with respiration of the abdominal wall. Major abdominal wall abnormalities such as omphalocele, gastroschisis, prune belly syndrome, and bladder extrophy will be obvious on initial inspection in the delivery room and may be diagnosed prenatally. A small omphalocele may produce only a slight widening of the umbilicus and proximal part of the umbilical cord (Fig. 25.18). If such an omphalocele is not detected postnatally before the umbilical cord is clamped and cut, the intestine within it may be damaged. Counting of the umbilical vessels is best done in the delivery room on the freshly cut cord.

The abdomen of the newborn ranges from flat to moderately protuberant, with substantial variation depending on feeding and the passage of gas and meconium. A markedly distended abdomen suggests the possibility of significant ascites, a large mass, or an intestinal obstruction. A proximal obstruction (e.g., esophageal or duodenal atresia) does not cause abdominal distention. A sunken or scaphoid abdomen may be seen in the newborn with respiratory distress caused by a diaphragmatic hernia.

The umbilicus should be inspected for meconium staining, signs of infection, visible discharge of urine caused by a patent urachus, and the rare occurrence of pallor and edema. At 1–2 days after birth, slight redness of the periumbilical skin is common, because of irritation from the cord clamp, and needs to be distinguished from an omphalitis or cellulitis. Bowel sounds should be auscultated before one proceeds to palpate the abdomen if the newborn is asleep or resting quietly.



• **Fig. 25.18** A small omphalocele that could be injured if the cord were to be clamped too close to its insertion. (From Rudolph AJ. *Atlas of the Newborn*. Vol 4. Hamilton, Ontario, Canada: BC Decker; 1997:109.)

Palpation should initially be gentle and superficial, to detect any signs of tenderness and the presence of an enlarged liver or spleen. Tenderness must be distinguished from the tendency of the newborn to stiffen the abdominal muscles in reaction to the touch of the examiner's fingers, which are usually colder than the newborn's skin. If the examiner's fingers remain in gentle contact within the same spot, the temperature difference quickly dissipates, and the baby will usually relax and allow the examiner to proceed without struggle. In the healthy newborn, the liver edge may be at or slightly above the right costal margin or palpable 1–2 cm below it. The spleen is rarely palpable unless it is enlarged. Gentle palpation of the lower abdomen can detect an enlarged bladder, which is the most common cause of a midline abdominal mass in newborns. Deep palpation to detect small masses or enlargement of the kidneys is most easily done soon after birth, before significant feeding, and when the newborn is quiet. However, a satisfactory examination can be done even in a crying newborn by one keeping the fingers in position and gradually increasing the depth of palpation each time the newborn briefly relaxes the abdominal muscles while taking a breath between cries. It is helpful to support the flank with one hand while palpating the abdomen to detect the kidney with the other or to palpate the abdomen with the thumb while supporting the flank with fingers of the same hand.

Percussion of the abdomen is not particularly helpful in routine examination, but it can sometimes help to define the boundaries of an enlarged liver or bladder.

Genitalia and Perineum

Brief inspection after delivery is usually sufficient to identify the newborn as male or female. (Evaluation and management of the newborn with ambiguous genitalia are discussed in detail in Chapter 97.) In both male and female newborns, a soft swelling or bulge in the inguinal area may be due to an inguinal hernia. The bulge typically appears or increases in size during crying and is easily reduced with gentle pressure when the newborn relaxes.

The perineum is inspected to locate the anus and assess the tone of the anal sphincter. Absence of a normal anal opening should be detected as part of the initial evaluation in the delivery room. However, external observation of an apparently normal anus does not guarantee internal patency of the anus. That is best confirmed by the normal passage of meconium.

The genitalia are mainly examined by inspection, supplemented by palpation, with the newborn in a supine, frog-leg position.

In the male newborn the foreskin normally covers the entire head of the penis, which is adherent to the glans. The urethral opening is usually hidden by the foreskin and need not be visualized if the foreskin is intact. The foreskin is typically incomplete if hypospadias is present, which allows the abnormal position of the urethral opening to be identified easily.

Congenital chordee, a ventral angulation of the head of the penis, may accompany hypospadias or occur in isolation. Chordee can be missed unless the examiner straightens the penis by gently retracting the skin along the shaft toward the base of the penis. In newborns who have a generous pad of subcutaneous fat at the base of the penis, this maneuver also helps to avoid a false impression that the penis is short.

Dribbling of urine or a weak stream is suspicious of bladder dysfunction or urethral obstruction.

The scrotal sac and inguinal areas are palpated to locate the testes and assess their size. Scrotal rugae usually appear at approximately 36 weeks' gestation and cover the entire scrotum at term. Enlargement of the scrotal sac is most commonly caused by a

hydrocele. Transillumination can help to distinguish a hydrocele from swelling because of congenital testicular torsion or other masses. Bowel sounds may be audible in a scrotum enlarged by an inguinal hernia.

In the female newborn the examiner must gently retract the labia majora laterally to allow full visualization. The sizes and positions of the labia minora, clitoris, urethra, and vaginal opening should be noted. Relative prominence of the labia minora is normal in preterm newborns.

Partial labial fusion and an increase in the size of the clitoris may represent virilization caused by congenital adrenal hyperplasia or related endocrine abnormalities. The posterior fourchette should be at least 1 cm from the anal opening.

Enlargement of the uterus because of hydrometrocolpos may produce a protruding perineal mass. Vaginal tags and mucoid vaginal discharge are common at birth, resulting from exposure to maternal estrogen. A slightly bloody vaginal discharge (pseudomenses) caused by hormonal withdrawal is common in healthy female newborns during the first week after birth.

Back

The back is inspected for asymmetry or abnormal positioning of the shoulders, ribs, and hips. The spine is inspected for straightness and palpated for the integrity and alignment of the posterior spinous processes. Major neural tube defects and large masses such as a large sacrocccygeal teratoma will often be detected prenatally or will be obvious on initial inspection in the delivery room. In the routine examination, the lumbosacral area should be inspected carefully for the presence of deep or unusual dimpling of the skin over the sacrum, for sinus tracts, for unusual tufts of hair, and for small masses such as a lipoma or hemangioma. Any of these findings may be associated with spina bifida occulta or tethering of the spinal cord.

Musculoskeletal System

The newborn's posture, muscle mass, and movements should be observed. Any localized swelling or tenderness should be noted. Major limb or skeletal malformations will usually be appreciated on initial inspection in the delivery room, but minor anomalies such as supernumerary digits, syndactyly, or nail hypoplasia can be missed. Limited movement of an extremity can be caused by trauma (most commonly a fractured humerus or clavicle) or by an intrinsic abnormality of the joint or limb. Although they generally require no treatment, fractures of the clavicle are sufficiently common that the clavicles should be specifically examined in every newborn. Crepitus and tenderness at the site of a clavicle fracture may be more easily detected if the examiner palpates a clavicle with one hand while using the other to elevate and rotate the ipsilateral shoulder.

Deformations of the extremities caused by in utero positioning are not always easy to distinguish from malformations on an initial examination. Mild inward bowing of the lower legs and feet (genu varum) is common in newborns. Congenital talipes equinovarus (club foot) is unlikely if an inward-turning foot can be brought easily to a neutral position. The angulation of the foot can be expected to normalize spontaneously.

Hips. Assessment for developmental dysplasia of the hip (DDH) is an important component of the examination for all newborns as the incidence is estimated at approximately 1 in 1000 live births. Risk factors for DDH include a family history, breech presentation in the third trimester, female sex, abnormal hip examination at birth, and incorrect lower extremity swaddling (Mulpuri et al.,

2015; Shaw and Segal, 2016) DDH may not be present at birth. Therefore surveillance for DDH should continue during infancy until the child is walking with the goal of preventing late DDH presentation (after 6 months of age).

The newborn hip examination begins with general inspection of the newborn. It includes attention to the resting posture and spontaneous movements. Any asymmetry or unusual positioning of the legs should be noted. Asymmetric gluteal or femoral skin folds may be signs of unilateral hip dislocation. In older children, a unilateral dislocation may also produce an apparent inequality of leg length, seen either with the legs in extension (Thomas sign) or with the feet flat on the bed and the knees bent (Galeazzi sign). A restricted range of motion, particularly abduction, is a clue that may detect either unilateral or bilateral dislocations.

The examiner may then proceed to performing the Barlow and Ortolani maneuvers. It is essential that *each hip is examined separately*. Minimal force should be used in performing either maneuver. The dislocation of the femoral head during a Barlow maneuver or its relocation during an Ortolani maneuver produces what is termed a clunk. The key element defining the *clunk* is a distinct sensation of abrupt movement of the femoral head as it passes over the rim of acetabulum and drops into or out of the socket. A dislocated or dislocatable hip has the distinctive clunk, whereas a subluxable hip is characterized by a feeling of looseness or sliding without a distinct clunk (Committee on Quality Improvement, 2009). Both maneuvers are performed with the newborn supine, starting with the legs held in neutral rotation and the hips flexed to 90 degrees but not more.

The Ortolani maneuver is the most important test for determining hip dislocation in a newborn. For this maneuver, the index and middle fingers of the examiner's hand are placed along the greater trochanter, with the thumb placed along the inner thigh near the knee. The other hand stabilizes the pelvis. The examiner gently abducts the hip by rotating the thumb outward while lifting the hip anteriorly with the fingers. A distinct sensation of movement is felt when a posteriorly dislocated hip relocates during abduction (visit <http://www2.aap.org/sections/ortho/> and scroll down to the Barlow–Ortolani video).

A positive Ortolani maneuver test finding prompts referral to an orthopedist. An ultrasound examination is not necessarily required/needed. Newborns with positive Barlow test results at either the newborn examination or the 2-week examination may have spontaneous resolution. However, these newborns should have sequential follow-up examinations as part of DDH surveillance (Shaw and Segal, 2016). For newborns with equivocal examination findings, the pediatrician should remain alert for the presence of other warning signs such as asymmetric creases, limited abduction, and apparent or true leg length discrepancies. Controversy remains regarding when additional diagnostic testing should be performed. Selective ultrasound screening may be performed in any newborn (male or female) if there were concerning findings on the initial newborn examination and/or in patients with significant risk factors (e.g., family history, breech presentation at birth, improper swaddling) because an ultrasound examination after 6 weeks of age and/or radiographs at 4–6 months of age may assist in detecting clinically silent DDH in the “high-risk” infant (Shaw and Segal, 2016).

Neurologic Examination

The neurologic system is assessed during the course of the general newborn examination and by the examiner performing some specific maneuvers to elicit neonatal reflexes. Because the healthy newborn's

responses vary with the state of alertness, and tolerance for prolonged examination is limited; elicitation of a perfect response to each maneuver should not be expected.

The newborn screening examination includes assessment of the newborn's alertness, spontaneous activity, posture, muscle tone and strength, head control, and responses to manipulation and handling. When an examination raises doubts, repeating selected parts at a later time may be more helpful in clarifying findings if the patient is otherwise stable.

The typical newborn will be alert for several hours after delivery but frequently becomes "sleepy" and uninterested in feeding for the remainder of the first 24 hours. If the newborn continues to be easily aroused and the examination findings remain otherwise normal, feeding will likely improve on the second day.

Alertness, tone, and activity are important components of the entire examination:

1. Normal newborns are easily awakened from sleep and remain alert through the remainder of the routine examination, shifting among states of quiet alertness, active alertness, and crying. The cry is vigorous when the newborn is upset, but a newborn should be able to self-console or should be consoled with holding, sucking, or feeding.
2. Diminished alertness, tone, or spontaneous activity are sensitive but nonspecific indicators of illness (e.g., neonatal sepsis, inborn errors of metabolism) rather than suggestive of a neurologic abnormality. A newborn who is stuporous or difficult to arouse is abnormal and needs further evaluation, as does a newborn who is unusually irritable or inconsolable.
3. Decreased tone or alertness occurring more than 24 hours after birth in a previously vigorous newborn is abnormal and requires prompt investigation for sepsis or other problems, including inborn errors of metabolism that can cause progressive neurologic signs because of accumulating toxic metabolites.

Sensory assessment relies on observation of the strength and quality of the newborn's movements in response to handling during the examination and to specific local stimulation, such as the elicitation of the palmar and plantar grasp reflexes, rooting reflex, and sucking reflex. This is obviously necessary because the newborn cannot verbally answer questions or voluntarily respond to commands.

Vision assessment is limited to observation of the following:

1. Pupillary constriction and blinking response to light (subcortical responses)
2. Newborn's visual attentiveness. A healthy term newborn is expected to visually fixate on and follow the examiner's face, but this can take considerable time to elicit.

Hearing assessment can be evaluated behaviorally by observation of the newborn's responses to the ringing of a bell or other sounds. However, this detects only profound, bilateral hearing loss, and it has been effectively replaced in the routine newborn examination by automated hearing tests ([American Academy of Pediatrics, 2007](#); [US Preventive Services Task Force, 2008](#)).

Motor function assessment relies on the observation of the newborn's posture and spontaneous movements, observation of the newborn's general responses to stimulation and handling, and the elicitation of specific reflexes. The scarf sign, forearm recoil, square window of the wrist, heel-to-ear maneuver, and popliteal angle are measures of tone and flexibility commonly scored in the GA assessment ([Dubowitz et al., 1970](#); [Ballard et al., 1991](#)).

In normal resting posture the elbows, hips, and knees are strongly flexed in the healthy term newborn.

Active tone and strength of upper extremity muscle groups are routinely assessed by elicitation of the palmar grasp and by arm traction during the pull-to-sit maneuver. Active tone and strength of lower extremity muscle groups are assessed during the general examination of the hips and feet and by testing for the supporting reaction, stepping reflex, Babinski reflex, and ankle clonus.

Neck flexors may be evaluated during the standard pull-to-sit maneuver or by the lifting of the shoulders to pull the baby to a sitting position. The neck *extensors* can be evaluated by the tilting of the newborn forward from a sitting position or can be evaluated along with truncal tone by the newborn being suspended in a prone position with the examiner's hand under the chest (ventral suspension maneuver).

During the examination, one should note any asymmetry and pay attention to qualitative characteristics such as the relative smoothness versus jerkiness or tremulousness of movement. Unusual jitteriness may be a sign of hypoglycemia or hypocalcemia, especially in newborns of diabetic mothers, newborns who are small or large for their GA, or newborns who were exposed to opiates or other drugs in utero and are experiencing withdrawal.

Passive restraint of an extremity should inhibit jittery movements but may not stop rhythmic contractions of seizure activity. The signs of neonatal seizures include:

1. Clonic movements
2. Tonic posturing
3. Repetitive stereotypical movements of the face or extremities
4. Tonic horizontal eye deviation or nystagmoid jerking
5. Staring or blinking
6. Apnea (distinct from apnea of prematurity, particularly for a term newborn)
7. Unexplained changes in heart rate or blood pressure

It is uncommon to observe an actual seizure during a routine newborn examination, but the pediatrician may be called to evaluate a newborn with a report of unusual movements.

In the otherwise healthy newborn who is fully alert and responsive, jerky or abrupt movements that are evoked by stimulation (e.g., startles), that can be suppressed by passive restraint (e.g., jitteriness), and that are not accompanied by autonomic changes or changes in alertness are unlikely to be seizures. Transient episodes of disconjugate gaze are also not unusual in normal newborns, particularly when the newborn is entering or awakening from sleep.

Neonatal Reflexes. The neonatal or primitive reflexes frequently tested during routine examination of the newborn include the Moro reflex, the asymmetric tonic neck reflex, truncal incurvation (Galant reflex), the palmar and plantar grasp reflexes, the Babinski reflex, and the placing and stepping reflexes.

The Moro reflex can be elicited following the pull-to-sit maneuver, by the lowering of the newborn until there is only a slight space between the neck and bed, and then allowing the newborn to fall back suddenly. Alternatively, the Moro reflex can be elicited by the "drop" method: the examiner lifts the baby completely off the bed, supporting the head and trunk with both hands and keeping the baby supine, and then rapidly lowers the baby by approximately 4–8 inches. The complete Moro reflex involves a quick bilateral abduction of the arms and extension of the forearms with full opening of the hands, followed by smoother and slower return of the hands toward the midline, with curling of the fingers.

The startle reflex is similar to the Moro reflex, but without full extension or hand opening, and may occur spontaneously or may be evoked by a sudden noise or movement.

The asymmetric tonic neck reflex demonstrates how neck position affects the tone of the extremities. This should be kept in mind during observation of the newborn's spontaneous movements, because it can cause a false impression of asymmetry if the position of the neck is not taken into account. To test the asymmetric tonic neck reflex, turn the newborn's head 90 degrees to one side for 15 seconds, keeping the newborn lying on the back with the shoulders horizontal. In a complete response, the ipsilateral arm and leg will extend and the contralateral arm and leg will flex, producing the "fencing" posture. The test is then repeated with the head turned to the other side. Observation of the complete response is reassuring, but its absence is not necessarily abnormal (partial and unidirectional responses are common too). However, an unusually sustained or exaggerated response is abnormal.

The truncal incurvation reflex is elicited with the newborn held in ventral suspension by the examiner stroking lightly down the back on one side and then the other. The normal response is for the newborn to curve the spine strongly, concave toward the stimulated side.

The supporting, placing, and stepping reflexes are elicited with the newborn held upright. The supporting reaction is elicited by the examiner lowering the newborn vertically until both feet touch the surface of the bed or table. A positive response, usually seen after a slight delay, is partial extension at the hips and knees, as though the newborn is attempting to stand and support his or her weight. The stepping reaction is tested by the examiner lowering the newborn so that one foot touches the surface, with the newborn tilted slightly forward. The newborn should flex that leg and extend the other, as though taking a step. The placing reaction is elicited by the examiner lifting the newborn to bring the dorsum of one foot in contact with the underside of a table or bassinet edge. In a positive response, the newborn lifts the foot up and places it on the top surface.

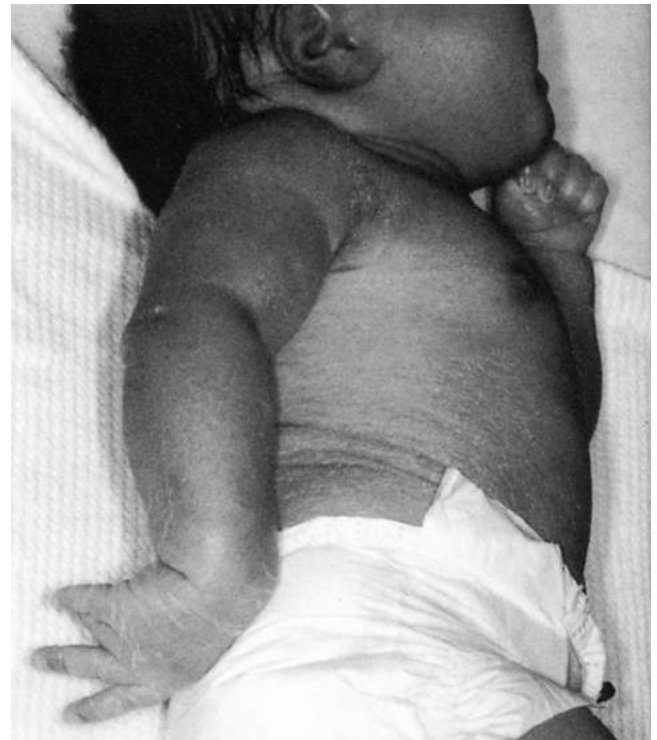
The Babinski reflex consists of dorsal flexion of the big toe and spreading of the other toes in response to stroking of the foot laterally (normally observed in newborns).

Firm pressure on the sole of the foot elicits the plantar reflex.

The deep tendon reflexes are usually not elicited during routine examination of the well newborn, but they are helpful as part of a more complete examination if neurologic abnormalities are suspected. The pectoralis, biceps, brachioradialis, thigh adductor, crossed adductor, knee jerk, and ankle jerk reflexes are the most readily elicited (Volpe, 2008). Ankle clonus may be elicited by quick dorsiflexion of the foot, which in a healthy term newborn should produce no more than approximately five beats of alternating extension and flexion with rapidly decaying intensity.

Brachial Plexus Injury. Although not common, brachial plexus injury is one of the more frequent neurologic abnormalities found in the otherwise healthy newborn, occurring in about 0.5–2 per 1000 live births. Often there is a history of difficult delivery because of shoulder dystocia. Brachial plexus injury can occur in isolation or in conjunction with fractures of the clavicle or humerus. Typical findings in Erb palsy are an inability to abduct and externally rotate the shoulder, flex the elbow, and supinate the forearm because of injury to C5–C6 (Volpe, 2008). If C7 is involved, wrist and finger extension are also weak (Fig. 25.19). A distal brachial plexus injury involving C8–T1 causes weakness of wrist and finger flexion.

Cranial Nerves. Observation and maneuvers during the routine examination provide at least partial assessment of all the CNs (Volpe, 2008) except for the olfactory nerve (CN I), which is not routinely tested. The corneal reflex can be tested by the examiner



• **Fig. 25.19** Hand and arm position in a newborn with Erb palsy involving C5, C6, and C7. (From Fletcher MA. *Physical Diagnosis in Neonatology*. Philadelphia: Lippincott-Raven; 1998:450.)

touching the cornea with a wisp of sterile cotton, but this is also not part of the routine newborn examination. Similarly, the masseter or jaw-jerk reflex can be elicited by the examiner placing the forefinger of one hand on the newborn's relaxed chin and tapping it with the forefinger of the other hand, but this is not routinely done either. Lastly, taste, mediated by CN VII (anterior two-thirds of the tongue) and CN IX (posterior third), is not evaluated during the routine examination.

Visual attentiveness, the ability to fix and follow, eyelid closing in response to light, and the pupillary light reflex require the optic nerve (CN II). The pupillary light reflex also tests the oculomotor nerve (CN III). CNs III, IV, and VI control extraocular movements.

Elicitation of rooting or a facial grimace in response to touching the face tests the sensory portion of the trigeminal nerve (CN V). The motor portion of CN V controls the muscles of mastication, which are involved in the jaw-closing phase of the suck and are assessed during elicitation of the suck when the newborn bites down on the examiner's finger.

Vertical gaze is difficult to assess in neonates, but horizontal gaze in both directions can usually be verified by observation of spontaneous eye movements or by the rotation test. The examiner performs the rotation test by turning in place while holding the newborn upright and supporting the back of the head.

If vestibular functions are intact, the newborn will turn the head in the direction of rotation or, if the head is restrained, turn the eyes in that direction. The doll's eye maneuver may also be used to assess CN VIII. The auditory portion of CN VIII is best assessed with use of brainstem auditory evoked responses, rather than behavioral responses to auditory stimulation.

CNs V, VII, IX, X, and XII are all involved in normal sucking and swallowing.



• **Fig. 25.20** Unilateral Facial Weakness in Two Newborns. There is mild facial asymmetry with flattening of the nasolabial folds at rest (A, C) and more obvious asymmetries of the grimace and eye closing during crying (B, D). The weakness is on the newborn's left side in (A) and (B) and on the newborn's right side in (C) and (D). (From Fletcher MA. *Physical Diagnosis in Neonatology*. Philadelphia: Lippincott-Raven; 1998:457.)

The facial nerve (CN VII) is required for pursing of the lips in sucking, as well as normal facial expression and tone. In utero positioning or forceps injuries that compress the facial nerve are common causes of unilateral facial weakness in the newborn period (Fig. 25.20).

CNs IX and X are needed for normal swallowing and the gag reflex. CN XII is involved in the milking action of the tongue during sucking and swallowing. CN XI is involved with sternocleidomastoid function and is assessed by evaluation of head flexion and lateral rotation. Because head control is often poor in normal newborns, it can be difficult to detect an abnormality unless it is unilateral. However, an asymmetric abnormality of a sternocleidomastoid is caused more commonly by torticollis than dysfunction of CN XI.

Suggested Readings

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Online Resources

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Pediatric neurologic examination, Available at http://library.med.utah.edu/pedineurologic-exam/html/home_exam.html. A tutorial with video demonstrations and descriptions by PD Larsen and SS Stensaas.

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26

Newborn Nursery Care

JAMES A. TAYLOR, JEFFREY A. WRIGHT, AND DAVID WOODRUM

KEY POINTS

- Interventions for newborns during the newborn nursery stay should be limited to those for which the evidence of benefit is clear.
- The short-term and long-term benefits of breastfeeding are clear. The effects on breastfeeding rates from interventions such as formula supplementation, frenotomy, and restriction of pacifiers are controversial.
- Comprehensive guidelines for management of common newborn conditions such as jaundice, risk of sepsis, and hypoglycemia are available. However, whether use of these guidelines improves outcomes is unclear.
- Online aids are available to clinicians to help guide individualized management of jaundice and weight loss in both breastfed and bottlefed newborns.
- With the implementation of universal maternal screening for group B streptococcus and the use of intrapartum antibiotic prophylaxis, rates of sepsis in term newborns have fallen significantly. Online aids are available to guide clinicians in assessing the risk of sepsis.
- Prenatal ultrasonography can diagnose multiple newborn conditions early. However, the natural history of many common ultrasound findings is variable, and the findings may, or may not, represent markers for serious disease.
- Pulse oximetry screening for critical congenital heart disease is recommended for healthy newborns. False-positive rates are low, but there is a substantial false-negative rate.

Two central paradoxes underlie the care of normal newborns. First, although birth is, almost by definition, the most natural of all human processes, until very recently the newborn mortality rate has been extraordinarily high. The second paradox in providing newborn care is that neonates are both the healthiest and the most vulnerable patients in medicine. Recent medical history is replete with examples of the pendulum swinging too far in each direction around these paradoxes. The promotion of scheduled feeding using infant formulas rather than breastfeeding is an example of the overmedicalization of neonatal care. Conversely, recent resistance to treatments that prevent uncommon, but disastrous, conditions represent a denial of the benefits provided by medical care.

Thus optimal care of a normal neonate is an attempt to balance these competing forces. Systems of care should be designed to support the concept that newborns are extraordinarily healthy and require little intervention beyond promotion of breastfeeding. Those interventions for which there is clear evidence that the benefits outweigh the risk should be provided as unobtrusively as possible. Simultaneously, while promoting “natural” care for these newborns,

healthcare providers need to be vigilant for the early identification of neonates who are at risk of conditions such as dehydration, sepsis, and severe hyperbilirubinemia.

The goal of this chapter is to provide an evidence base for the promotion of normal newborn care by parents, the rationale for monitoring term neonates for various conditions, a risk–benefit analysis of common treatments, and the significance of common prenatal and postnatal findings. Rather than providing a comprehensive prescription on how to care for these newborns, we hope that the reader will integrate the information provided in this chapter with expert opinion and his or her own clinical experience to determine the proper management of normal newborns.

Initial Assessment

The timing of the initial assessment of a term newborn is dependent on the condition of the newborn and parental preference. In most instances a healthcare professional who is present at the birth will make a general appraisal of the newborn and alert the child’s provider if there is an acute problem necessitating an immediate evaluation. Usually the neonate will be healthy, and the assessment can be timed so as not to interfere with breastfeeding, bonding with the family, and routine care.

Before a well newborn is examined, the mother’s medical history should be reviewed to identify issues that could affect the care or prognosis of the newborn. For example, a history of diabetes in the mother would lead to glucose testing in the neonate. Maternal drug use should be assessed for possible teratogenic effects, possibility of symptoms of withdrawal in the newborn, and compatibility with breastfeeding. It is important to review the pregnancy history, focusing on estimated gestational age (GA), the results of screening for genetic conditions, and the results of prenatal ultrasound examinations. Perinatal events such as the type of delivery, length of time that membranes were ruptured, and Apgar scores should also be reviewed. Finally, it is critical to review the mother’s social history to ensure that the newborn will be raised in a nurturing environment and to identify high-risk situations for which interventions are needed before, or shortly after, discharge from the newborn nursery.

The results of several laboratory tests commonly performed on pregnant women will determine the need for treatment and/or monitoring during the newborn nursery stay. These include maternal HIV and hepatitis B (HBV) surface antigen status and syphilis testing. The mother’s blood type, Rhesus (Rh) status, and antibody test results are useful in identifying newborns with an increased risk of hyperbilirubinemia. It is important to note the results of testing for maternal colonization with group B streptococcus (GBS)

and the type and timing of antenatal antibiotic prophylaxis in mothers who are GBS positive.

The newborn's weight, length, and head circumference should be measured shortly after birth and plotted on a standardized chart. Although the most common reason for a significant discrepancy between weight, height, and head circumference percentiles is an inaccurate measurement, a valid discrepancy warrants close clinical observation or testing. Glucose testing may be indicated for newborns found to be small or large for their GA. If the estimated GA of the newborn is inconsistent with the growth parameters, then a formal evaluation by a Dubowitz–Ballard GA assessment may be helpful (Ballard et al., 1979).

When a newborn is examined for the first time, the initial focus is directed toward an overall assessment of the child's health. Observation and auscultation of the chest allow detection of an irregular heart rate, murmur, or acute lung condition such as pneumothorax. The heart rate and respiratory rate can be measured. Normal values for heart and respiratory rate in a newborn are 100–160 beats per minute and 35–60 breaths per minute respectively. Evaluation of skin color may be useful for the identification of a neonate with cyanotic congenital heart disease or pulmonary conditions. If uncertainty exists about the presence of cyanosis, oxygen saturation can be quickly measured with a pulse oximeter. The newborn's tone, general posture, and movement should be assessed; abnormalities may be indicative of an acute or chronic central nervous system problem or sepsis.

Routine Testing

Glucose

The fetal blood glucose level is approximately 70% of that of the maternal level. Following birth and separation from its major energy supply, the newborn's glucose level falls, on average, by a factor of two. Over the next several hours, it gradually increases to a level approaching that of older newborns (Fig. 26.1). The critical factors involved in this normal adaptive process include transient inhibition of the newborn's insulin secretion and an increase in levels of the counterregulatory hormones: growth hormone, cortisol, catecholamines, and glucagon (Adamkin, 2015). The effect of this is to promote liver glycogen breakdown, gluconeogenesis, and tissue lipolysis. Clinical scenarios that might indicate the need for early glucose screening and possible therapeutic intervention include:

- Any newborn who demonstrates clinical signs of hypoglycemia (Box 26.1)
- Newborns of diabetic mothers/large for GA newborns
- Newborns demonstrating intrauterine growth retardation
- Premature newborns
- Newborns delivered after in utero and/or intrapartum distress
- Family history of congenital hyperinsulinism

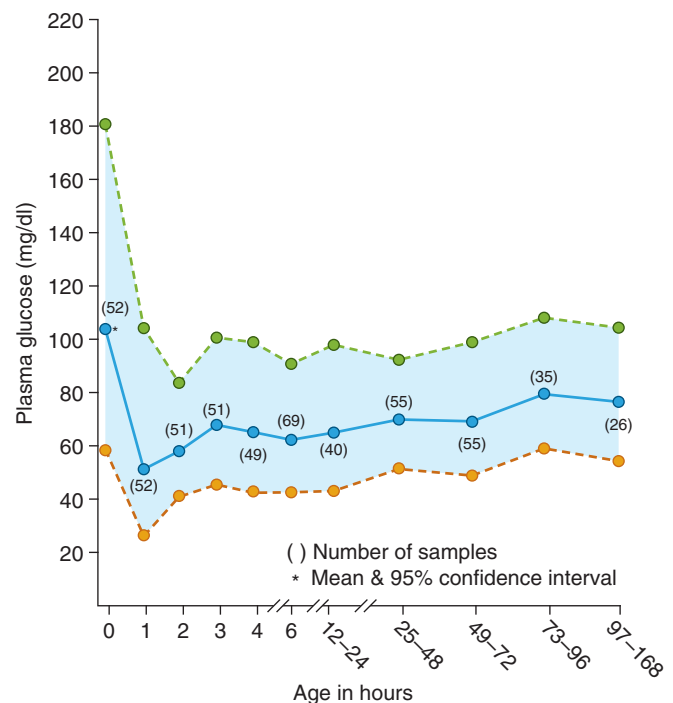
• BOX 26.1 Clinical Signs Compatible With Hypoglycemia

Poor feeding
Lethargy
Hypotonia
Irritability
Tremor
Seizure-like activity
Apnea

- Newborns with midline facial anomalies that might be markers for pituitary deficiency

The treatment approach to confirmed hypoglycemia depends on the glucose level and/or the presence of clinical signs. Newborns with symptomatic hypoglycemia require immediate intervention (Committee on Fetus and Newborn and Adamkin, 2011). However, while it is apparent that severe and symptomatic hypoglycemia result in brain injury leading to developmental and other issues, the effects of less severe and asymptomatic hypoglycemia on the neonatal brain are much less clear (Burns et al., 2008; Rozance and Hay, 2012). Thus there is considerable debate regarding the definition of *hypoglycemia* and an actionable glucose level in this early transition period in asymptomatic newborns. In an attempt to provide a rational and standardized approach, the American Academy of Pediatrics (AAP) published a guideline on neonatal hypoglycemia in 2011 (American Academy of Pediatrics. Committee on Fetus and Newborn and Adamkin, 2011). It is recommended that asymptomatic, at-risk term and late preterm newborns who have glucose levels below 25 mg/dL in the first 4 hours after birth or levels below 35 mg/dL at 4–24 hours of age should receive intravenous glucose. Early feeding and retesting are suggested for those with glucose levels between 25 and 45 mg/dL, depending on age. Whereas a “target” glucose level greater than 45 mg/dL before routine feeds is recommended, the guideline authors acknowledge that there is no clear evidence that this is the appropriate threshold for defining a normal glucose level in newborns (American Academy of Pediatrics. Committee of Fetus and Newborn and Adamkin, 2011).

Following publication of the guideline, two large studies on neonatal hypoglycemia have had somewhat contradictory results.



• **Fig. 26.1** Predicted plasma glucose values during the first week of life in healthy term neonates with birthweight appropriate for their gestational age. Green and orange lines denote upper and lower limits of 95% confidence interval, respectively, around the mean values (blue line). (Modified from Srinivasan G, Pildes RS, Cattamanchi G, Voora S, Lilien LD. Plasma glucose values in normal neonates: a new look. *J Pediatr*. 1986;109:114–117.)

First, among a group of almost 1400 fourth grade children who had universal glucose screening during their birth hospitalizations, those with transient hypoglycemia, defined as a glucose level below 35 mg/dL, below 40 mg/dL, or below 45 mg/dL, were significantly more likely to score below grade level on standardized testing for literacy and mathematics proficiency than those without hypoglycemia. The odds ratios for lack of proficiency related to hypoglycemia ranged from 1.28–2.33, and there was some suggestion of a “dose effect” with severer hypoglycemia leading to worse outcomes (Kaiser et al., 2015). Conversely, in a prospective study of 528 newborns at risk of hypoglycemia, no association between hypoglycemia (defined as a glucose level <47 mg/dL) and developmental assessment at 2 years of age was found (McKinlay et al., 2015). In this study, newborns were managed with a goal of maintaining a glucose level greater than 47 mg/dL; neither the number of hypoglycemia episodes nor the lowest glucose concentration was associated with abnormal development. Further, study participants underwent continuous interstitial glucose monitoring, but the results of this monitoring were not available to the treating clinicians. Nearly 25% of study newborns had undiagnosed and untreated hypoglycemia identified with continuous monitoring, some for several hours. These episodes of unrecognized hypoglycemia were also not associated with developmental delays.

Taken in their entirety, the results of these two large studies suggest that it is “safe” to follow the AAP hypoglycemia guideline; screening and treatment protocols designed to maintain glucose levels greater than 45 mg/dL are associated with good developmental outcomes. It is much less clear if this is the “best” approach. The higher the threshold for defining hypoglycemia, the more testing and treatment there will be in asymptomatic newborns. It is possible that, in the absence of a clear benefit, more screening and more treatment will actually lead to more harm than good (McKinlay and Harding, 2015). In addition, although the rate of hypoglycemia is greater in newborns at risk, at any given time in a typical newborn nursery most newborns with hypoglycemia will be term newborns without any risk factors, simply because there are so many more of these neonates (Kaiser et al., 2015). Thus most hypoglycemia in a newborn nursery goes undiagnosed if screening is limited to those at risk.

Newborn Metabolic Screening

Newborn screening (NBS) for metabolic disorders began in 1962 when 29 states participated in a trial of testing for phenylketonuria (PKU). With the implementation of screening programs, criteria were proposed for determining which conditions should be included in screening programs. It was recommended that only disorders that were important health problems be included in screening programs. The condition should be detectable before the onset of significant symptoms. Importantly, a specific treatment to prevent adverse clinical consequences from the disorder should be available, and the screening program for the condition should be cost effective (Tarini, 2007). On the basis of these criteria, conditions such as congenital hypothyroidism and congenital adrenal hyperplasia were slowly added to NBS tests in many states, and subsequently conditions such as sickle cell disease were added. Although there is no specific treatment for sickle cell disease, there was evidence that the use of an NBS program to identify newborns with the disorder led to early initiation of penicillin treatment, which resulted in fewer deaths from sepsis than when newborns were identified at the onset of symptoms (Vichinsky et al., 1988). Given the demonstrable effectiveness of early identification, sickle cell disease met the criteria for newborn screening.

The advent of tandem mass spectrometry in the 1990s revolutionized newborn metabolic screening. With this technology it is possible to test for a multitude of conditions on a very small sample of blood. In 1995 the average number of conditions included in state-mandated screening programs was 8; by 2005 this had increased to 19, with some states testing for up to 46 conditions. Unfortunately, this increase in NBS has been controversial. Some of the conditions included do not meet the long-established criteria for screening in that there are no known effective treatments, and, in some cases, it is not known whether the targeted condition always leads to disease. In addition, with increasing numbers of tests come increasing numbers of false-positive results, leading to increased parental anxiety and potential for overuse of medical services (Tarini et al., 2006; Berry, 2015).

In an attempt to define a rational list of disorders for which NBS is appropriate, the American College of Medical Genetics used an iterative process to identify 29 “core conditions” that should be included in mandatory screening programs (Watson et al., 2006). Subsequently, federal legislation was passed to facilitate standardization of NBS across the United States. This legislation led to the development of the Recommended Universal Screening Panel, which initially included 29 core disorders. All US states currently provide testing for all of these disorders (Berry, 2015). The recommended screening panel has now been expanded to include 32 core disorders and 26 secondary disorders. In addition to newborn hearing screening and screening for critical congenital heart disease (CCHD), there are nine organic acid, five fatty acid oxidation, six amino acid, two endocrine, three hemoglobin, and four other conditions included in the list of core disorders. Information on the screening program in each state in the United States and on specific disorders can be found at <http://www.babysfirsttest.org>.

The most common disorders included on newborn metabolic screens in the United States are congenital hypothyroidism (1 case per 3000–4000 newborns) and sickle cell disease (Kaye et al., 2006; Hertzberg et al., 2011). The incidence of PKU is approximately 1:15,000 (Serving the family from birth to the medical home, 2000). For many of the core conditions for which screening is now recommended, the incidence rates are in the 1:100,000 to 1:200,000 range (Kaye et al., 2006). For some disorders, the incidence rate is unknown.

Hearing Screening

Newborn hearing screening has become universal in the United States, with more than 97% of newborns screened in 2013. Every state and territory in the United States has now established an early hearing detection and intervention program and is required to provide tracking data. Newborns who do not “pass” the hearing screen in the newborn nursery should be referred for more definitive testing in a timely manner. Ultimately, in 4.8%–10.3% of newborns who do not pass the hearing screen, permanent hearing loss is diagnosed; the current rate of hearing loss in the United States is approximately 1.5 per 1000 newborns screened (Williams et al., 2015; Summary of 2013 National CDC EHDI Data, 2016). Many other countries have adopted or are in the process of adopting universal hearing screening. Experts from the World Health Organization endorsed universal newborn hearing screening in 2009. It is reported that 80% of early childhood hearing loss is congenital and that most cases have genetic origins and/or are a result of cytomegalovirus infection (Declau et al., 2008; Lammens et al., 2013).

There is growing evidence that early intervention with amplification or cochlear implants can improve childhood reading, language,

and communication skills (Vohr et al., 2008; McCann et al., 2009; Ohmori et al., 2015; Stika et al., 2015). These treatments are effective when implemented by 6 months of age, preferably younger. This creates time urgency to verify an initial screen with a test and make referrals to specialists who can provide treatment. This process is particularly challenging in rural areas and in countries with limited access to these services (Bush et al., 2015).

A significant challenge is to avoid labeling a child as abnormal with this screening process. Of the newborns who fail to pass their newborn hearing screen, more than 80% will be found to have normal hearing on follow-up testing (Nelson et al., 2008). Given this false-positive rate, approximately 8–10 newborns with normal hearing will be referred for follow-up testing to identify newborns with hearing loss (Nelson et al., 2008). The risk of labeling a child as possibly abnormal can cause permanent alteration of the parent–child relationship, a condition dubbed *vulnerable child syndrome* (Pearson and Boyce, 2004).

To decrease the risk of false-positive tests and vulnerable child syndrome, it is recommended that the term *refer* be used instead of *fail* when the screen results are being discussed. Babies have an increased “refer” rate when born by cesarean delivery or screened during the first day of life, so it is best to wait until the third or fourth day to screen newborns whenever possible (Lupoli Lda et al., 2013; van Dyk et al., 2015; Xiao et al., 2015). Many nurseries have adopted a two-step process using an automated otoacoustic emissions (OAE) test for the first step followed by a brainstem auditory evoked potential test in those who do not pass the automated OAE test. This process has been shown to decrease the false-positive rate (Papacharalampous et al., 2011; Caluraud et al., 2015). All newborns who are at high risk of early hearing loss should be sent directly for auditory brainstem response (ABR) screening. High-risk factors include premature birth, family history of early childhood or infant hearing loss, craniofacial anomalies or abnormal ear examination findings (includes microtia but not tags), and exposure to aminoglycoside antibiotics.

False-negative screens are also a concern but the rate is low. A screen will be falsely negative in 0%–2% of newborns (Johnson et al., 2005; Cebulla et al., 2014). With use of ABR screening, the false negative rate is lower.

Screening for Critical Congenital Heart Disease

It has been estimated that approximately 25% of newborns with congenital heart disease have “critical” lesions, defined as a lesion requiring surgery and/or cardiac catheterization in the first year of life (Mahle et al., 2009). Overall, CCHD is diagnosed in less than half of newborns prenatally, and 25%–30% are not identified as having CCHD during the birth hospitalization (Peterson et al., 2014). Further, some neonates with lesions that are amenable to surgical intervention who are not identified as having CCHD before discharge from their birth hospitalization may die from their CCHD before a clinical diagnosis is made (Peterson et al., 2014). Because of this, it is now recommended that all newborns be screened with pulse oximetry for CCHD before discharge from their birth hospitalization (Mahle et al., 2012).

Pulse oximetry screening is based on the concept that most, but not all, CCHD lesions lead to hypoxemia in the affected newborn. To minimize false-positive results, pulse oximetry screening should be delayed until newborns are 24 hours or older, if possible. Conversely, since some newborns with CCHD have different oxygen saturation levels when measured preductally or postductally, false negatives results are reduced by screening newborns both in the right hand (preductal) and in a lower extremity (postductal). Newborns with

a measured oxygen saturation level of less than 90% at either site are classified as having a positive screen and should be evaluated by a pediatric cardiologist on an urgent basis. Repeated screening is recommended for newborns with oxygen saturation levels of 90% or greater and less than 95% or with a difference of 3 or more percentage points between the right hand and the lower extremity; if either of these findings persists after three screenings, the screen is considered positive (Kemper et al., 2011).

There were initially concerns that wide-scale implementation of universal pulse oximetry screening would result in a high proportion of false-positive results, leading to unnecessary and expensive evaluations of normal newborns. Thankfully, these concerns have been largely unfounded, with observed false-positive rates of less than 0.5%. However, the sensitivity of pulse oximetry screening is only approximately 77% (Thangaratinam et al., 2012). Sophisticated models have indicated that the number of “true” positives might be approximately matched by the number of false-negative results (Ailes et al., 2015). Given this, a diagnosis of CCHD should be seriously considered in a young infant with signs or symptoms of one of the lesions that screening is designed to identify despite the presence of negative screening results during the birth hospitalization.

Prenatal Ultrasound Screening for Birth Defects

Ultrasound screening for fetal anomalies has become increasingly routine. Major fetal organ system abnormalities can, for the most part, be identified, and the mother can be referred for appropriate fetal and neonatal management. There are, however, a number of ultrasound findings that have a variable natural history, which may or may not be markers for serious conditions and do not always result in a definitive prenatal work-up. These findings often do not fit within the pediatric lexicon. They can present a challenge to the pediatrician when it comes to parent counseling and/or determining management in the neonatal period.

Central Nervous System Findings

Choroid plexus cysts are found in 2%–4% of second trimester fetal ultrasound examinations. They are transient, functionally benign in nature, and generally resolve spontaneously before term. If one or more choroid plexus cysts are found in isolation on prenatal ultrasound examination, no adverse effect on fetal growth and development has been noted. Thus without other risk factors, no further evaluation is needed in an infant with this isolated finding who has had a benign prenatal and a normal postnatal course (Ebrashy et al., 2016). Choroid plexus cysts are believed to be a “soft marker” for aneuploidy (particularly trisomy 18) when associated with other fetal anomalies or with maternal risk factors, such as advanced maternal age. In such situations current recommendations are to begin an appropriate prenatal evaluation, that is, karyotyping (DiPietro et al., 2006; Lopez and Reich, 2006; Sohaey, 2008a).

Agensis of the corpus callosum is reported to occur in 0.3%–0.7% of unselected postnatal populations. Aneuploidies have been reported in 10%–20% of children with this prenatal ultrasound finding. Major organ system abnormalities are reported to occur in up to 60% of such fetuses. Notably, when absence of the corpus callosum is an isolated fetal ultrasound finding, the reported rate of a relatively normal developmental outcome ranges from 50% to 75%. However, well-conducted long-term follow-up studies are lacking. It is recommended that fetal magnetic resonance imaging be considered when one is confronted with this diagnosis since other abnormalities have been identified in more than 50% of such fetal patients. Postnatal management for infants with a

history of agenesis of the corpus callosum on prenatal ultrasound examination should include, at a minimum, close clinical assessment and indicated work-up (Fratelli et al., 2007; Chadie et al., 2008; Winter, 2008; Sotiriadis and Makrydimas, 2012).

Mild, isolated ventriculomegaly is a relatively uncommon fetal ultrasound finding that may be a soft marker for aneuploidy, fetal infection, or other central nervous system abnormalities. As such, it is recommended that serial imaging studies and, in some cases, more extensive work-up be undertaken. In the presence of a benign fetal assessment, most newborns appear to do reasonably well following delivery. It is important to consider close developmental follow-up and serial imaging studies (Leitner et al., 2009; Melchiorre et al., 2009; Devaseelan et al., 2010).

Cardiac Findings

Echogenic cardiac focus is an incidental ultrasound finding in 3%–4% of normal fetuses. Notably, there is an increased incidence (10%–30%) in Asian populations. It is said to be a soft marker for chromosomal abnormalities (trisomy 21 and trisomy 13) when associated with other screening abnormalities. Further work-up may be indicated in high-risk populations. If the physical examination findings for a newborn are normal and there are no other ultrasound findings, no further evaluation is suggested (Borgida et al., 2005; Koklanaris et al., 2005; Ouzounian et al., 2007; Sohaey, 2008b Rodriguez et al., 2013).

Gastrointestinal Findings

Echogenic bowel when noted to be present during a second trimester ultrasound examination and determined to be grade 0 or 1 (i.e., less echogenic than bone) is considered a normal variant with a good prognosis. No special prenatal or postnatal work-up is recommended. Anything of density equal to or greater than that of bone (grade 2 to 3) is abnormal and potentially a marker for cystic fibrosis, trisomy 21, gastrointestinal anomalies, in utero infection, bowel ischemia or bleeding, intrauterine growth restriction, and/or impending in utero demise. Appropriate work-up is indicated (Goetzinger et al., 2011; Ebrashy et al., 2016).

Cholelithiasis is an uncommon third trimester fetal ultrasound finding that needs to be differentiated from hepatic calcification. Cholelithiasis is considered a benign condition requiring no special evaluation or treatment but careful clinical follow-up. An imaging examination at 1 year of age for a child with this prenatal finding may be helpful in documenting expected resolution (Sohaey, 2008d; Triunfo et al., 2013).

Hepatic calcifications are uncommon fetal ultrasound findings. They are often isolated, single and, in a low-risk mother, of no significance. However, when numerous, hepatic calcifications may be markers for fetal aneuploidy, infection, meconium peritonitis, hepatic tumor, or vascular insult. A significant percentage are associated with some form of fetal disease. Neonatal management depends on the prenatal work-up and the clinical presentation in the newborn period (Simchen et al., 2002; Pata et al., 2012).

Urinary Tract Findings

Mild fetal pelviectasis is one of the more common abnormalities detected by second trimester ultrasound examination, with a reported incidence of 0.5%–5% in unselected pregnant populations. Diagnostic criteria differ but generally include a second trimester renal pelvis diameter of more than 4 mm and less than 10 mm and a third trimester renal pelvis diameter of more than 7 mm and less than 10 mm. Renal pelvis diameters of 10 mm or greater are always considered abnormal. Some experts consider mild fetal

pelviectasis to be a soft marker for aneuploidy, especially trisomy 21. When mild, fetal pelviectasis is an isolated finding, the prognosis is good, and the condition often resolves either in utero or during early childhood. In a metaanalysis it was reported that only 11% of children with a history of mild fetal pelviectasis demonstrated postnatal disease. The authors of a prospective cohort follow-up study reported uropathy in 18% of their cases. Authorities thus recommend a postnatal follow-up renal ultrasound examination approximately 1 week after birth and, if necessary, at 1 month of life to document resolution (Lee et al., 2006; Coelho et al., 2007; Nguyen et al., 2010; Sohaey, 2008d; Ebrashy et al., 2016).

Car Seats and the Newborn Car Seat Challenge

All states have laws that require the use of car seats for children. It is important that parents purchase only approved car seats, so bargain-hunting at used child equipment stores should be done with caution. Lists of approved seats are available online at websites such as <https://www.healthychildren.org/English/safety-prevention/on-the-go/Pages/Car-Safety-Seats-Product-Listing.aspx>.

The observation that preterm newborns had episodes of hypoxia when monitored in car seats led the AAP to recommend in 1991 that preterm newborns be observed and monitored for apnea, bradycardia, or oxygen desaturation in their car safety seat before hospital discharge—the so-called car seat challenge (American Academy of Pediatrics Committee on Injury and Poison Prevention and Committee on Fetus and Newborn 1991, 1996; American Academy of Pediatrics Committee on Injury and Poison Prevention, 1999). In the United States, the car seat challenge expanded to include late preterm newborns, most of whom did not have respiratory problems during their hospital stay. It has been reported that 25% of late preterm newborns do not fit securely into standard car safety seats, and 12% of healthy late preterm newborns have apneic or bradycardic events in their car seats (Merchant et al., 2001). The rate of failure (about 4%) has been found to be about the same in small for GA babies as those born late preterm.

The authors of a Cochrane systematic review questioned whether or not car seat trials actually prevent morbidity or death and if there might be adverse effects from not passing this “screen,” such as prolonging the hospital stay or creating parental anxiety. Their review did not discover any randomized trials, and they concluded that “it is unclear whether undertaking a car seat challenge is beneficial or indeed whether it causes harm” (Pillely and McGuire, 2006). Since then, there has been one randomized trial in healthy term newborns comparing car seats with car beds, and no differences were found in the rates of oxygen desaturation or apnea events (Kinane et al., 2006). A study comparing a polysomnogram with the car seat challenge showed that the challenge has a low negative predictive value when compared with polysomnogram (Schutzman et al., 2013).

Routine and Common Medical Treatments

Prevention of Ophthalmia Neonatorum and Conjunctivitis

Approximately 15%–20% of babies will develop conjunctivitis in the first few weeks of life. Conjunctivitis can be caused by a sexually transmitted bacterium, normal skin or nasopharyngeal flora, or chemical irritation (Krohn et al., 1993). In addition, eye discharge

can be caused by obstruction of the nasolacrimal duct rather than from conjunctivitis. The most worrisome infection is that of *Neisseria gonorrhea*, which can invade the cornea in a matter of hours and lead to blindness. Despite effective preventive measures known since the 1880s, thousands of children are still blinded by this infection worldwide each year.

Most states in the United States have laws or regulations requiring administration of topical antibiotic ointment to the conjunctivae of babies within a few hours of birth. This practice has been effective in reducing the cases of blindness caused by gonococcal conjunctivitis. It is moderately effective in preventing conjunctivitis caused by chlamydia. The main risk of antibiotic ointment application is that it may cause a chemical conjunctivitis. Silver nitrate solution instilled into both eyes immediately after birth was the standard of care for many years, but it caused a high rate of chemical conjunctivitis.

Parents may question the need to expose their babies to eye medication, especially if the mother has been tested and found to be without gonorrhea or chlamydia. Some countries have stopped routine administration of eye prophylaxis. In those countries, an increase in infection, primarily caused by chlamydia, has been noted.

There has been an ongoing search for alternative prophylaxis that causes less chemical conjunctivitis and is not in an ointment base. A variety of prophylactic treatments have been recommended, including 1% nitrate solution, 1% tetracycline solution, 1% erythromycin solution, 2.5% povidone–iodine solution, fusidic acid, and freshly expressed breast milk. Among those, tetracycline has been reported as most effective (Zuppa et al., 2011). There is lay literature recommending the instillation of colostrum or breast milk into the eyes of babies to prevent or treat conjunctivitis. Although colostrum has antimicrobial action, its efficacy has not been adequately studied.

Povidone–iodine solution has been shown to be more effective and cause less irritation than erythromycin ointment. It is also less expensive but is not yet approved for this use by the US Food and Drug Administration (Ali et al., 2007). A major concern is that medical errors can occur if povidone–iodine soap is mistakenly substituted for the solution; it can cause eye damage. Fusidic acid has been used for preoperative prophylaxis for a number of surgical procedures in adults. However, data on its use in newborns are limited (Zuppa et al., 2011).

Vitamin K

Vitamin K is necessary for biologic activation of several human proteins, most notably coagulation factors II (prothrombin), VII, IX, and X. Since placental transfer is limited, umbilical cord blood levels of vitamin K₁ (phyloquinone) are 30-fold lower than maternal levels. Intestinal bacteria synthesize menaquinone (vitamin K₂), which has 60% of the activity of phyloquinone. However, neonates have a decreased number of bacteria in their gut that manufacture vitamin K₂; levels of this form of vitamin K are not found in the livers of infants until they are 2–3 months old. Thus newborns are deficient in vitamin K at birth and are at risk of significant bleeding. Fortunately, intramuscular (IM) vitamin K rapidly activates clotting factors, greatly decreasing this risk.

Three presentations of vitamin K–deficient bleeding (VKDB) have been described. “Early” VKDB presents in the first 24 hours after birth, is not prevented by postnatal administration of vitamin K, and usually occurs in newborns born to mothers who are taking medications that inhibit vitamin K. Common medications that

inhibit vitamin K include many anticonvulsants, isoniazid, rifampin, warfarin, and some antibiotics, such as cephalosporins. Early VKDB is frequently serious because of intracranial and/or intraabdominal hemorrhage. It is estimated that in neonates at risk of early VKDB the incidence is as high as 12% (Van Winckel et al., 2009).

“Classic” VKDB occurs in newborns during the first week of life. Although the presentation is often mild, blood loss can be significant, and intracranial hemorrhages have been reported. Although estimates differ, the incidence of classic VKDB, in the absence of vitamin K supplementation, is 0.25%–1.7% (American Academy of Pediatrics Committee on Fetus and Newborn, 2003).

“Late” VKDB occurs between the ages of 2 and 12 weeks and is usually severe. The mortality rate from late VKDB is approximately 20%, and 50% of infants with this disorder develop intracranial hemorrhages. Late VKDB is associated with exclusive breastfeeding. Human milk contains only 1–4 µg of vitamin K per liter, while commercially available formula contains 50 µg/L or more. In exclusively breastfed neonates who do not receive supplemental vitamin K, the incidence of late VKDB is estimated at 4.4–7.2 per 100,000 (or 1 per 15,000 to 1 per 20,000) (Van Winckel et al., 2009).

Vitamin K administered shortly after birth is effective in preventing classic and late VKDB. Since 1961, the recommended dose of vitamin K for term newborns born in the United States has been 1 mg given intramuscularly. However, the results of a study suggesting an association between intramuscularly administered vitamin K given at birth and childhood cancer created controversy regarding this practice (Golding et al., 1990, 1992). The results of subsequent studies strongly suggest that there is no increased risk of solid tumors in children given vitamin K intramuscularly (Puckett and Offringa, 2000).

Because of those previous concerns regarding an increased risk of childhood cancers, a switch to orally administered vitamin K occurred in some countries but not in the United States. It is apparent that a single oral dose of vitamin K has efficacy similar to that of an IM dose in preventing classic VKDB but offers less protection against late VKDB. Repeated doses of an oral vitamin K preparation until an infant is 8–12 weeks old increases the efficacy of this route of administration. However, it is not clear that even multiple doses of an oral formulation of vitamin K are as effective as a single IM dose given at birth. In a multinational review, the rates of late VKDB in infants receiving various regimens of orally administered vitamin K were mostly in the range of 1.2–1.8 per 100,000 compared with no cases in 325,000 children receiving an IM dose (Cornelissen et al., 1997). The oral regimens assessed included a birth dose of 1 mg. The reported rates of VKDB in newborns who received 2 mg orally at birth, with repeated doses subsequently, are lower but still somewhat higher than in neonates treated with intramuscularly administered vitamin K (Von Kries et al., 2003; Busfield et al., 2007). Early data from the Netherlands where infants received 1 mg orally at birth and 25 µg daily for up to 12 weeks suggested that this regimen was as efficacious as an IM dose (Cornelissen et al., 1997). However, in a subsequent study from the Netherlands, the rate of late VKDB was 3.2 per 100,000 in a group of infants with undiagnosed biliary atresia who had been treated with this dosing schedule (van Hasselt et al., 2008). Unrecognized cholestatic liver disease is a significant risk factor for VKDB. Finally, no cases of late VKDB were found among 396,000 Danish infants who received an oral dose of 2 mg of vitamin K at birth and 1 mg weekly until the age of 3 months (Hansen et al., 2003).

Highlighted by the reports of intracranial hemorrhages in four newborns from Tennessee who did not receive vitamin K at birth, there are concerns that the rate of parental refusal of vitamin K in the United States is increasing (Centers for Disease Control and Prevention, 2013; Schulte et al., 2014). Although there has been little widespread surveillance on the rates of vitamin K refusals, recent studies suggest that the rates in North America may be in the 0.3%–0.8% range; the rates are higher for newborns born at birthing centers rather than in a hospital (Sahni et al., 2014; Hamrick et al., 2016). Rather than being concerned about the reports linking vitamin K with childhood cancers, parents who refuse vitamin K treatment for the newborn are also more likely to refuse vaccines for their children at later ages and share many of the beliefs of other parents refusing vaccines for their children (Hamrick et al., 2016). In one study the most commonly cited reason for parents refusing vitamin K treatment for their newborns was “synthetic or toxic ingredients,” followed by concerns about an “excessive dose” and side effects; only 7% of those surveyed were concerned about the risks of cancer (Hamrick et al., 2016).

The risks from intramuscularly administered vitamin K include pain at the injection site and the possibility of a serious medication error. The risks of a significant complication from the injection are probably negligible; in one study, zero significant complications were reported after 420,000 injections (Von Kries, 1992). In the United States, oral administration is complicated by the lack of an oral vitamin K preparation licensed for newborns. In some settings, infants have received the IM preparation orally. However, tolerability may be a problem, and the efficacy of this preparation when given orally may not be comparable with the oral formulations used in Europe. In addition, adherence with repeated doses of orally administered vitamin K in infants may be suboptimal. Finally, it is unknown whether the use of repeated administration of an oral vitamin K preparation in the dose range of 1–2 mg each week is associated with an increased risk of childhood cancers.

For parents who have questions regarding the best method to prevent classic and late VKDB, the clinician is advised to discuss the pros and cons of IM versus oral administration of vitamin K. If the parents choose oral administration, a dose of 2 mg of vitamin K should be given shortly after birth, with subsequent doses until the newborn is at least 4 weeks old if he or she is breastfed. In a policy statement, the AAP suggests that if an oral vitamin K formulation becomes licensed for use in the United States, a 2-mg dose may be given at birth and repeated at 1–2 weeks of age and at 4 weeks of age for neonates whose parents decline IM vitamin K treatment (American Academy of Pediatrics Vitamin K Ad Hoc Task Force, 1993).

Circumcision

Neonatal circumcision is a polarizing issue for both healthcare professionals and parents. Those who favor routine circumcision highlight health benefits such as decreased risk of urinary tract infections (UTIs), reduced risk of penile cancer, and possibly lower rates of sexually transmitted infections, including HIV (Schoen, 2008). Those who oppose the procedure point out that the number of circumcisions needed to be performed to prevent one of these outcomes (number needed to treat) is large, that the risks of the procedure balance out the benefits, that circumcision leads to loss of sexual sensation, and that subjecting a neonate to a painful procedure without clear benefits may be unethical (Andres, 2008). In 2012 the AAP published a policy statement concluding that the benefits of circumcision outweigh the risks of the procedure.

However, these health benefits were not great enough to recommend routine circumcision in all male neonates (American Academy of Pediatrics Task Force on Circumcision, 2012a).

It is clear that circumcision reduces the risk of UTI by threefold to 10-fold (American Academy of Pediatrics Task Force on Circumcision, 2012b). However, given the low incidence of UTI in male newborns, 100 boys need to be circumcised to prevent one UTI. Similarly, although circumcision has been shown to prevent penile cancer, this is an extremely rare condition, and the number needed to treat is about 900 (Christakis et al., 2000). The results of studies in three African countries indicate that circumcision reduces the risk of HIV infection by 56% (Mills et al., 2008). In the United States, where HIV infection rates are lower, it has been estimated that circumcision might decrease the acquisition of HIV through heterosexual transmission by 16%; 298 boys would need to be circumcised to prevent one case of HIV infection (Sansom et al., 2010). There is limited evidence suggesting that circumcision might reduce the risk of other, selected, sexually transmitted infections, including syphilis and genital herpes. However, there is no compelling evidence that circumcision reduces the risk of chlamydia or gonorrhea (American Academy of Pediatrics Task Force on Circumcision, 2012b).

Circumcision is generally a safe procedure. Although some increased bleeding is reported after 1% of circumcisions, the rate of significant complications is about 0.2% (Gee and Ansell, 1976; Wiswell and Geschke, 1989; Christakis et al., 2000). Bleeding, sometimes requiring suturing of a vessel, is the most common significant complication, followed by penile injury and infection. Infection is more common following a circumcision using a Plastibell rather than a Gomco clamp; the incidence of hemorrhage is reportedly similar after either technique (Gee and Ansell, 1976).

Circumcision is an uncomfortable experience for the neonate. Small amounts of sucrose solutions can be offered to the baby for soothing. Pain from the actual surgery can be significantly decreased with the use of a dorsal penile nerve block or ring block (American Academy of Pediatrics Task Force on Circumcision, 2012b). In one study, 65% of newborns who had received a dorsal nerve block had no or minimal response to the initial clamping of the foreskin (Taeusch et al., 2002). However, the results of a randomized controlled trial suggest that ring block provides superior analgesia compared with dorsal penile nerve block (Lander et al., 1997). Although topical anesthesia may be better than no anesthesia, it provides inferior pain relief compared with dorsal penile nerve block (Brady-Fryer et al., 2004).

A poor cosmetic outcome can be caused by removal of too little foreskin. It has been estimated that 1%–9.5% of circumcisions are redone because of parental concern regarding the appearance. In a prospective study among boys younger than 3 years who had been circumcised with use of either a Plastibell clamp or a Mogen clamp, the glans was fully exposed in only 35.6%. However, in older circumcised boys, the glans was fully exposed in more than 90% (Van Howe, 1997). This suggests that parents of a circumcised infant should be counseled that the vast majority of properly done circumcisions will lead to an acceptable cosmetic appearance over time.

In the United States the Gomco clamp is the most commonly used apparatus for performing circumcisions, followed by the Plastibell clamp and the Mogen clamp (Stang and Snellman, 1998). The use of the Mogen clamp, which was designed by a Jewish mohel, leads to shorter procedures and, reportedly, less pain and bleeding than the other techniques (Reynolds, 1996; Kurtis et al., 1999; Taeusch et al., 2002). However, less foreskin is removed

with the use of the Mogen clamp than with the other two techniques (Alanis and Lucidi, 2004).

Hepatitis B Vaccine

The implementation of routine HBV immunization during infancy has been associated with a dramatic decrease in the incidence of this infection. Between 1990 (before routine vaccination of infants) and 2004 the overall incidence of acute HBV in the United States declined by 75% and by 94% among children and adolescents (Centers for Disease Control and Prevention, 2005). Both the Centers for Disease Control and Prevention and the AAP recommend that the initial dose of the three-dose HBV immunization series be given in the newborn nursery. However, this recommendation is not universally followed; the rate of receipt of a birth dose of HBV vaccine in United States was estimated at 72.4% in 2014. The rates differ substantially across the United States, ranging from 88.4% among newborns born in North Dakota to 48.4% among those born in Vermont (Hill et al., 2015).

There are at least two advantages of providing the first dose of HBV vaccine during the newborn nursery stay. First, newborns who receive a birth dose are more likely to complete their HBV immunization series on time than those who receive a first dose later (Yusuf et al., 2000). Secondly, since a dose of HBV vaccine given within 12 hours of birth can prevent vertical transmission of HBV infections in 75%–90% of cases, early provision of immunization serves as a “safety net” in cases where there has been an error in identifying a mother who is HBV surface antigen positive (Centers for Disease Control and Prevention, 2005).

The main disadvantage of providing a dose of HBV vaccine during the nursery stay is that it may complicate documentation of HBV immunization status in a child by increasing the number of vaccination providers. There is no evidence that administration of a birth dose of HBV vaccine leads to more evaluations for sepsis because of adverse events related to the immunization.

Ongoing Care

Umbilical Cords

Umbilical Cord Variants

At the time of delivery a description of the umbilical cord should be documented in the medical record as there may be long-term implications for care of the newborn if there are cord abnormalities. A great deal has been written about cords with a single umbilical artery, but other variations are also important.

Short cords are associated with decreased fetal movement and may indicate an underlying neuromuscular disorder or genetic syndrome. This decreased fetal movement may also cause a decrease in bone density in the newborn (Krakowiak et al., 2004; Wright and Chan, 2009).

Long cords are associated with large and active babies and more often are found in males. They are associated with an increased risk of cord compression, entrapment, knotting, and nuchal cords (Sornes, 2000). True knots may cause problems for the fetus during pregnancy and may also compress at the time of delivery, causing additional compromise. In the presence of chronic cord compression, neonates have an increased risk of brain imaging abnormalities (Baergen et al., 2001). Velamentous insertions of the umbilical cord are associated with an increase in obstetric complications such as vasa previa and cord rupture (Hasegawa et al., 2006).

A single umbilical artery is detected in about 4 in 1000 births and is associated with a number of congenital anomalies, including renal or genitourinary malformations, cardiac malformations, and chromosomal anomalies, including Down syndrome, but most newborns with a single umbilical artery are normal. In this era of near universal prenatal fetal ultrasonography, many of the associated anatomic abnormalities are discovered before delivery (Johnson and Tennenbaum, 2003; Deshpande et al., 2009). Unless there are additional problems noted on physical examination of the newborn, there is no need to repeat diagnostic ultrasound examinations after the birth of a newborn with a single umbilical artery.

Prevention of Omphalitis

The recommendations for umbilical cord care may range from “dry cord care” to use of dyes and/or cleansing with alcohol, soap and water, or antiseptics. Concerns over the possible toxic effects of dye and antiseptics led many hospitals in the United States to adopt the “dry cord care” method of cord care. Unfortunately, this may be causing an increase in the risk of omphalitis (Janssen et al., 2003; Simon and Simon, 2004). In one review a 50% reduction in omphalitis and a 12% reduction in neonatal mortality was found when chlorhexidine was used versus standard dry cord care methods (Sinha et al., 2015). This was the same conclusion reached in a review of home births in developing countries, but no clear difference was found in developed countries (Imdad et al., 2013). If the dry cord method is used, parents and healthcare providers should watch for redness around the umbilical cord stump.

Tetanus neonatorum, with the infection occurring via the umbilical cord, continues to be reported in more than 20 developing countries, resulting in 58,000 neonatal deaths per year. The condition is related to low vaccination rates in women of childbearing age, home deliveries, and certain cultural care practices. Public health efforts focusing on effective vaccination programs and use of “clean bed” deliveries are needed to eliminate the disease (Thwaites et al., 2015).

Delayed Cord Clamping

The results of several studies suggest a benefit to delaying clamping of the umbilical cord at birth. This practice has been shown to benefit term as well as preterm newborns. The babies are more hemodynamically stable, have greater red cell mass and iron stores during infancy, and have improved neurodevelopmental outcome during childhood. These findings have led to the endorsement of policies to delay cord clamping by both the AAP and the American College of Obstetrics and Gynecology (McAdams, 2014). With delayed cord clamping, there is an increase in the risk of mild polycythemia and an increase in risk of jaundice but not increased treatment for these conditions.

While current guidelines call for a 30–60-second delay before clamping, it seems beneficial to wait for the baby to show physiologic readiness. Babies who cry vigorously may be ready for clamping sooner than quiet babies. An ultrasound study of blood flow through the umbilicus showed arterial and venous flow may not stop for 3 minutes (Boere et al., 2015).

Umbilical Cord Blood Banking

The practice of banking umbilical cord blood is increasing worldwide. This service is offered by both public and privately supported entities; however, the quality of service is better regulated in public blood banks. While there are controversies, the potential for use of stem cells is growing, and there will likely be an increased demand for this service (Yoder, 2014). One of the greatest barriers

to more consistent collection of umbilical cord blood is that current hospital practices generally do not support this service (Broder et al., 2013).

Breastfeeding

Benefits of Breastfeeding

There is voluminous evidence that the optimal nutrition for normal neonates is human milk provided via the mother's breast. Growing evidence supports the role of human milk in prevention of early onset of allergies, prevention of adult obesity, reduction in severity and frequency of infections (including those leading to hospitalization in developed countries and those leading to death in developing countries), and increased intellectual functioning (Section on Breastfeeding, 2012). It is a public health imperative and incumbent on our society to provide systems that support breastfeeding (Christakis, 2013).

Support of Breastfeeding

Breastfeeding is not always the “easy and natural” undertaking it is touted to be. Primiparous mothers report more difficulties than multiparous mothers. Breastfeeding support begins with encouragement and education at prenatal visits. After birth, in-person lactation support is helpful in promoting both initiation and persistence of nursing (Renfrew et al., 2012). Places of employment should provide support by having adequate maternity care leave policies, improving facilities, and having policies allowing time and space for nursing and pumping for lactating women at the workplace (Sattari et al., 2013). Fathers and grandparents should provide a supportive social network by performing homecare tasks to facilitate rest for lactating mothers (Alvarez et al., 2015). Problems with nursing should trigger additional intervention with lactation specialist evaluation and advice.

In 1991 the World Health Organization and the United Nations Children's Fund developed a program to promote breastfeeding called the Baby-Friendly Hospital Initiative (BFHI). As a comprehensive program, implementation of the 10 steps of the BFHI (Box 26.2) has been shown to significantly increase the rates of breastfeeding (Kramer et al., 2001). In addition, there is evidence of a “dose–response” relationship between the number of BFHI steps that women are exposed to and improved breastfeeding outcomes (Perez-Escamilla et al., 2016).

• BOX 26.2 Baby-Friendly Hospital Initiative: Ten Steps to Successful Breastfeeding

1. Maintain a written breastfeeding policy that is routinely communicated to all healthcare staff.
2. Train all healthcare staff in the skills necessary to implement this policy.
3. Inform all pregnant women about the benefits and management of breastfeeding.
4. Help mothers initiate breastfeeding within 1 hour of birth.
5. Show mothers how to breastfeed and how to maintain lactation, even if they are separated from their newborns.
6. Give newborns no food or drink other than breast milk unless medically indicated.
7. Practice “rooming-in”—allow mothers and newborns to remain together 24 h a day.
8. Encourage unrestricted breastfeeding (breastfeeding on demand).
9. Give no pacifiers or artificial nipples to breastfeeding newborns.
10. Foster the establishment of breastfeeding support groups, and refer mothers to them on their discharge from the hospital or clinic.

For some of the individual steps of the BFHI, such as excluding the use of pacifiers, the evidence is contradictory (Cramton et al., 2009; O'Connor et al., 2009). There are a number of epidemiologic studies showing cessation of breastfeeding is associated with pacifier use, but the few randomized prospective trials done give different results. The authors of a review concluded that among mothers who were motivated to breastfeed, pacifier use did not significantly affect the prevalence or duration of breastfeeding (Jaafar et al., 2012). Sucking is a primitive brain self-soothing process. Babies with certain temperaments may benefit more than others by using sucking to self-soothe. Pacifier use has been shown in some studies since the 1970s to decrease the risk of sudden infant death syndrome (SIDS), and in premature infants, nonnutritive sucking actually enhances weight gain. The AAP developed a policy statement supporting pacifier use but recommended waiting until approximately 1 month of age (Section on Breastfeeding, 2012). The important issue is whether or not the use of a pacifier is replacing feedings, so if a mother is motivated to breastfeed and maintains a frequency of 8–12 feedings per day, the use of a pacifier between meals seems reasonable.

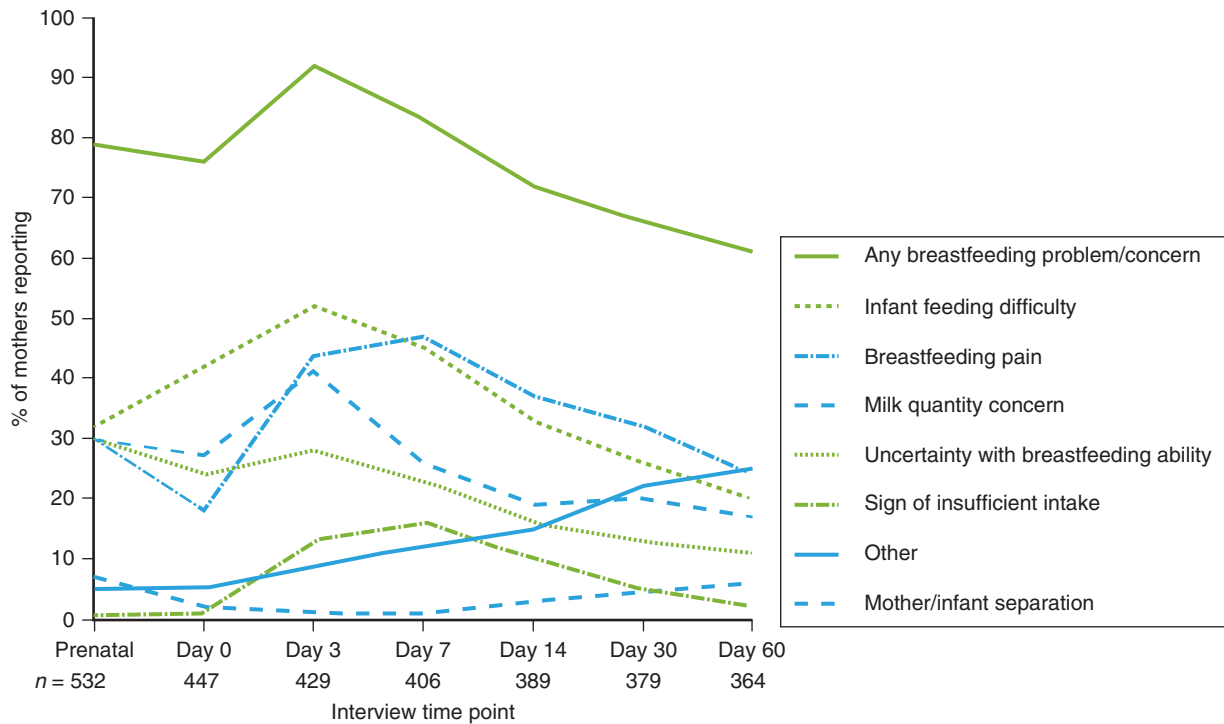
Another contentious issue is the use of supplemental formula during the initial newborn period. The results of a randomized prospective trial indicated that use of limited supplemental formula was associated with increased breastfeeding rates at 3 months of age (Flaherman et al., 2013). That seems contradictory to earlier studies showing a decline in nursing when formula samples or discharge packages were given to families. One key difference is that in the trial, formula use was limited to 10 mL after feedings, administered via a syringe, and supplement was discontinued once mature milk was produced. More research is needed to determine which mother–infant dyads will benefit from supplement while avoiding sabotage of breastfeeding.

From a practical standpoint there are several evidence-based interventions during the newborn nursery stay that increase the rate and/or duration of breastfeeding. These include the use of frequent demand feedings as opposed to a rigid feeding schedule, early skin-to-skin contact between the mother and the newborn, professional advice on breastfeeding techniques, and exclusion of commercial formula from discharge packs (Donnelly et al., 2000; Renfrew et al., 2000; Anderson et al., 2003; Britton et al., 2007).

In an era of early hospital discharge for healthy newborns, it is particularly important to have in-home or clinic follow-up at age 3–4 days. This is when the newborn's weight reaches its nadir, jaundice peaks, lactogenesis II is starting (see later), and mothers are sleep deprived because of dealing with the around-the-clock needs of the newborn. Breastfeeding mothers may need extra encouragement during this time and continued vigilance to ensure that breastfeeding is established.

Breastfeeding Problems

Breast milk development is divided into two or three phases: lactogenesis I occurs during pregnancy with breast enlargement due to proliferation of ducts and lobules and later in pregnancy with colostrum production. Lactogenesis II occurs usually about 56–72 hours after birth; gonadotropin and progesterone levels decline and prolactin level increases (Dewey et al., 2003). This phase is characterized by the rapid increase in milk volume—sometimes this is exuberant to the point of engorgement. Lactogenesis III occurs generally after 1 month of nursing when the milk composition and volume are responsive to the reciprocal relationship between the mother and her baby—a demand and



• **Fig. 26.2** Prevalence of reported breastfeeding concerns by mothers by newborn age. (Modified from Wagner EA, Chantry CJ, Dewey KG, Nommsen-Rivers LA. Breastfeeding concerns at 3 and 7 days postpartum and feeding status at 2 months. *Pediatrics*. 2013;132:e865–e875.)

supply feedback loop. Some experts combine lactogenesis II and lactogenesis III into one phase.

Delays in lactogenesis II have been shown to occur after cesarean birth, in poorly controlled diabetic mothers, when there is stress during delivery, when there are retained placental fragments, and when there is pituitary failure. There are some situations when no milk production occurs, leading to frustration and feelings of failure in mothers. The timing of lactogenesis II is a biologic clock that cannot be accelerated by pumping or frequent nursing (Chapman et al., 2001; Flaherman et al., 2012a).

More than 90% of mothers report concern and difficulty with nursing during the first 10 days after delivery (Fig. 26.2) (Wagner et al., 2013). Combined with hormonal changes and sleep deprivation, this can compound the risk of postpartum depression and early cessation of nursing. Postpartum depression should be screened for at health supervision visits until the infant is 6–12 months of age (McLearn et al., 2006).

Common issues that may lead to early cessation of nursing include nipple pain, newborn jaundice, excessive weight loss or poor weight gain, concern about maternal medications, and lack of social support. There are also conditions associated with low milk volume production, including maternal factors (lack of social support, prenatal confidence and expectations about breastfeeding, timing of return to work, inadequate frequency of nursing, inadequate breast tissue, flat or inverted nipples, or very large breasts) and baby factors (hypotonia, drug withdrawal, asymmetric jaw, high arch palate, poor tongue motor abilities, and temperamental issues).

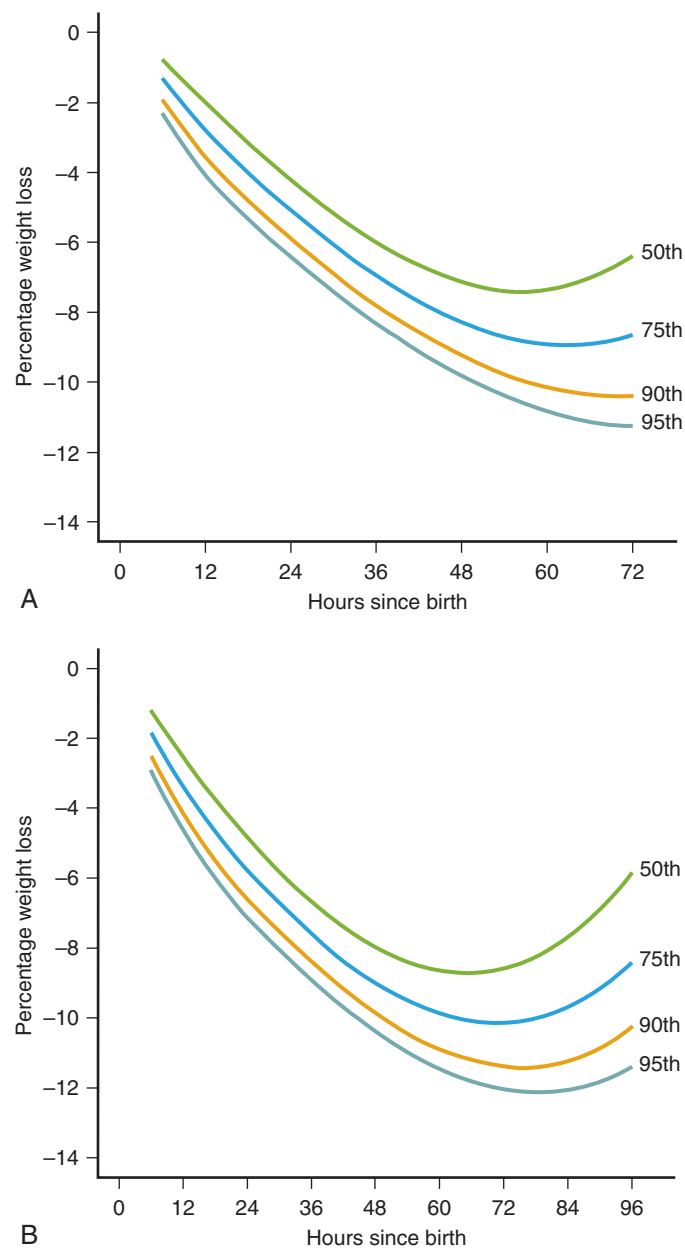
For nipple pain there is no treatment that is clearly advantageous (e.g., hydrogel, lanolin, breast milk, shields), but education on latch position is helpful. For most, the pain decreases within the first 7 days regardless of the treatment selected (Dennis et al., 2012). In a review of nipple shields, McKechnie and Eglash (2010)

found a lack of randomized trials but expressed concern about shields interfering with milk transfer.

There has been a recent increase in the use of frenotomy to alleviate pain with nursing, presumably due to a tight lingual frenulum (tongue-tie or ankyloglossia). With use of objective rating scales, the rate of tongue-tie in newborns is about 1%–4%, but more infants are undergoing frenotomy, and there are concerns that this may be more due to anecdotal reports rather than more rigorous study (Power and Murphy, 2015). Sometimes frenotomy is done to alleviate the frustrations of mothers (and lactation specialists) who are dealing with breastfeeding problems of unknown origin. There are only a few prospective studies on frenotomy, and the results are contradictory (Power and Murphy, 2015). It may be prudent to wait until the physiologic process of lactogenesis II and early nipple pain have passed before frenotomy is considered. Although the procedure is simple and relatively free of side effects, unnecessary interventions or labeling of a newborn as abnormal should be avoided in newborn care whenever possible. Frenotomy for posterior tongue-tie has received extra scrutiny because evidence is lacking that supports the diagnosis and outcome from treatment, and the procedure is more invasive (Douglas, 2013).

Jaundice has become more prevalent with the resurgence of breastfeeding. Along with more visible jaundice, there has been an increase in concern and anxiety about the risks associated with jaundice. Most jaundice is physiologic and resolves with onset of lactogenesis II. It is important for providers who care for newborns not to overestimate the risks, while maintaining vigilance for the exceptionally rare case when there is true risk. The bilirubin level should serve as a call for action to emphasize lactation support rather than lead to a separation of the newborn from the mother and artificial feeding to lower the bilirubin level.

The normal newborn is born with a surplus of extracellular free water, and in cesarean delivery births, mothers are often given



• **Fig. 26.3** Nomograms of weight loss in exclusively breastfed newborns born vaginally (A) or by cesarean delivery (B). (Modified from Flaherman VJ, Schaefer EW, Kuzniewicz MW, et al. Early weight loss nomograms for exclusively breastfed newborns. *Pediatrics*. 2015;135:e16–e23.)

additional boluses of fluids that may further hydrate the newborn (Chantry et al., 2011). It is normal, expected, and perhaps preferable that babies will lose this free water in the first 72 hours of life. This free water is protective of the newborn's fluid balance while the mother's milk is awaited (Mulder and Gardner, 2015). In cases of extra hydration, extra weight loss may be expected. The average term newborn loses about 7% of birthweight, with 12% of newborns born vaginally losing more than 10% of birthweight (Fig. 26.3) (Flaherman et al., 2015). The loss during the first 24 hours of life can predict those who will lose more. This is not a state of dehydration but is a normal physiologic adaption to extrauterine life, so healthcare providers should not alarm parents or suggest that there is something wrong with their baby.

With the onset of copious production of mature milk, neonates begin to gain weight and their serum sodium levels fall (Marchini and Stock, 1997). Newborns fed human milk regain their birthweight, on average, by the age of 8.3 days; 97.5% have regained their birthweight by 21 days (Macdonald et al., 2003). In newborns who lose substantially more than 10% of their birthweight because of breastfeeding difficulties, there is the potential for significant hypernatremia (Oddie et al., 2013).

Supplementation of Breastfeeding

It is usually unnecessary to provide any nutrition or fluid to term breastfed newborns beyond human milk. Dextrose water or commercial formula may be needed in neonates with hypoglycemia who are not responsive to breastfeeding. Supplementation may also be indicated in newborns who have lost more than 10% of their birthweight and/or have decreased urine and stool output or in the presence of significant hyperbilirubinemia. Supplementation should be considered a temporary intervention, and its provision should not interfere with the onset of successful breastfeeding.

Temporary supplemental formula or expressed breast milk when available can be provided via a supplemental nursing system, finger feeding, or a bottle. Of greatest importance is close monitoring of the change in weight of the baby and continued lactation support. The use of banked or donor milk is increasing, but there has also been increased concern about the use of the unregulated milk that may be available. Some milk has been diluted with formula and may contain infectious diseases. Publicly supported milk banks that follow the recommendations of the World Health Organization use pasteurization and quality control to provide safer milk (Kair et al., 2014; Steele et al., 2015).

Contraindications to Breastfeeding

The few, absolute contraindications to breastfeeding include maternal HIV infection, untreated tuberculosis in the mother, evidence of current cocaine use,¹⁵⁸ use of antimetabolite drugs, and galactosemia in the neonate (Gartner et al., 2005; Field, 2008). There are a myriad of drugs for which concern exists regarding long-term neurodevelopmental outcomes in the infant. Selective serotonin reuptake inhibitors are commonly used to treat depression and anxiety in young women. Among drugs in this category, sertraline and paroxetine are thought to be the safest for use in breastfeeding mothers, while fluoxetine and citalopram are felt to have the most potential for toxicity in the neonate (Field, 2008). Overall, few adverse effects have been noted with use of any of these drugs, and generally the potential risks associated with these medications are thought to be outweighed by the benefits of breastfeeding (Field, 2008). Similarly, although methadone is detectable in the breast milk of women receiving this medication, serum levels in neonates are quite low and unlikely to have a significant effect (Jansson et al., 2008). Online references are available that have the current status of the effects of toxins and medications in breast milk: <http://toxnet.nlm.nih.gov/newtoxnet/lactmed.htm>.

Hepatitis C virus RNA has been found in the milk of mothers infected with this virus. Despite this, transmission of infection via breastfeeding has not been documented. Maternal hepatitis C is not considered a contraindication to breastfeeding (Gartner et al., 2005).

Bottle feeding

Commercial formula that provides adequate nutrition, vitamins, and minerals is available for infants of mothers who do not wish

to breastfeed their infants or in those rare instances when breastfeeding is contraindicated or impossible. There are three major categories of formula used in neonates: cow's milk-based, soy, and hydrolyzed formula. Of these, cow's milk-based formula is the most commonly used. The main carbohydrate in cow's milk-based formula is lactose. Soy formulas were developed for infants with suspected cow's milk allergy. Because the main carbohydrate in soy formulas is sucrose or corn syrup, soy formula can be used in neonates with suspected galactosemia. Protein hydrolyzate formulas were initially developed for use in infants who are highly intolerant to cow's milk protein (Kleinman, 2009b). They are purported to lead to fewer allergies in babies and children than cow's milk-based formula, but the evidence for this is limited (Osborn and Sinn, 2006). All extensively hydrolyzed formulas are lactose free (Kleinman, 2009b). Extensively hydrolyzed formulas are indicated in infants with definitive evidence of cow's milk protein allergy because 10%–14% of such children also have soy allergy (Bhatia and Greer, 2008).

Traditionally, standard preparations of formulas available for use in healthy term neonates provide 0.67 kcal/mL. This caloric density was based on the calories in human milk. However, the results of some studies indicate that the average caloric density of human milk may be closer to 0.64–0.65 kcal/mL. Because of this, some infant formulas have been modified to provide 0.643 kcal/mL (19 kcal/oz) (Marriage et al., 2015). Most formulas are fortified with iron at a concentration of 10–12 mg/L. Vitamin D at a concentration of approximately 400 IU/L is provided in all of the commercially available formulas (Kleinman, 2009a).

Mothers who elect to bottlefeed their babies report feeling unsupported in their decision by healthcare professionals, and up to 50% feel pressured to breastfeed (Lakshman et al., 2009). Although the benefits of breastfeeding should be provided to mothers who have not decided how to feed their babies, the role of healthcare providers is also to support the decision of those who have elected to bottlefeed their babies. It is also important to provide practical education about bottlefeeding to these parents; this is frequently not done in many newborn nurseries (Lakshman et al., 2009).

Newborns who are bottlefed can feed *ad lib* beginning shortly after birth. The average formula intake in term newborns during the first day of life is 15–20 mL/kg and is 40–45 mL/kg during the second day (Dollberg et al., 2001). Term newborns who are formula fed during their birth hospitalization typically lose less weight than breastfed infants (Flaherman et al., 2015; Miller et al., 2015). The median weight loss in formula-fed term newborns at 48 hours of life has been reported to be 2.9% of birthweight for those born vaginally and 3.7% among those born by cesarean delivery; weight loss of 7% or more during a typical newborn nursery stay in formula-fed infants is uncommon (Miller et al., 2015).

Anticipatory Guidance

A primary duty of newborn care providers is to ensure that the newborn's caregivers (usually the parent or parents) have the knowledge and skills to provide for their baby's normal growth and development. Parents who are well informed about normal newborn development and behavior have more realistic expectations about the work involved and look on their child with more fondness. Conversely, it is important to assess the parents' ability to provide a safe and nurturing environment for the neonate before discharge from the newborn nursery. Parents showing concerning behaviors, possibly leading to abuse or neglect, should have supervision and interventions to help them, or their parental rights should be terminated (Wattenberg et al., 2001; Davidson-Arad et al., 2003).

There are several major challenges faced by parents of normal newborns: sleep deprivation, learning how to calm a crying newborn, dealing with significant life changes, and the new worries that come with being responsible for a totally dependent being. Postpartum depression is more common and of longer duration than previously thought and is present in at least 10% of mothers. Depression also occurs in about 10% of fathers during early infancy and is related to maternal depression. This condition is related to sleep deprivation and has a major and long-lasting impact on infant homeostasis and development (Chaudron, 2003). Depression screening of parents should occur at all well-child visits during the first 6–12 months of life.

Anticipatory guidance should be given to help prepare new parents for the common tasks of newborn care and to educate them about the many normal variations in newborn behavior. Learning how to soothe a baby is one of the earliest needed parenting tasks; providers can help give suggestions both to reduce crying and to better cope with those newborns who are more sensitive and harder to soothe (Barr et al., 2009).

Most parents have questions about feeding, elimination, bathing, cord care, genital care, jaundice, and common rashes. There are numerous checklists of educational topics that can be overwhelming to new parents and providers. In addition, learning styles differ, with some preferring written materials and others preferring audiovisual materials or hands-on demonstration. Ideally, education should be targeted toward the topics of interest and with the appropriate materials for the learning style (Dusing et al., 2008).

Mothers are often not in a good learning state in the immediate postpartum period because of pain, postpartum hormonal changes, and the stress of being in a hospital. However, this is also a period of heightened receptivity to change, so attempts to teach or make lifestyle changes (e.g., smoking cessation) may be more effective. There is some evidence that providing parental education using tools such as interactive videos and computers may be superior to traditional teaching (Trepka et al., 2008; Snowdon et al., 2009). Also, DVDs provided in the nursery or along with well-child visits have been shown to be helpful. A randomized controlled trial of a 15-minute anticipatory guidance DVD linked to a well-child visit led to more parents in the DVD group feeling prepared to care for their baby after the visit, having high confidence in bathing their baby, and having high confidence in recognizing congestion when compared with the control group. Those in the DVD group also had fewer additional office visits between birth and 2 months (Paradis et al., 2011). Another effective teaching method is to perform home visits for education of parents. A metaanalysis comparing 60 home visit programs revealed improvement in parenting behaviors, faster parental return to work or school, and lower rates of child abuse (Sweet and Appelbaum, 2004).

Given the obstacles to providing meaningful education during the nursery stay, it is probably better for practitioners to focus on a few key points of anticipatory guidance rather than reciting a long litany of instructions. There is also a philosophical choice in deciding whether to emphasize the overall health of a newborn or to concentrate on prevention or identification of illness. There is little evidence about the efficacy of most anticipatory guidance provided to parents during the newborn nursery stay. A notable exception is the successful Back to Sleep campaign to reduce the risk of SIDS, discussed in the next section.

There is also emerging evidence that education regarding the normality of inconsolable crying in newborns helps parents deal with this stressful situation and may reduce the risk of shaken baby syndrome (Barr et al., 2009). A DVD entitled "Period of

Purple Crying” is provided at some newborn nurseries to parents to help prevent shaken baby syndrome by teaching calming and coping techniques. Early evaluations of the DVD indicate that it may lead to better parental knowledge about newborn crying; however, in one population-based study, provision of the DVD in newborn nurseries was not associated with a decrease in the incidence of abusive head trauma in infants (Zolotor et al., 2015).

Sleep Position

With the exception of immunizations, no child health intervention in the past 3 decades has resulted in a larger decrease in post-neonatal infant mortality than the Back to Sleep campaign. The remarkable change in the predominant sleep position of infants from prone to supine has led to a 30%–50% reduction in the rate of SIDS in the United States (American Academy of Pediatrics Task Force on Sudden Infant Death Syndrome, 2005). A multipronged effort including brochures, public service announcements, and education provided by healthcare professionals was used to affect the change in sleep position (Willinger et al., 2000). Obviously, education provided to parents during the newborn nursery stay is a crucial determinant of the sleep position of an infant. In addition to providing education, there is evidence that parents model sleep position for their babies after how they saw nurses and physicians place their neonate in his/her bassinet in the newborn nursery (Colson and Joslin, 2002; American Academy of Pediatrics Task Force on Sudden Infant Death Syndrome, 2005). There is no evidence that placing newborns on their side during the first few hours after birth decreases the risk of aspiration (American Academy of Pediatrics Task Force on Sudden Infant Death Syndrome and Moon, 2011). Therefore it is crucial that neonates are placed on their backs to sleep in the newborn nursery. In addition, there is an additive effect from both physicians and nurses recommending and demonstrating the supine sleep position (Willinger et al., 2000).

In addition to the supine position, there are other factors related to the sleep environment that may impact the risk of SIDS. It is recommended that infants sleep on firm surfaces and without excessive bedding such as pillows. Although a controversial topic, the results of a metaanalysis strongly indicate that cosleeping increases the risk of SIDS by nearly threefold. The risk of cosleeping is highest in infants of mothers who smoke (Vennemann et al., 2012). In addition, although use of a pacifier has been found to reduce the risk of SIDS, there is a reluctance to recommend these devices because of concerns about reducing breastfeeding (American Academy of Pediatrics Task Force on Sudden Infant Death Syndrome, 2005; American Academy of Pediatrics Task Force on Sudden Infant Death Syndrome and Moon, 2011).

Discharge and Follow-Up

The average length of the initial hospital stay for US newborns born at 35 weeks’ gestation or later is 48–52 hours (Datar and Sood, 2006; Paul et al., 2006; Kuzniewicz et al., 2009). Since approximately 50% of newborns delivered vaginally are discharged before 48 hours after birth and up to 40% of those born by cesarean delivery are discharged before 72 hours after birth, a large proportion of newborns are discharged before 3–4 days after birth, which is when bilirubin levels typically peak and breastfeeding is well established (Paul et al., 2006). It is recommended that newborns discharged before 48 hours after birth have a follow-up appointment with a provider within 48 hours (American Academy of Pediatrics

Committee on Fetus and Newborn, 2004). This follow-up can be accomplished either by a visit to a healthcare provider or by a home nursing visit.

The risk factors for readmission following an initial hospital stay of less than 48–72 hours include GA of less than 39 weeks (and especially <37 weeks), primiparous mother, and Asian race (presumably because of an increased risk of hyperbilirubinemia) (Liu et al., 1997; Grupp-Phelan et al., 1999; Paul et al., 2006; Burgos et al., 2008). Consideration of a longer nursery stay is suggested for newborns with one or more of these risk factors. In addition, early discharge is not recommended for term newborns who have not voided, passed at least one stool, or demonstrated adequate breastfeeding (American Academy of Pediatrics Committee on Fetus and Newborn, 2004). However, there is little evidence to support these recommendations.

Common Problems During the Nursery Stay

Hypothermia and Hyperthermia

Being too cold or too hot causes metabolic stress to the newborn, so efforts to maintain a steady and neutral thermal environment should be made. The best practice is to dry the baby immediately after delivery and place the newborn skin-to-skin with the mother. Although the AAP and the American College of Obstetricians and Gynecologists jointly recommend keeping newborns’ core temperatures within the narrow range of 36.5°C to 37°C, in one study of healthy term newborns the average temperature was 36.5°C, with a normal range from 36.0°C to 37.9°C (Takayama et al., 2000). Thin babies tend to have lower body temperatures, and heavier babies tend to have higher body temperatures. Bathing a newborn often causes hypothermia. This is less likely when bathing is performed from trunk to head or when a bath is used versus washing with a cloth.

Standard practice at most nurseries is to measure axillary temperatures, probably because of reports in the 1960s and 1970s of rare perforations caused by rectal thermometers; however, axillary temperatures may not always accurately reflect core temperature (Hutton et al., 2009). Importantly, axillary temperatures are not the standard used in studies of sepsis in infants younger than 2–3 months of age.

Hypothermia

On leaving the womb a newborn is immediately challenged with maintaining a normal body temperature. If a neonate is not quickly dried at birth, he or she may lose up to 1°C body temperature per minute. Healthy term babies are able to increase heat production through glycogenolysis and nonshivering thermogenesis for minutes to a few hours, depending on environmental conditions (Aylott, 2006). Babies typically experience a decline in body temperature during the first hour after birth, with a gradual increase during the following 12 hours (Li et al., 2004). By the second day the newborn’s body temperature becomes more stable, but heat loss can occur again with bathing or other stresses (Takayama et al., 2000).

Many nurseries worldwide have adopted policies to delay bathing to avoid hypothermia, to allow time for initial bonding, and to promote breastfeeding. Early skin-to-skin contact between the newborn and the mother is useful both to prevent and to treat early temperature loss, but attention to positioning and frequent checks by nursing staff are required (George et al., 2015). Hypothermia should be managed by the baby being placed skin-to-skin with a parent or under a radiant warmer.

Hyperthermia

An elevated body temperature at birth generally reflects the intrauterine temperature and is not usually a sign of sepsis (Baumgart, 2008). Isolated hyperthermia during labor is associated with neonatal encephalopathy, occurring in approximately 1 in 2000 births (Blume et al., 2008). After the first 3–4 days of life, increased temperatures are most likely caused by dehydration from suboptimal breast milk supply (Maayan-Metzger et al., 2003). A single increased temperature in an otherwise normally behaving newborn is not a strong predictor of infection but has been reported as a sign of intracranial hemorrhage (Fang et al., 2008).

Elimination

Urination

Approximately 15% of healthy newborns void at the time of delivery, and 95% void by 24 hours of age. Delayed voiding is likely a consequence of stress on the newborn during labor and delivery, which is a protective mechanism for the baby (Vuohelainen et al., 2007, 2008). Normally, no intervention is needed once homeostatic adaptation to extrauterine life has been established.

The differential diagnosis of delayed voiding (defined as no urine output by 24–48 hours of age) includes renal and postrenal causes. With the frequent use of prenatal ultrasound examination, it is unusual for a significant renal anomaly to be unknown before birth. Most newborns with bilateral renal agenesis have other findings, such as oligohydramnios or Potter sequence. Unilateral renal agenesis does not usually give signs of decreased urine output. Renal vascular thrombosis can cause anuria, and babies with this condition are usually ill. Severe cystic kidney disease can involve urinary outflow obstruction. The diagnosis of cystic kidneys is usually made after the newborn period or is found incidental to evaluation of other anomalies and not because of delayed voiding.

Postrenal causes of delayed voiding include neuropathic bladder dysfunction and anatomic obstruction of urinary flow by anomalies in ureters, the bladder, or the urethra. Persistent or recurrent bladder distention after catheterization is found with occult lower spinal cord anomalies. Presacral teratoma or other tumors can cause compression and urinary blockage as well. In male newborns, there is the possibility of posterior urethral valves. Congenital lower urinary tract obstruction occurs in 1 in 3000 births, with two-thirds of these due to posterior urethral valves. Physical findings of loose abdominal skin or musculature and a distended bladder suggest this diagnosis (Malin et al., 2012).

In a healthy-appearing newborn who has a normal physical examination and a history of a normal prenatal ultrasound, allowing up to 72 hours for a spontaneous first void will avoid excessive testing. In fussy neonates and neonates with other genitourinary abnormalities, enlarged kidneys, or a distended bladder, testing should begin immediately. Ultrasound examination of the bladder, kidneys, and posterior urethra is often diagnostic.

Normal newborns have decreased renal concentrating ability and excessive extracellular free water at birth. As a result, they will continue to void despite low intake of fluids. This process is normal. Newborns maintain normal hydration despite weight loss. Conversely, delayed voiding is not indicative of dehydration in the first 72 hours after birth. When mothers undergo cesarean section, the fluid boluses given to prevent hypotension may result in additional free water in their newborn. This can lead to greater than 7% birthweight loss with extra voiding (i.e., a physiologic diuresis)

(Mulder et al., 2010). Hearing about weight loss in their newborn creates stress, guilt, and anxiety in parents, which may be counterproductive to breastfeeding success. It is important for providers to emphasize the normalcy of weight loss (Flaherman et al., 2012b).

Defecation

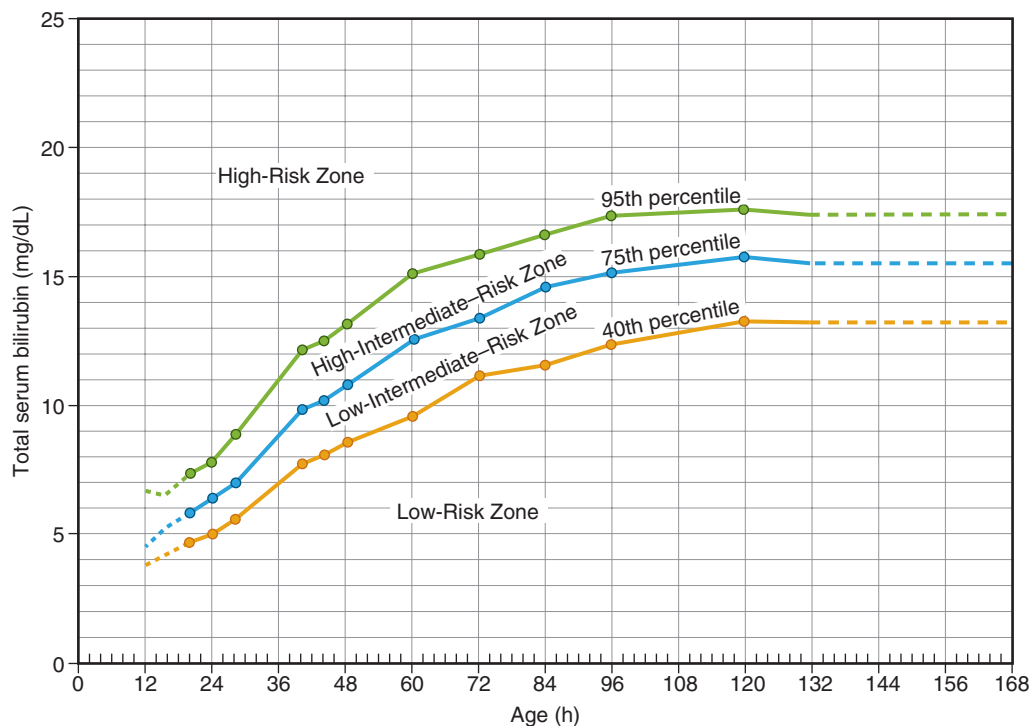
Similarly to the first void, the first passage of meconium occurs by an average of 7 hours of age. One-third of newborns pass meconium before their first feeding. Late preterm newborns tend to pass meconium later than term newborns, and 32% of preterm newborns do not pass meconium within 48 hours after birth. Although intake is not well correlated with meconium output, the number of wet and soiled diapers does reflect adequacy of breast milk production by day 4. Fewer than four soiled diapers on day 4 correlates with inadequate milk production (Nommsen-Rivers et al., 2008). By 2 weeks of age, breastfed newborns pass feces more frequently than bottlefed newborns; they also have larger variability in the time between bowel movements (Sievers et al., 1993; den Hertog et al., 2012). After the first month, breastfed and formula-fed infants have about the same rate of defecation.

Because 99.7% of healthy newborns pass meconium by 34 hours of age, those whose passing of meconium is delayed beyond that time deserve extra vigilance during examination to avoid obstructions being missed, such as an imperforate anus (Metaj et al., 2003). A baby with abdominal distention or vomiting and delayed stooling deserves evaluation for a possible gastrointestinal tract obstruction.

Jaundice

As many as 60%–84% of newborns develop visual jaundice in the first few days after birth (Bhutani et al., 2013a; National Institute for Health and Clinical Excellence, 2016). Despite this almost ubiquitous nature, there are few conditions in newborns that create as much controversy and clinician and parental angst as hyperbilirubinemia. Since the discovery of phototherapy in 1956 and its integration into medical care in the 1970s, the standard management of neonatal jaundice in the United States has gone through three distinct phases. Until the early 1990s, clinicians visually monitored term neonates during their 2–5-day newborn nursery stay and obtained serum bilirubin levels on those with significant jaundice. Phototherapy was initiated when the total serum bilirubin (TSB) level reached 15 mg/dL, and an exchange transfusion was indicated if the level rose to 20 mg/dL (Watchko and Oski, 1983). The wisdom of this approach was challenged by reviews of data on jaundice in term newborns without hemolytic disease indicating that the risk of kernicterus in such newborns was extraordinarily low (Watchko and Oski, 1983; Newman and Maisels, 1992).

On the basis of this evidence a “kinder and gentler” approach to the management of hyperbilirubinemia in term newborns was advocated, leading to the AAP practice parameter in 1994 (Provisional Committee for Quality Improvement and Subcommittee on Hyperbilirubinemia, 1994). Under this guideline, phototherapy for a healthy, 72-hour-old term newborn was not definitively recommended unless the TSB level was 20 mg/dL or greater. Unfortunately, publication of this guideline coincided with a shortening of the nursery stay by term newborns to as little as 24 hours. Thus newborns were discharged home before their bilirubin levels “peaked” at 3–4 days, and there were numerous reports of newborns with extremely high bilirubin levels and a general



• **Fig. 26.4** “Bhutani nomogram” for total serum bilirubin levels in newborns born at 36 weeks’ gestation or later. (Modified from Bhutani VK, Johnson L, Sivieri EM. Predictive ability of a predischARGE hour-specific serum bilirubin for subsequent significant hyperbilirubinemia in healthy term and near-term newborns. *Pediatrics*. 1999;103:6–14.)

impression that the incidence of kernicterus was increasing as well. In retrospect, it does not appear that the incidence of kernicterus did increase, but case reports and anecdotal evidence led to significant consternation by clinicians, parents, and quality assurance organizations (Burke et al., 2009; Brooks et al., 2011).

Since newborns are usually discharged well before their bilirubin levels reach their peak, it was clear that predictive models were needed to assess risk in newborns who are discharged early. Bhutani et al. (1999) developed a nomogram based on data from neonates in whom serum bilirubin levels were measured multiple times (Fig. 26.4). Those newborns who had initial bilirubin levels above the 95th percentile at any time point (in hours) were more likely to have “significant hyperbilirubinemia” when the levels were measured subsequently than those with lower levels initially. These data were used in the development of the 2004 AAP practice guideline (American Academy of Pediatrics Subcommittee on Hyperbilirubinemia, 2004). With this iteration of the guideline, clinicians are provided hour-by-hour guidance on TSB levels for which phototherapy or exchange transfusion is indicated. Separate curves for “low-risk,” “medium-risk,” and “high-risk” neonates have been developed on the basis of the presence of factors (e.g., GA less than 38 weeks, hemolytic disease, serum albumin level less than 3.0 g/dL, glucose 6-phosphate dehydrogenase deficiency, sepsis, and acidosis) that might increase the risk of brain damage in an neonate who has an extremely high TSB level (Maisels et al., 2009). Internet-based tools are available in which the appropriate management for a newborn with a specific bilirubin level at a specific hour after birth is provided.

There are numerous neonatal conditions that increase the risk of hyperbilirubinemia (Dennerly et al., 2001). Chief among these is hemolysis secondary to maternal antibodies to red blood cell

antigens. Although hemolysis secondary to antibodies to Rh factor is now quite rare in the United States and other high-income countries because of proper management of Rh-negative mothers, it has been estimated that as many as 114,000 newborns die each year in locations in the world where resources are not adequate to prevent Rh sensitization (Bhutani et al., 2013b). A common cause of hemolysis in newborns is an ABO incompatibility. Since there is no way to prevent hemolysis from an ABO incompatibility, it may be useful to test umbilical cord blood from neonates born to mothers with blood type O for blood type and the presence of antibodies on their red cells (i.e., Coombs test) at birth. The increase in bilirubin level secondary to ABO compatibility, even with a positive direct Coombs test, is highly variable. Some newborns will have an early and dramatic rise in serum bilirubin level and evidence of hemolysis, while in others no effect can be detected clinically. In addition to ABO incompatibility, some women will have antibodies to “minor” red cell antigens that can usually be diagnosed prenatally. In most instances the increases in bilirubin level associated with antibodies against minor antigens are mild.

Other neonatal conditions that are risk factors for hyperbilirubinemia include bruising secondary to birth trauma and polycythemia. Decreased intake of breast milk may lead to decreased passage of stool. Because intestinal bacteria break down conjugated bilirubin to the unconjugated form, a decrease in stool frequency may lead to increased reabsorption of this unconjugated bilirubin (enterohepatic circulation). Breastfeeding is a significant risk factor for hyperbilirubinemia particularly when intake is limited. The propensity for developing significant jaundice is variable in different racial groups. Asian and Native American newborns are at the highest risk of significant hyperbilirubinemia (Dennerly et al., 2001).

Finally, late preterm newborns are at significantly increased risk of both significant hyperbilirubinemia and kernicterus.

Traditionally, visual assessment has been used to judge whether or not a newborn has significant jaundice. Unfortunately, visual assessment is only relatively accurate; reported correlations between estimated bilirubin levels and TSB levels are in the range of 0.36–0.75, and clinicians tend to underestimate the severity of newborn jaundice ([National Institute for Health and Clinical Excellence, 2016](#)). Because of this, it is recommended that all newborns be screened for jaundice before discharge from the birth hospitalization with a TSB level or a transcutaneous bilirubin (TcB) measurement using a transcutaneous bilirubinometer, a noninvasive method for estimating bilirubin levels ([Maisels et al., 2009](#)).

Although TcB level offers a reasonable approximation of the gold standard TSB level, with published correlations ranging from 0.77 to 0.93, it is designed to be used only as a screening tool ([Maisels et al., 2009](#); [National Institute for Health and Clinical Excellence, 2016](#)). If the TcB level is above a certain threshold, it is recommended that blood be obtained for a TSB measurement to be used for clinical decision making. Several decision rules have been recommended for use in newborns during their birth hospitalization. These include:

1. Plot TcB level results on the Bhutani nomogram. If the TcB level is in the high-intermediate or high-risk zone, the screen is “positive.”
2. Determine the phototherapy threshold with use of the AAP hyperbilirubinemia guideline. If the TcB level is within 70% of this threshold, the screen is positive.
3. Determine the phototherapy threshold. If the TcB level is within 3 mg/dL of this threshold, the screen is positive ([Maisels, 2006](#); [Maisels et al., 2009](#)).

With each of these rules it is recommended that a TSB level be obtained in a newborn with a positive TcB screen. The utility of these three decision rules has been evaluated in a large multisite study ([Taylor et al., 2016](#)). The main outcome was accurate identification of a newborn with a TSB level at or above the phototherapy threshold. The false-negative rates with each of the rules was less than 10%, but no rule correctly identified all neonates with a TSB level requiring phototherapy. These results indicate that use of TcB level for screening is not foolproof; a blood draw for a TSB level may be needed if clinical evaluation suggests that the level of jaundice in a neonate is more severe than that indicated by TcB measurement.

Unless levels are high enough to require an exchange transfusion, phototherapy is effective for treatment of a newborn with significant hyperbilirubinemia. Although repeated measurements of direct bilirubin level are not cost-effective, one assessment is helpful before, or just after, initiation of phototherapy to rule out direct hyperbilirubinemia ([Newman et al., 1991](#)). An assessment of the potential for hemolysis as the cause of the elevated bilirubin level, possibly including a review of maternal and infant blood type, direct Coombs test result, hematocrit, reticulocyte count, and red cell morphology, may also be useful.

There is no conclusive evidence as to whether continuous phototherapy leads to more rapid reduction in serum bilirubin levels than intermittent treatment ([Lau and Fung, 1984](#); [American Academy of Pediatrics Subcommittee on Hyperbilirubinemia, 2004](#); [Sachdeva et al., 2015](#)). Unless bilirubin levels are approaching exchange transfusion levels, it is probably reasonable to discontinue treatment for several minutes to 1 hour at frequent intervals to allow parents to feed and hold their baby. Serial bilirubin measurements are needed to determine the adequacy of therapy and to

determine when phototherapy can be discontinued. A “rebound” bilirubin level obtained 24 hours after discontinuation of phototherapy may be helpful in some clinical situations.

Respiratory Complications

The term or late preterm fetus accomplishes transition from dependency on the placenta to the newborn cardiorespiratory system, for the most part, without incident. After birth, pulmonary blood flow increases, fetal shunts reverse and begin to close, spontaneous breathing effort is initiated, and fetal lung fluid is cleared. Effective cardiorespiratory function, as represented by an absence of respiratory distress (nasal flaring, grunting, chest wall retractions, a respiratory rate of greater than 60 per minute) and an oxygen saturation in the mid-90s, is established by several hours of age ([Levesque et al., 2000](#); [O'Brien et al., 2000](#)).

This normal sequence of events fails to occur in 2%–8% of newborns born at 34 weeks' gestation or later ([Hansen et al., 2008](#); [Yoder et al., 2008](#); [Farchi et al., 2009](#)). It is important to keep in mind that the initial presenting symptoms are relatively nonspecific. [Agrawal et al. \(2003\)](#) studied a large number of consecutively born neonates in an attempt to determine the frequency and nature of different early-onset respiratory disorders, and found that more than half of the cases did not meet specific diagnostic criteria. When confronted with early-onset respiratory symptoms, the most important diagnostic considerations to consider include:

- Complex structural cardiac system anomalies; incidence estimated to be between 0.11% and 0.17%; often, but not always, identified by in utero imaging studies ([Oster et al., 2013](#))
- Diaphragmatic hernia; incidence estimated to be between 0.04% and 0.08%; commonly identified by second trimester ultrasound examination ([de Buys Roessingh and Dinh-Xuan, 2009](#))
- Respiratory distress syndrome (RDS); incidence estimated to range between 0.45% and 2.4% depending on the population studied; risk is increased in late preterm newborns and newborns born by cesarean delivery particularly if this happens before labor ([Bertin et al., 1996](#); [Yoder et al., 2008](#); [Jain et al., 2009](#))
- Persistent pulmonary hypertension of the newborn; incidence between 0.1% and 0.3%; often occurs in association with other acute respiratory conditions; a recent large study suggests a slightly increased risk with maternal selective serotonin reuptake inhibitor treatment ([Konduri and Kim, 2009](#); [Huybrechts et al., 2015](#))
- Meconium aspiration syndrome; incidence reported to range between 2% and 9% among newborns delivered through meconium-stained amniotic fluid (7%–20% of all deliveries); risk is increased in newborns delivered after 40 weeks' gestation and/or with intrapartum distress (routine tracheal intubation to facilitate suction is no longer recommended) ([Liu and Harrington, 2002](#); [Bhutani, 2008](#); [Perlman et al., 2015](#))
- Spontaneous pneumothorax; incidence between 0.1% and 0.8%; newborns born by cesarean delivery may be at increased risk ([Zanardo et al., 2007](#); [Benterud et al., 2009](#))
- Transient tachypnea of the newborn (TTNB); incidence ranges between 0.3% and 3.9%; risk factors include late prematurity and cesarean delivery; initial diagnosis is sometimes difficult to differentiate from pneumonia and early respiratory distress ([Gugliani et al., 2008](#); [Yoder et al., 2008](#); [Jain et al., 2009](#); [Tita et al., 2009](#))
- Pneumonia; incidence difficult to determine, one recent retrospective report estimated it to be 0.3%; risk factors include maternal chorioamnionitis and prolonged rupture of membranes;

sometimes it is difficult to differentiate it from RDS and/or TTNB (Yoder et al., 2008)

Review of the maternal history, particularly pregnancy, labor, and delivery, may provide useful diagnostic information. For example, the results of a second trimester ultrasound examination could reveal the possibility of a cardiac defect or diaphragmatic hernia. A positive maternal GBS test result without adequate treatment, prolonged rupture of amniotic membranes, and/or evidence of chorioamnionitis suggests the possibility of pneumonia. For newborns with respiratory distress born by cesarean delivery before the onset of labor a diagnosis of RDS should be considered and GA should be estimated. Finally, TTNB is a diagnosis of exclusion; it is prudent to rule out other causes before this is considered to be the cause of respiratory distress in a term neonate.

In most cases, minimum initial diagnostic efforts for a term newborn with unsuspected respiratory distress should include a chest X-ray and assessment of the arterial oxygen saturation. The results of these studies, in combination with the maternal history, should provide information helpful to (1) establish the initial management, such as the need for supplemental oxygen and/or continuous monitoring, (2) determine the need for further work-up or treatment, possibly including an echocardiogram, laboratory testing, and treatment for possible sepsis, or (3) in severe cases, refer the newborn for further specialty consultation and/or intensive care.

Cardiovascular Issues

Congenital heart disease is a relatively common condition in newborns, with an estimated incidence of 81 cases per 10,000 live births (Reller et al., 2008). Ventricular septal defect (VSD) is, by far, the most common defect, accounting for more than 30% of all cases. The increasing accuracy of prenatal ultrasound examinations has greatly improved the early diagnosis of complex congenital heart disease (CHD). The results of population-based reviews indicate that the sensitivity of routine prenatal ultrasound examinations in identifying selected congenital defects is as high as 70% and is as high as 85% for hypoplastic left-sided heart syndrome (Rasiah et al., 2006; Chew et al., 2007). For mothers at high risk of delivering a newborn with CHD, the use of fetal echocardiography is helpful for delineating the anatomy and significance of specific lesions. However, many of the most common defects, particularly VSD, are not typically detected prenatally.

In the absence of a prenatal diagnosis, detection of CHD is via physical examination and oxygen saturation screening (see the section entitled “Screening for Critical Congenital Heart Disease”). Even if the oxygen saturation screen is normal, it is important to continue to consider the possibility of CCHD since some lesions, particularly coarctation of the aorta, may not be detected with screening (Lannering et al., 2015).

At birth, many babies have loud murmurs that are thought to be from either a closing ductus arteriosus or tricuspid regurgitation (Silberbach and Hannon, 2007). These murmurs are transient and not indicative of disease. Conversely, murmurs associated with VSDs may not be heard for several days, when the pulmonary vascular resistance has dropped enough to permit a significant shunting of blood from left to right. Although the ratio of pathologic to benign murmurs is higher in newborns than in older children, most of the murmurs heard during the newborn nursery stay in a healthy neonate are not clinically significant. Characteristics that increase the likelihood that a murmur signifies the presence of CHD include an intensity of 0.5 or more, a harsh quality, occurrence

during all of systole or into diastole, and being heard best at the lower sternal border or right upper border (Mackie et al., 2009). In a healthy newborn the most common presentation of CHD is a somewhat harsh systolic murmur that is heard best at the lower left sternal border in an asymptomatic newborn, indicative of a VSD.

In addition to auscultation, it is helpful to assess a newborn with a murmur for dysmorphic features and/or other anomalies as these findings increase the likelihood that the murmur is indicative of CHD. It is important to evaluate the adequacy of femoral pulses to rule out coarctation of the aorta. Femoral pulses may be hard to feel in a neonate; if there is uncertainty, upper and lower extremity blood pressures can be measured. Chest radiographs and electrocardiograms are usually of limited value in evaluating healthy newborns with murmurs (Oeppen et al., 2002; Mackie et al., 2009).

Term neonates frequently have alterations in cardiac rhythm and rate. Heart rates in term newborns may be as high as 200 beats per minute (particularly when agitated) or as low as 80 beats per minute (particularly when asleep). These values are usually indicative of normal variation and are not clinically meaningful unless there are other signs of illness and/or if there is a lack of variability in rate with stimulation or attempts at calming the newborn. Arrhythmias are also not uncommon, occurring in approximately 1% of newborns (Southall et al., 1981). By far the most common arrhythmia in a well-appearing term newborn is from premature atrial contractions (PACs) (O'Brien et al., 2000; Larmay and Strasburger, 2004). These are almost always benign and usually transient. If there is concern about an irregular rhythm in a newborn, an electrocardiogram can be obtained. With PACs the irregular beat is initiated by a P wave. Although the QRS complex may be widened, it is always preceded by the P wave. In most cases no further work-up is needed. Cardiology consultation may be warranted if PACs are persistent or if widened QRS complexes are seen on the electrocardiogram.

Possible Neonatal Sepsis

Although early-onset sepsis (EOS) is a distinctly rare occurrence in term neonates, early identification of a newborn with EOS is a central focus of newborn care and a source of considerable anxiety for clinicians. This anxiety is driven by two unique features of EOS. First, the phenomenon of an apparently healthy newborn becoming moribund from overwhelming sepsis in a matter of hours is well described. Secondly, the earliest signs of infection are frequently subtle and nonspecific. Fortunately, with the advent of screening mothers for GBS colonization prenatally and providing intrapartum antibiotics for those colonized, the rates of EOS have fallen substantially in the past 25 years. In the 1980s and 1990s the rates of neonatal sepsis in the United States were estimated at 2.0–2.5 cases per 1000 live births. By 2008 the overall rate was estimated at 0.8–1.0 cases per 1000 live births (Isaacs et al., 1995; Cordero et al., 2004; Stoll et al., 2011; Weston et al., 2011).

The rate of EOS is lower among term newborns. In one large surveillance study, conducted in the era of nearly universal screening for maternal GBS infection, the rate of sepsis in newborns with birthweights greater than 2500 g was 0.57 cases per 1000 live births (Stoll et al., 2011). However, even with the reduction of GBS cases, this bacterium accounted for most cases of EOS in newborns with birthweights of 2500 g or greater, with a rate of 0.37 cases per 1000 live births. The rate of EOS caused by *Escherichia coli* among newborns with birthweights greater than 2500 g was 0.07 cases per 1000 live births, with the remainder of cases caused

by a variety of other bacteria. Of note, in this study, among a group of almost 400,000 newborns, only two cases of EOS caused by *Listeria monocytogenes* were identified.

Given the current rates of EOS and distribution of pathogens, the decision of whether to initiate empiric antibiotic therapy for possible EOS in a term newborn is dependent on a few key variables. The easiest of these variables to quantify are the maternal GBS screening results, the drugs used, and the timing of intrapartum antibiotic therapy provided to mothers who screen positive. In addition to GBS status, other “risk factors” related to labor and delivery can be used to develop a numeric estimate of a newborn’s risk of EOS at birth (Puopolo et al., 2011; Escobar et al., 2014). In some cases the estimated risk of EOS is sufficiently high at birth such that empiric antibiotic therapy for possible sepsis is begun regardless of the appearance of a neonate. In newborns who are not initially treated with antibiotics, clinicians use vital sign, physical examination, and laboratory test findings in combination with the newborn’s estimated risk at birth to determine when to begin empiric antibiotic therapy for EOS. Unfortunately, although the overall appearance of a newborn is said to be the best predictor of EOS, there are only a handful of specific, objective, and evidence-based vital sign and physical examination findings that are useful, and laboratory test results are, disappointingly, of only moderate help. The utility and limitations of these variables are discussed in the following sections

Group B Streptococcus Screening and Intrapartum Antibiotic Prophylaxis

Since the 1970s GBS has been a major cause of neonatal sepsis (Schuchat, 1998). The implementation of intrapartum antibiotic prophylaxis (IAP) to prevent early-onset GBS disease in neonates has been associated with a more than 80% decrease in the rate of infection (Phares et al., 2008; Centers for Disease Control and Prevention, 2016). Recent surveillance data from the United States suggest that the overall rate of EOS caused by GBS is now in the range of 0.25 cases per 1000 live births. Unfortunately, the rates of GBS disease are more than twice as high among African-American neonates in the United States than in newborns of other races (Centers for Disease Control and Prevention, 2016).

The currently recommended strategy to prevent GBS disease is to obtain rectovaginal cultures on all pregnant women at 35–37 weeks’ gestation and to administer IAP with penicillin or ampicillin during labor to those colonized with the bacteria. In situations where the mother’s GBS status is unknown before the onset of labor, IAP is advised for those with certain risk factors for neonatal infection. IAP is not needed in women who undergo cesarean delivery before the onset of labor with intact amniotic membranes (Verani et al., 2010).

The effectiveness of IAP in preventing early-onset GBS disease in neonates born to colonized mothers is dependent on the drugs used and the timing of administration. Penicillin or ampicillin, with at least one dose given more than 4 hours before delivery, is the most effective treatment. It is estimated that the efficacy of this regimen in preventing GBS disease in term newborns is 91% (95% confidence interval 63%–98%). Although ampicillin or penicillin administered less than 4 hours before delivery has been shown to be effective in reducing vertical transmission, in one surveillance study the effectiveness of ampicillin or penicillin administered less than 2 hours before delivery in preventing early-onset GBS disease was only 47%, and the effectiveness of a dose administered more than 2 hours but less than 4 hours before delivery was estimated at 38%. The effectiveness of clindamycin,

administered to women who had a history of penicillin allergy, was estimated at 22%, although antimicrobial susceptibility testing was infrequently performed on the GBS strains cultured from the mothers in this study (Fairlie et al., 2013). It is recommended that clindamycin be used only for IAP in mothers who are colonized by a strain of GBS that is documented to be sensitive to this antibiotic (Verani et al., 2010). Although there are fewer data on the effectiveness of cefazolin for IAP, on the basis of its pharmacologic profile it is likely to have an effectiveness similar to that of penicillin in preventing GBS disease.

Overall, about 75% of women who are screened for GBS are classified as GBS negative (Van Dyke et al., 2009). However, approximately 10% of these classifications are false negatives. Ironically, because of this false-negative rate, and since mothers with negative GBS screening results do not commonly receive IAP, more than 60% of newborns in the United States who develop early-onset GBS disease are born to women classified as GBS negative (Van Dyke et al., 2009). Thus although the risk of EOS from GBS is very low, clinicians should consider this possibility in term newborns born to “GBS-negative” mothers who are displaying signs and symptoms of the disease.

Term newborns whose mothers have received IAP for a positive GBS screen at least 4 hours before delivery (termed *adequate* IAP) can be safely discharged at 24 hours of age if there are no signs or symptoms of infection (Verani et al., 2010). One area of consternation is how long to observe term newborns when the only doses of antibiotics were administered less than 4 hours before delivery (“inadequate” IAP). Most newborns with sepsis present early in life—many at birth, and nearly all by 12 hours of age (Escobar et al., 2000). Among a group of 172 term newborns with documented early-onset GBS infection, 95% had presenting symptoms within 24 hours after delivery (Bromberger et al., 2000). There is the theoretical concern that newborns whose mothers receive some but inadequate IAP to prevent early-onset GBS disease will have a delayed or muted initial presentation because of the antibiotics received. Perhaps because of this concern, in current guidelines it is recommended that such newborns be monitored in the hospital for at least 48 hours after birth (Verani et al., 2010). However, evidence to date suggests that newborns who develop GBS disease despite IAP, either inadequate or adequate, continue to present within the first 24 hours of life and have the same presentation as those with EOS caused by GBS whose mothers did not receive IAP (Bromberger et al., 2000). These data suggest, but do not definitively indicate, that some term newborns born to GBS-positive mothers who received IAP less than 4 hours before delivery may be discharged at less than 48 hours of age. This decision is best made on an individual basis considering all risk factors, examination of the baby, vital sign stability, the results of any available laboratory tests, and parental wishes.

Evaluation of Perinatal Risk Factors

Because the initial signs and symptoms of EOS can be difficult to distinguish from normal newborn variation, the estimate of a newborn’s risk of sepsis at birth is crucial for determining appropriate monitoring, laboratory testing, and/or treatment. Several prenatal and perinatal conditions are known to substantially increase the chance that a newborn will develop EOS, including preterm birth, prolonged rupture of amniotic membranes, maternal GBS infection and IAP, maternal fever, chorioamnionitis, and history of a sibling with EOS (Verani et al., 2010; Wortham et al., 2016). In particular, the clinical diagnosis of chorioamnionitis may increase the risk of EOS by twofold to sixfold (Benitz et al., 1999; Escobar et al.,

2000). Because of this, both the Centers for Disease Control and Prevention and the AAP recommend blood cultures be obtained and *all* newborns born to mothers with diagnosed chorioamnionitis be treated with empiric intravenous antibiotics pending the results of the blood cultures.

However, guideline recommendations to initiate treatment with intravenous antibiotics on all *term* newborns born to women with chorioamnionitis remain controversial. Many of the large studies that documented the increased risk of EOS in the setting of chorioamnionitis were conducted before universal GBS screening and IAP (Taylor and Opel, 2012). Although exact figures are elusive, it is estimated that chorioamnionitis complicates approximately 3% of births in the United States (Verani et al., 2010). Given the low current rate of EOS, it has been estimated that 80–210 newborns born to mothers with chorioamnionitis would require empiric antibiotic treatment in order to “catch” one neonate with EOS (i.e., the number needed to treat is 80–210). For term newborns who appear well, the estimated number needed to treat is 450 (Wortham et al., 2016).

Investigators from Boston and northern California have adopted a different approach for estimating the risk of EOS at birth in newborns born at 34 weeks’ gestation or later. Using data from more than 600,000 deliveries, they developed a model for assigning a numeric risk of EOS in a newborn based on the following criteria: GA, maximal maternal temperature during labor, hours of rupture of membranes, GBS screening results in the mother, and use and timing of IAP (Puopolo et al., 2011). Of note, the specific presence or absence of chorioamnionitis is not a variable considered in the model. Using a website (<http://www.newbornsepsiscalculator.org>), clinicians can enter the appropriate data for these variables, and a numeric estimate of sepsis at birth in a particular newborn is provided. The risk of sepsis is further refined on the basis of the clinical appearance of the newborn (well-appearing, equivocal presentation, or evidence of clinical illness). Specific criteria for these classifications are provided. The estimated risk for newborns in each of these categories is provided. The clinician can then consider this risk estimate and determine the appropriate management for the baby. Alternatively, the originators have recommended that empiric antibiotic therapy be initiated if the estimated risk of EOS is 1.54 or more cases per 1000 live births (Escobar et al., 2014). However, there is no rationale for this threshold other than the opinion of the investigators.

Clinical and Laboratory Evaluation

Early identification of a newborn with EOS based on clinical and laboratory findings is difficult. A number of signs and symptoms have been reported to be present in neonates with EOS, including fever, hypothermia, temperature instability, hypotension, lethargy, irritability, pale or mottled skin, cyanosis, apnea, hypoglycemia, acidosis, tachycardia, tachypnea, grunting respirations, nasal flaring, and inspiratory retractions (Escobar et al., 2014; Wortham et al., 2016). Unfortunately, with a few exceptions there has been little investigation on how frequently these signs and symptoms are present early in the course of EOS in term newborns, and it is unclear whether the presence of each of these signs and symptoms is more common in those with EOS versus noninfected babies. Thus the utility of most of these signs and symptoms in helping to diagnose EOS is unknown.

It is apparent that most newborns with EOS have clinical signs of illness and that the rate of EOS is significantly lower in well-appearing neonates who are at risk of infection because of maternal and/or perinatal risk factors. In a compilation of studies the rate

of EOS among well-appearing term and preterm newborns was 0.04 per 1000 “at-risk” infants versus 2.9% in symptomatic term and late preterm neonates (Benitz et al., 2015). In another study, of 2785 newborns with birthweights greater than 2000 g who underwent a laboratory evaluation for sepsis, the absence of clinical signs of sepsis reduced the risk of EOS by almost fourfold (Escobar et al., 2000).

The developers of the newborn sepsis calculator (described in the previous section) identified four specific and objective clinical signs of sepsis: temperature of 38.0°C or greater or less than 36.4°C; heart rate of 160 or more beats per minute; respiratory rate of 60 or more breaths per minute; and respiratory distress (grunting, flaring, and retractions) (Escobar et al., 2014). Importantly, for one of these signs to be “present” it needs to be identified at least twice, with the two instances separated by more than 2 hours. If one or more of these signs are present in a newborn previously thought to be “well-appearing,” the newborn is not categorized as having “equivocal” examination findings for EOS. This classification system is useful; the sepsis calculator provides different numeric estimations for the risk of sepsis in a newborn with a given risk at birth based on whether the examination findings are normal or equivocal.

Several laboratory test findings, including a low absolute neutrophil count, a high ratio of immature to total neutrophil counts, a high ratio of immature to total neutrophil counts divided by total neutrophil count, and an elevated C-reactive protein level, are statistically associated with an increased risk of EOS (Benitz et al., 1998; Escobar et al., 2000; Newman et al., 2010; Newman et al., 2014). Unfortunately, many, if not most, newborns with EOS have normal laboratory screening test results (Benitz et al., 1998; Escobar et al., 2000; Newman et al., 2010). Further, the negative predictive values of these tests are very high, but since the prevalence of EOS in term newborns, even those with risk factors for sepsis, is low, a normal laboratory test finding, in isolation, is of limited help in aiding clinical decision making.

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27

Newborn Screening

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KEY POINTS

- Newborn screening (NBS) provides an opportunity for early identification of newborns with disorders in which the clinical complications develop postnatally and may remain undetected before irreversible clinical damage.
- NBS is a “screen,” and individuals should not be labeled as having a disorder before diagnostic testing confirms the condition.
- Once a disorder has been confirmed, however, treatment should be started without delay to prevent irreversible clinical complications.
- False-positives are inevitable; false negatives are possible.
- The clinical spectrum of disorders is wider than expected.
- The disorder detected is not always the one that was sought.
- Numerous methods are used in NBS, ranging from isoelectric focusing to enzymatic assays, tandem mass spectrometry, and high-throughput genomic sequencing.
- Advances in technology result in modifications in the panel of disorders screened and/or the biomarkers analyzed to screen newborns for them.

Newborn screening (NBS) is directed at disorders in which the clinical complications develop postnatally. In metabolic diseases the complications result from biochemical abnormalities that appear after birth, when the infant is no longer protected by fetal–maternal exchange. For example, the infant with phenylketonuria (PKU) has a normal blood phenylalanine level at birth but within a few hours demonstrates hyperphenylalaninemia. The infant with congenital hypothyroidism (CH) is also protected in utero, most likely from placental transfer of maternal thyroxine (T_4). If the hyperphenylalaninemia in PKU is not controlled by diet or the hypothyroidism in CH is not corrected by supplemental T_4 , the infant begins to show signs of developmental delay and subsequently becomes intellectually disabled. If therapy begins during the first few weeks of life, intellectual disability in both disorders is prevented.

PKU was the first metabolic disorder known to benefit from dietary therapy. This fact was established by the mid-1950s. By the late 1950s it was evident that dietary therapy could prevent intellectual disability if initiated in the neonatal period. Detecting PKU in all affected newborns at that early age, before irreversible brain damage occurred, then became the challenge. Meeting this challenge required neonatal screening for a biochemical marker of the disease, which was accomplished in 1962 when Guthrie developed a simple bacterial assay for phenylalanine requiring only

a few drops of blood saturating circles on filter paper (Guthrie and Susi, 1963). Therefore newborns in newborn nurseries could be routinely tested for PKU in blood specimens obtained by the lancing of the heel and the blotting of the drops of blood onto a filter paper card. This filter paper blood specimen (dried blood spot specimen) could be mailed to a central laboratory for PKU testing. An increased concentration of phenylalanine in the specimen indicated PKU in the infant. By the mid-1960s many states had established routine NBS programs for PKU using the Guthrie method. Infants with PKU were identified in larger numbers than anticipated and showed normal development while receiving treatment (O’Flynn, 1992).

The success of PKU screening led to the addition of tests for other metabolic diseases, including galactosemia, maple syrup urine disease (MSUD), and homocystinuria. These additional tests could be performed on the same blood specimen obtained for PKU screening. Within a decade, NBS expanded to include the endocrine disorders CH and congenital adrenal hyperplasia (CAH), the hematologic disorder sickle cell disease, and later biotinidase deficiency, cystic fibrosis, and other diseases.

In 1990, the technology of tandem mass spectrometry (MS/MS) was applied to the dried blood spot specimen, opening a new era in NBS (Millington et al., 1990; Levy, 1998). This method allowed the accurate detection of numerous biochemical markers for metabolic disorders with a single assay, thereby replacing several assays traditionally used in screening for metabolic disorders and adding additional biomarkers not detectable by previous methods, thus greatly expanding the spectrum of conditions identifiable in the neonate (Levy and Albers, 2000). By the early 1990s, Naylor (Chace and Naylor, 1999) and Rashed et al. (1995) began using MS/MS to routinely screen neonates for more than 20 biochemical disorders with high specificity that resulted in an extremely low rate of false-positive results. Currently all programs in the United States and many screening programs in Europe and elsewhere have integrated MS/MS into NBS; some are screening newborns for more than 60 individual conditions (<http://www.babysfirsttest.org/newborn-screening/states>).

In 2006, in an attempt to standardize screening panels nationwide, the American College of Medical Genetics (2006) recommended a panel of 29 core conditions for screening and an additional 25 secondary conditions for which test results could be reported. These secondary conditions, are those that are identified in the course of screening for the 29 core conditions, but either their clinical spectrum is not well known or effective treatment is unavailable. Currently the task of reviewing and recommending

conditions nominated for inclusion in the Recommended Uniform Screening Panel (RUSP) is performed by the Advisory Committee on Heritable Disorders in Newborns and Children. The committee completes a systematic evidence-based review, deliberates on the evidence available, and votes to recommend or not recommend adding the nominated condition to the RUSP for consideration by the Secretary of Health and Human Services. The secretary makes the final decision on whether to add, or not to add, a recommended condition to the RUSP. At time of writing the RUSP includes 34 core disorders and 26 secondary disorders (Tables 27.1–27.2) (<http://www.hrsa.gov/advisorycommittees/mchbadvisory/heritabledisorders/recommendedpane/>).

Molecular diagnostic techniques (i.e., DNA analysis) are also used in NBS, predominantly as “second-tier” tests to specify diseases such as cystic fibrosis (CF) or medium-chain acyl-coenzyme A (CoA) dehydrogenase deficiency (MCADD) that are suspected because of a primary screening abnormality. Molecular testing substantially increases the positive predictive value of a primary screening result based on metabolite testing (Ranieri et al., 1994; Wilcken et al., 1995; Ziadeh et al., 1995). With the initiation of screening for severe combined immunodeficiency (SCID) syndrome, wherein the markers measured are DNA molecules (i.e., T-cell receptor excision circles [TRECs]), the role of molecular technology extended into primary marker analysis (Vogt, 2008; Baker et al., 2009). The use of molecular testing in screening will most likely expand further with advances in DNA technology.

Screening Procedure

Currently almost all disorders on the RUSP are screened by laboratory analysis of the dried blood spot specimen of the neonate. The two exceptions are a point-of-care hearing test and pulse oximetry performed in the nursery to screen newborns for hearing loss and critical congenital heart disease (CCHD) respectively.

Specimen

The blood specimen is generally obtained from the heel of the infant. This simple sampling method, conceived and introduced by Guthrie and Susi (1963), has made NBS feasible, since blood is easily obtained and can be easily and inexpensively delivered to a central testing facility by mail or courier. There are no serious complications from obtaining these newborn specimens, contrary to early fears that their collection would lead to infection or result in excessive bleeding.

Specimen Collection

The blood specimen should be obtained from the lateral or the medial side of the heel (Fig. 27.1). Blood should be applied to only one side of the filter paper card, but it should saturate each circle on the card. Contamination of the filter paper specimen with iodine, alcohol, petroleum jelly, stool, urine, milk, or a substance such as oil from the fingers can adversely affect the results of the screening tests. In addition, exposure to heat and humidity can inactivate enzymes and produce false results. The specimen should be dried in air at room temperature for at least 3 hours before being placed in an envelope.

Specimens are sometimes collected in capillary tubes, by venipuncture of a dorsal vein or from a central line, and then spotted on filter paper. There is little or no substantial difference in analyte levels between blood collected directly from the heel



• **Fig. 27.1** Hatched areas on the medial and lateral sides of the heel of the sole indicate the proper sites for a heel stick in the newborn.

and that collected by any of these other methods (Lorey and Cunningham, 1994). However, there is the danger of introducing amino acids into the specimen in infants receiving total parenteral nutrition (TPN) if the blood is collected from a central line, resulting in a false-positive increase in the levels of amino acids or interference in some molecular assays by the heparin within the line. In general, it is preferable that blood for screening be spotted on filter paper directly from the heel.

Timing of Collection

Specimen collection timings differ around the world. In the United States most specimens are collected 24–48 hours after birth. In Europe and Australia, however, screening specimens are collected within 48–72 hours, and in the United Kingdom the specimen is not collected until the newborn is 5–8 days old (Public Health England, 2013). The specimen should be obtained from every newborn before nursery discharge or by the third day of life, whichever is first. In newborns whose initial specimen was obtained within the first 24 hours after birth, as may happen with the practice of early nursery discharge, a second blood specimen should be obtained at no later than 7 days of age to be certain that a diagnosis is not missed.

NBS encompasses a gamut of conditions, each with its own ideal screening period during which there is the greatest chance of diagnosing the disorder and before the onset of symptoms. As a result, it is worth noting that recommendations on the timing of specimen collection, although appropriate for most conditions, may not be ideal for all conditions on the screening panel. For instance, for CAH, in which the symptoms can manifest themselves within the first week of life, the optimal time for collection of the specimen is within 24–48 hours after birth. Formerly there was concern that with the NBS specimen collected early, often during the first day of life, some newborns with metabolic disorders or with CH might not have a sufficient degree of abnormality for identification. However, the MS/MS method with its increased sensitivity and specificity has considerably increased the reliability of screening for metabolic conditions in very early specimens (Chace and Naylor, 1999). Furthermore, use of thyroid-stimulating

**TABLE
27.1****Core Disorders in the Recommended Universal Screening Panel**

| Disorder | Acronym | Primary Marker |
|--|----------|---------------------------------------|
| Metabolic Disorders Detected by Tandem Mass Spectrometry (Amino Acid and Acylcarnitine Markers) | | |
| Organic Acid Disorders | | |
| β-Ketothiolase deficiency (mitochondrial acetoacetyl-CoA thiolase deficiency) | BKT | C5:1 |
| Cobalamin defects A, B, | CBL A,B | C3 |
| Isovaleric acidemia ^a | IVA | C5 |
| Glutaric aciduria I | GA-I | C5DC |
| 3-Hydroxy-3-methylglutaryl-CoA lyase deficiency ^a | HMG | C5OH |
| Multiple carboxylase deficiency ^a | MCD | C3; C5OH |
| 3-Methylcrotonyl-CoA carboxylase deficiency | 3MCC | C5OH |
| Methylmalonic acidemia (mutase) ^a | MMA-MUT | C3 |
| Propionic acidemia ^a | PA | C3 |
| Fatty Acid Oxidation Defects | | |
| Carnitine uptake defect (carnitine transporter defect) | CUD | C0 (reduced) C0 |
| Long-chain hydroxyacyl-CoA dehydrogenase deficiency ^a | LCHAD | C16OH/C18:1OH |
| Trifunctional protein deficiency ^a | TFP | C16OH/C18:1OH |
| Medium-chain acyl-CoA dehydrogenase deficiency | MCAD | C8 |
| Very long chain acyl CoA dehydrogenase deficiency | VLCAD | C14:1 |
| Amino Acid Disorders | | |
| Argininosuccinic aciduria (argininosuccinate lyase deficiency) ^a | ASA | Argininosuccinic acid |
| Citrullinemia I (argininosuccinate synthase deficiency) ^a | CIT-I | Citrulline |
| Phenylketonuria | PKU | Phenylalanine |
| Maple syrup urine disease ^a | MSUD | Leucine |
| Homocystinuria | HCY | Methionine |
| Tyrosinemia type I | TYRI | Succinylacetone |
| Other Metabolic Disorders | | |
| Biotinidase deficiency | BIOT | Biotinidase activity |
| Galactosemia ^a | GALT | Total galactose, GALT activity |
| Glycogen storage disease type II (Pompe disease) | GSD-II | Lysosomal acid α-glucosidase activity |
| Mucopolysaccharidosis type I | MPS-I | α-L-Iduronidase activity |
| X-linked adrenoleukodystrophy | X-ALD | C26:0 lysophosphatidylcholine |
| Endocrine Disorders | | |
| Congenital adrenal hyperplasia ^a | CAH | 17-Hydroxyprogesterone |
| Congenital hypothyroidism | CH | T ₄ , TSH |
| Hemoglobin Disorders | | |
| S,S disease (sickle cell anemia) | HbSS | Hb variants |
| S,C disease | HbS/C | Hb variants |
| Hemoglobin S/β-thalassemia | HbS/β-Th | Hb variants |
| Other Disorders | | |
| Cystic fibrosis | CF | Immunoreactive trypsinogen |
| Severe combined immunodeficiency | SCID | T-cell receptor excision circles |
| Hearing | HEAR | Failed hearing test |
| Critical congenital heart disease | CCHD | Pulse oximetry |

GALT, Galactose 1-phosphate uridylyltransferase; Hb, hemoglobin; T₄, thyroxine; TSH, thyroid-stimulating hormone.^aCan manifest itself acutely in the first week of life.

TABLE 27.2 Secondary Disorders Recommended for Universal Screening

| Disorder | Acronym | Primary Marker |
|--|---------------|----------------------------------|
| Metabolic Disorders Detected Using Tandem Mass Spectrometry (Amino Acid and Acylcarnitine Markers) | | |
| Organic Acid Disorders | | |
| Cobalamin defects C, D | CBL C, D | C3 |
| Isobutyrylglycinuria | IBG | C4 |
| Malonic aciduria | MAL | C3DC |
| 2-Methylbutyrylglycinuria | 2MBG | C5 |
| 3-Methylglutaconic aciduria | 3MGA | C50H |
| 2-Methyl 3-hydroxybutyric aciduria | 2M3HBA | C50H |
| Fatty Acid Oxidation Defects | | |
| Carnitine palmitoyltransferase IA deficiency | CPT-IA | C0 |
| Carnitine palmitoyltransferase II deficiency | CPT-II | C16, C18:1 |
| Carnitine–acylcarnitine translocase deficiency | CACT | C16, C18:1 |
| 2,4-Dienoyl-CoA reductase deficiency | DE RED | C10:2 |
| Glutaric aciduria II/multiple acyl-CoA dehydrogenase deficiency | GA II | C4, C5, C5DC, C8, C14, C16 |
| Medium-chain ketoacyl-CoA thiolase deficiency | MCAT | C8 |
| 3-Hydroxyacyl-CoA dehydrogenase deficiency (listed as medium/short-chain 3-hydroxyacyl-CoA dehydrogenase deficiency) | HAD (M/SCHAD) | C40H |
| Amino Acid Disorders | | |
| Arginase deficiency | ARG | Arginine |
| Citrullinemia II | CIT-II | Citrulline |
| Benign hyperphenylalaninemia | H-PHE | Phenylalanine |
| Biopterin defect—cofactor biosynthesis | BIOPT (BS) | Phenylalanine |
| Biopterin defect—cofactor regeneration | BIOPT (REG) | Phenylalanine |
| Tyrosinemia type II | TYR-II | Tyrosine |
| Tyrosinemia type III | TYR-III | Tyrosine |
| Other Disorders | | |
| Galactose epimerase deficiency | GALE | Total galactose |
| Galactokinase deficiency | GALK | Total galactose |
| Various other hemoglobinopathies | Var Hb | Hb variants |
| T-cell–related lymphocyte deficiencies | — | T-cell receptor excision circles |

Hb, Hemoglobin.

hormone (TSH) as the primary marker for CH, or as a second-tier test when T_4 is the primary marker, has similarly allowed early screening for CH to be reliable.

Special circumstances require specific attention to newborn blood specimen collection. Premature newborns or those with very low birth weight, as well as newborns who are sick and those in neonatal intensive care units (NICUs), are at risk of unreliable screening owing to factors such as the unique physiology of the newborn, therapeutic interventions, and a focus on critical activities in caring for the very sick neonate. Consequently, a single specimen is inadequate for screening newborns in this subpopulation, and

additional specimens should be collected for retesting. Serial screening with collection of three specimens—on admission to the NICU, between 24 and 48 hours after birth, and at discharge or at 28 days of age, whichever is sooner—has been proposed as an adequate and efficient protocol for this population ([Clinical and Laboratory Standards Institute, 2009](#)). In addition, some programs recommend that screening be performed every month until discharge for babies who continue to remain in the NICU.

A blood specimen should be collected from any infant who is being transferred to a different hospital or to an NICU, regardless of age. The first specimen should be collected before transfer, and

a second specimen should be collected at the receiving hospital by 4 days of age. This dual collection policy covers the child from whom a newborn specimen might not have been obtained in the turmoil that frequently accompanies the transfer of neonates.

In a newborn who is to receive a blood transfusion within 24 hours of birth precluding collection of an ideal routine specimen, a screening specimen should ideally be collected before transfusion, and a second specimen should be collected 2 days after the transfusion. In addition, a third screening specimen should be obtained 2 months after the last transfusion, when most of the donor red blood cells have been replaced, to ensure reliable testing for analytes present in red blood cells if a pretransfusion specimen has not been obtained.

Screening Tests

NBS tests are usually performed in a centralized state, provincial, or regional laboratory. In a regional program the specimens may be received by the state program and then delivered to the regional state or private laboratory, or they may be sent directly to the regional laboratory. In either case the individual state programs serve as the state data and follow-up centers.

The testing procedure begins with the punching of small discs (each usually 3 mm in diameter) from the screening specimen. These small discs are then analyzed by various methods for the individual markers being sought. Amino acids and acylcarnitines, the markers for most of the screened metabolic conditions, are simultaneously measured by MS/MS (Rinaldo et al., 2004). As implied at the beginning of this chapter, MS/MS is superior in terms of accuracy of measurement of the individual analytes when compared with alternative methods originally used for screening amino acids, such as bacterial assays or fluorometric techniques. Immunoassays, including fluorimmunoassay and enzyme-linked immunosorbent assays, are used to test for endocrinopathies such as CH and CAH, for infectious diseases such as congenital toxoplasmosis, and for cystic fibrosis. Hemoglobin analysis of blood eluted from the filter paper can be performed either by high-performance liquid chromatography (HPLC) or by isoelectric focusing to identify abnormal hemoglobins associated with the hemoglobinopathies sickle cell disease (hemoglobin S) and thalassemia (hemoglobin A₂, hemoglobin H). An enzyme assay is often used to screen newborns for galactosemia and is always used to screen newborns for biotinidase deficiency. A molecular assay (quantitative polymerase chain reaction) is applied to identify SCID by quantifying TRECs, a marker of newly formed, antigenically naïve thymic emigrant T cells (Chan and Puck, 2005). TREC analysis was the first NBS test to use DNA as the primary analyte. Before implementation of screening for SCID, molecular assays were used only as second-tier tests to detect targeted mutations in disorders such as CF following an out-of-range biomarker. Several platforms, DNA microarrays, and microsphere-based assays can multiplex several molecular and immunologic assays for high-throughput screening and are being used by screening programs (McCabe and McCabe, 1999; Dobrowolski et al., 1999; Green and Pass, 2005). Next-generation sequencing (NGS) technologies with their new high-throughput and massively parallel DNA sequencing technologies have substantially reduced the cost and time required for sequencing and have made it possible to sequence the whole exome and entire coding regions of a gene/genes (Landau et al., 2014). NGS has already been adopted by a few screening laboratories to provide supplemental genotyping information as a second-tier test (Baker et al., 2016) and offers the prospect of

becoming a first-tier test to screen newborns for genetic disorders that do not have a biomarker identifiable by current screening (see the section entitled “The Future” at the end of this chapter).

Secondary Tests

An abnormal finding on an NBS test is not diagnostic. Abnormalities in the newborn specimen can be transient or artifactual. Accordingly, when an abnormality is identified, the original specimen is retested for the analyte that was abnormal. Additional tests can be performed by the screening laboratory to substantiate the finding and increase the specificity of screening (Rinaldo et al., 2006; Matern et al., 2007).

In screening newborns for CH, many programs initially measure T₄. Specimens in which a low T₄ level is found are further tested for an increased level of TSH, which would indicate CH. A normal thyroid stimulating hormone (TSH) level suggests transiently low T₄ levels, a common finding in premature infants, or T₄-binding globulin deficiency. Some screening programs have adopted screening protocols in which the primary analysis is for TSH, and T₄ is measured as a second-tier test in specimens with high concentrations of TSH. Similarly, in screening newborns for galactosemia, an elevated galactose measurement in a specimen can trigger the analysis of galactose 1-phosphate uridylyltransferase (GALT) activity as a second-tier test.

Second-tier molecular testing is also performed in some screening laboratories. For example, in screening newborns for cystic fibrosis, an initial out-of-range primary marker prompts DNA analysis to identify several specific pathogenic mutations (Wilcken et al., 1995; Comeau et al., 2004; Rock et al., 2005). Screening programs following this two-tier immunoreactive trypsinogen–DNA approach can identify up to 99% of patients with CF and report a positive predictive value ranging between 1/9.5 and 1/25 (Grosse et al., 2004). Molecular assays to detect disease-causing mutations are currently used as second-tier tests for several other disorders, and their use is likely to expand with advances in DNA technology. Some examples include testing for the prevalent c.985A>G mutation in MCADD screening, a panel of several GALT mutations in galactosemia screening, and sequencing of the *ABCD1* gene in X-linked adrenoleukodystrophy (X-ALD) screening.

The final interpretation of the screening results is based on the primary analysis and, if available, the results of second-tier testing. However, it is important to realize that screening is not intended to be diagnostic; abnormal screening results must be supported by confirmatory investigations. These studies require additional specimens and are performed by clinical laboratories or sometimes by the screening laboratory.

Physician Contact for Abnormal Results

Table 27.3 indicates disorders or other reasons for abnormal screening results, sorted according to the primary analyte usually used to screen the newborn for the condition. For example, a low T₄ level together with an elevated TSH concentration indicates CH, and a marked elevation of 17-hydroxyprogesterone (17-OHP) level indicates the likelihood of CAH. An elevation of an acylcarnitine level could indicate an organic acid or fatty acid oxidation disorder.

Any infant for whom such a screening result is reported should be evaluated by the primary care provider as soon as possible to facilitate the next steps toward the confirmation and management of the disorder. However, several conditions screened for are

**TABLE
27.3****Disorders or Other Reasons for Abnormal Screening Results Sorted by Primary Marker Analyzed**

| Marker | Concentration | Disorders | Possible Causes of False-positives |
|---|---------------|---------------------------------|--|
| Markers Analyzed Using Tandem Mass Spectrometry | | | |
| Free carnitine | Low | CUD | Poor feeding, maternal CUD |
| Free carnitine | High | CPT-I deficiency | Carnitine supplementation |
| Propionylcarnitine | High | MMA, PA, CBL (A, B), CBL (C, D) | Hemolysis, ^a maternal biotin deficiency, carrier of associated disorders |
| Butyrylcarnitine | High | SCADD/EE/IBDD | Hypoglycemia from other causes, ^a FIGLU level elevation |
| Tiglylcarnitine | High | BKT deficiency | VLBW neonate |
| Isovalerylcarnitine | High | IVA | Antibiotics containing pivalic acid, IVA/MBCD carrier, VLBW neonate, neonate receiving total parental nutrition, FAS hemoglobin profile ^a |
| Glutaryl carnitine | High | GA-I | MCADD carrier, twin or multiple births ^a |
| 3-Methylglutaryl carnitine | High | HMG deficiency | Severe respiratory distress, neonates receiving ECMO ^a |
| Hydroxyisovalerylcarnitine | High | 3MCC deficiency | Maternal 3MCC deficiency, maternal biotin deficiency |
| Octanoylcarnitine | High | MCADD | MCADD carrier, MCT supplementation |
| Tetradecenoylcarnitine | High | VLCADD | Carrier |
| Hydroxyhexadecanoylcarnitine | High | LCHADD/TFP | Carrier, renal dysfunction |
| Hexadecanoylcarnitine | High | CPT-II deficiency | Severe hemolysis ^a |
| Arginine | High | Arginase deficiency | Hyperalimentation |
| Argininosuccinic acid | High | ASA | — |
| Citrulline | Low | OTC/CPS/NAGS | Poor feeding ^a |
| Citrulline | High | CIT-I, CIT-II | Carriers |
| Phenylalanine | High | PKU | Hyperalimentation, specimen contaminated with artificial sweetener ^a |
| Leucine | High | MSUD | Hyperalimentation, hydroxyprolinemia (benign disorder) |
| Methionine | High | HCY | Hyperalimentation, liver dysfunction |
| Tyrosine | High | TYR-II | Prematurity, transient immaturity of enzyme |
| Succinylacetone | High | TYR-I | — |
| Markers Analyzed by Assays Other Than Tandem Mass Spectrometry | | | |
| Biotinidase activity | Low | Biotinidase deficiency | Exposure of specimen to heat, improper drying |
| Total galactose | High | Galactosemia | Contamination with milk/cream |
| GALT activity | Low | Galactosemia | Exposure of specimen to heat |
| 17-Hydroxyprogesterone | High | CAH | Physiologic stress (seen commonly in NICU babies), VLBW, EDTA in specimen |
| T ₄ /TSH | Low/high | CH | Neonates in NICU, maternal thyroid medications |
| Immunoreactive trypsinogen | High | CF | VLBW neonate, NICU |
| T-cell receptor excision circles | Low | SCID | Other immunodeficiencies, Di George syndrome, heparin in specimen ^a |
| α-L-Iduronidase | Low | MPS I | Pseudodeficiency |
| Lysosomal acid α-glucosidase | Low | GSD-II | Pseudodeficiency |
| C26:0 lysophosphatidylcholine | High | X-ALD | Zellweger spectrum disorders |

3MCC, 3-Methylcrotonyl-CoA carboxylase; ASA, argininosuccinic aciduria; BKT, β-ketothiolase deficiency; CAH, congenital adrenal hyperplasia; CBL, cobalamin defect; CF, cystic fibrosis; CH, congenital hypothyroidism; CIT-I, citrullinemia I; CIT-II, citrullinemia II; CPS, carbamoyl phosphate synthetase deficiency; CPT-I, carnitine palmitoyltransferase I; CPT-II, carnitine palmitoyltransferase II; CUD, carnitine uptake defect; ECMO, extracorporeal membrane oxygenation; EDTA, ethylenediaminetetraacetic acid; EE, ethylmalonic encephalopathy; FAS, hemoglobin electrophoretic profile indicative of sickle cell trait; FIGLU, formiminoglutamic acid; GA-I, glutaric aciduria I; GSD-II, glycogen storage disease type II; HCY, homocystinuria; HMG, 3-hydroxy-3-methylglutaryl-CoA lyase; IBDD, isobutyryl-CoA dehydrogenase deficiency; IVA, isovaleric acidemia; LCHADD, long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency; MBCD, 2-methylbutyryl-CoA dehydrogenase deficiency; MCADD, medium-chain acyl-CoA dehydrogenase deficiency; MCT, medium-chain triglycerides; MMA, methylmalonic aciduria; MPS I, mucopolysaccharidosis type I; MSUD, maple syrup urine disease; NAGS, N-acetylglutamate synthetase deficiency; NICU, neonatal intensive care unit; OTC, ornithine transcarbamylase deficiency; PA, propionic acidemia; PKU, phenylketonuria; SCADD, short-chain acyl-CoA dehydrogenase; SCID, severe combined immune deficiency; T₄, thyroxine; TFP, trifunctional protein deficiency; TSH, thyroid-stimulating hormone; TYR-I, tyrosinemia Type I; TYR-II, tyrosinemia Type II; VLBW, very low birth weight; VLCADD, very long chain acyl-CoA dehydrogenase deficiency; X-ALD, X-linked adrenoleukodystrophy.

^aAssociations observed in the New England Newborn Screening Program.

extremely rare, and primary healthcare professionals might not have sufficient information available to be able to direct appropriate intervention in screen-positive infants. To overcome the challenge, readily accessible, one-to-two page explanations of the possible disorders represented by the abnormality and the recommended confirmatory tests, known as ACTion (ACT) sheets and confirmatory algorithms (Kaye et al., 2006), are available on the website of the American College of Medical Genetics (https://www.acmg.net/ACMG/Publications/ACT_Sheets_and_Confirmatory_Algorithms/ACMG/Publications/ACT_Sheets_and_Confirmatory_Algorithms/ACT_sheets_Homepage.aspx). Similar explanations are also often included with the screening report.

Although all specimens with a metabolite concentration that crosses its threshold are considered screen positive, all screen-positive results are not associated with the same likelihood of being associated with a disorder. Most infants with a positive screening result that is only mildly abnormal are less likely to have a disorder (see the later discussion of false-positive results) than are infants with analyte concentrations that are severalfold above the cutoff. Applying a uniform approach for all positive results in terms of urgency of intervention or a battery of tests suggested can result in unnecessary parental anxiety and medical costs. However, if recommendations for further action and work-up are customized in accordance with the potential significance of the abnormality, both parental anxiety and the costs associated with false-positive results can be reduced. To achieve this goal, some programs subcategorize positive screening results. The New England NBS Program uses primary marker concentrations, second-tier analyses, biomarker profiles for markers analyzed by MS/MS, and acuity of the likely disorder to subcategorize out-of-range screening results (Sahai et al., 2007). The primary care providers are supplied with category-based, customized fact sheets when a positive screening result is reported (Sahai and Eaton, 2008). These sheets include information on disorders associated with the marker, the estimated likelihood of being affected, clinical presentations of likely disorders, factors contributing to false-positives, and recommendations for further management. The follow-up recommendations can range from immediate admission to a hospital, where further evaluation and therapy for the illness can be initiated without delay, to simply repeating the filter paper analysis on a sample collected a few days later. Other programs approach this problem differently, but with the same goal in mind, providing the primary care providers with the information needed to put the result in the appropriate context for the family.

Any infant for whom an abnormal screening result is reported should be seen as soon as possible and evaluated with a careful history and physical examination. When specific guidelines based on the individual results are not provided by the screening program and the infant is ill or the likely disorder manifests itself acutely within the first few days of life (see Tables 27.1–27.2), a specialist should be contacted. The infant may need to be admitted to the hospital, where further evaluation and therapy for the illness can be initiated without delay.

If the infant is active and alert with good feeding and shows no abnormal signs on initial evaluation and the suspected disorder does not require immediate attention, a second filter paper blood specimen can be obtained and sent to the screening laboratory for repeat testing, or confirmatory testing can be performed on a less urgent basis. In many cases, confirmatory testing or referral to a specialist is required only if the second test indicates the presence of a disorder. However, the follow-up of an initial positive screening result can differ. In some programs, more specific confirmatory testing is the first response to a presumptive positive newborn

screen, with a less intense time frame for individuals in whom the level of suspicion is lower.

The physician should contact the screening laboratory when an infant whose screen has been reported as normal or whose screening results have not yet been reported has symptoms that suggest a metabolic disorder. The screening laboratory can check the results in the infant's newborn specimen. If the testing has been completed and the newborn specimen is retained in storage, the laboratory may wish to recover the specimen and repeat the tests. The physician should also contact the screening laboratory for the results of repeated tests and inform the family of the results as soon as possible. If the second result is normal, the duration of the family's anxiety may be shortened.

Screened Disorders

Brief summaries of the categories of the most common disorders detected by NBS are provided in the following sections. There is no attempt to describe any of the disorders in detail or their rare variants.

Metabolic Disorders

Amino Acid Disorders

The amino acid disorders are caused by an enzymatic defect in the catabolic pathway of amino acids, with consequent accumulation of specific amino acids above the block. Screening relies on the detection of these elevated amino acids in the newborn specimen. The clinical manifestations may be a result of the toxic effects of the accumulating amino acid and metabolites produced by alternative pathways, a deficiency of the products of the normal pathway, or both. PKU is the best known example of an amino acid disorder and is the paradigm for screened disorders in the newborn.

In addition to PKU, MSUD and homocystinuria are historically significant in the context of screening because they are among the original metabolic conditions for which screening was performed before the expansion of NBS through the introduction of MS/MS technology. Screening for other amino acid disorders such as the urea cycle defects became possible only with the advent of MS/MS technology.

Phenylketonuria

In PKU the cardinal screening feature is an increased level of phenylalanine. PKU should always be identified by NBS. If untreated, patients with PKU experience severe intellectual disability and other neurologic abnormalities. The average incidence of the disorder is approximately 1 in 12,000 live births. With screening by MS/MS, PKU can reliably be identified as early as the first day of life (Chace et al., 1998). Not all infants with an elevation of phenylalanine level have PKU. Occasionally, an infant with elevated phenylalanine level will have one of the cofactor deficiency (pterin) disorders that can result in severe neurologic disease with choreo-athetosis and seizures as well as profound intellectual disability unless this is diagnosed and treated specifically. Therefore on evaluation every infant with increased phenylalanine level must have a blood and urine test to determine whether a pterin disorder rather than PKU is the diagnosis. Mild phenylalanine level elevations not due to a pterin disorder can indicate mild hyperphenylalaninemia, usually a benign condition. Liver disease, such as that associated with galactosemia, tyrosinemia type I, or citrin deficiency, can also produce increased levels of phenylalanine. For all of these reasons, treatment of PKU should never be started on the basis

of a positive screening test result alone. Every identified infant should be referred directly to a metabolic center for confirmatory testing and prompt consideration of dietary treatment. If the screening level of phenylalanine is only slightly increased, a second NBS specimen could be collected and sent to the screening program to determine whether the first result was persistent or transient before a diagnostic work-up is initiated.

Maple Syrup Urine Disease

The primary indicator for MSUD in the newborn blood specimen is an increase in leucine level. To increase the specificity for MSUD of an abnormal leucine measurement, some screening programs also report the ratio between leucine and a reference amino acid or alloisoleucine analysis as a second-tier test (Matern et al., 2007). The average incidence of classic MSUD is 1 in 185,000. MSUD can be a fulminant disease presenting initially with nonspecific findings such as vomiting, irritability, and lethargy, and it can progress rapidly to coma and death. Consequently, the finding of a substantially increased leucine level in the newborn blood specimen should prompt an immediate telephone call from the screening program to the attending physician. If the infant is sick, he or she should be transported immediately to a NICU at a medical center with a metabolic specialist. Confirmatory plasma and urine specimens should be obtained, and emergency therapy should be initiated. Plasma amino acid analysis in an infant with MSUD will show marked increases in the levels of leucine, isoleucine, valine, and alloisoleucine level (the branched-chain amino acids). Unlike the other classic organic acidurias (discussed later), pronounced acidosis, ketosis, hyperammonemia, or other abnormalities on routine laboratory tests may not be present. The primary biochemical abnormalities are the substantially increased levels of branched chain amino acids (leucine, isoleucine, valine, and alloisoleucine) in plasma and large quantities of the branched-chain ketoacids (2-ketoacids) in the urine. The 2-ketoacids are detectable by urinary organic acid analysis or by the 2,4-dinitrophenylhydrazine test. The characteristic odor, reminiscent of maple syrup, which appears earliest in cerumen and only later in urine, will probably be detected on a cotton-tipped swab inserted in the infant's ear.

Milder variants of MSUD can be missed by NBS (Bhattacharya et al., 2006). A newborn with the intermediate variant might not have a blood leucine level elevation, or the increase may be so mild as to be below the cutoff value. In the intermittent variant, the blood leucine concentration is normal in the newborn period, becoming elevated only in later infancy or childhood during acute metabolic episodes precipitated by febrile illness or surgery. Hydroxyproline at an increased level can be mistakenly identified as leucine in NBS. This would indicate a benign metabolic disorder known as hydroxyprolinemia. Quantitative plasma amino acid analysis will differentiate this from MSUD.

Homocystinuria

Individuals with homocystinuria are clinically normal at birth but, if untreated, may develop ectopia lentis (dislocation of the lens), thromboembolism, osteoporosis, and intellectual disability. The worldwide frequency of all forms of homocystinuria has been estimated at 1 in 344,000 but may be considerably higher (Skovby et al., 2010). The newborn blood screening marker for homocystinuria is an increased level of methionine. Homocysteine can be measured in a second-tier analysis to increase specificity (Matern et al., 2007). The diagnosis of homocystinuria may be missed if the blood methionine concentration is not sufficiently elevated to be reported by the screening program at the time the newborn

specimen is collected, but reducing the cutoff value for methionine can avoid this false-negative occurrence (Peterschmitt et al., 1999).

A high methionine level alone is not diagnostic of homocystinuria. Liver disease of a nonmetabolic nature can produce a strikingly high methionine level, as can isolated hypermethioninemia (methionine *S*-adenosyltransferase I/III deficiency), a metabolic disorder that is largely benign (Chien et al., 2015). Two additional rare disorders also produce hypermethioninemia: glycine *N*-methyltransferase deficiency associated with liver disease (Mudd et al., 2001; Luka et al., 2002) and *S*-adenosylhomocysteine hydrolase deficiency, which may result in developmental delay and hypotonia (Baric et al., 2004). Confirmation of the disorder requires quantitative amino acid analyses of plasma and urine. In the infant with homocystinuria, homocystine may be detectable in plasma and urine, methionine level is increased, and cystine level is reduced. Most strikingly, plasma total homocysteine level is markedly increased. In isolated hypermethioninemia, methionine level is markedly increased in plasma, but there is no detectable homocystine in plasma or urine, and the plasma cystine concentration is normal. Plasma total homocysteine level is also normal or slightly increased. Hypermethioninemia secondary to liver disease owing to tyrosinemia type I or to non-specific liver disease is usually accompanied by increased tyrosine level.

Urea Cycle Disorders

The three urea cycle disorders routinely screened by MS/MS analysis are citrullinemia, argininosuccinic acidemia, and arginase deficiency. All three may produce hyperammonemia in the neonate, accompanied by poor feeding, tachypnea, lethargy, and vomiting. Respiratory alkalosis is characteristic. Severe hyperammonemia in the newborn is a medical emergency and should trigger prompt consultation with a metabolic specialist or referral to the NICU at a metabolic center. Discontinuation of protein administration and the provision of intravenous fluids with high caloric content are the first steps to take. Citrullinemia and argininosuccinic acidemia require L-arginine, as well as the "scavenger drugs" sodium phenylbutyrate and sodium benzoate. In arginase deficiency, however, arginine supplementation is to be avoided. Hemodialysis is required to control the neurotoxic hyperammonemia if the blood ammonia level reaches 300 $\mu\text{mol/L}$ to prevent irreversible brain damage, coma, and death. Early identification through NBS with presymptomatic or early symptomatic therapy is critical to protecting patients with urea cycle disorders. Arginase deficiency usually presents in childhood with neurologic features such as spasticity and developmental delays in infancy and mild hyperammonemia rather than severe hyperammonemia in the neonate (Crombez and Cederbaum, 2005).

Tyrosinemia

Tyrosinemia type I is an amino acid disorder that can be diagnosed in NBS by the finding of an elevation of succinylacetone level in MS/MS analysis (Allard et al., 2004). This disorder leads to liver and renal tubular disease and can later result in hepatocellular carcinoma. It is treated with administration of 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (Orfadin) (Swedish Orphan Biovitrum [sobi]) and a diet low in phenylalanine and tyrosine. Tyrosinemia types II and III may be identified by an increased level of tyrosine in NBS. Tyrosinemia type II can result in intellectual disability, painful hyperkeratoses, and keratoconjunctivitis and is treated by a low tyrosine–low phenylalanine diet. Tyrosinemia type III seems to be benign, although developmental

delay has been reported (Ellaway et al., 2001). Transient tyrosine level elevations are common in neonates.

Medium-Chain Acyl-Coenzyme A Dehydrogenase Deficiency and Other Fatty Acid Oxidation Disorders

The fatty acid oxidation disorders include those in which the long-chain fatty acids cannot traverse the mitochondrial membranes to be oxidized within the mitochondrial matrix and those in which there are defects in fatty acid oxidation per se (see Tables 27.1–27.2). In either category the problem is the inability to fully oxidize fatty acids. Fatty acid oxidation is essential to supply energy as adenosine triphosphate via the Krebs cycle and as ketones in the presence of a low supply of glucose. The disorders involving defective transport concern carnitine, whereas those with defective oxidation are named according to the enzyme that is deficient (see Tables 27.1–27.2). The major clinical consequence of these disorders is fasting intolerance resulting in hypoketotic hypoglycemia, lethargy, hyperammonemia, metabolic acidosis, hepatomegaly, and sometimes sudden death. Cardiomyopathy is an additional feature of very long chain acyl-CoA dehydrogenase deficiency (VLCADD) and long-chain hydroxyacyl-CoA dehydrogenase deficiency (LCHADD). Each fatty acid disorder is associated with a specific or almost specific acylcarnitine pattern on MS/MS analysis.

The most common fatty acid oxidation disorder is MCADD. Tragically, before NBS was available, this disorder was often diagnosed only retrospectively after a sudden unexplained death, usually when postmortem examination revealed a fatty liver. This devastating outcome and a frequency of 1:15,000 to 1:20,000, comparable with that of PKU, made MCADD the primary reason for the addition of MS/MS technology to NBS (Zytkovicz et al., 2001). The primary finding in NBS is an elevation of octanoylcarnitine level. To specify MCADD, some programs have added molecular testing of the c.985A>G medium-chain acyl-CoA dehydrogenase mutation as second-tier screening. Because this mutation occurs in as many as 90% of individuals with MCADD, this additional analysis of a newborn blood specimen substantially increases the predictive value of the octanoylcarnitine level elevation (Zytkovicz et al., 2001).

The fatty acid oxidation disorder is treated by avoidance of fasting with high-carbohydrate, low-fat feedings and, of critical importance, prompt attention to acute illnesses in which vomiting occurs (Yusupov et al., 2010). Carnitine supplementation may be beneficial. Medium-chain triglycerides (i.e., MCT oil) is given for the long-chain disorders VLCADD, LCHADD, and trifunctional protein deficiency. Any infant with a fatty acid oxidation disorder should be evaluated at a metabolic center. Most of these disorders are treatable, but screening enables early diagnosis and genetic counseling for the family even when early treatment may not be effective, such as in neonatal carnitine palmitoyltransferase II deficiency (Albers et al., 2001). Short-chain acyl-CoA dehydrogenase deficiency (SCADD) is likely benign, although before NBS it was considered to be a serious disorder (Waisbren et al., 2008).

Organic Acid Disorders

Organic acid disorders are a heterogeneous group of disorders with a combined frequency of approximately 1 in 50,000 (Zytkovicz et al., 2001). Many of them can be identified through MS/MS screening (see Tables 27.1–27.2). The marker for this disease group, as for the fatty acid oxidation disorders, is an abnormal acylcarnitine pattern. If a screening result suggests an organic acidemia, a metabolic specialist should be consulted immediately. The major organic acid disorders identified in NBS are propionic acidemia, the

methylmalonic acidemias, the cobalamin (vitamin B₁₂) defects, and isovaleric acidemia.

The organic acidemias can manifest themselves in the neonatal period with a life-threatening, sepsis-like picture of feeding difficulties, lethargy, vomiting, and seizures. Metabolic acidosis virtually always accompanies this presentation, and hyperammonemia is common. In this situation, protein administration should be discontinued and replaced by the administration of intravenous fluids with high caloric content and carnitine. The hyperammonemia rarely requires specific treatment since control of the organic acid metabolites will almost always result in resolution of the hyperammonemia. The long-term benefits of early diagnosis and treatment for the clinical and neurologic development of individuals affected by an organic acid disorder are under investigation (Dionisi-Vici et al., 2006).

Galactosemia

Galactosemia typically manifests itself in the neonatal period as failure to thrive, vomiting, and liver disease (Hughes et al., 2009). Death from bacterial sepsis, usually caused by *Escherichia coli*, occurs in a high percentage of untreated neonates (Levy et al., 1977). The average incidence of the disorder is 1 in 62,000 (Levy and Hammersen, 1978).

Some screening programs use a metabolite assay for total galactose (galactose and galactose 1-phosphate) to detect galactosemia, other programs screen the newborn specimen with a specific semiquantitative enzyme assay for activity of GALT, which is usually undetectable in classic galactosemia, and a few programs use both tests as a primary screen. The enzyme assay identifies only galactosemia, whereas the metabolite assay also identifies other galactose metabolic disorders, such as deficiencies of galactokinase and epimerase. Severe neonatal liver disease and portosystemic shunting caused by anomalies in the portal system can also increase the galactose level. NBS programs that use total galactose as the primary screen usually perform second-tier GALT testing in specimens with elevated galactose level. If the newborn specimen has markedly reduced or absent GALT activity, some screening programs then perform targeted molecular testing for the most frequent mutations associated with galactosemia, particularly gln188arg and asn314asp (Elsas and Lai, 1998).

Infants having increased galactose and reduced GALT activity in NBS or those with no detectable activity when only the GALT assay is performed should immediately be seen at a metabolic center. This is particularly important in galactosemia since neonatal sepsis with meningitis, almost always due to *E. coli*, is a major threat to the galactosemic infant. If the infant is breastfeeding or receiving a regular lactose-containing formula, urine should immediately be tested for reducing substance, and blood should be tested for red blood cell GALT activity and galactose 1-phosphate. Infants with a strongly positive urine-reducing substance finding (3+ or 4+) should also have tests for liver function, including prothrombin time and partial thromboplastin time for coagulopathy, and glucose level for evaluation of hypoglycemia, especially if they are showing clinical signs of galactosemia, such as jaundice, hepatomegaly, poor feeding, and/or lethargy. Breastfeeding or lactose-containing formula feeding should be discontinued, and appropriate intravenous fluids with glucose should be given as needed. If the urine test is negative for reducing substance, the NBS result is most likely to be false-positive or indicative of a benign GALT variant (e.g., Duarte variant). Nevertheless, urine-reducing substance may be absent in infants with clinically significant variants of galactosemia. Consequently, follow-up testing should be performed

for all newborns with an initial positive galactosemia screening result.

Markedly increased galactose and normal GALT activity in NBS suggest the possibility of galactokinase deficiency. This disorder produces early-onset cataracts, which are prevented by removal of lactose from the diet. Moderately increased galactose with normal or somewhat reduced GALT activity could indicate uridine diphosphate (UDP)-galactose 4-epimerase deficiency, a largely benign disorder.

Biotinidase Deficiency

Biotin recycling is necessary for the maintenance of sufficient intracellular biotin to activate carboxylase enzymes. Biotinidase is a key enzyme in biotin recycling. Lack of biotinidase activity results in reduced carboxylase activities and an organic acid disorder known as *multiple carboxylase deficiency* (Wolf and Heard, 1991). The clinical features of the disorder are developmental delay, seizures, hearing loss, alopecia, and dermatitis. The developmental delay and seizures usually manifest themselves at 3–4 months of age. Death during infancy has also been reported.

Initiation of biotin therapy in early infancy, when the disorder is presymptomatic, seems to prevent all the features of biotinidase deficiency. For this reason, a screening test has been developed and added to NBS in a number of NBS programs throughout the world (Hart et al., 1992). The frequency of newborns identified in these programs has a wide range, from 1 in 30,000 to 1 in 235,000. The average frequency seems to be approximately 1 in 60,000 (Wolf, 2012). With presymptomatic biotin treatment, virtually all identified infants have remained normal.

Lysosomal Storage Disorders

Lysosomes are organelles required for cellular turnover and contain more than 50 acid hydrolases that catabolize macromolecules. Deficiency of the individual enzyme or a combination of enzymes and transporters can result in accumulation of the substrate and progressive cellular and organ dysfunction. The disease phenotype is a consequence of the type of substrate and its sites of turnover, and severity generally correlates with the amount of residual enzyme activity. The incidence of these disorders as a group is estimated to be 1 in 7700 to 1 in 10,000 births. Direct assay of lysosomal enzymatic activity in dried blood spots by MS/MS or fluorometry techniques is currently feasible for several lysosomal storage disorders (LSDs): Fabry disease, Gaucher disease, Krabbe disease, mucopolysaccharidosis type I (MPS I), mucopolysaccharidosis type II, Niemann–Pick A/B disease, and Pompe disease (Gelb et al., 2015). Pompe disease and MPS I are the two currently included in the RUSP.

Pompe disease, also known as *glycogen storage disorder II*, is characterized by accumulation of lysosomal glycogen, predominantly in muscles, resulting from the decreased activity of lysosomal acid α -glucosidase (GAA) due to pathogenic variations in the corresponding *GAA* gene (Leslie and Tinkle, 2013). Pompe disease exhibits a broad spectrum in regard to age of onset, cardiac involvement, and progression of skeletal muscle dysfunction. The severe infantile form manifests itself within the first few months of life and is characterized by severe progressive muscle weakness. Cardiomyopathy is present in the classic form, and without treatment death occurs within the first year of life. Late-onset Pompe disease manifests itself clinically after 1 year of age, often not until adolescence or adulthood, with a slowly progressive myopathy and minimal cardiac involvement. In general, lower GAA levels are associated with earlier onset and greater severity of the disease, although the correlation is not absolute, and diversity among

individuals with identical GAA genotypes has also been observed, suggesting the effect of other modifying factors (Kroos et al., 2012). In addition to supportive care and nonspecific treatment, enzyme replacement therapy (ERT) is available and should be started as soon as the diagnosis is established. Clinical trials have shown that infants in whom ERT was initiated before the age of 6 months, and before the need for ventilatory assistance, showed improved survival and ventilator-independent survival as compared with untreated historical controls. The overall incidence of Pompe disease is 1 in 28,000, with 28% being the infantile forms. NBS offers the opportunity to detect the infantile forms early (22 days vs 3.6 months by clinical ascertainment) and thus justifies its inclusion in the RUSP. Decreased GAA activity in the screening blood spot should prompt molecular analysis for confirmation. Homozygosity for a “pseudodeficiency” allele c.(1726G>A; 2065G>A) is associated with low GAA activity similar to that seen in patients with Pompe disease but does not cause disease. This genotype, seen in approximately 4% of individuals in the Asian population, will result in false-positive screens in the first-tier enzymatic assays.

MPS I is a progressive multisystem disorder with features ranging over a continuum of severity. It is caused by deficiency of the lysosomal enzyme α -L-iduronidase (IDUA) (encoded by the *IDUA* gene), which leads to an accumulation of glycosaminoglycans (or mucopolysaccharides) within lysosomes of the affected cells (Beck et al., 2014). MPS I is broadly categorized into Hurler syndrome (MPS I H; severe, incidence 1 in 100,000), Hurler–Scheie syndrome (MPS I H/S; attenuated; incidence 1 in 500,000), and Scheie syndrome (MPS I S). Newborns typically appear normal at birth. The severe form manifests itself within the first year of life, and the early findings are quite nonspecific (umbilical hernia, recurrent upper respiratory tract infections). Subsequently, coarsening of the facial features and gibbus deformity of the lower spine may be observed. The severe form is characterized by progressive skeletal dysplasia (dysostosis multiplex) involving all bones, decreased linear growth, and progressive and profound intellectual disability. Corneal clouding and hearing loss are common. Death, typically caused by cardiorespiratory failure, usually occurs within the first 10 years of life. The clinical onset in the attenuated forms is usually between 3–10 years of age. The severity and rate of disease progression span a spectrum, ranging from death in the teens or 20s to a normal life span complicated by disability from progressive joint manifestations and cardiorespiratory disease. Hearing loss and cardiac valvular disease are common. Neurologic and psychomotor involvement are limited in the attenuated forms. Hematopoietic stem cell transplantation (HSCT), considered the standard of care for severe MPS I, increases survival (<5% vs 65% at 10 years) and reduces facial coarseness, increases growth, improves cardiac function, and relieves hepatosplenomegaly. However, it has limited effects on skeletal and valvular manifestations or corneal clouding. It delays cognitive decline, but its impact on other neurologic complications is uncertain. Maximum benefit is achieved if it is performed before 2 years of age. ERT, available for treatment of the non-CNS manifestations of MPS I in individuals with attenuated disease, increases joint mobility, growth, and visual acuity, relieves hepatomegaly, and reduces pain. The outcomes of both HSCT and ERT are significantly impacted by disease burden at the time of initiation of treatment. Decreased IDUA activity as measured by MS/MS or fluorometry in the NBS suggests the possibility of MPS I and needs to be confirmed by molecular analysis of the *IDUA* gene and/or confirmatory biochemical testing (urinary glycosaminoglycans, IDUA activity in leukocytes or fibroblasts). Unfortunately the residual IDUA activity is not predictive of the

phenotype, and the presence of pseudodeficiency alleles renders interpretation of IDUA activity more difficult. Genotyping may help predict the expected phenotype if it reveals mutations with good genotype–phenotype correlation (Clarke, 2016).

X-Linked Adrenoleukodystrophy

X-ALD is one of the most common monogenic forms of an inherited neurodegenerative disease. It is inherited in an X-linked manner, with a prevalence of 1 in 21,000 in males and 1 in 16,800 in males and presenting females. As the name suggests, it affects the white matter and adrenal cortex. The clinical phenotype is highly variable but the neurologic manifestations are present in nearly all males by adulthood (Engelen et al., 2012). Two main neurologic presentations are seen in affected males:

1. The cerebral form manifests itself insidiously between the age of 4 years and the age of 8 years, and the initial symptoms resemble attention-deficit disorder or hyperactivity. The ongoing inflammatory brain demyelination results in progressive behavioral, cognitive, and neurologic deficits leading to a vegetative state and death within 2–5 years after onset. Among males, 35%–40% develop the childhood cerebral form of X-ALD, and an additional 20% may develop it in their lifetime.
2. Adrenomyeloneuropathy is a slowly progressive axonopathy affecting both sensory and motor spinal cord tracts manifesting itself as paraparesis, sphincter disturbances, and sexual dysfunction; all symptoms are progressive over decades. The penetrance for this form is 100% in males who escape the cerebral form initially and 65% in females.

X-ALD may present as a primary adrenocortical insufficiency without evidence of neurologic abnormality in about 10% of males, and neurologic involvement occurs subsequently. Overall, adrenal function is abnormal in 90% of neurologically symptomatic boys and in 70% of men with adrenomyeloneuropathy.

HSCT has been shown to arrest the inflammation in the early stages and thus provide an efficient treatment for the inflammatory form of X-ALD. However, it is recommended only for individuals with evidence of brain involvement by magnetic resonance imaging (MRI) but minimal neuropsychological findings and normal clinical neurologic examination findings, as HSCT can accelerate neurologic decline and is likely to be unsuccessful if it is performed after cerebral demyelination has started. Detection of X-ALD by NBS allows periodic reevaluation of adrenocortical function and MRI for detection of males with early cerebral disease, thereby providing an ideal window of opportunity for HCST to treat the cerebral form. C26:0 lysophosphatidylcholine (C26:0 LPC) measured by HPLC–MS/MS in the dried blood spot is the primary biomarker used to screen individuals for X-ALD (Matern et al., 2013). Individuals with a positive screen require plasma very long chain fatty acid (VLCFA) analysis and sequencing of the *ABCD1* gene to confirm the diagnosis. However the phenotype cannot be predicted by VLCFA plasma concentration or by the nature of the *ABCD1* pathogenic variant as the same pathogenic variant can be associated with each of the known phenotypes. Increased concentrations of C26:0 LPC in the screening specimen will also be noted in other peroxisomal disorders, such as Zellweger spectrum disorders, peroxisomal acyl-CoA oxidase 1 deficiency, and D-bifunctional enzyme deficiency.

Endocrine Disorders

Congenital Hypothyroidism

CH is the most common disorder identified by routine NBS. It is found in 1 in 3000 to 1 in 5000 screened infants (Dussault,

1993). The major clinical features of untreated CH are growth retardation and delayed cognitive development leading to mental deficiency. If treatment with pharmacologic doses of T_4 is initiated early, growth and mental development are normal.

Two screening approaches are used (Pass and Neto, 2009). One method is primary screening for low T_4 levels with secondary screening for high TSH levels. The second method is primary screening for high TSH levels. Either procedure identifies CH. Nevertheless, affected infants can be missed with either approach. This situation may be due to a lack of the identifying marker abnormality at the time of specimen collection. Specifically, the T_4 level during the first 24 hours of life in an affected newborn might not yet be sufficiently decreased for identification because of persistence of maternally transmitted T_4 . Moreover, in the premature newborn with CH, it might take 2 weeks or more for a TSH level elevation to develop (Larson et al., 2003).

The reported false-positive rates of screening for CH range from approximately 0.05% to as high as 4% (Pass and Neto, 2009). Newborns with false-positive results have transiently low T_4 or elevated TSH levels. Many of those with low T_4 levels are premature neonates with a normal TSH concentration or infants with perinatal stress and elevated TSH levels. To avoid missing CH, screening programs require a second blood specimen from each of these babies. In addition to false-positive results, a low T_4 level with a normal TSH value can result from benign T_4 -binding globulin deficiency (Dussault, 1993; Mandel et al., 1993) or hypothyroidism secondary to pituitary deficiency.

Newborns with a positive screening test result should not be labeled as having CH until diagnostic testing confirms the disorder. This is especially true if the TSH concentration reported by the screening program is normal. If CH is confirmed, however, administration of T_4 should be started without delay to prevent irreversible brain damage.

Congenital Adrenal Hyperplasia

CAH caused by steroid 21-hydroxylase deficiency occurs in 1 in 16,000 to 1 in 20,000 births (White, 2009). Infants with the salt-losing form of CAH can rapidly become hyperkalemic and die precipitously, often without a specific diagnosis. The clinical diagnosis may be suspected in the newborn girl because of ambiguous genitalia. However, the diagnosis is usually not initially suspected in boys or in girls with atypical forms of CAH in which ambiguous genitalia may not occur. Moreover newborn girls with ambiguous genitalia might not be recognized as having CAH if the ambiguity is not obvious, or they could be misassigned as boys if the ambiguity is advanced. Because accurate sex assignment and initiation of hormone therapy as soon as possible are critical to a favorable prognosis in CAH, NBS is important for early diagnosis and prompt therapy with pharmacologic doses of hydrocortisone. Consequently, testing for CAH has been incorporated into routine NBS in most programs.

Screening is based on identifying elevated levels of 17-OHP, the preferred substrate for 21-hydroxylase, and is usually measured with an immunoassay. Unfortunately, compared with other neonatal screening assays, the specificity of the CAH assay is low, and false-positive results in NBS for CAH are relatively common, with the rate often as high as 0.5%. The finding may be due to a truly increased 17-OHP level, as in perinatal stress and early specimen collection (within the first 24 hours of life), or may be due to cross-reacting steroids, such as in prematurity and low birth weight (al Saedi et al., 1996). Cross-reacting steroids are produced by residual fetal adrenal cortex or result from decreased metabolic clearance by an immature liver.

A decidedly increased level of 17-OHP suggests CAH, and the infant should be referred to a pediatric endocrinologist for management. A second blood specimen is usually requested from infants found to have a slightly to moderately increased 17-OHP level. If the infant shows signs of illness or has ambiguous genitalia, serum electrolyte levels should be measured. If these results indicate hyponatremia and hyperkalemia, the infant should be hospitalized without delay, and the electrolyte imbalance should be corrected immediately. Pediatric endocrinology consultation should also be sought. It may be possible to increase the positive predictive value by second-tier screening using DNA-based methods or liquid chromatography followed by MS/MS, but currently these methods are not widely used by NBS programs.

Sickle Cell Disease

In most NBS programs in the United States, the blood specimen is routinely tested for hemoglobin abnormalities. The major goal of this testing is to identify newborns with sickle cell disease so that they can be given penicillin prophylaxis to prevent pneumococcal septicemia. Additional benefits of early detection are early referral to a comprehensive sickle cell program and early education and genetic counseling for parents (Smith and Kinney, 1993). Unfortunately, the long-term complications are not yet preventable.

Sickle cell screening is usually performed by separation of variant and abnormal blood hemoglobins eluted from the Guthrie specimen by electrophoresis or HPLC. This procedure identifies sickle cell disease, sickle cell trait, and several other hemoglobin abnormalities. Other than sickle cell disease, most of these abnormalities are benign. It is especially important to differentiate the common and benign sickle cell trait from the much rarer sickle cell disease (homozygosity for hemoglobin S). For example, sickle cell disease affects approximately 1 in 600 African-American individuals, whereas sickle cell trait (carrier status for hemoglobin S) is present in 1 in 12. Infants with sickle cell trait do not have complications and should not be stigmatized as having sickle cell disease.

When sickle cell disease is confirmed, penicillin prophylaxis should be initiated as soon as possible, and the infant should be referred to a sickle cell disease center or hematologist. The combination of screening and careful follow-up has been highly effective in prevention of pneumococcal sepsis in infants with sickle cell disease.

Cystic Fibrosis

The frequency (1 in 2000 to 1 in 3000) and severity of CF explain its inclusion in routine NBS. As with sickle cell disease, therapy that can prevent the most serious complications of CF is not yet available. However, early and usually presymptomatic diagnosis through screening leads to early nutritional therapy, pancreatic enzyme replacement, and antibiotic prophylaxis for pulmonary infection. Data suggest that children with CF who are identified by NBS have better growth, prevention of early vitamin deficiency, and some advantage in terms of pulmonary status later in life (Farrell et al., 2001; McKay and Wilcken, 2008; Southern et al., 2009). Other benefits of NBS are identifying the parents' genetic potential for producing additional children with CF and, through presymptomatic identification, allowing the family to avoid months or years of delay in making the correct diagnosis for a child with chronic respiratory problems or poor growth (Farrell and Mischler, 1992).

The analyte marker in NBS for CF is increased immunoreactive trypsinogen (IRT) level. Transient increases in IRT level are common in healthy newborns as a result of perinatal stress or for unknown reasons. Consequently, the rate of false-positive results in CF

screening using only IRT is relatively high. To reduce this rate, screening programs have adopted a second-tier DNA analysis for a panel of CF mutations when the specimen has increased IRT level (Ferec et al., 1995). Despite this two-tiered approach to screening, a substantial number of infants who do not have CF must undergo a sweat test or complete DNA sequencing of the CF gene before the diagnosis can be ruled out.

Severe Combined Immunodeficiency

SCID includes more than 15 genetic disorders characterized by the absence of both humoral and cellular immunity and is estimated to occur in 1 in 50,000 to 1 in 100,000 births. Approximately 50% of cases are due to mutations in the *IL2RG* gene, localized on the X chromosome. Disease presentation differs, but as protection from maternal antibodies wanes during the first few months after birth, infants with SCID will usually develop infections due to both common and opportunistic pathogens. Treatment and prevention of infections can prolong life but are not curative. Early HSCT before the onset of severe infections offers the best chance of cure and has been made feasible by NBS.

As SCID is characterized by absent or extremely low production of antigenically naïve T cells from the thymus, low concentration of TRECs in the NBS specimen (marker of newly formed, thymic emigrant T cells) with a valid result for the internal control (e.g., ribonuclease P) is used to screen newborns for SCID (Comeau et al., 2010). A high-risk positive SCID NBS result prompts further diagnostic testing such as flow cytometry to measure specific T-cell markers and specialized immune function tests before the diagnosis is established or excluded. The most low-risk positive SCID NBS results are from neonates in the NICU and, depending on the testing laboratory's algorithms, may require only repeated screening. Non-SCID lymphopenias and infants who have had a thymectomy (most often during cardiac surgery) can show low TREC values.

Hearing Loss

Hearing loss is the most common birth defect, with 1 in 500 newborns having confirmed hearing loss. Infants with hearing loss not identified before 6 months of age have delays in speech and language development, while intervention before 6 months of age allows an infant with impaired hearing to develop normal speech and language. This rationale prompted the National Institutes of Health to recommend screening for congenital hearing loss for all newborns in 1993. Typically automatic auditory brainstem response or otoacoustic emissions testing is performed to detect hearing loss in the neonate. To maximize the benefits, the hearing screen should be performed before 1 month of age, preferably before hospital discharge; infants who screen positive should have a diagnostic audiologic evaluation before 3 months of age, and all infants identified with hearing loss should receive appropriate early intervention services before 6 months of age (medical, audiologic, and early intervention). Approximately 1.6 % of all newborns do not pass the hearing screen and are referred for an audiologic evaluation, and among those 10.3% are confirmed as having hearing loss. Enrollment in early intervention services among children diagnosed with hearing loss is estimated to be 61.7%. For a more detailed discussion, readers are referred to Chapter 109.

Critical Congenital Heart Disease

Congenital heart disease (CHD) is the most common cause of death in the first year of life, accounting for 3% of all infant deaths.

CHD affects about 7 in 1000 to 9 in 1000 live births; a fourth of these can be classified as CCHD, a term that encompasses the heart defects that cause severe and life-threatening symptoms and require intervention within the first year of life ([Centers for Disease Control and Prevention, 2012](#)). Currently pulse oximetry, in conjunction with a clinical examination, is the recommended screening approach and will usually identify CHD that results in hypoxemia. This group accounts for approximately 17%–31% of CHD cases and includes hypoplastic left-sided heart syndrome (HLHS), pulmonary atresia with intact septum, tetralogy of Fallot, total anomalous pulmonary venous return, transposition of the great arteries, tricuspid atresia, and truncus arteriosus. Each condition has palliative and surgical options, with a spectrum of standard interventions for each. Overall, the 5-year survival rates after intervention range from 65% to 97%, with five of the seven conditions having 5-year survival rates greater than 85%. The two conditions with the lowest long-term survival rates are pulmonary atresia with intact septum and HLHS. The sensitivity of screening for CCHD is 30.8%, 47%, and 77% by pulse oximetry, clinical examination alone, and both, respectively. For a more detailed discussion, readers are referred to Chapter 55.

Specific Issues in Newborn Screening

Criteria for Newborn Screening

Under the auspices of the World Health Organization, [Wilson and Jungner \(1968\)](#) published a set of criteria for screening for conditions that have generally been accepted as required for population screening. The ten criteria state that (1) the condition be an important health problem, (2) there must be accepted treatment, (3) facilities for diagnosis and treatment must be available, (4) there must be a recognizable latent or early symptomatic stage, (5) there should be a suitable test, (6) the test should be acceptable to the population, (7) the natural history of the condition should be understood, (8) there should be a policy prescribing whom to

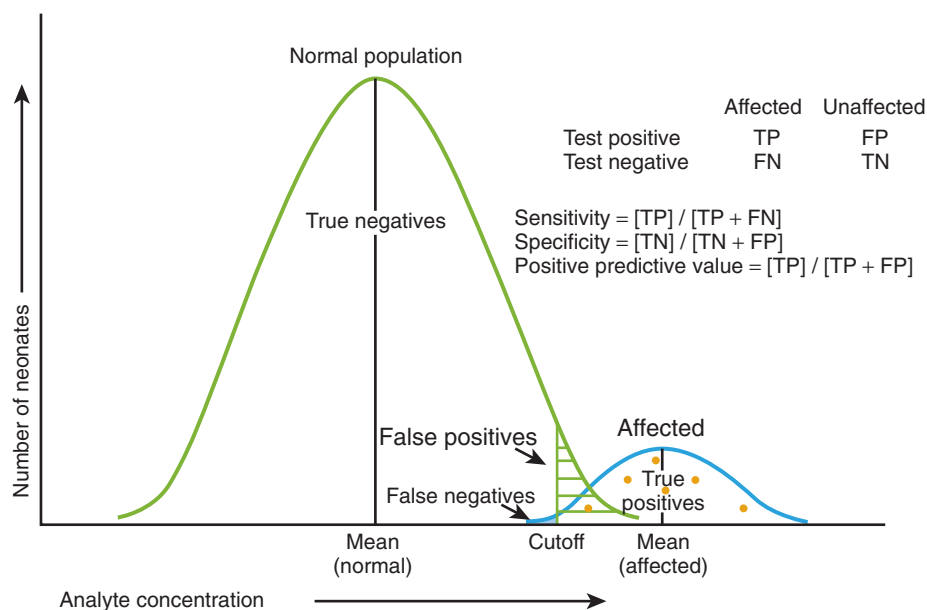
treat, (9) the cost of case finding should be economically balanced in relation to medical care as whole, and (10) case finding should be a continuing process.

These criteria were developed at a time when NBS was in its beginning stages and with screening for adult disorders in mind. For example, regarding the first criterion, Wilson and Jungner recognized that the term *important* is relative; whereas diabetes was prevalent and treatment might not influence outcome, PKU was rare, but it warranted screening because of the serious consequences that would be prevented by early diagnosis and treatment.

There is a question as to the current relevance of the Wilson–Jungner criteria for NBS. Ideally, all the criteria should be applied to NBS. However, advances in technology have caused this application to be questioned ([Green and Pollitt, 1999](#); [Levy, 1999](#)). As an example, screening using MS/MS allows detection of serious disorders for which there may not be acceptable or agreed on therapy or for which the natural history is largely unknown, challenging the criterion that any disorder included in screening should have acceptable treatment. How is this dilemma resolved? The answer is not yet available. It is hoped that the experience and findings from expanded NBS will be used to develop a new set of criteria that will apply to NBS. These criteria will likely retain the essence of the Wilson–Jungner compilation but with important modifications that could be applied to any new screening venture.

False-positive Results

The majority of positive results in NBS, particularly when the result is only mildly or moderately abnormal, are not due to a disorder. Unfortunately, because screening is primarily based on a quantitative measure, false-positive results must be addressed. Metabolite concentrations differ among individuals, and the distribution curves of the markers from affected and unaffected populations are expected to be different ([Fig. 27.2](#)). In screening, a value that separates the two distribution curves is established as a cutoff.



• **Fig. 27.2** Distribution of quantitative markers measured in screening. For most conditions the distribution in the unaffected or normal population overlaps that in the affected individuals. The number of false-positives (FP), false negatives (FN), true positives (TP), and true negatives (TN) depends on the established cutoff.

The cutoff values of the quantitative biomarkers are established by the individual screening laboratories, and they can differ among the different laboratories because of variations in the testing technology. In general, the cutoff value should be set such that it is different (greater than or less than, depending on the condition) than the 99th percentile of the concentration in normal neonates and the 5th percentile of the concentration in affected neonates (Rinaldo et al., 2006). However, for disorders that are extremely rare, the population of affected neonates may be so small that establishing an appropriate cutoff becomes a challenge. In such cases, the laboratory may empirically set a cutoff at three to four standard deviations from the population mean and adjust the values with experience to minimize the false-positives without compromising the sensitivity. Specimens in which the concentration crosses the established cutoff are considered screen positive. For disorders characterized by an increased metabolite concentration, the concentrations in most unaffected infants are below the cutoff value, but in a small proportion the metabolite concentration crosses this threshold. These latter screening results are considered false-positives. Because of some degree of overlap in the distribution curves of the affected and unaffected populations, these false-positives cannot be entirely eliminated without compromising the sensitivity of screening. Furthermore, other physiologic factors, such as immaturity of metabolic enzymes, stress, and therapeutic interventions, can skew the concentrations of certain metabolites and lead to false-positive screening results. Currently, the indicators measured by immunoassays (e.g., 17-OHP in CAH and T_4 in CH) or enzymatic activity (low GALT activity in galactosemia) are associated with the highest false-positive rates.

The false-positive results are more common in preterm and low birth weight infants than in term infants. For example, up to 85% of preterm infants have transiently low T_4 levels (Paul et al., 1998). Transient increases in 17-OHP level are another common abnormality in infants who are preterm or have low birth weights or have experienced perinatal stress (Pang and Shook, 1997). In addition, transient tyrosinemia is commonly observed in preterm and low birth weight infants, although it can also occur in term infants (Levy et al., 1969).

Artifacts produced in the collection or transport of the Guthrie specimen account for some false-positive results. As mentioned in the discussion of the specimen collection procedure, obtaining the specimen from a central line can result in mixing with the amino acids in the TPN solution and consequently a false increase of the levels of amino acids in the specimen. Even with correct specimen collection, TPN can produce a transient amino acid level increase. Contamination with milk (or any drink containing milk) can result in a false elevation in galactose level and the mistaken suspicion of galactosemia. Prolonged exposure to heat can reduce the activity of GALT in the specimen and produce a false impression of galactosemia when the enzyme assay is used to screen an individual for this disorder. This error is common during the summer, especially when the specimen remains in a mailbox for some time. Some factors known to be associated with false-positives are shown in Table 27.3 (Sahai and Marsden, 2009).

With the substitution of MS/MS for the traditional bacterial or specific assays, such as those for PKU and MSUD, the number of false-positive results is distinctly lower. For PKU screening, the false-positive rate is reported at 0.05%, compared with 0.23% for earlier methods (Levy, 1998). In addition, with MS/MS the multiplexed approach allows a profile of analytes rather than a single analyte level (e.g., phenylalanine/tyrosine ratio vs only a

phenylalanine level for PKU identification), further increasing the specificity of screening (Chace et al., 1998; Schulze et al., 1999). Therefore the average positive predictive value for primary markers analyzed by MS/MS, previously reported to be 8%–10% (Schulze et al., 2003; Wilken et al., 2003), can be increased substantially if the marker is evaluated in context with other metabolites that are screened or when a tiered approach is applied (Frazier et al., 2006; Sahai et al., 2007; Marquardt et al., 2012).

Second-tier assays, such as molecular assays for CF or secondary immunoassays for hypothyroidism, are commonly performed to reduce the false-positive rates for primary markers analyzed by immunoassays or enzymatic assays. Nevertheless, false-positive results cannot be entirely eliminated; therefore it is important to reassure the parents that not every abnormal result of NBS inevitably implies a disorder and that transient or nonspecific abnormalities are common. Although all infants with an abnormal screening result must undergo repeated testing, the families should be informed that an initial positive result might have no medical implications. This approach can alleviate excessive anxiety and prevent unnecessary diagnostic procedures and treatment.

Increased Detection by Screening

For certain disorders screened by MS/MS, many more infants are identified than the numbers previously expected solely on the basis of clinical identification. The greatest increases are in the fatty acid oxidation disorders, including SCADD, MCADD, VLCADD, and carnitine uptake defect and in three organic acid disorders (glutaric acidemia type I, 3-methylcrotonyl-CoA carboxylase deficiency, and 3-ketothiolase deficiency; Wilken et al., 2003).

Although individuals who are symptomatic but with no diagnosis could account for some of the clinically identified cases, it is likely that the greater part of the excess represents infants with benign or milder forms of the disorders who did not come to clinical attention. Notably, Spiekerkoetter et al. (2003) identified a frequent mutation in asymptomatic patients with VLCADD detected by NBS, and Ensenauer et al. (2004) found a common, mild, and perhaps asymptomatic mutation in patients with isovaleric acidemia identified by screening. More recently, Spiekerkoetter et al. (2010) have demonstrated that most infants assumed from NBS to have VLCADD are either false-positive or have an enzymatically very mild form of the disorder that is most likely to be benign.

Missed Cases

A few infants with CH, PKU, intermittent MSUD, glutaric acidemia type 1, tyrosinemia type I, and other screened disorders have been missed by NBS. Laboratory or program errors were reported as the most common cause of these missed cases (Holtzman et al., 1974). In some instances, a specimen was never collected, such as when infants were transferred to another hospital. However, in screening for a multitude of disorders, each with its own biomarker that varies with time and physiologic states, an occasional affected neonate may have normal biomarker concentrations in the newborn specimen simply because the timing of collection was not ideal for that particular condition. Therefore physicians must exercise clinical judgment and not fall into the trap of excluding a diagnosis because an infant has presumably been screened. Specific testing should be performed in any patient with symptoms that suggest the presence of a disorder, regardless of the assumed or actual NBS result.

The Future

Many factors are impinging on NBS, including rapidly advancing technology, new and increasingly available therapeutic approaches to previously untreatable disorders, and advocacy by family support groups and influential citizens or legislators who are heavily invested in individual disorders that may or may not be ready for inclusion in screening tests. To address these pressures, the Advisory Committee on Heritable Disorders in Newborns and Children meets regularly to study newly proposed NBS tests (and those currently recommended) to judge their suitability for inclusion in the screening panels. This process has resulted in the addition of SCID, Pompe disease, MPS I, and X-ALD to the RUSP. Other candidate conditions being considered include fragile X syndrome and Smith–Lemli–Opitz syndrome. These disorders will be judged on the basis of frequency, severity, availability of preemptive therapies, and the cost and robustness of the screening test itself. Some disorders have been included in isolated state panels on the basis of the political influences mentioned earlier.

The most controversial and potentially the most far-reaching possible addition to NBS is genetic screening. This possibility is the result of next-generation sequencing, a dramatic technological advance in allowing the sequencing of nucleotides in the genome as well as an equally dramatic reduction of the cost for this sequencing (Landau et al., 2014). Genetic screening could be whole-exome sequencing, which could include all of the exons in selected genes or even in the entire genome or whole-genome sequencing, which would include exons and introns of selected genes or the entire genome. Any of these approaches could possibly be applied to the DNA in the screening specimen and result in an enormous expansion of NBS, potentially identifying mutations for many genetic disorders, nonmetabolic as well as metabolic, not currently covered by NBS. It could be additive to current screening or could replace to a degree as well as add to current NBS (Levy, 2014; Howard et al., 2015).

Nevertheless, the challenges of genetic screening would be formidable. The most formidable initial difficulty would be interpreting the clinical implications of the data. An enormous amount of bioinformatics would be required. Even with a huge degree of bioinformatics, the clinical significance of most of the sequence changes would be unknown (variation of unknown significance). The time required to process and interpret the data would increase the turnaround time of NBS from the current 2–4 days, thus potentially delaying the initiation of treatment for disorders in which very early treatment may be critical. Assuming these and other difficulties would be resolved, there would remain the problem of genetic screening based on the recognition of mutations, greatly increasing the number of infants with benign variations of disorders who would receive otherwise unnecessary medical tests and perhaps unnecessary treatments. These challenges do not even address the medical genetic services needed that would far exceed the current availability, the greatly increased need for confirmatory tests, and the huge amount of uncertainty and anxiety in families that genetic screening would generate.

A much more limited genetic screening, however, could be envisioned. Some degree of specified NGS of a few key genes in which the mutations and their implications are well known could be added to current NBS with a two-stage NBS reporting procedure in which the current screening results are reported promptly and the genetic screening results reported later. The disorders targeted would be those in which early treatment might not be critical or

even available but in which diagnosis would be important for defining the origin of illness and/or for family considerations. This screening would require informed consent and would have to be very carefully constructed. To examine these questions, the National Institute of Child Health and Human Development and the National Human Genome Research Institute have funded four major 5-year pilot projects to examine the potential of genetic NBS. These projects include the technical feasibility of applying genomic sequencing to the NBS specimen, to examine the medical effectiveness of sequencing in a neonatal setting, and to address the ethical, legal, and societal implications of sequencing in NBS (<https://www.nih.gov/news-events/news-releases/nih-program-explores-use-genomic-sequencing-newborn-healthcare>).

Finally, there is the issue of retention of blood spots for future study. Despite multiple safeguards to protect the identity and anonymity of individuals, parents and civil libertarians are concerned that retention of these blood spots poses a threat to the privacy of individuals and that the specimens should be destroyed. If this view prevails, a resource of great value in the development of new and more effective tests, and one that is increasingly recognized as the avatar of personalized medicine, will be lost.

Suggested Readings

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28

Neonatal Transport

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KEY POINTS

- Regionalization of neonatal critical care improves outcomes.
- Highly effective neonatal transport systems feature centralized communications, experienced medical oversight, and skilled teams trained to care for sick neonates.
- Appropriate transport care depends on the competency of the transport teams.
- The care delivered to the patient should not decrease in sophistication at any time during the transport process.
- The referring provider is responsible for ensuring the adequacy of the chosen mode of transport.

Controversies

- Optimal transport team composition and configuration
- With proliferation of midlevel neonatal intensive care units, determining or anticipating which patients require transfer to higher levels of care and when
- Optimal method to achieve and monitor neonatal therapeutic hypothermia during transport

Regionalization of medical care enhances the ability to centralize resources and has improved patient outcomes. For optimal coordinated care, however, an adequate medical transport infrastructure needs to be developed and continually refined to enable delivery of patients to regional centers and for specialized care to be available for and delivered to patients in need. For centers that provide basic or specialized service, there will be times when subspecialty care is required, and for those who deliver subspecialty care, there may be times when transfer is indicated for reasons such as capacity or extraordinary care (i.e., extracorporeal membrane oxygenation [ECMO]). For hospitals that do not have birthing centers but to which neonates are brought for care, transfer may be indicated, and potential morbidity and mortality of these patients depend on high-quality and efficient neonatal transport. Although transfers to neonatal centers in the 1960s and early 1970s often occurred in an ad hoc manner, such as in a police car or a general ambulance, transfer programs today offer a more sophisticated level of care. Many transport services and centers have grown around individual center needs, without clear attention to coordination and regionalization of

services. Competitive systems, often located in similar areas or vying for similar patient populations, have resulted in the duplication of services at the ground and air levels and at times increased risk and cost to patients and providers as part of the efforts to maintain or increase patient volume and revenue. Furthermore, national and international standards of care in neonatal and pediatric transport medicine are evolving.

When considering transport of neonatal patients, several situations can occur: *intrafacility* for specialty services within a particular institution and *interfacility*, often between lower and higher levels of service capability, as well as between relatively equivalent levels of service because of capacity or other issues. Transported patients may be of high acuity, relatively stable, or in various stages of convalescent care. Each type of transport requires anticipatory planning, skilled, qualified, and certified transport personnel, adequate modalities (e.g., transport vehicles), and strong communication and relationships between referring and receiving providers. As noted in the next section, transfer agreements may help minimize inefficiencies and enable rapid approval and eventual transport of patients.

This chapter will review considerations and requirements for neonatal transport; discuss issues involved in transport team operation, including equipment, personnel, mode of transport, and medical legal issues; and present general and specific topics, including quality improvement opportunities, that might be encountered in a neonatal transport system (Cornette, 2004; American Academy of Pediatrics, 2016).

Regionalization of Neonatal Care, Care in the Community, and Transfer Agreements

Historical Perspective

The concept of regionalization of neonatal care and transport developed from the formation of neonatal stations, areas within hospitals, in the 1920s and 1940s (Oppenheimer, 1996) that had additional resources to care for premature neonates. One consequence of the formation of these stations was the development of equipment and protocols to transport premature neonates from other area hospitals to those with specialty services.

In the 1960s and 1970s, as interest in neonatal care grew, so did the number of hospitals offering services for premature infants.

To help optimize the care being delivered, the March of Dimes produced *Toward Improving the Outcome of Pregnancy: Recommendation for the Regional Development of Maternal and Perinatal Health Services* in 1976 (Committee on Perinatal Health, 1976). The report stratified maternal and neonatal care into levels based on complexity, and it proposed the referral of high-risk patients to centers with sufficient personnel and resources to provide care. The goal was to create standard definitions so that comparisons of health outcomes, resource utilization, and costs among regional institutions could be made. High-risk maternity patients would be able to actively participate in selecting a delivery service, and businesses would be able to select appropriate healthcare resources for their employees. The subsequent March of Dimes publication in 1993 *Toward Improving the Outcome of Pregnancy: The 90s and Beyond* reiterated the importance of regionalized care and further delineated care levels (Committee on Perinatal Health, 1993). The concepts of regional care were adopted and incorporated into the Guidelines for Perinatal Care (American Academy of Pediatrics Committee on Fetus and Newborn and Bell, 2007; Woodward et al., 2007). Whereas the original driving forces for regionalization in the 1970s were the shortage of centralized trained personnel to care for low birth weight (LBW) neonates and the economic expense to maintain these skills, during the late 1980s and 1990s, technology and clinical expertise disseminated outside the regional tertiary centers, resulting in proliferation of the number of intermediate-care neonatal intensive care units (NICUs). This proliferation has blurred many of the original distinctions between various care systems. Whether driven by third-party payers or other factors, with various interpretations and applications of what “regional care” means, the results have been the creation of a variety of care options (Lainwala et al., 2007) but without necessarily improved or optimal outcomes for all patients. For example, Kastenber et al. (2015) found that very low birth weight (VLBW) neonates with necrotizing enterocolitis had significantly increased mortality when cared for in low-volume mid-level NICUs compared with higher-volume units and those with higher acuity status. While there is limited robust evidence that demonstrates a clear causal relationship between improved outcomes and regionalized systems of neonatal care (Rashidian et al., 2014), understanding the impact of deregionalized systems on quality of care and patient outcomes will help inform which neonates require transfer to regional, high-volume, high-acuity centers and how to optimize use of the neonatal transport system to meet these needs.

Care in the Community and Back Transport

Regionalization of care, space limitations, and longitudinal care coordination contribute to a current system in which neonates are being both transported to higher levels of care for acute and/or critical needs and also transported back (“back transport”) to appropriate centers, perhaps closer to home, once their critical condition has resolved or stabilized (Lynch et al., 1988; Donovan and Schmitt, 1991; Attar et al., 2005). Regionalization guidelines should support the return to community facilities for patients who no longer need the highest level of care. Patient selection for back transport and care in another facility should match the capabilities and expertise of the community hospital (Stark and American Academy of Pediatrics Committee on Fetus and Newborn, 2004). The increasing application of telemedicine and technologies that allow real-time assessment and medical guidance may enhance not only the capacities of more remote facilities and providers during initial stabilization but also those for ongoing care of high-risk

neonates. In addition, telemedicine applications may augment the assessment and care provided by the transport team when at the patient bedside when they are able to communicate in real time with specialty providers at a receiving facility. Kim et al. (2013) conducted a prospective study to assess the impact of telemedicine in Arkansas on regionalized care and infant mortality. They found that there were fewer VLBW neonates born outside hospitals without NICUs and an overall decline in infant mortality statewide. Webb et al. (2013), in a multicenter study assessing the impact of telemedicine on neonates with suspected congenital heart disease (CHD), observed improvements in the diagnosis of CHD, fewer patient transfers, reductions in the length of stay, and use of inotropes and indomethacin. Telemedicine is a rapidly growing area in medicine, with increasing presence in NICUs, and an area of continued opportunity for development for transport.

Transfer Agreements

Although third-party payers often drive decision making, transport relationships can develop between various institutions by formalized transfer/preferred provider agreements and/or by historical and personal relationships (Attar et al., 2005). Transfer agreements can help to define the roles, understanding, and expectations between institutions and the transport service; they also help frequently to detail reimbursement issues. These agreements set the expectations for participating facilities, with the ultimate goal of the timely movement of patients from one facility to another (Woodward et al., 2007; American Academy of Pediatrics, 2016). Agreements should be reviewed by legal counsel to ensure compliance with state law (American Academy of Pediatrics, 2016).

A neonatal transport system must also determine if maternal transport is part of its purview. It is clear that preterm neonates who are born outside hospitals and require transfer to tertiary care centers or transfer between tertiary care centers have worse outcomes, which include increased mortality and morbidity such as intra-ventricular hemorrhage (Janse-Marec and Mairovitz, 2004; O'Brien et al., 2004; Jony and Baskett, 2007; O'Hara et al., 2008; Baskett and O'Connell, 2009).

Several investigators have shown that mortality is lowest for deliveries of VLBW neonates that occurred in hospitals with tertiary care NICUs (Robertson et al., 1994; Chien et al., 2001; Phibbs et al., 2007; Rautava et al., 2007). Clark (2003) supported the idea that, whenever possible, women in early preterm labor should be moved to the regional hospital rather than the neonate being transferred after birth. The study was insufficiently powered, however, to make a recommendation regarding the difference between regional and large community NICUs (Clark, 2003). In trying to discern which LBW neonate might be better cared for in tertiary regional care centers, Vieux et al. (2006) showed that there were factors associated with both increased and decreased need for neonatal intensive care. These data can help inform guideline development for transporting women in preterm labor and optimize the use of transport and local community resources to ensure timely transfer and optimal patient outcome.

Transport Communication

When one is developing and maximizing transport capabilities, a key concept is centralized communication, often in the form of a communication center. While a call from a referring provider directly to a receiving provider might seem to be the most efficient way to initiate a clinical conversation about a potential transport,

• BOX 28.1 Features of a Highly Functional Centralized Communication Center

- Centralized number with immediate access to transport system or center personnel 24/7
- Contact number advertised, easily recalled, monitored 24/7
- Single call or point of contact for referring provider to accomplish all transport needs
- Dedicated communication center personnel with transport-specific training
- Recorded calls for quality assurance and quality improvement activities

Data from Southard PA, Hedges JR, Hunter JG, Ungerleider RM. Impact of a transfer center on interhospital referrals and transfers to a tertiary care center. *Acad Emerg Med*. 2005;12:653–657.

there are more effective means of communication. Those who have transported or referred patients in systems without centralized access understand the challenges in working through operators, unit clerks, multiple providers, and often multiple services to enable a singular transport. This process is time-consuming and often frustrating for the referring provider, and the time could be better spent in direct assessment and care of the patient. When one is communicating with the transport system, there are key elements that must be considered and appreciated on both sides to initiate and complete a successful patient transfer:

1. Appreciation and recognition of the need for transfer
2. Awareness of appropriate and available transport modality and options
3. Identification of appropriate receiving facility and acceptance by receiving provider
4. Verification of regional and local bed capacity
5. Review of current medical issues
6. Determination of required transport services, including personnel
7. Dispatch of transport team and potential limitations

Centralized access through a communication center can allow all those functions to occur simultaneously, enabling more rapid transport response and appropriate involvement of all individuals required for the care of a particular patient ([American Academy of Pediatrics Committee on Fetus and Newborn and Bell, 2007](#); [Woodward et al., 2007](#)) (Box 28.1).

Medical Supervision

A key requirement for any system is to have appropriately skilled and immediately available medical command physicians (MCPs) (also referred to equally as *medical control physicians*) ([American Academy of Pediatrics Committee on Fetus and Newborn, 2007](#); [Woodward et al., 2007](#); [American Academy of Pediatrics, 2016](#)). An MCP should be literate and expert in the medical area of concern, as well as up to date on transport capabilities. In most cases involving neonatal transport, this provider should be a neonatologist. There may be instances, however, when the referring or receiving physicians may request or desire additional medical expertise. For example, a cyanotic newborn with CHD may be temporarily stabilized by the referring provider and additional medical advice may be provided by the receiving MCP, as well as a partnering cardiac intensive care physician. A communication center can facilitate an initial call in which multiple providers are linked, allowing the highest level of advice to be presented and discussed among providers. These telephone calls should ideally be recorded for quality control and/or for review if verification of

TABLE 28.1 Characteristics of Mode of Transport

| Transport Modality | Distance to Receiving Facility (Miles) | Features |
|--------------------|--|---|
| Ground | 10–20+ | <ul style="list-style-type: none"> • Fewer weather restrictions • Door-to-door • Well-lit care environment • Space for family, providers • Efficient in urban, short-range transfers |
| Rotor wing | >20–100 | <ul style="list-style-type: none"> • More expeditious >50 miles, ideal efficacy 50–150 miles • Can access less accessible areas • Can potentially be door-to-door |
| Fixed wing | >100 | <ul style="list-style-type: none"> • Expeditious over long distances, ideal efficacy >150 miles • Can circumvent weather issues • Potential space for family, provider • Ability to pressurize cabin • Requires ground transport to and from airports |

information is needed and should include the transport personnel so that background information and care plans are communicated directly.

Mode of Transport

Once the transport referral has been made and discussions have been started with the MCP, the transport process begins in earnest. A decision on mode of transport is an important consideration at this juncture and is ultimately the responsibility of the referring provider, although it can be appropriately influenced by the MCP. (Table 28.1).

In addition to distance from the referring and receiving facilities, which will impact the total transport time, the decision regarding the mode of transport is influenced by several additional considerations when aiming to arrange and dispatch the most appropriate team for a given patient:

- Available mode of transport
- Staffing and medical expertise of providers involved in each mode
- Patient's current stability and potential illness progression during the projected transport time
- Capabilities of referring facility and personnel
- Urgency of need for intervention and definitive care of patient
- Geography and weather

In a study examining the decision-making factors around the mode of transport, [Quinn et al. \(2015\)](#) found that the decision to activate a helicopter versus a ground unit was made in the face of not only prolonged distance (>45–60-minute drive time) but also the presence of perceived high-risk clinical conditions, specifically neurovascular and respiratory concerns, even more so than blood pressure or heart rate.

Currently, there are guidelines but no national absolute criteria or standards to direct the choice of ground versus air transport. Each modality has its own risks and benefits. First, with both air

and ground transfer, concerns include potential physiologic stress and discomfort experienced by the neonate secondary to stimuli such as vibration and noise (Schierholz, 2010; Sittig et al., 2011; Bouchut et al., 2011; Harrison and McKechnie, 2012; Karlsson et al., 2012; Prehn et al., 2015). Therefore adjuncts to minimize the stress and discomfort, such as gel mattresses and earmuffs, should be used as much as possible. Air transport can also present specific stressful stimuli such as gravitational forces during acceleration and deceleration, temperature variations, and decreased humidity with altitude and introduces issues related to altitude physiology that can affect patients with respiratory issues or air trapping as well as air-containing equipment (e.g., endotracheal tube cuffs, laryngeal mask airways) (Woodward and Vernon, 2002; Wilson et al., 2008; Woodward et al., 2006; Schierholz, 2010). Dalton's law recognizes that ambient oxygen partial pressure decreases as altitude increases; therefore there may be a need for pressurization and augmentation with increased fraction of inspired oxygen. Boyle's law states that as altitude increases, the volume of a gas also increases, as barometric pressure is inversely related to the volume of the gas. Thus consequences of this law are potentially a serious issue for patients with an enclosed gas collection, such as a simple or developing pneumothorax or pneumatosis in suspected cases of necrotizing enterocolitis.

Secondly, weather and physical distance can make each mode of transport more or less accessible or reasonable at a given time. For instance, while ground transport may be more readily available than air transport because of fewer weather constraints and an increased number of vehicles, the overall transport time may be too long given the clinical needs of the patient.

Finally, each mode of transport has occupancy limitations associated with select vehicles, especially rotary air transport. These limitations can preclude extra passengers such as parents or family members or potentially crew members if weight and balance is an issue.

Transport Personnel, Education, and Team Composition

Awareness of the capabilities of the transport system and of the personnel involved is imperative in decisions regarding the mode of transport. Although it is ultimately the responsibility of the referring physician to identify the appropriate mode and personnel for transport, per the federal Emergency Medical Treatment and Active Labor Act (EMTALA), opportunities exist for tertiary care and referral centers to help inform the referring providers regarding optimal transport planning and use (Bolte, 1995; Woodward, 1995). In general, issues influencing transport decisions include the patient's current level of care, urgency for a different level of medical capability or equipment, current provider capabilities, stability of the patient, options available to the provider and patient, and efficiency and quality of the transport process. Ideally, these issues are key determinants of appropriate transfer; however, referring providers are often overwhelmed by the severity or acuity of the patient, and their primary desire may be to have the patient removed from their facility as quickly as possible. The providers may focus on a transfer process based solely on the speed of transport rather than the quality of care. It is imperative for the receiving and tertiary care centers to educate the referring providers regarding the importance of stabilization, initiation and quality of the primary response, transport options, and definitive care to maximize patient outcome. When examining the transfer of patients, providers should

ask a simple question: Are we trying to deliver the patient to tertiary care, or are we trying to deliver tertiary care to the patient? In most high-functioning transport and referral centers, the latter is true. The referring physician should expect to have tertiary care advice and direction delivered at the moment of the referral call and continued throughout the transport process (Woodward, 1995; American Academy of Pediatrics Committee on Fetus and Newborn and Bell, 2007; Woodward et al., 2007).

When considering the transport team composition, it is important to consider the quality of the personnel, their expertise and experience, and their ability to work in the transport environment (King et al., 2001; King and Woodward, 2002a; King et al., 2007). There are many variations of transport teams in the United States and abroad (Karlsen et al., 2011). These teams can be composed of a combination of physicians, nurse practitioners, nurses, respiratory therapists, paramedics, and other healthcare providers. Regardless of the formal educational background of an individual, there are several criteria that must be met to be optimally effective in the transport environment. First, the provider must have adequate certification, be licensed for the care he/she delivers, and be able to provide the assessments and interventions that a patient currently or potentially requires during the transport process. For example, a neonatal retrieval service must be able to manage acute and critical airways in the neonatal population, both at a referring hospital and during the transport. While transport team providers might not be credentialed to provide certain skills within their home hospital (i.e., intubation), they have been certified to provide them in the ambulance environment. In general, this must be done under the auspices of a physician's care, which may be from an accompanying physician or via online medical control (real-time medical advice during the transport process) or protocol-based care (off-line medical control). It is important to recognize that the transport time frame is somewhat limited; therefore the personnel may not need to have the longitudinal or differential diagnosis expertise of a fully trained neonatologist. However, these personnel must have the acute care assessment abilities and intervention skills of an experienced neonatal expert.

From the transport and pediatric literature, patient outcomes are improved with specialty providers. While multiple studies have examined this particular issue (Mullane et al., 2004; Belway et al., 2006; Borrows et al., 2010; Kuch et al., 2011), the most compelling is the study by Orr et al. (2009), which examined transport by variable providers within the same system. This study compared outcomes in patients whose care was delivered by specialized pediatric critical care teams with those whose care was delivered by general providers. Both teams had the same medical command oversight, equipment, and modalities. Patient outcomes were worse for those whose care was not delivered by specialty teams and was much improved for those whose care was delivered by specialty teams. While the study by Orr et al. and studies by others are compelling, a recent Cochrane review that focused specifically on neonatal specialty teams (Chang et al., 2015) concluded that in the absence of randomized controlled studies there is not good evidence to refute or support the use of neonatal specialty teams for high-risk neonates. Evidently, specialty teams and patient outcomes is an area for ongoing investigation.

In addition to training the transport personnel, MCPs should understand the opportunities and limitations of the transport services, the environment, and the risks and challenges that referring personnel can potentially encounter with situations and patients who exceed their own or their facility's management abilities. It is imperative that MCPs have clear and efficient communication,

not only with referring providers and those from different disciplines but also within the transport team.

Quality Improvement

Throughout medicine, there is an increasing focus on measuring and improving the quality of care provided across all healthcare domains and patient experiences. Transport medicine offers an opportunity for potential quality improvement activities within the inpatient arena, in the transport system, and at the referring facilities (Chen et al., 2005; Browning Carmo et al., 2008; Lim and Ratnavel, 2008; McPherson et al., 2008; Ramnarayan, 2009). While there are guidelines of care and process recommendations such as those provided through the American Academy of Pediatrics and the Commission on Accreditation of Medical Transport Services, formal national transport benchmarks or standard quality-of-care metrics are still evolving. A consensus document on behalf of the American Academy of Pediatrics Section on Transport Medicine was recently published that offers 12 core quality metrics for neonatal and pediatric transport (Schwartz et al., 2015). The proposed neonatal and pediatric transport national benchmark and quality metrics are as follows:

1. Unplanned dislodgement of therapeutic devices
2. Verification of tracheal tube placement
3. Average mobilization time of the transport team
4. First-attempt tracheal tube placement success
5. Rate of transport-related patient injuries
6. Rate of medication administration errors
7. Rate of patient medical equipment failure during transport
8. Rate of CPR performed during transport
9. Rate of serious reportable events (http://www.qualityforum.org/Topics/SRE/List_of_SREs.aspx)
10. Unintended neonatal hypothermia on arrival at destination
11. Rate of transport-related crew injury
12. Use of standardized patient care handover

The authors propose that these metrics serve as benchmarks and help guide individual program quality improvement. Details of their recommendations can be accessed at <http://www.aap-sotm.org>.

Neonatal and pediatric airway management may be one of the most important aspects of transport clinical care and historically is one of the more challenging areas for emergent and prehospital management. Examples of quality-based investigations reflect these challenges and provide information to guide improvements. Bigelow et al. (2015) retrospectively assessed first-pass intubation success for neonatal and pediatric patients across nine transport programs over a 6-month period. The overall success rate was 64%, with a range of 35%–87%, but was highest for teams that had live-patient training for initial competency and lowest for those using simulation alone. Smith et al. (2015) considered risk factors for intubation failure among neonatal and pediatric specialty teams and found higher rates of intubation failure for neonates compared with pediatric patients, especially for the smallest neonates necessitating the use of tube sizes of 2.5 mm or less. Additional risk factors for failure were the use of uncuffed tubes and the failure to use sedation and the lack of neuromuscular blockade.

Quality improvement activities in the transport domain are diverse and range from assessment of the transport process by review of recorded calls to the monitoring of intubation success and vital signs in transport to improving communication through standard handovers (Weingart et al., 2013). However, this glimpse into facility/provider medical sophistication and capability is one that is privileged and should be used to identify educational

opportunities for all providers involved rather than used as a judgmental review. Education by receiving physicians and transport teams can have a significant effect on the quality and outcome of patient care and the volume of future referrals. Ideally, once a referral call is made, a receiving physician or MCP will direct the care so that the job of the transport team is to verify that an appropriate working diagnosis has been made and adequate stabilization has been achieved. Systems that do not gather adequate information or offer appropriate advice, or in which the referring facilities do not follow that advice or choose not to perform needed interventions, can put the patient at risk by delaying potentially necessary interventions, prolonging the transport process, and delaying delivery of definitive care. The transport team that has invested several hours at a bedside stabilizing a newborn with medical or surgical issues may be spending time in a facility that is not ideal, has a limited number of skilled personnel, and has minimal backup, thus prolonging the transport process, delaying definitive care, and potentially putting that individual patient and the transport team at risk (Chen et al., 2005; Haji-Michael, 2005). Furthermore, during this prolonged stabilization time, the entire system becomes at risk because the valuable resource of specialized neonatal transport personnel is not available for another patient.

Ideally, care delivery would be the same at referral and receiving centers, and the development of practice guidelines can help in this regard. Guidelines that are evidence based, developed by regional and local experts, and disseminated to referring centers and transport teams will help standardize and promote consistent care across variable locations. It is necessary, however, to assess and reassess the quality of the guidelines and the competency of their use to ensure optimal results. Even in the best of hands, near-miss or realized adverse events may happen. It is clear that identification of those events, discussion with families (where appropriate), and root cause analysis are imperative. Several studies have examined adverse events in transported patients. Van den Berg et al. (2015) looked at adverse events over 13 years with their neonatal transport team in northern Sweden. They found that such events had differing significance (53% low risk to 11% high or extreme risk), were common, and were often related to transport logistics and equipment failure. Ligtenberg et al. (2005) noted that one-third of patients had an adverse event, and 50% of adverse events resulted from the advice of the MCP not being followed. Of that group, 70% of events were avoidable and 30% involved logistical issues. In a review of the London Neonatal Transfer Service, Lim and Ratnavel (2008) noted that 36% of their patients had one or more adverse events and that two-thirds of those were due to human error. Half of the events occurred before the team arrived at the referral center and were due to patient preparation and communication issues.

Transport Administration

As a hospital develops and optimizes a neonatal transport program, experts in transport medicine are integral to the success of the program (American Academy of Pediatrics Committee on Fetus and Newborn and Bell, 2007; Woodward et al., 2007). A quality medical director and program director, often a nurse or respiratory therapist, are essential for understanding the potentially complicated and challenging environment of transport medicine. These leaders should be instrumental in identifying expectations, roles, and responsibilities for the entire transport process, including oversight of the communication center and developing and disseminating referral center expectations.

The responsibilities of the referring center when transferring patients are to:

- Stabilize and prepare the patient before transport.
- Make an appropriate decision to transfer the patient.
- Choose an appropriate transport process and destination.
- Obtain family consent for transport, including the mode and receiving facility.
- Discuss and initiate a plan for stabilization with the medical command physician.
- Communicate clearly when suggested interventions are beyond the scope of the referring center or cannot be done.
- Be present and participate in the transition of care to the transport team and the receiving service.

In turn, there are also clear expectations of the receiving center and transport team.

- Immediate availability for patient care consultation
- Able and qualified to provide clear and concise recommendations
- Quick determination if able to accept the patient for transfer
- Ensure that receiving facility staff are prepared for both patient and transport team arrival.
- Document interaction with and recommendations to the referring facility.

Most importantly, the team needs to ensure that appropriate skills and therapeutics are available and delivered throughout the process, from the referral call through definitive placement, and ensure seamless transition at each point of care. The team needs to communicate well with the patient's physicians and document their advice, interventions, and activities in a clear, concise fashion to enable appropriate patient care and provide protection for the transport service.

Transport Safety

Safety of the transport system and its providers is paramount and must be assessed and ensured before any patient is transported. Vehicles must be safe and meet the standards for air or ground transport; the personnel must be trained and skilled in the care of neonates, licensed, and competent; and the patients must be managed in the most appropriate and professional fashion. In addition, the logistics of travel must include a safe environment, including helmets and fire-retardant suits for those who fly in helicopters, three-point restraints, and appropriate ambulance seating arrangements. Providers should not put themselves at risk by being unrestrained or being in an area where unsecured debris or inappropriately placed equipment may cause harm to them or the patient. Adherence to all rules and regulations of air and ground travel is essential (Clawson, 2002; King and Woodward, 2002b; Levick, 2006; Greene, 2009; National Highway Traffic Safety Administration, 2009).

It is important to recognize that there are risks with both air and ground transport. The air transport industry saw a spike in tragic and fatal air accidents (Greene, 2009; National Transportation Safety Board Accident Database, 2009). This increase caused the industry, and the US government, to investigate these incidents and offer recommendations to improve transport safety (Fact Sheet—FAA Initiatives to Improve Helicopter Air Ambulance Safety, 2014; Flight Safety Foundation/Aerosafetyworld, 2008). Requirements such as duty hours for pilots, weather restrictions, flight under instrument flight rules with terrain avoidance equipment, and night vision goggles can help to minimize transport risk. While ground ambulances are used much more frequently and the risk of injury and death is evident, the fatality rate is lower in ambulance accidents than it is in aircraft accidents (King and Woodward,

2002b; Becker, 2003; Becker et al., 2003). Many systems do not allow ambulances to exceed posted speed limits and allow them to use lights and sirens only as a way to identify an emergency response not to enable the vehicle to circumvent or ignore standard traffic laws (Clawson, 2002). Appropriate equipment for ambulances is required as well, and the most recent joint statement by the American College of Surgeons Committee on Trauma and the American Academy of Pediatrics regarding appropriate equipment for ambulances should be reviewed by the providers of all transport systems (American Academy of Pediatrics, American College of Emergency Physicians, American College of Surgeons, et al., 2014).

One challenge for transport teams is that differentiation of medical resources, such as a neonatal specialty team, likely means that there may be a scarcity of resources and a potential need to ration those resources. It is possible to develop teams with a variety of personnel with complementary cognitive and procedural skill sets and work toward appropriate triage of transport requests to ensure the optimal level of onsite patient care and safe transport. There have been multiple attempts to develop triage tools for pediatric and neonatal care providers, including the Mortality Index for Neonatal Transport, the Modified Clinical Risk Index for Babies, the Risk Score for Transported Patients, and the Transport Risk Index of Physiologic Stability (TRIPS) (Lee et al., 2001; Broughton et al., 2004a, 2004b; Markakis et al., 2006). Notably, TRIPS is a validated tool that can be calculated in a single assessment and has been shown to correlate with NICU mortality. The original authors of TRIPS recently validated TRIPS-II, the application of TRIPS over 12–24 hours after NICU admission, and found it correlates with illness severity not only at admission but also up to 24 hours (Lee et al., 2013). Given the simplicity and ease of use, TRIPS and possibly TRIPS-II are examples of scoring tools that can be applied in both the transport environment and the hospital environment reflecting clinical deterioration or improvement over time.

Family-Centered Care

Transport team research has shown that family-oriented care, as in other areas of health care, is an important component of transport (Woodward and Fleegler, 2000, 2001; Granrud et al., 2014; Mullaney et al., 2014; Joyce et al., 2015; American Academy of Pediatrics, 2016). Families who have been formally surveyed appreciate the opportunity to participate in the care of their child and express increased stress and anxiety when they do not accompany their child during transport (Mullaney et al., 2014; Joyce et al., 2015). In neonatal transport, however, there are times when there are two patients who may require care in two disparate locations. A mother who has had a cesarean delivery and has delivered an acutely ill neonate who requires transfer to a specialty pediatric facility with neonatal intensive care capability is one such example. Transport teams should be sensitive to the challenges and opportunities for the family members and include them in the process when possible. It is evident that when parents attend or accompany transport team members during critical care transports, they are there not to assess the medical skill set of the provider but to provide support to their child. It is also a great opportunity for the transport team to demonstrate to the family that their patient is in focused, professional, caring, and capable hands.

Medical Legal Issues

There are many medical legal issues in transport medicine, as elsewhere in the medical system (Williams, 2001; Woodward,

2003; Hedges et al., 2006; Fanaroff, 2013; American Academy of Pediatrics, 2016). The Health Insurance Portability and Accountability Act is a required component of transport planning and delivery. Discussion of patients should not happen in a public area or via public communication airways, where patient-specific information could be overheard. As noted earlier, a requirement of EMTALA is that the referring physician choose the appropriate mode of transport and ensure that the transport process and receiving hospital are appropriate for the particular patient. Patients should not be transferred if they are unstable and the ability to further stabilize them is available at the initial site of care. If a patient must be transferred for care while in an unstable condition—a frequent scenario for critically ill patients who need care not available at the referring institution—consent must be obtained from the family, which acknowledges their understanding of the potential risks and benefits of the process. In practice, there are often patients in an unstable condition who are transferred from lower to higher levels of care because the level of care that can be provided at the referring center is not optimal for the child. This reason is appropriate for transfer as compared with transfer of acutely unstable patients because of financial or other economic incentives.

The medical liability for transport is a shared process. Before the referring center contacts a receiving facility or transport team, the entire medical responsibility lies with the referring provider. Once the receiving team has accepted the patient and offered advice, medical liability becomes a shared process. The referring physician maintains most of the liability, as well as medical control of the patient, throughout the process until the transport team has left the referring hospital. It is important to recognize that most transport teams and personnel do not have privileges at referring hospitals and are working under the guidance and supervision of the referring physician team. Transport teams that act independently, or referring providers who are not available when the transport team arrives, put not only the patient but also the referring provider and transport team at risk.

There will be times, however, when there is disagreement regarding the optimal care to be delivered. This situation can be challenging, and it must be handled appropriately. It is never appropriate to have obvious provider conflict occur at a patient's bedside in front of family members. The appropriate way to handle a situation that cannot be easily mitigated is to involve the MCP with a telephone call to the referring physician in a discussion at a peer-to-peer level. Transport teams have been known to comply with the wishes of the referring providers to not perform advanced procedures at the referring hospital, only to perform those procedures in the ground or air ambulance, which is a much less desirable location. Ideally, all disagreements and considerations of different therapies should be discussed in a collegial fashion.

As noted previously, documentation of all information received and advice offered is imperative. If there is a review or there are challenges regarding the care delivered before or during transport, clear and appropriate documentation should stand alone as an excellent defense. In addition, many centers use recorded (i.e., digital, tape, other retrievable recording process) intake and advice lines; this is another way to review, educate, and ensure that appropriate information is delivered in an effective communication style. The use of recorded lines with frequent review, for educational and quality assurance purposes, can be invaluable. Review with legal advisors can help define the length of time the recorded materials should be maintained for quality improvement or patient record addendum.

Patient Care During Transport

The primary clinical goals for any neonatal transport include, but are not limited to, the following and should be established during the initial stabilization phase before departure from the referring facility and maintained until handover at the receiving facility:

- Secure and patent airway
- Adequate ventilation and oxygenation
- Thermoregulation, especially for premature neonates, goal 36°C–37°C (except as indicated for hypoxic–ischemic encephalopathy)
- Normoglycemia, goal glucose level 50–200 mg/dL
- Adequate blood pressure and perfusion
- Appropriate condition-specific care such as for myelomeningocele

A team's ability to achieve these care goals may be impacted by the patient's clinical status but will also depend on the team's preparedness and skill and experience caring for critically ill neonates. Therefore the general approach to any neonatal transport includes ensuring the availability of appropriate equipment, such as an isolette and endotracheal tubes for the smallest premature neonates, that there are skilled team members who can optimally care for a sick neonate, that there are appropriate medications for specific situations such as prostaglandin E₁ (PGE₁), and that there is smooth communication with the MCP.

Extreme Prematurity and the Limits of Viability

Of the approximately 4 million live births that occur annually in the United States, approximately 10% are preterm (<37 weeks' gestation), and approximately 1.5% are VLBW (<1500 g) (Hamilton et al., 2015). The most effective method of transporting extremely premature neonates is before delivery with the mother serving as the transport vehicle, but sometimes this is just not possible and a very premature baby is delivered unexpectedly at a referral center. Neonates born at 23–26 weeks outside tertiary centers have higher mortality and morbidity than those delivered within specialty centers (Cifuentes et al., 2002; Phibbs et al., 2007; Rautava et al., 2007; EXPRESS Group et al., 2009; Maheshwari and Luig, 2014). When delivered at a referral hospital, the premature neonate is exposed to all the variability of the extrauterine environment, with the added complexity of having to be transported to another facility capable of meeting the neonate's needs. Increased transport duration, lower gestational age, and severe acidosis before transfer have been associated with increased risk of clinical deterioration and mortality among transported VLBW neonates (Wilson et al., 2012; Arora et al., 2014). In one study the odds of deterioration increased 1.33 for every 5 minutes of transport time (Arora et al., 2014). Most neonatal transport teams are regarded as extensions of the NICU. The team initiates and provides much of the same level of complex neonatal care as the receiving hospital, but in a changing environment. It is this changing environment that poses unique challenges for both the patient and the caregiver. These issues would be amplified in the case of a natural disaster with care and transfer required for premature neonates (Gershanik, 2006; Espiritu et al., 2014).

Human viability is limited largely by the physiology of pulmonary development and currently appears to be at approximately 22–24 weeks' gestation (Pignotti and Donzelli, 2008) and 400 g (American Academy of Pediatrics, 2016). Unfortunately, for transport teams and providers at referring hospitals, it may be difficult to determine which neonates born at the margins of viability should be resuscitated and treated with aggressive neonatal care and which should not (American Academy of Pediatrics Committee on Fetus and Newborn and Bell, 2007; Buchanan, 2009). These decisions are

best made collaboratively with the family, transport team members, and the referring and receiving physicians (Tyson et al., 1996; Gunderman and Engle, 2005; Ahluwalia et al., 2008) and may ultimately result in patient transport, even when the likelihood of survival is minimal. Telemedicine may be useful to help with decision making in these difficult cases.

Thermoregulation

Problems in neonatal thermoregulation continue to be a major contributor to neonatal morbidity and mortality worldwide and can be especially problematic in neonatal transport. During transport, neonates often cross into and out of multiple different environments with wide temperature and humidity variations. Although a normal term neonate may be capable of significant homeothermic response by using its sympathetic nervous system to vasoconstrict peripherally, preterm neonates lack the subcutaneous fat insulation to protect their core temperature. Several recent studies using TRIPS found that temperature instability accounted for clinical deterioration during transport, with as many as 57% of neonates demonstrating clinical decline (Goldsmith et al., 2012; Romanzeira and Sarinho, 2015). In one study, increased mortality was observed among those neonates with clinical deterioration (Goldsmith et al., 2012). Skiöld et al. (2015) identified factors associated with temperature instability before transport and found that VLBW and the presence of respiratory support were predictors of temperature instability on arrival at the receiving center. Clearly, steps should be taken to minimize temperature instability during transport, such as adequate isolette temperature and use of chemical gel packs and polyethylene occlusive skin wrapping to help maintain temperature, especially of VLBW neonates (Vohra et al., 2004).

While humidity contributes to neonatal temperature control, especially for LBW neonates, it is also important for gas delivery among those receiving mechanical ventilator support (Sousulski et al., 1983). Ventilation with dry gases affects the airway epithelium in VLBW neonates and can result in hypothermia secondary to their large surface area to body mass ratio and their relatively large respiratory minute volume (Fassassi et al., 2007). Whereas ventilator complications can be reduced and thermoregulation can be improved by provision of exogenous heat and humidity to the gases, active heated humidification systems are used infrequently during neonatal transport. Passive hygroscopic heat and moisture exchangers have been used for short-term conventional mechanical ventilation and with some types of high-frequency ventilation (Schiffmann et al., 1997; Schiffmann et al., 1999; Fassassi et al., 2007).

For all newborns, an equally important condition to avoid is hyperthermia. Although elevated temperatures in neonates occur with increased metabolic rates, prolonged seizures, dehydration, or infection, the most common cause of neonatal hyperthermia is high ambient air temperature and humidity (Baumgart, 2008).

Surfactant

Surfactant replacement therapy has proven to be one of the most significant advances in neonatal critical care (Liechty et al., 1991; Horbar et al., 1993; Schwartz et al., 1994; Rojas-Reyes et al., 2012). Benefits are achieved with both prophylactic (defined as within 10–30 minutes of birth) and rescue (within 12 hours of birth) surfactant administration, especially for extremely LBW neonates (Kendig et al., 1991; Soll, 2000; Rojas-Reyes et al., 2012). Surfactant administration can be complicated by airway obstruction, right

mainstem bronchus or esophageal instillation, bradycardia, hypotension, and rarely pulmonary hemorrhage. These complications led the American Academy of Pediatrics Committee on Fetus and Newborn to recommend that “preterm and term neonates who are receiving surfactant should be managed by nursery and transport personnel with the technical and clinical expertise to administer surfactant safely and deal with multisystem illness” (Engle and American Academy of Pediatrics Committee on Fetus and Newborn, 2008).

Given the potential for rapid physiologic changes and the risk of complications, surfactant administration should generally not be initiated en route between facilities. While investigations are still ongoing as to the optimal manner of surfactant administration, future technology might eventually allow surfactant to be delivered without tracheal intubation. The administration of surfactant via a small intratracheal catheter while the neonate breathes spontaneously while receiving continuous positive airway pressure (CPAP), a technique known as *minimally invasive surfactant therapy* (MIST) (Kribs et al., 2007), has been shown to be equally effective as techniques involving tracheal intubation and in some studies resulted in reduced mortality and morbidity (Aguar et al., 2014; More et al., 2014; Göpel et al., 2015; Kribs et al., 2015). Several studies, including NICU-based randomized controlled trials (Rojas et al., 2009; Dani et al., 2010) have shown success with the INSURE method (Verder et al., 1994; Keszler, 2009): *intubate–surfactant–extubate* to CPAP to avoid the potential sequelae and complications of prolonged mechanical ventilation in preterm neonates. This method has not been studied in the transport environment; however, there are preliminary investigations into parameters that may serve as eligibility criteria to identify in which neonates extubation to CPAP could be safe and successful before they depart from the referring center (Priyadarshi et al., 2015). Aerosolized surfactant has yet to be shown to be superior to endotracheal tube administration (Berggren et al., 2000; Mazela et al., 2007).

Currently, the most frequently used surfactant preparations in the United States are derived from animal lung extracts. These surfactants require refrigeration and are usually carried by transport teams in small containers cooled by gel packs. Lucinactant, a synthetic surfactant, requires a special warming cradle to convert it from a gel to a liquid before administration, adding a level of difficulty to its administration during neonatal transport (Kattwinkel, 2005; Moya et al., 2005). Because smaller community hospitals may not have surfactant readily available, the transport team should carry it as part of their medical supplies.

After the surfactant has been administered, monitoring pulmonary compliance and adjusting the patient’s ventilator while still at the referring facility may extend the transport time but also limit avoidable clinical complications.

Hypoxic Respiratory Failure

Hypoxic respiratory failure describes a heterogeneous group of neonatal disorders that have in common impaired oxygenation and the need for assisted ventilation, most commonly respiratory distress syndrome, meconium aspiration syndrome, and persistent pulmonary hypertension of the newborn (PPHN). Interfacility transport of the newborn with hypoxic respiratory failure is potentially hazardous, with a high risk of patient deterioration and complications of therapy.

Assisted ventilation during neonatal transport can be accomplished with a variety of devices, although not all modes of mechanical ventilators have been modified for use in a mobile

setting. Newborns, including very preterm neonates born at 28–32 weeks' gestation with milder degrees of illness, may be managed successfully with CPAP (Murray and Stewart, 2008) with a variety of interfaces, ranging from a face mask to nasal prongs or a nasal cannula (Jani et al., 2014). A review of the limited literature (Trevisanuto et al., 2005) suggests a potential role for neonatal laryngeal mask airway during transport. This modality is particularly interesting since the success of neonatal intubation is highly variable across transport programs, specialties, and individuals. The use of sedation and neuromuscular blockade has been shown to increase intubation success (Smith et al., 2015) and is recommended by the American Academy of Pediatrics (Kumar et al., 2010). However, caution is required when considering paralytic agents if the ability to establish the airway and/or ventilate the patient is at all in question, such as with a provider inexperienced in managing a neonatal or difficult airway.

Inhaled nitric oxide (iNO) is approved for use in term and near-term newborns with hypoxic respiratory failure with clinical or echocardiographic evidence of pulmonary hypertension. Administration of iNO to newborns with PPHN reduces the need for ECMO (Clark et al., 2000; Lowe and Trautwein, 2007). With increasing availability to community NICUs, iNO therapy is often initiated before transport to a tertiary care center. Once iNO is administered, abrupt cessation of therapy can result in rapid clinical deterioration from rebound pulmonary hypertension, so it is essential to continue this therapy throughout the transport (Kinsella et al., 1995). Transport teams may also consider initiating iNO therapy for term and near-term newborns with hypoxic respiratory failure. If the referring facility does not have the capability to perform echocardiography, the transport staff should carefully consider the possibility of congenital heart lesions that can lead to clinical decompensation with iNO, including total anomalous venous return and lesions dependent on right-to-left ductal flow such as critical aortic stenosis.

The rationale for initiating iNO therapy during transport is to reduce pulmonary vasoreactivity and increase stability during transport. However, there have been no prospective studies to determine whether this practice affects patient outcome. During transport iNO has been delivered by a number of different systems, such as the aeroNOX (Aeronox Technology Corporation, Quezon City, Philippines) iNOvent, and INOmax DS (INO Therapeutics, Hampton, New Jersey, United States) transport systems (Kinsella et al., 1995; Tung, 2001; Kinsella et al., 2002; Lutman and Petros, 2008). The use of iNO in combination with high-frequency ventilation in non-ECMO centers can complicate the transfer process. If the transport team does not have the capability to provide mobile high-frequency ventilation, it is recommended that the referring hospital perform a trial of conventional mechanical ventilation before transport to establish that the neonate can tolerate the transition.

Both high-frequency jet ventilation (Bunnell ventilators; Bunnell, South Salt Lake, Utah, United States) and high-frequency flow interrupter ventilation (Bird ventilators; CareFusion, Yorba Linda, California, United States) have been configured and used with nitric oxide during transport (Honey et al., 2007; Mainilli et al., 2007). The high-frequency oscillator (SensorMedics 3100A; CareFusion, Yorba Linda, California, United States) that is commonly used in many NICUs is impractical for ground transport and is not configured for helicopter or fixed-wing transport. However, even with these technologies, the conditions of 30%–40% of critically ill neonates improve only temporarily with iNO therapy, and they will ultimately require a higher level of care (Kinsella et al., 2002; Fakioglu et al., 2005).

For patients with pulmonary or cardiac failure who are unresponsive to maximal medical therapy, conventional ECMO is often used as a bridge therapy to allow either the lungs or heart, or both, to recover. Ideally, centers without ECMO capability have prospective criteria to guide the transfer of newborns before the need for ECMO cannulation. In some cases the patient's condition is so unstable that conventional transport cannot be conducted safely. Select programs have the capability to provide mobile ECMO, during which patients are cannulated for ECMO either before transport or when the transport team arrives, in this case usually including ECMO surgical and medical specialty providers as part of the transport team. In a recent study the most common diagnoses associated with the need for neonatal ECMO transport were meconium aspiration syndrome, persistent pulmonary hypertension, sepsis, and congenital diaphragmatic hernia (Broman et al., 2015). Mobile ECMO can also benefit patients already receiving ECMO at a tertiary facility who are in need of transfer for advanced quaternary therapies, such as heart or heart–lung transplants (Wilson et al., 2002; Wagner et al., 2008; Cabrera et al., 2011). Fixed-wing and ground ambulance transport predominate as primary modalities for ECMO transport, which is logical given the size of the equipment and the team involved. In a recent study, however, 5% of ECMO transport occurred in a helicopter (Broman et al., 2015).

The resources and skill set necessary to safely and consistently perform ECMO in the transport environment have, by their complexity, restricted the number of transport programs with mobile ECMO capabilities (Coppola et al., 2008; Cabrera et al., 2011; Broman et al., 2015), and there are a limited number of centers worldwide that have long-standing, relatively high volume pediatric/neonatal ECMO transport programs. While there are no published data to set criteria for the number of ECMO transports needed to maintain competency or impact mortality, recent literature suggests that centers with at least 20–30 neonatal and pediatric ECMO cases annually have higher survival rates than centers with a lower volume (Cabrera et al., 2011; Freeman et al., 2014; Broman et al., 2015). These data support the regionalization of ECMO transport services to optimize transport team skills and performance as well as patient outcomes given the high-risk, low-frequency nature of these transports.

Neurologic Issues

Hypoxic–ischemic encephalopathy affects approximately 1 in 1000 to 2 in 1000 term neonates (du Plessis and Volpe, 2002; Shah, 2010). Several randomized trials of therapeutic hypothermia (generally sustained core temperature of 33.5°C for 72 hours) for term neonates and a recent Cochrane review have demonstrated a reduction in the combined outcome of death and neurodevelopmental disability up to 7 years of age when neonates were cooled compared with a control population (Gluckman et al., 2005; Shankaran et al., 2005; Simbruner et al., 2010; Shankaran et al., 2012; Jacobs et al., 2013; Azzopardi et al., 2014; Natarajan et al., 2014). Clinical trials and current guidelines suggest that cooling should be initiated within 6 hours of insult. For patients with a delay in referral or who are being transported from a distant center, waiting to start hypothermic therapy until their arrival at the receiving center potentially delays management shown to be beneficial. Initial larger clinical trials did not address these issues with regard to critical care transport, but several case (Anderson et al., 2007), pilot (Eicher et al., 2005), single-center studies and one recent multicenter randomized control trial (Zanelli et al., 2008; Azzopardi et al., 2009; O'Reilly et al., 2013; Akula et al.,

2015; McNellis et al., 2015) have demonstrated the feasibility and safety of controlled hypothermia during transport. Given the potential benefits of therapeutic hypothermia initiated in a timely manner, transport teams should strongly consider initiating therapeutic hypothermia, if possible, before arrival at the referral center (Box 28.2).

If the criteria for therapeutic hypothermia are met, cooling is achieved by either passive (no active warming) or active methods such as the placing of wrapped disposable cooling packs next to the trunk and head and continuous monitoring of rectal or esophageal temperature. One of the challenges is reliable temperature regulation and monitoring during the transport process with either cooling method, and it has been observed that 50% or more of neonates do not reach the target temperatures during transport (O'Reilly et al., 2011; Akula et al., 2013). A recent randomized controlled study (Akula et al. 2015) investigated the use of a servo-controlled cooling device during transport and found not only improved temperature control (target temperatures reached 89% of the time compared with 49% for traditional passive and active methods), but also therapeutic hypothermia was achieved more rapidly compared with traditional passive and active cooling methods (Akula et al. 2015). In the United Kingdom, 70% of transport teams now use servo-controlled devices, and none use non-servo-controlled active cooling methods (Sharma, 2015). Currently, the only US Food and Drug Administration approved servo-regulated cooling devices weigh between 35 and 59 kg, significantly more than the device used in the United Kingdom (6.8 kg) and also in the study by Akula et al. (2013). Considering the limitations for weight and balance on any given flight, the heavier devices could be a significant, and potentially prohibitive, addition for most flight programs.

Therapeutic hypothermia is the standard of care for perinatal asphyxia, and with additional studies reliable means of continuing or initiating this practice during transport may become clearer.

Congenital Heart Disease

Transport of the neonate with CHD follows the same general approach as for the transport of any critically ill neonate. However, neonates with complex CHD often need therapeutic intervention, requiring the support of multiple subspecialty services, including a pediatric cardiothoracic surgeon and cardiologist (Stark et al., 2000; Allen et al., 2003), which may necessitate transport to a specialized center (Castaneda et al., 1989; Penny and Shekardemian, 2001). Importantly, the preoperative care of these patients affects their postoperative outcomes and mortality (Wernovsky et al., 1995; Mahle and Wernovsky, 2000; Mahle et al., 2000; Wernovsky et al., 2000; Robertson et al., 2004; Simsic et al., 2007). In some cases the neonate will have a prenatal diagnosis of CHD and the team will have disease-specific management plans, for instance, initiation of PGE₁ therapy on arrival for a neonate with a clinically stable ductal-dependent lesion. In other cases, CHD may be suspected in a critically ill neonate with signs of cardiovascular collapse, and the transport team must be prepared to initiate treatment, such as PGE₁ therapy, or limit others, such as treatment with fluids and oxygen, to manage a potential but not yet confirmed diagnosis. Regardless of whether the presence of CHD is known or suspected, the transport team must be prepared to manage it.

To optimize management, early communication with the receiving specialty center is vital. Successful transport involves two phases: referring hospital staff to the transport team and subspecialists and referring transport staff to the accepting hospital

• BOX 28.2 Criteria for Neonatal Therapeutic Hypothermia on Transport

1. Patient *must* meet the following:
 - ≥ 36 weeks' gestation
 - Birth weight >1800 g
 - Less than 6 h of age
 - First-hour blood gas, if available: pH ≤ 7.15 and base deficit ≥ 10
 - No severe congenital anomaly
 - Plan for continued full care
 2. Criteria additional to above eligibility:
 - And first-hour blood gas: pH ≤ 7.0 or base deficit or base deficit ≥ 16
 - Or no blood gas available or first-hour blood gas: pH 7.0–7.15 or base deficit 10–16
- And both:*
- 10-min Apgar score ≤ 5 or assisted ventilation at birth ≥ 10 min
 - Acute perinatal event
 - Intrauterine distress
 - Cord rupture or prolapse
 - Uterine rupture
 - Maternal trauma/hemorrhage
 - Cardiopulmonary arrest
3. *And either of the following:*
 - Moderate to severe encephalopathy
 - Seizures

From McNellis E, Fisher T, Kilbride HW: Safety and effectiveness of whole body cooling therapy for neonatal encephalopathy on transport. *Air Med J.* 2015;34(4):199–206 modified from Shankaran S, Laptook AR, Ehrenkranz RA, et al. Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. *N Engl J Med.* 2005;353:1574–1584.

TABLE 28.2 Steps Toward Optimal Transport of the Neonate With Congenital Heart Disease

| All Patients | Intubated Patients |
|---|--|
| <ul style="list-style-type: none"> • Secure vascular access. • Ensure vascular access for volume resuscitation that is not infusing inotropes or PGE₁. • Avoid interruption of PGE₁ infusion. • Maintain normothermia to minimize oxygen consumption. | <ul style="list-style-type: none"> • Secure and record endotracheal tube position. • Place nasogastric or orogastric tube for decompression. • Maintain NPO status including fluids and medications. • Provide appropriate sedation. |

NPO, Nil per os; PGE₁, prostaglandin E₁.

staff. The need for precise and thorough communication between respective teams cannot be overemphasized. Whenever possible, the pediatric cardiologist, neonatologist, or intensivist at the accepting hospital should be included in formulating the transport management plan while the neonate is still at the referring hospital, thereby guiding the timing and urgency of the transport, line placement, and recommendations for airway management and supplemental oxygen therapy (Table 28.2).

The neonatal resuscitation algorithm is applicable in the presence of CHD (Johnson and Ades, 2005) but should be modified in certain circumstances. In presentations with hypoxemia that is unresponsive to supplemental oxygen, congestive heart failure, or shock, simultaneous attention is devoted to the basics of neonatal advanced life support and to assurance of a patent ductus arteriosus.

A stable airway must be maintained, allowing adequate alveolar oxygenation and ventilation before transport. In critically ill neonates with known CHD presenting with severe cyanosis or circulatory collapse, intubation should be performed by a skilled provider after premedication with sedation and neuromuscular blockade. Reliable venous access is important, and arterial monitoring is helpful for ongoing assessment of blood pressure, acid–base status, and gas exchange. Volume resuscitation, inotropic support, and correction of metabolic acidosis may be required to maximize cardiac output and tissue perfusion. In neonates with suspected but not confirmed CHD, an evaluation for sepsis is typically considered simultaneously, and empiric antibiotic therapy is initiated.

Supplemental Oxygen

Supplemental oxygen is a potent pulmonary vasodilator and systemic vasoconstrictor, and it can adversely affect the physiology of neonates with a single ventricle, as well as those with two ventricles with an unrestrictive ventricular septal defect or great vessel communication. The oxygen-induced pulmonary vasodilation can decrease pulmonary vascular resistance and increase pulmonary blood flow at the expense of systemic blood flow, thus reducing systemic output in some cases. For known CHD, the use of oxygen therapy and goal oxygen saturations should be determined with the use of established guidelines for specific lesions or in consultation with an MCP. Titrating oxygen via a nasal cannula or face mask to a target peripheral saturation of 75%–85% usually corresponds to adequate blood flow in both the pulmonary system and the systemic system. Higher oxygen saturations are typically not necessary and in fact may ultimately result in decreased oxygen delivery to the peripheral tissues. In cases of undifferentiated neonatal hypoxemia where CHD is suspected, oxygen therapy should be continued until CHD is ruled out by echocardiogram since severe pulmonary hypertension is also in the differential diagnosis and supplemental oxygen therapy is indicated.

Prostaglandin E₁ Therapy

PGE₁ therapy is the standard of care for stabilization of known ductal-dependent CHD (DDCHD) but is often initiated in undifferentiated hypoxic neonates when DDCHD is possible but not confirmed. In a study examining the diagnostic accuracy for CHD based on the clinical findings of a transport team, [Gupta et al. \(2014\)](#) found that there was no single finding predictive of DDCHD, concluding that making this diagnosis outside a specialty center was challenging. In a separate study, the same authors ([Gupta et al., 2013](#)) studied the effects of PGE₁ administered to both neonates with DDCHD and those with PPHN and found that when PGE₁ was administered to neonates with persistent pulmonary hypertension, they recovered faster and no adverse events were observed during transport.

In the instance of prenatally diagnosed CHD, ductal dependency is often already determined. In these cases, maintaining ductal patency is key. Whenever possible, PGE₁ infusions should be prepared ahead of arrival at the referring center and started promptly. PGE₁ may be given via a central or peripheral intravenous catheter or in emergent situations where intravenous access cannot be obtained via an umbilical catheter or intraosseous line. For neonates with suspected CHD whose duct has begun to close, usually after the first 24 hours of life, PGE₁ is often required and response is immediate if ductal patency is central to the hemodynamics of the neonate. In all cases, failure to respond to appropriate dosing may mean that the initial diagnosis of DDCHD is incorrect, the ductus

TABLE 28.3

Adverse Effects of Prostaglandin E₁ Infusion

| Most Common | Less Common |
|---|---|
| <ul style="list-style-type: none"> • Hypotension due to vasodilation • Apnea • Rash • Fever | <ul style="list-style-type: none"> • Seizures • Gastric outlet obstruction • Cortical hyperostosis • Leukocytosis |

Data from Kramer HH, Sommer M, Rammos S, Krogmann O. Evaluation of low dose prostaglandin E₁ treatment for ductus dependent congenital heart disease. *Eur J Pediatr*. 1995;154:700–707; Lewis AB, Freed MD, Heymann MA, Roehl SL, Kensey RC. Side effects of therapy with prostaglandin E₁ in infants with critical congenital heart disease. *Circulation*. 1981;64:893–898; Arav-Boger R, Baggett HC, Spevak PJ, Willoughby RE. Leukocytosis caused by prostaglandin E₁ in neonates. *J Pediatr*. 2001;138:263–265; and Teixeira OHP, Carpenter B, MacMurray SB, Vlad P. Long-term prostaglandin E₁ therapy in congenital heart defects. *J Am Coll Cardiol*. 1984;3:838–843.

is unresponsive to PGE₁ therapy (which may occur in older neonates), or there is no ductus arteriosus present. Often the condition of the patient with a ductal-dependent lesion will improve greatly with the initiation of PGE₁ therapy, and the patient may not need to be rushed to the cardiac referral center as an emergent case.

On rare occasions, the neonate may have progressive instability after initiation of PGE₁ therapy. This important diagnostic finding strongly suggests a congenital heart defect with obstructed blood flow out of the pulmonary veins or the left atrium.

The following are types of CHD unresponsive to PGE₁ therapy:

- Hypoplastic left-sided heart syndrome with a restrictive foramen ovale or intact atrial septum
- Variants of mitral atresia with a restrictive foramen ovale
- Transposition of great arteries with an intact ventricular septum and restrictive foramen ovale
- Total anomalous pulmonary venous return with obstruction of the common pulmonary vein

If the neonate clinically deteriorates despite receiving PGE₁ therapy, urgent echocardiography is indicated, followed by prompt transfer to a cardiac unit ([Penny and Shekerdeman, 2001](#)). Controversy exists as to whether PGE₁ therapy should be continued in these rare instances, but most importantly, the apparent lack of response is a marker for rare forms of CHD that do not respond to medical management and require urgent surgical or catheter intervention.

Although PGE₁ is critical in the management of DDCHD, there are a number of potential adverse effects associated with PGE₁ that must be anticipated, particularly in the premature neonate, in which they occur more commonly ([Table 28.3](#)). Apnea and hypotension usually manifest themselves during the first few hours of administration but can occur at any time during the infusion. Judicious fluid resuscitation will generally normalize the blood pressure in cases of hypotension ([Kramer et al., 1995](#)). If hypotension is refractory to fluid administration, an alternative cause of hypotension should be considered (e.g., a restrictive ductus, pericardial effusion, myocardial dysfunction, sepsis), and inotropes should be prepared. Hypotension is a late finding in neonatal cardiogenic shock and ideally should be managed before transport during the stabilization phase.

The potential side effect profile of PGE₁ mandates the need for ongoing cardiorespiratory monitoring during transport; however, it does not in and of itself usually require intubation and mechanical ventilation without the presence of significant or recurrent apnea ([Kramer et al., 1995](#); [Browning Carmo et al., 2007](#)). Low-dose PGE₁ infusions are unlikely to cause apnea requiring mechanical

TABLE 28.4 Features of High-Lying Versus Low-Lying Umbilical Artery Catheters

| High | Low |
|--|---|
| <ul style="list-style-type: none"> • Catheter tip above diaphragm, below left subclavian artery, ~T6–T10 • Decreased risk of vascular complications • No significant increase in adverse sequelae | <ul style="list-style-type: none"> • Catheter tip below renal arteries, above aortic bifurcation, ~L3–L5 |

Data from Barrington KJ. Umbilical artery catheters in the newborn: effects of position of the catheter tip. *Cochrane Database Syst Rev*. 2000;(2):CD000505; and Hermansen MC, Hermansen MG. Intravascular catheter complications in the neonatal intensive care unit. *Clin Perinatol*. 2005;32:141–156.

ventilation, and neonates can be safely transported without an artificial airway being established in most cases (Carmo and Barr, 2005).

Vascular Access

Umbilical venous catheters and umbilical arterial catheters are useful in the stabilization and transport of critically ill neonates, including those with CHD. However, there is a time window in which each of the vessels can be accessed: typically, an umbilical venous catheter (UVC) can be placed up to 1 week of age, while placement of an arterial catheter is challenging beyond approximately 72 hours. In addition, placement of these lines is not without risk. Beyond the infectious risks, newborns with CHD are at a higher risk of thromboembolic events because of their underdeveloped clotting mechanisms, small-vessel lumens, and low flow states. There is also an ongoing risk of systemic embolization of air and particulate matter in babies with an intracardiac right-to-left shunt. There has been much controversy surrounding the optimal placement of an umbilical arterial catheter (UAC) in premature neonates, although there are scant data regarding term neonates with CHD (Table 28.4).

Catheter levels between high and low designations are associated with increased risks of complications, as are placements below the L5 level (Hermansen and Hermansen, 2005). The UVC should be placed, if possible, at the inferior vena cava/right atrial junction or in the atria. It might not be necessary to obtain ideal placement of either the UAC or the UVC for stabilization and transport, and manipulation of lines and reconfirmation can delay transport to the tertiary care center. The risk–benefit relationship for the use of umbilical lines in the neonate with ductal-dependent circulation has not been well delineated. The need for central access should be judged on the basis of the clinical status of the neonate and stability for transport.

Surgical Emergencies

Because most births occur in hospitals without a NICU or neonatal surgical services, the need for surgical evaluation or intervention is a common reason for interfacility transport. Whereas some surgical conditions are relatively nonurgent, there are several diagnoses that represent truly life-threatening conditions for which stabilization and transport require expertise and specialized resources.

Congenital Diaphragmatic Hernia

Congenital diaphragmatic hernia (CDH) presents along a spectrum of severity, is associated with pulmonary hypoplasia

and hypertension, and can be isolated or accompanied by other congenital malformations. Advances in ultrasound technology have resulted in prenatal diagnosis in up to 60% of fetuses with CDH (Gallot et al., 2007). A recent systematic review showed that outcome was improved for newborns in whom CDH was diagnosed prenatally or who were born in a tertiary care center (Logan et al., 2007b). Newborns with undiagnosed CDH are at high risk of complications.

The following are important in the transport of critically ill neonates with known or suspected CDH (Logan et al., 2007a, 2007b; Grisaru-Granovsky et al., 2009):

- Secure the airway with endotracheal intubation if respiratory distress is present.
- Ventilation approach: gentle ventilation
 - Allow permissive hypercapnia.
 - Limit peak airway pressures.
 - Use low tidal volumes.
 - Allow spontaneous breathing as much as possible, with judicious use of sedation and neuromuscular agents.
- Decompress the bowel with a nasogastric or orogastric tube connected to low intermittent suction.
- Maintain adequate systemic blood pressure with fluid and inotropes as needed.

Although there is still controversy over the role of ECMO for newborns with CDH, transport to a high-volume center with ECMO capabilities should be strongly considered (Grushka et al., 2009; Morini et al., 2006).

Abdominal Wall Defects

The proper management of a newborn with gastroschisis or an omphalocele is critical during the first several hours of life, and delivery in a tertiary care center has been associated with improved outcome (Quirk et al., 1996). In addition to the need for initial resuscitation and cardiorespiratory support, the correct treatment of the exposed bowel or sac may improve the newborn's chances of a successful repair and long-term intestinal function.

While current guidelines aim for at least 38 weeks' estimated gestational age for elective cesarean delivery, previously, the mean gestational age for newborns with gastroschisis was 36.6 weeks (Lausman et al., 2007). Many affected neonates are small for their gestational age (Baerg et al., 2003), and as with other mildly premature and growth-restricted neonates, patients with gastroschisis are at risk of hypothermia and hypoglycemia. Heat loss is exacerbated by the large surface area of the exposed intestines, which can also serve as a significant source of fluid loss. Prevention of heat and fluid loss can be accomplished by placement of the lower part of the neonate's body, including the intestines, into a transport bag (i.e., bowel bag or Lahey bag) before placement of the neonate into the heated transport isolette. Significant fluid losses can occur through the exposed mucosa, and the patient may require aggressive fluid replacement (120–150 mL/kg per day). The use of antibiotics should be considered if risk factors for sepsis are present after review with a pediatric surgeon.

Neonates with gastroschisis are at risk of intestinal vascular compromise, because the vascular pedicle containing the arterial supply and venous drainage from the bowel must pass through the relatively small abdominal wall defect. Transport personnel must closely monitor the appearance of the bowel to detect signs of venous congestion or ischemia. Transporting the neonate in the lateral position, with support of the exposed intestines to avoid tension or torque, is recommended. The use of intestinal pulse oximetry has been described for monitoring the bowel for ischemia

through a transparent silo but has not been studied as a tool during interfacility transport (Kim et al., 2006). Vascular compromise of the intestine is a surgical emergency, and communication with the receiving facility is essential to coordinate urgent intervention.

The transport of a neonate with an omphalocele has similar considerations, although unless the sac has ruptured, there is minimal risk of heat and fluid loss. Neonates with an omphalocele are more likely than those with gastroschisis to have other birth defects (e.g., CHD). Furthermore, neonates with giant omphaloceles often have respiratory insufficiency caused by diaphragmatic dysfunction, pulmonary hypoplasia, or both and may require ventilatory assistance.

All neonates with gastroschisis or an omphalocele require placement of a large-bore nasogastric or orogastric tube because of the functional ileus or intestinal obstruction that may occur with associated stenoses or atresias. In general, cannulation of the umbilical vessels is not recommended unless other methods for vascular access are not successful.

Esophageal Atresia and Tracheoesophageal Fistula

Esophageal atresia, with or without tracheoesophageal fistula, is typically diagnosed within the first day of life because of increased secretions, poor feeding, and respiratory distress. General transport considerations include placement of a large-bore sump-type tube for continuous aspiration of the proximal esophageal pouch, positioning (prone with the head of the bed elevated), and respiratory support as indicated. Direct aspiration of secretions into the trachea may occur with either a proximal or a distal tracheoesophageal fistula. Transport providers should be aware that neonates with a distal tracheoesophageal fistula (type C), characterized by the presence of air in the intestinal tract, are at risk of gastric and intestinal insufflation via the fistula when receiving positive pressure ventilation. Bag-mask ventilation and CPAP should be avoided. If the neonate requires endotracheal intubation, the endotracheal tube should be positioned as close to the carina as tolerated in an effort to position the distal tip beyond the fistula and minimize direct inflation of the distal esophageal segment with pressurized gas. In extreme cases, gastric rupture with pneumoperitoneum has been reported, requiring emergency paracentesis, laparotomy, or both (Maoate et al., 1999).

Midgut Volvulus

Malrotation with midgut volvulus can be a catastrophic event resulting in intestinal ischemia and shock and represents a surgical emergency. The most common clinical presentation of midgut volvulus is bilious vomiting, which is a nonspecific sign of intestinal obstruction. Expedient evaluation of the newborn with bilious vomiting is essential to facilitate prompt surgical intervention to prevent progression of vascular insufficiency to actual intestinal necrosis. An upper gastrointestinal tract series is the radiologic test of choice to diagnose malrotation and midgut volvulus, although some practitioners have reported success with the use of ultrasound examination to identify the relationship of the superior mesenteric vessels (Lampl et al., 2009; Shew, 2009).

A neonate with suspected midgut volvulus should be rapidly cared for in a facility with pediatric radiology and surgical capabilities. Care of the neonate with suspected midgut volvulus during interfacility transport is primarily supportive and includes circulatory interventions with intravenous fluid repletion, correction of metabolic abnormalities, and gastric decompression with a large-bore nasogastric or orogastric tube.

Necrotizing Enterocolitis

Necrotizing enterocolitis is relatively common in premature neonates, and approximately 30% of newborns with necrotizing enterocolitis will require surgical intervention in the form of laparotomy or peritoneal drain placement (Guthrie et al., 2003). Neonates with moderate or severe necrotizing enterocolitis should be transported to a facility with pediatric surgical capabilities. Care during transport is primarily supportive and includes intravenous fluids, administration of broad-spectrum antibiotics, correction of metabolic abnormalities, and gastric decompression. Respiratory failure is common because of disordered control of breathing and elevation of the diaphragm from abdominal distention. With significant free intraperitoneal air or pneumatosis and changes in altitude, neonates may be at risk of intra-abdominal hypertension and abdominal compartment syndrome. Transport providers should be aware of this and discuss potential interventions, such as a peritoneal drain, with the MCP and referring provider before departure from the referring facility. Abdominal decompression before transport has been previously recommended for the transport of neonates with pneumoperitoneum (McAdams et al., 2008).

Meningomyelocele

Although in most newborns with neural tube defects diagnosis is prenatally because of an elevated alpha fetoprotein level, and they are therefore delivered in a tertiary care center, the unexpected birth of a baby with a meningomyelocele may be an indication for neonatal transport. For purposes of transport, the newborn should be placed in the prone position, and the spinal defect should be covered with moist sterile dressings as well as some form of plastic wrap to maintain moisture. The lesion can be covered with a moistened nonadherent dressing and then loosely encircled with a gauze roll “donut,” with the entire defect covered with a sterile drape. This dressing can be moistened as required during the transport process (Jason and Mayock, 1999). The use of latex gloves should be avoided during care of these patients. If cerebrospinal fluid leakage is observed or suspected, there is an increased risk of infection, and empiric antibiotics should be considered. Neonates with a meningomyelocele may or may not have accompanying hydrocephalus at birth; approximately 25% of affected patients will require shunting in the immediate newborn period, with up to 85% eventually undergoing shunt placement (Bowman et al., 2001). In one institution, the percentage of patients necessitating shunt placement was reduced when stricter criteria were used, including permissive mild ventricular dilatation and the presence of symptomatic hydrocephalus (Chakraborty et al., 2008). Furthermore, the results of the Management of Myelomeningocele Study, which demonstrated reduced need for shunt placement and corresponded with improved neurologic and motor outcomes at 30 months (Adzick et al., 2011) following in utero repair of the spinal defect, may lead to additional practice changes in postnatal shunt placement.

Future Directions

The field of transport medicine has grown substantially in the past several decades and will continue to grow and change, particularly in relation to special and vulnerable patient populations. The goals include rapid awareness of need, clear communications, and delivery of care that is similar to that in our level III/IV NICUs. Development of standards and guidelines will enable us to assess the impact of neonatal transport and the opportunity it provides for patients and clinicians. The role of developing technologies, such as

telemedicine, will likely have an increasing presence in referral hospitals as well as for use by transport teams. In addition, there will likely be increasing attention paid to cost and value, especially of high-cost air transport, that may lead to stricter criteria and a greater push toward national transport guidelines, and potentially even drones may play a role at least in equipment and medication delivery to outlying hospitals. The transport of critically ill neonates is most often a high-risk, low-frequency event in the context of all patient transfers. Therefore as systems move forward, it is imperative to ensure the highest level of skill and competence both in the transport team and in the facilities that refer and receive these patients be it through team specialization, regionalization of high-level neonatal critical care, or support of telemedicine and outreach initiatives. Most likely it will be achieved through all of these.

Suggested Readings

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29

Temperature Regulation

W. ALAN HODSON

KEY POINTS

- Providing an appropriate and stable thermal environment is important for newborns regardless of size or gestational age.
- The thermal neutral environment (TNE) refers to the ambient temperature necessary to maintain normal metabolism.
- Newborn (admission) hypothermia continues to be a global challenge, particularly in resource-limited settings.
- Radiant warmers, warm blankets or thermal mattresses, head covering, plastic wrap (without drying), delayed bathing, and skin-to-skin care have been recommended to reduce neonatal hypothermia.
- Interhospital transport, particularly for critically ill and/or very-low birth weight (VLBW) neonates, increases the risk of thermal instability.

All newborns are at risk of experiencing cold stress. Providing an appropriate and stable thermal environment is important for newborns regardless of size or GA. Most of the temperature needs of the full-term infant will be associated with birth or in rare circumstances with the development of an illness or instability. It is imperative that providers involved with delivery or subsequent care understand the various causes and consequences of heat loss, the mechanisms of heat production by the infant, and the multiple management options for providing the correct thermal environment. A variety of different clinical conditions present different management challenges depending on the degree of immaturity, birthweight, and concurrent illness such as respiratory distress, sepsis, or asphyxia. Temperature regulation is an essential and important component of neonatal intensive care.

More than 50 years ago and before the development of intensive care nurseries, research demonstrated an association between temperature control and increased survival in premature infants. Incubators first appeared in the 19th century in Europe, but it was not until the 1930s that they were incorporated into the care of premature infants at Michael Reese Hospital in Chicago. However, the concept that premature infants were unharmed by hypothermia (acting as if they were similar to a poikilothermic animal) prevailed until controlled trials (Silverman et al., 1958) demonstrated the associated morbidity and mortality of cooling in various birthweight groups. A number of studies (Karlberg, 1962; Day et al., 1964; Scopes and Ahmed, 1966; Dahm and James, 1969; Hey, 1969) demonstrated the metabolic cost of cold stress, resulting in a doubling or tripling of oxygen consumption, particularly in

early-gestation infants. These studies were the first attempts at defining the optimal environmental temperature resulting in a normal body temperature—the so-called *thermal neutral environment* (TNE). This quest has resulted in ever-increasing refinements of protective management techniques, including hybrid incubators, radiant heaters, heated gel mattresses, plastic wrapping, heat shields, a laminar flow device, clothing, caps, room temperature control, and protocols for intermittent skin-to-skin care (SSC). The appropriate incorporation of the many management options will depend on the needs of individual babies related to their birthweight, maturity, degree of illness, and postnatal age.

Mechanisms of Heat Loss

The most vulnerable time for heat loss occurs during the first minutes after birth. It is of interest to consider the thermal environment of the fetus and how a stable body temperature is maintained despite variations in fetal metabolic activity associated with sleep cycles, muscular activity, respiratory movements (fetal breathing), and changes in maternal temperature. Periods of “heat excess” probably occur as maternal temperature varies; the fetal temperature follows closely and maintains a differential of approximately 0.5°C above maternal temperature. What is the mechanism of fetal heat transfer? It is estimated that 85% of heat exchange occurs through the placenta, and the remaining 15% occurs through conduction from the skin to amniotic fluid and then to the uterine wall (Rudelstorfer et al., 1981). The heat flux across the skin will increase if there is compromised placental function. Significant elevations in maternal temperature have the potential to cause fetal harm, hence the standard advice given to pregnant women to avoid hot tubs. Maternal fever as a result of inflammation or infection is most likely of higher risk to the fetus than maternal exercising or ambient heat stress. The temperature gradient between the fetus and the mother may increase beyond 0.5°C during labor or uterine contractions and widens slightly with advancing GA.

There are four major mechanisms of heat loss that will vary over progressive days of nursery care (depending on the maturity of the baby, growth, illness, and environmental factors). These include evaporation, radiation, convection, and conduction, each with variable contributions to the heat loss (Table 29.1).

Evaporation

The evaporative losses at birth may result in a fall of 2°C–3°C within the first 30–60 minutes after birth if the newborn is extremely

TABLE 29.1 Four Major Mechanisms of Heat Loss in Newborns: Estimated Percentages of Each, Based on the Environmental Temperature

| | ENVIRONMENTAL TEMPERATURE | | |
|-------------|---------------------------|------|------|
| | 30°C | 33°C | 36°C |
| Radiation | 43% | 40% | 24% |
| Convection | 37% | 33% | 5% |
| Evaporation | 16% | 24% | 56% |
| Conduction | 4% | 3% | 1% |

TABLE 29.2 Relative Skin Surface Area in the Adult and in Low Birth Weight Infants

| | Relative Skin Surface Area (cm ² /kg) |
|---------------|--|
| Adult | 250 |
| 1500-g infant | 870 |
| 1000-g infant | 1000 |
| 500-g infant | 1400 |

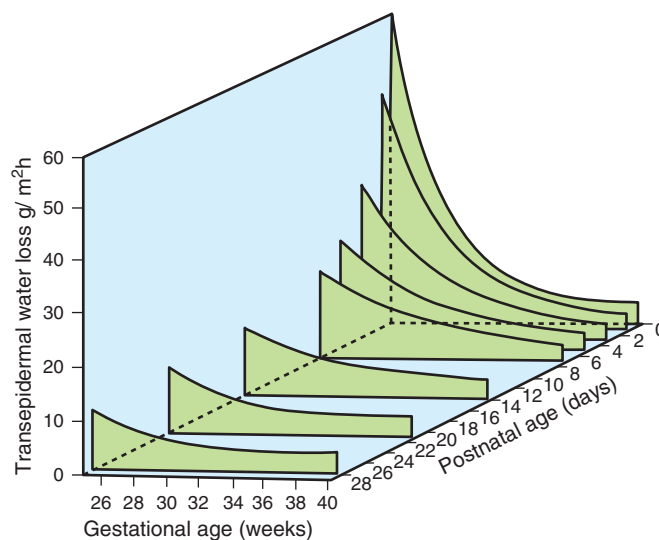
premature or no wrapping, drying, or clothing is applied in a larger newborn. Delays in warming may occur if resuscitation or other medical care delays drying. The first minutes after birth are already stressful because of the physiologic adaptations for the onset of breathing, absorption of fetal lung fluid, circulatory changes, and the transient drop in arterial partial pressure of oxygen below 15 Torr.

The major heat loss from evaporation is due to exposure of the large surface area of wet skin relative to the baby's body mass and to the duration of exposure while naked and wet from the amniotic fluid. The more immature the newborn, the larger the relative surface area (Table 29.2). In full-term newborns the evaporation of water from the skin decreases until a body temperature of 36.6°C–37.1°C is reached. The concomitant increase in skin blood flow does not appear to influence evaporative heat loss.

Insensible water loss through the skin continues to contribute to evaporative heat loss and decreases over the first few days after birth. The rate of evaporative water loss depends on the ambient humidity and increases at humidity levels below 50%. Evaporation is somewhat greater under a radiant heater compared with an incubator. Studies have demonstrated large losses from transepidermal water loss (evaporative) over the first few days after birth in extremely low birth weight newborns (Fig. 29.1). This suggests that the skin permeability of these very premature neonates, especially during the first hours after birth, is very different from that of neonates born beyond a GA of 34 weeks (Hammarlund and Sedin, 1982).

Radiation

Radiation is a major source of heat loss, so it has been the focus of most research studies and has led to the development of many devices to minimize that loss and to protect the infant from excessive energy expenditure. Radiant heat loss from the skin can be



• **Fig. 29.1** Transepidermal water loss in relation to gestational age at birth and at different postnatal ages in appropriately grown infants. (Redrawn from Sedin G, Agren J. Water and heat—the priority for the newborn infant. *Ups J Med Sci.* 2006;111:45–59.)

responsible for 40% or more of the daily heat loss (when air movement is low). Many variables can influence the degree of heat loss, including body surface area, environmental temperature, the type of external heat source, clothing, blankets, caps, heat shields, and swaddling. The full-term infant should not present ongoing concerns with radiant heat loss if commonsense measures of care are provided, including a room temperature of 24°C–26°C, appropriate SSC, clothing, and blankets. Premature infants and those who are undergrown or ill with cardiorespiratory distress, sepsis, asphyxia, or other disorders will be at continued risk of radiant heat losses. This mechanism of heat loss is influenced by the mean temperature of the skin and the mean temperatures of the surrounding walls, as well as the temperature gradient to a nearby object of lesser warmth and temperature even if the object is outside but near the incubator or the warmer.

Convection

Heat loss due to convection is determined by the airflow around the infant, the mean temperature of the ambient air, the mean temperature of the skin, and the exposed surface area of the infant. Different incubators have different airflow, humidity, and wall temperature capacities that can influence the degree of convective heat loss.

Conduction

Conductive heat losses contribute minimally to energy expenditure and depend on the thermal conductivity of the mattress, which is low in incubators and under radiant warmers. Skin adjacent to a colder object such as an X-ray plate or other cold instruments will result in some conductive heat loss.

Mechanisms of Thermoregulation

How does the infant sense temperature? Does the newborn have sensing mechanisms similar to those of the adult, and are there comparative developmental changes in the relative contribution

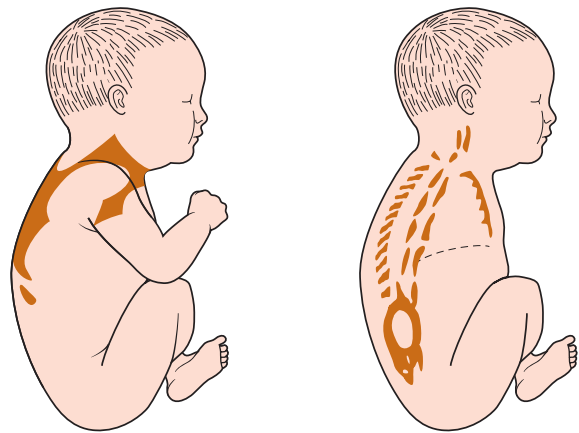
from skin or central nervous system receptors in the extremely premature infant and the full-term infant? Temperature sensors are located in the skin at many sites. Some are more dominant than others in influencing the infant's heat production. More information is needed on the relative contribution of peripheral versus central temperature receptors in controlling core temperature.

The optimal temperature for a specific infant cannot be defined by a single central temperature; rather, it is defined by the range of measurable skin temperatures that are associated with minimal heat production. This implies an optimal central or core temperature. The recommended range for normal rectal and axillary temperatures is 36.5°C–37.5°C for full-term infants, 35.6°C–37.3°C for preterm infants, and 36.7°C–37.3°C for very low birth weight (VLBW) infants. Measurement sites include the axilla, rectum, and skin. The small size of the ear canal precludes tympanic measurement. Axillary temperature measurement is intermittent, with minimal errors related to placement of the thermometer, and rectal temperatures have a risk of trauma and inaccuracies because of the depth or duration of insertion as well as passage of stool. Continuous temperature monitoring with an infrared skin probe placed over the midline of the upper abdomen or on the back below the scapulae permits accurate assessment of core temperature under various external heating arrangements.

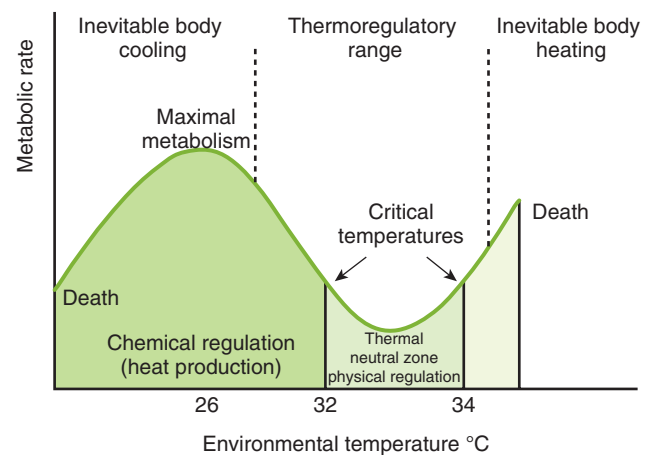
The “thermal neutral zone” or TNE refers to the ambient temperature necessary to maintain normal metabolism. For the average naked adult it is 25°C–27°C, and for the full-term naked newborn it is 32°C–34°C (Hey, 1970). For premature infants it will differ with GA. Infants of a GA of 25 weeks or less will require an ambient temperature close to that in utero (i.e., 35°C–37°C) until they are placed in an appropriate protective environment. The tiniest infants are more susceptible to temperature instability because of the absence of subcutaneous fat for insulation, decreased levels of brown fats, large body surface area to body weight, poor vasoconstrictor control, decreased spontaneous muscle activity, increased evaporative losses, and transepidermal insensible water loss. Small for GA infants will have a higher metabolic rate for weight. An increase in activity and ingestion of food in growing preterm infants will increase metabolic rate through altered energy expenditure.

Nonshivering Thermogenesis

Since the newborn is unable to reduce heat loss or increase heat production by shivering or increasing voluntary muscle activity, nonshivering thermogenesis produces energy output and hence heat production through the oxidation of fatty acids, largely from brown fat. Glucose or glycerol appears to be an alternative fuel utilized for this purpose. Brown fat comprises most of the fat content of the newborn. Brown fat deposits have been located in six major sites (Merklin, 1974) and show increased oxidation within minutes of birth (Fig. 29.2). It appears as early as 25 weeks in the fetus but is probably not an efficient participant in thermogenesis at this stage of development. Temperature receptors sensing a low temperature stimulate increased sympathetic output from the central nervous system, resulting in norepinephrine release, which in turn stimulates β -adrenergic receptors in brown fat, increasing cyclic AMP production (Hull and Seagall, 1965). A major uncoupling protein (thermogenin) is found exclusively in brown fat. The release of cytoplasmic stores of triglycerides and fatty acids increases metabolism, resulting in increased oxygen consumption and heat production. Other endocrine factors such as thyroxine,



• **Fig. 29.2** Distribution of brown fat deposits in the newborn from 28 to 42 weeks' gestation. (Redrawn from Aherne W, Hull D. The site of heat production in the newborn infant. *Proc R Soc Med.* 1964;57:1172–1173.)



• **Fig. 29.3** Variations in metabolic rate with changes in environmental temperature in human newborns, including the thermal neutral zone and extremes of cooling and heating. (Redrawn from Baumgart S. Incubation of the human newborn infant. In: Pomerance JJ, Richardson CJ, eds. *Neonatology for the Clinician*. Norwalk: Appleton and Lange; 1993:139–150.)

triiodothyronine, and thyroid-stimulating hormone may play a role in this process, but further study is needed (Houstek, 1993).

During the 1960s, considerable clinical and animal research helped to define the TNE for newborns in relation to changes in environmental and core temperatures. Environmental temperatures were progressively decreased below 34°C in experiments that resulted in a steady increase in oxygen consumption to values that were two to three times the resting value. If environmental cooling exceeds the infant's thermoregulatory response of energy output, the core temperature will drop along with a decrease in oxygen consumption (Fig. 29.3). This “ Q_{10} effect” of decreased metabolism when the environmental temperature falls below a critical point is the basis for the use of induced hypothermia in other clinical situations. The increase in oxygen consumption and the increase in metabolic output are considered important contributors to increased morbidity and mortality especially in extremely low birth weight infants. Extended periods of cold stress have been

associated with hypoglycemia, respiratory distress, hypoxia, metabolic acidosis, necrotizing enterocolitis, and death.

Thermal Management Strategies

Understanding the many causes of heat loss and the resulting response of nonshivering thermogenesis with the consequences of cold stress have led to a variety of protective and therapeutic options. Much controversy has arisen about various warming devices and techniques, resulting in new or modified equipment and strategies. The goal of estimating and maintaining the TNE for each infant is supported by evidenced-based trials. The indication and timing of interventions have resulted in management protocols applicable to various clinical settings, including transport, delivery rooms, full-term nurseries with “rooming-in” policies, and neonatal intensive care units (NICUs).

Delivery Room Environment

Hypothermia soon after birth has been associated with increased morbidity and mortality, prompting organizations such as the Neonatal Resuscitation Program and the World Health Organization to stress the importance of preventing early postnatal hypothermia (Lapcharoensap and Lee, 2016). The maternal temperature at delivery should be noted on the baby’s birth record, along with an axillary temperature. The room temperature should be kept at 28°C. Ideally, a preheated gel mattress, with a warm bassinet, warm blankets, and a radiant heat source should be in place. Immediate drying, swaddling (once stabilized), and placement of a cap will reduce evaporative heat loss. The full-term newborn will maintain a normal body temperature with appropriate clothing and blankets in an environment of at least 24°C. Preterm newborns need additional protection from heat losses, including higher ambient temperature and the use of plastic wraps (without drying). Early SSC, once the infant has been stabilized, can be very effective in preventing hypothermia—particularly in resource-limited settings.

Delayed umbilical cord clamping in very preterm newborns has raised concern regarding thermal management. However, a metaanalysis of several studies done in newborns of less than 32 weeks’ GA was reassuring, with no differences noted in admission temperature (Backes et al., 2014).

Care of the Extremely Low Birth Weight Infant

The neonate born before 28 weeks’ gestation presents a major challenge in the prevention of heat loss. Hypothermia is reported to occur in the most extremely low birth weight neonates on their admission to an NICU and is especially hard to prevent in neonates born at less than 25 weeks’ gestation. Furthermore, it is associated with an increased risk of early and late neonatal death (Wilson et al., 2016). Recent studies support the use of plastic bags and caps in the delivery room (Knobel et al. 2005). The newborn is placed immediately in a plastic bag up to the neck without drying and placed under a radiant warmer. The head is dried and covered with a plastic cap. Resuscitation can proceed with the bag in place. This provides initial stability for transfer to the NICU or transport to another facility. The first 12 hours is important in preventing further heat loss. Possible contributors to cold stress are the temperature of the inspired airflow, evaporative loss under radiant heat, cold hands, weighing scales, the temperature of intravenous infusions, fluid boluses within syringes, and intervention procedures such as intubation, X-rays, and vessel cannulation.

The duration of vessel cannulation determines the length of time the newborn is covered with a sterile drape and will interfere with warming. Expediency is therefore important in completing this procedure.

Warming Babies

Both incubators and radiant warmers have been in use in NICUs for several decades and remain the focus of heat management. Each of these warming devices has undergone sophisticated evolution and remains effective in providing a TNE for infants of differing sizes and with differing illnesses. Both provide enhanced observation and access when needed. There are slight differences between incubators and radiant warmers in the degree of evaporative and radiant heat loss, but no significant differences in outcomes have been demonstrated.

Incubators Versus Radiant Warmers

The prototype of the modern incubator, using the principal of convective heating of air, came into widespread use in nurseries in the 1960s. Since then there have been many modifications allowing precise control of air temperature, airflow, humidity, and limitation of heat losses due to convection, radiation, and conduction within the incubator (Antonucci et al., 2009). Comparisons of single-walled and double-walled incubators have shown that both are effective, with appropriate monitoring. There are three options to achieve a stable and desirable skin temperature of 36.0°C–36.5°C:

1. Servo control of incubator air temperature
2. Servo control of abdominal skin temperature (using an abdominal skin probe)
3. Manual control of incubator temperature with knowledge of abdominal skin temperature

The advantage of servo control with an abdominal skin probe is that it ensures maintenance of a constant body temperature despite perturbations caused by a decreased environmental temperature from open portholes or opening of the incubator top. Overheating can occur if the probe becomes detached or covered with a blanket.

Heat shields and thermal blankets have been used over the infant within the incubator and can reduce insensible water and radiant heat loss. Heat shields will not increase metabolic rate and do not need to be used with single-walled incubators. The rationale for creating the double-walled incubator was to reduce heat loss. Several studies demonstrate small differences in heat loss and heat production and have not shown a difference in clinical outcome if skin temperature servo control is used (Deguines et al., 2012).

Overhead radiant heaters were first used in delivery rooms, particularly for support during resuscitation procedures. They then evolved into the Sieracin Cradle Warmer, which fit over an open bassinet. This warmer was used to observe naked babies for signs of distress over the first few hours after birth, during the so-called transition or observation period. The “observation nursery” is now a thing of the past and has been replaced by the “intermediate care nursery” for infants with a minor degree of instability such as mild respiratory distress, irritability, jaundice, or risk for sepsis. Monitoring these infants with temperature probes as well as by means of vital signs has facilitated the provision of a TNE without needing a NICU admission. This device and the primitive delivery room heaters led to the development of the current radiant warmers now in common use in NICUs. They produce overhead heat in the infrared range distributed in a uniform fashion to the infant and controlled by an abdominal skin thermistor. The temperature

probe itself, not the skin temperature, controls heat output. Insensible water loss is slightly greater and exaggerated if humidity falls below 50%. Open access under the warmer is excellent and advantageous in the assessment and management of the seriously ill infant on admission.

Comparisons of the superiority of the incubator over the radiant warmer have shown no differences in outcome. Both methods are effective and safe and appropriate for the care of the extremely low birth weight newborn, with recognition of small differences in insensible water loss. The more recent development of a hybrid incubator allows the intermittent use of a vertically adjustable radiant heater as well as drop-down incubator walls to improve access. Advocates for its use suggest that the incidence of severe bronchopulmonary dysplasia may be lessened and that better fluid balance and growth velocity may be achieved (Kim, 2010). Further studies are needed.

Weaning to an Open Crib

The continuing need for an incubator is relevant to discharge planning and home care. Body weight attainment serves as a useful indicator as the infant approaches a weight of 1600 g. Infants weighing 1500 g transferred to an unheated open crib demonstrate satisfactory growth velocity and a stable temperature without other adverse effects (Berger et al., 2014). However, earlier transfer to an open crib does not necessarily relate to earlier discharge. Crib nursing of full-term infants requires a room temperature of at least 24°C, while infants of 1500 g should be in an environment of 26°C–28°C. The smaller infants should be fully dressed, including a head covering, and may require a room temperature of 30°C.

Skin-to-Skin Care

“Kangaroo mother care” was originally used as an effective way for mothers to keep their full-term babies warm while breastfeeding and subsequently as an alternative method of caring for low birth weight (LBW) babies in resource-limited countries. In these original versions, the infant is placed skin-to-skin in a vertical position between the mother’s breasts and under her clothes and is exclusively (or almost exclusively) breastfed. More recently, intermittent SSC, provided by the mother or father, has been introduced in resource-rich countries for babies requiring neonatal intensive care—even extremely premature infants and those on ventilators. Enhanced parental bonding, facilitation of breastfeeding, better sleep patterns, and procedural pain relief are some of its purported benefits. Several guidelines regarding SSC in the NICU have been published, but since it requires intensive staffing support, resources, and parent participation, development of individualized unit guidelines has been recommended (Baley and Committee on Fetus and Newborn, 2015).

Temperature regulation during SSC has been a concern for both care providers and parents and may limit its use. Karlsson et al. (2012) evaluated thermal balance in extremely preterm neonates before, during, and after SSC during the first week after birth. Neonates maintained normal body temperature, although transfer to and from the parent was associated with a drop in skin temperature, and insensible water loss through the skin was higher during SSC. The authors concluded that conduction of heat from parent to infant is sufficiently high to compensate for the increase in evaporative and convective heat loss. A metaanalysis reported a statistically (but not clinically) significant increase in body temperature and decrease in oxygen saturation in term and preterm

infants receiving SSC compared with incubator care, although this was more evident in lower-resource than in higher-resource settings, and in cold environments (Mori et al., 2010).

In a 2015 clinical report regarding SSC in the NICU, the Committee on Fetus and Newborn of the American Academy of Pediatrics recommended that NICU infants should be continuously monitored during SSC and that “any infant who requires careful temperature regulation or a high-humidity environment might have SSC delayed until he or she is more stable” (Baley and Committee on Fetus and Newborn, 2015).

Additional Considerations

Transport

Temperature instability during transport to a NICU remains a challenging problem particularly for extremely low birth weight neonates. Neonates born at less than 25 weeks’ gestation, even those delivered within a tertiary care center, are commonly noted to have a drop in core temperature within 12 hours of admission to a NICU (Costeloe et al., 2000). A review of several thousand LBW infants in the National Institute of Child Health and Human Development Neonatal Research Network who were admitted directly from the delivery room to the NICU revealed an association between lower admission temperature and lower birthweights as well as the need for immediate intubation. Admission temperature was inversely related to mortality (28% increase per 1°C decrease) and the incidence of late-onset sepsis (11% increase per 1°C decrease) (Laptook et al., 2007).

Interhospital transport, particularly for critically ill and/or VLBW neonates, increases the risk of thermal instability. Infants may be exposed to a cooler, draftier non-NICU environment at the referring hospital and in transfer to and from the transport incubator. Evaluation and stabilization efforts—often accompanied by the movement of multiple care providers and equipment—can also increase convective heat losses. Intubation equipment, vascular access instruments, cold inspired air, unheated intravenous solutions or medications, and cold hands may increase conductive heat losses. One level III referral NICU recently used transport risk index of physiologic stability (TRIPS) scores to evaluate the impact of transport on the physiologic stability of VLBW infants. It was found that more than half the transported infants demonstrated deterioration in their TRIPS score, primarily because of a decrease in temperature, and the likelihood increased with longer duration of transport. It was concluded that better temperature regulation during transport may decrease the risk of clinical deterioration in such infants (Arora et al., 2014).

As discussed previously, the use of occlusive plastic wraps and a hat is advocated while evaluation, resuscitation, and/or stabilization measures are occurring. However, additional warming methods are often needed and may be provided by a heated gel mattress and a radiant warmer. The urgency of cardiopulmonary stabilization and other life support measures may take priority over the many details of heat loss prevention, and development of standard temperature care protocols and team training in their use—for every neonatal transport—is recommended.

Hypothermia

Induced hypothermia by controlled cooling of the body or head for the treatment of neonatal encephalopathy is discussed in Chapter 62.

Once infants have a decrease in their core temperature, they have exceeded their ability to generate sufficient heat by increasing metabolism. How best and how quickly should rewarming occur to reach a core temperature of 36°C without increasing oxygen consumption or metabolism? Few data exist to support the optimal rate of rewarming. Current protocols favor a rapid return to a TNE with extra heat provided by radiant heat and a heated mattress or an incubator. A decrease in core temperature in VLBW infants, especially in the first hours of life, remains an important risk factor for increased morbidity and mortality. The World Health Organization, concerned that neonatal hypothermia is prevalent around the world as a contributor to inadequate newborn care, has developed a classification system to underscore the importance of intervention. Three levels are defined:

1. “Cold stress” or mild hypothermia: temperature 36.0°C–36.4°C
2. Moderate hypothermia: temperature 32.0°C–35.9°C
3. Severe hypothermia: temperature below 32°C

A retrospective study assessing these criteria found that approximately 56% of VLBW newborns were moderately hypothermic in the first few hours of life and had an associated adjusted risk factor for an increase in intracranial hemorrhage (odds ratio [OR] 1.3) and death (OR 1.5) (Miller, 2011). Therefore emphasis on the need for rewarming has been focused on the care of infants at risk of “moderate” hypothermia.

Rewarming involves setting a skin thermistor at a desired level of 37°C or 38°C, and under a radiant heat core temperature of 37°C can be achieved in 4 hours with a set point of 37°C or 2 hours with a set point of 38°C. Although there is minimal evidence for an increase in apnea or hypoglycemia, both should be monitored.

Hyperthermia

Overheating (core temperature above 37.3°C) can result in tachycardia, tachypnea, restlessness, and so-called heat stress. It is important to assess whether the cause is environmental or caused by infection such as sepsis. Measurement of the abdominal skin temperature and that of the foot or toe should normally reveal a difference of 2.0°C. As core temperature increases because of an

infection, vasoconstriction of the periphery will occur in an effort to support the body's effort to increase central temperature. Therefore a toe-to-abdominal skin temperature difference of greater than 2.5°C would indicate an infection rather than an environmental cause. However, it is unclear whether extremely immature infants are able to mount a vasomotor response. If the abdomen-to-toe temperature gap is less than 1°C or reversed, this indicates excess heat is being applied. Environmental temperature should be reduced by 0.5°C at intervals of about 30 minutes, with excess layers of clothing removed. Again, the abdomen-to-toe temperature gap in the extremely LBW infant may be unreliable because of impaired control of microvascular perfusion, and thus monitoring of other signs of overheating is more important.

Bathing

There is a tendency for all neonates, large and small, to be bathed soon after birth. In some cultures, customs and rituals associated with birth dictate the timing of naming, cord separation, first feed, and first bath, among other events. Early bathing is probably of limited value and can safely be delayed, but there is often a desire to rid the skin of its coating of vernix. The vernix is made up of a thick coat of mostly lipids, including triglycerides, free fatty acids, and cholesterol esters. Its function is not fully understood, but it probably provides barrier function, primarily for transepidermal water movement in utero, and may have a similar role after birth as well as providing mechanical protection. There is no evidence that it plays a role in thermal protection, but it may provide some benefit to the premature neonate.

There is recent evidence that one benefit of a vaginal delivery is the transfer of organisms in the maternal microbiome to the infant, leading to the suggestion that smearing infants born by cesarean delivery with vaginal secretions would be of benefit to establishing a microbiome pattern akin to that of the mother. Bathing would interfere with that goal. Bathing may also increase heat loss through evaporation. Therefore to minimize exposure it is recommended that newborns be bathed starting with the trunk and concluding with the head with its greater evaporative surface area, thus minimizing heat loss.

Summary

Body temperature homeostasis is but one element of the normal adaptation to extrauterine life for all newborns. Prevention of heat loss is especially important in reducing morbidity and mortality in premature newborns. Hypothermia in critically ill infants whether due to prematurity, respiratory distress, sepsis, asphyxia, congenital anomalies, or other abnormalities will result in increased energy expenditure and oxygen consumption. Protocols and guidelines have been developed for protective management applications based on clinical trials and physiologic rationale and are periodically revised by medical and technical staff. The use of sophisticated equipment requires constant monitoring of heat output and the neonates' core temperature with the goal of providing the appropriate thermal neutral environment. It is particularly important for care providers to understand the consequences of heat loss in fragile neonates.

Several challenges remain, particularly in the maintenance of body temperature of extremely LBW newborns in the delivery room, during transport to the NICU, and in the first few hours after birth. This is due in part to resuscitative and stabilization

efforts that may take priority over wrapping and other temperature control procedures. However, there is room for improvement in the coordinated and simultaneous approach to the prevention of unintended cooling without delaying or impeding necessary resuscitation and stabilization procedures. This should be emphasized within the current American Academy of Pediatrics Neonatal Resuscitation Program manual. There is a need for improvement in the standards for optimal humidification levels for incubators and radiant warmers as current assessment of nurseries indicate wide variations from below 50% up to 100%.

Future modifications of existing equipment will probably be directed at improving safety and efficacy in maintaining strict and accurate control of the appropriate thermal neutral environment. The use of noncontact infrared thermography to measure multiple skin sites simultaneously is being evaluated (Herman et al., 2013). Different areas of the skin may indicate temperature fluctuations under different conditions, such as movement from an incubator to SSC and back, effects of phototherapy, vasomotor instability, and sites of conductive heat loss. Detecting changes in the peripheral

perfusion index may provide a clue to early signs of the development of necrotizing enterocolitis. Infrared thermal imaging can provide minute-to-minute information from various sites and may be useful in studying the developmental changes in the neural control of temperature (central and peripheral receptors) with advancing gestational and postnatal age. It will require a handheld camera or video recorder for continuous monitoring.

The concept of substituting an individual laminar flow device over each infant has theoretical advantages over the existing incubator and radiant warmer (Perez et al., 2013). It would provide warmed convective and filtered air, an embedded humidifier,

microbial isolation, and open access without taking up any more space than an overhead warmer. Further development of such a device lies in the future; however, a very positive aspect is an apparent strong fidelity for providing excellent thermal and microbial protection as well as improved patient access.

Perhaps the larger and more important need for the future is to work toward reducing newborn morbidity and mortality in lower-income and middle-income countries. Improved temperature regulation may provide the stepping stone for introducing more advanced newborn care, as has occurred with the history of modern newborn care in the developed world.

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30

Fluid, Electrolyte, and Acid–Base Balance

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AND JACQUELYN R. EVANS

KEY POINTS

- Many pathologic states in the neonate are associated with disruptions in normal body fluid, electrolyte, and acid–base balance, which at times may in themselves be life threatening.
- The maintenance of normal fluid, electrolyte, and acid–base balance is a cornerstone of appropriate management of the sick neonate

Fluid and Electrolyte Balance

Maintenance of fluid and electrolyte balance is essential for normal cell and organ function during intrauterine development and throughout extrauterine life. Pathologic conditions in the newborn often lead to disruption of the complex regulatory mechanisms maintaining homeostasis. Therefore a thorough understanding of the physiologic changes in the neonatal period and the provision of appropriate therapies based on the principles of developmental fluid and electrolyte physiology are among the cornerstones of modern neonatal intensive care.

Developmental Changes Affecting Fluid and Electrolyte Balance in the Fetus and Neonate

Developmental Changes in Body Composition and Fluid Compartments

Dynamic changes occur in body composition and fluid distribution during intrauterine life, labor and delivery, and the early postnatal period. Thereafter the rate of change in body composition and fluid distribution gradually decreases, with subtler changes occurring especially after the first year of age (Friis-Hansen, 1961).

Changes During Intrauterine Development

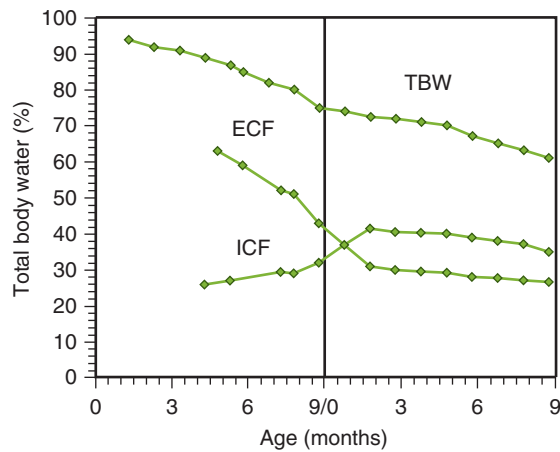
In early gestation, body composition is characterized by a high proportion of total body water (TBW) and a large extracellular compartment (Friis-Hansen, 1983; Brans, 1986). As gestation advances, rapid cellular growth, accretion of body solids, and fat deposition and developmental changes in the production of hormones regulating body water homeostasis result in gradual reductions in TBW content and extracellular fluid (ECF) volume, while the intracellular fluid compartment increases (Fig. 30.1)

(Friis-Hansen, 1983). In the 16-week-old fetus, TBW represents approximately 94% of total body weight; approximately two-thirds of the TBW is distributed in the extracellular compartment, and approximately one-third is distributed in the intracellular compartment. By term, TBW contributes only 75% of body weight, and approximately half of this volume is located in the intracellular compartment. Therefore premature newborns have excess TBW and a larger extracellular volume compared with their term counterparts, with most of the expanded extracellular volume being distributed in the interstitium (Bauer, 2011).

Changes During Labor and Delivery

Greater changes in TBW and its distribution occur during labor and delivery. Arterial blood pressure rises several days before delivery because of increases in plasma catecholamine, vasopressin, and cortisol levels and translocation of blood from the placenta into the fetus. This rise in arterial blood pressure, along with changes in the fetal hormonal milieu and an intrapartum hypoxia-induced increase in capillary permeability, results in a shift of fluid from the intravascular to the interstitial compartment and an associated approximately 25% reduction in circulating plasma volume in the human fetus during labor and delivery (Bauer, 2011). The postnatal increase in oxygenation and changes in vasoactive hormone production act to restore capillary membrane integrity and favor absorption of interstitial fluid into the intravascular compartment. The return of interstitial fluid into the bloodstream aids in maintaining intravascular volume during the first 24–48 hours postnatally, when oral fluid intake may be limited. However, prematurity, pathologic conditions, or both can disrupt this delicate process and interfere with the physiologic contraction of the ECF compartment in the immediate postnatal period.

In the fetus, body composition and fluid balance depend on the electrolyte and water exchange between the mother, fetus, and amniotic space (Brace, 1986). Antenatal events can have significant effects on postnatal fluid balance. Maternal indomethacin treatment or excessive administration of intravenous (IV) fluids during labor can result in neonatal hyponatremia with expanded extracellular water content (Rojas et al., 1984; vd Heijden et al., 1988). Placental insufficiency or maternal diuretic therapy can impair fetal extracellular volume, urine output, and amniotic fluid volume (Van Otterlo et al., 1977).



• **Fig. 30.1** Total body water content and its distribution between the extracellular fluid and intracellular fluid compartments in the human fetus, newborn, and infant from conception until 9 months of age. ECF, Extracellular fluid; ICF, intracellular fluid; TBW, total body water. (The data represent average values from Friis-Hansen B. Body water compartments in children: changes during growth and related changes in body composition. *Pediatrics* 1961;28:169–181.)

Effect of Timing of Cord Clamping

The timing of cord clamping after delivery is another important factor significantly affecting total circulating blood volume and extracellular volume in the neonate. Immediate cord clamping does not allow placental transfusion and negatively influences hemodynamic transition, especially in the preterm neonates (Hooper et al., 2015a). However, if the cord clamping is delayed, up to 25–50 mL of blood per kilogram is transfused into the neonate, representing an approximate 25%–50% increase in the total blood volume (Yao and Lind, 1974; Linderkamp, 1982). In a metaanalysis of term neonates, delayed cord clamping for at least 30 seconds was associated with increased iron stores and higher birth weight but with a higher need for phototherapy (McDonald et al., 2013). In a metaanalysis in preterm neonates, delayed cord clamping was associated with significantly decreased intraventricular hemorrhage (IVH), necrotizing enterocolitis, and need for inotropic support and blood transfusion (Rabe et al., 2012). Although concern about delaying neonatal resuscitation has been cited as a reason for not delaying cord clamping (Jelin et al., 2014), this concern may be decreasing (Katheria et al., 2016). Of note is that, according to recent animal data (Hooper et al., 2015b) and earlier observations in term neonates (Philip and Teng, 1977), the establishment of an appropriate functional residual capacity, usually with the onset of spontaneous respiration at birth, is the most relevant factor in determining the volume and rapidity of placental transfusion. Accordingly, neonates who do not establish effective respirations and who require resuscitation at birth may be at additional risk of lower circulating blood volumes. Although further follow-up is needed to understand whether increased blood volume at birth is associated with improved long-term outcomes, on the basis of current data, the American College of Obstetricians and Gynecologists recommends delaying cord clamping for 30–60 seconds after birth in preterm but not term neonates, whereas the World Health Organization recommends delaying cord clamping for 30–60 seconds in all neonates (World Health Organization, 2014).

Changes in the Postnatal Period

In the first few days and weeks after birth, the TBW content and distribution are affected by gestational and postnatal ages, pathologic conditions, the immediate environment (temperature, humidity), and the type of nutrition (enteral vs. parenteral). Normally, in the first few days after birth, an increase in capillary membrane integrity favors absorption of the interstitial fluid into the intravascular compartment (Modi, 2004). The ensuing rise in circulating blood volume stimulates the release of atrial natriuretic peptide (ANP) from the heart, which in turn enhances renal sodium and water excretion (Sagnella and MacGregor, 1984), resulting in an abrupt decrease in TBW and attendant weight loss. Although it is generally accepted that this postnatal weight loss is primarily due to the contraction of the expanded ECF compartment (Cheek et al., 1961), some water loss from the intracellular compartment can also occur, particularly in infants with extremely low birth weight (ELBW) and increased transepidermal water losses (TEWLs) (Costarino and Baumgart, 1991; Sedin, 1995; Modi, 2004).

Healthy term newborns lose approximately 10% of their birth weight during the first 4–7 days of age (Bauer et al., 2011), and thereafter they establish a pattern of steady weight gain. Because preterm neonates have an increased TBW content and extracellular volume, they lose approximately 10%–15% of their birth weight during this period (Shaffer and Meade, 1989; Bauer, 2011) and, depending on the degree of prematurity and associated pathologic conditions, these neonates only regain their birth weight by 10–20 days after birth (Fig. 30.2). Neonates who have intrauterine growth retardation have a smaller initial weight loss and more rapidly regain their birth weight than their normally grown counterparts, whether term or preterm (Bauer et al., 1993). Although the mechanisms for these differences have not been well studied, they appear to be associated with less diuresis in the infant with intrauterine growth retardation (Bauer et al., 1993; Méio et al., 2008).

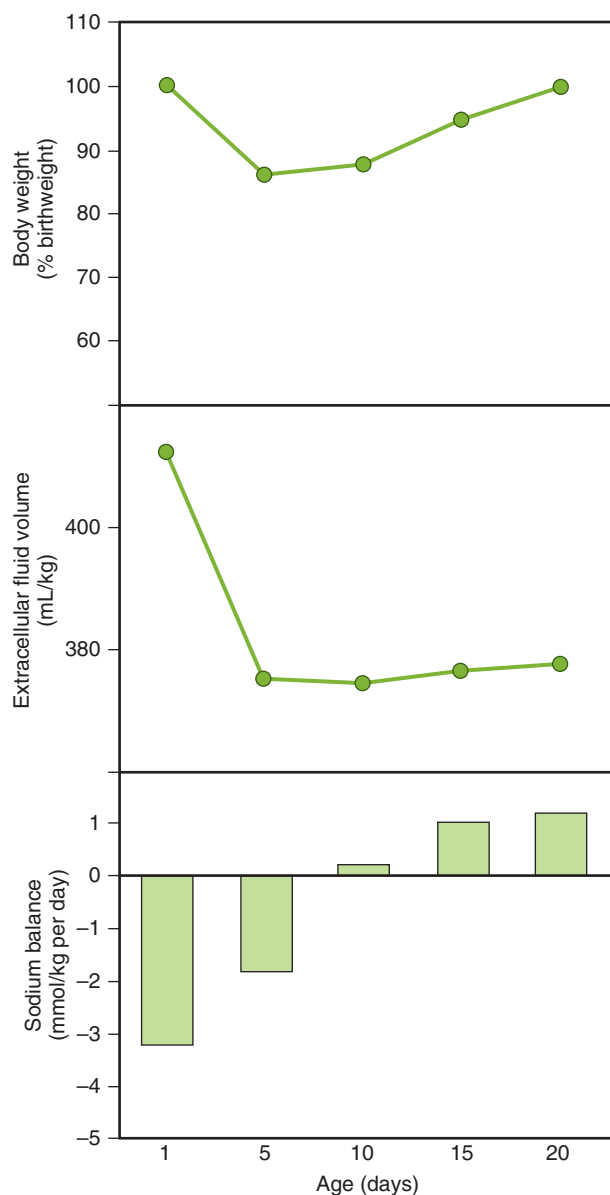
It is important in management of the neonate that the appropriate weight loss be anticipated and facilitated, if necessary, as lack of the early postnatal weight loss has been associated with higher rates of persistency of the patent ductus arteriosus (PDA), bronchopulmonary dysplasia (BPD), and necrotizing enterocolitis in low birth weight infants (Bell et al., 1979; Stonestreet et al., 1983; Saalmüller et al., 1994; Oh et al., 2005; Bell et al., 2014).

Physiology of the Regulation of Body Composition and Fluid Compartments

Although human cells have the ability to adjust their intracellular composition, extracellular volume and osmolality impact intracellular conditions, and if the changes are too dramatic, they may go beyond the cells' capacity to appropriately maintain the intracellular milieu. Therefore monitoring and active regulation of extracellular volume and osmolality are necessary in sick infants and those born prematurely.

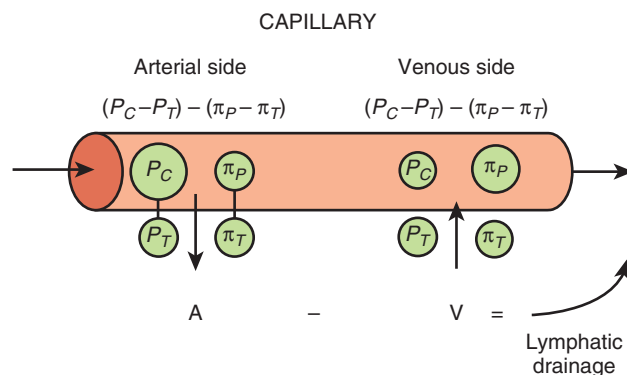
Regulation of the Intracellular Solute and Water Compartment

The major intracellular solutes are the proteins necessary for cell function, the organic phosphates associated with cellular energy production and storage, and the equivalent cations balancing the phosphate and protein anions (MacKnight and Leaf, 1977). Potassium is the major intracellular cation, and sodium is the major extracellular cation. The energy derived from the concentration differences for sodium and potassium between the intracellular and extracellular compartments is used for cellular work. Because changes in osmolality of the extracellular compartment are reflected



• **Fig. 30.2** Postnatal changes in body weight (expressed as a percentage of birth weight), extracellular fluid volume (estimated by the bromide dilution method), and sodium balance (defined as the difference between sodium intake and urinary sodium excretion). (From Shaffer SG, Weismann DN. Fluid requirements in the preterm infant. *Clin Perinatol*. 1992;19:233–250.)

as net movements of water into or out of the cell, regulation of ECF osmolality ultimately controls the intracellular compartment (MacKnight and Leaf, 1977). This physiologic principle must be kept in mind by the neonatologist managing sick term and preterm neonates with disturbances of sodium homeostasis. Rapid changes in serum sodium concentration and thus in extracellular osmolality directly affect the osmolality and size of the intracellular compartment and can lead to irreversible cell damage, especially in the central nervous system (CNS). Hyponatremia in the neonatal period has been associated with adverse long-term outcomes, especially in preterm neonates, while hypernatremia has been associated with short-term morbidities, including seizures and thrombosis (Morris-Jones et al., 1967; Escobar et al., 2007; Unal et al., 2008; Baraton et al., 2009; Moritz et al., 2009). These associations underscore



• **Fig. 30.3** Filtration and reabsorption of fluid along the capillary under physiologic conditions. A, Arterial; P_C , capillary hydrostatic pressure; P_T , tissue/interstitial hydrostatic pressure; V, venous; π_P , plasma oncotic pressure; π_T , tissue/interstitial oncotic pressure.

the importance of fluid and electrolyte homeostasis in the neonatal period.

Regulation of the Intracellular–Extracellular Interface: the Interstitial Compartment

In the healthy term neonate, hydrostatic (P_C , capillary hydrostatic pressure; P_T , tissue/interstitial hydrostatic pressure), and oncotic (π_P , plasma oncotic pressure; π_T , tissue/interstitial oncotic pressure) pressures are well balanced, with both being approximately half those in the adult (Sola and Gregory, 1981). In normal physiologic conditions, movement of fluid across the capillary is determined by the direction of the net driving pressure ($[P_C - P_T] - [\pi_P - \pi_T]$) and the water and protein permeability characteristics of the capillary wall (Fig. 30.3). At the arterial end of the capillary, intracapillary hydrostatic pressure (P_C) is high and plasma oncotic pressure (π_P) is relatively low, resulting in a net movement of fluid out of the capillary. As filtration of relatively protein-poor fluid continues along the capillary, plasma oncotic pressure rises and intracapillary hydrostatic pressure drops; therefore on the venous side, fluid moves from the interstitium into the capillary, so much of the filtered fluid is reabsorbed at the end of the capillary bed. The fluid remaining in the interstitium (arterial–venous side of the capillary) is drained by the lymphatic system. Interstitial hydrostatic (P_T) and oncotic (π_T) pressures remain virtually unchanged along the capillary bed. However, pathologic conditions readily disturb the delicate balance between the hydrostatic and oncotic forces, leading to an expansion of the interstitial compartment at the expense of the intravascular compartment. The increased interstitial fluid volume (edema) then further affects tissue perfusion by altering the normal function of the extracellular–intracellular interface. Box 30.1 summarizes the mechanisms for conditions resulting in interstitial edema formation in the neonate. There are also some important developmentally regulated differences between the newborn and the adult relating to the pathogenesis of edema formation. Capillary permeability to proteins is increased during the early stages of development (Gold and Brace, 1988; Bauer, 2011). Because neonatal capillary permeability is further increased under pathologic conditions (see Box 30.1), protein concentration in the interstitial compartment may approach that of the intravascular space, favoring further intravascular volume depletion and interstitial volume expansion. However, although the findings of most (Bignall et al., 1989; So et al., 1997; Oca et al., 2003) but not all (Lynch et al., 2008) clinical studies suggest that 0.9% saline administration for suspected hypovolemia is associated with less fluid retention and similar

• BOX 30.1 Mechanisms for Conditions Causing Interstitial Edema in the Neonate

Conditions Favoring Fluid Accumulation in the Interstitial Space by Causing a Disequilibrium Between Filtration and Reabsorption of Fluid by the Capillaries

Increased hydrostatic pressure
 Elevated capillary hydrostatic pressure
 Increased cardiac output
 Venous obstruction
 Decreased tissue hydrostatic pressure
 Conditions associated with changes in the properties of the interstitial gel (edematous states, effects of hormones including prolactin)
 Decreased oncotic pressure gradient
 Decreased capillary oncotic pressure
 Prematurity, hyaline membrane disease
 Malnutrition, liver dysfunction
 Nephrotic syndrome
 Increased interstitial oncotic pressure is usually the result of increased capillary permeability.
 Elevation of the filtration coefficient
 Increased capillary permeability
 Organs with large-pore capillary endothelium (liver, spleen)
 State of maturity (preterm infants > term newborns > adults)
 Production of proinflammatory cytokines (sepsis, anaphylaxis, hypoxic tissue injury, tissue ischemia, ischemia–reperfusion, soft tissue trauma, extracorporeal membrane oxygenation)
 Increased capillary surface area
 Vasodilation

Conditions Associated With Decreased Lymphatic Drainage

Decreased muscle movement
 Neuromuscular blockade and/or heavy sedation
 Central and/or peripheral nervous system disease
 Obstruction of lymphatic flow
 Increased central venous pressure
 Scar tissue formation (bronchopulmonary dysplasia)
 Mechanical obstruction (dressings, high mean airway pressure in mechanically ventilated newborns)

improvements in the cardiovascular status compared with 4.5% or 5% albumin infusion, the topic remains controversial.

Even in the presence of hypoalbuminemia, when sick neonates are treated with frequent albumin boluses, much of the infused albumin rapidly leaks into the interstitium. This creates a vicious cycle of intravascular volume depletion and edema formation, resulting in vasoconstriction and disturbances in tissue perfusion and cellular function, exacerbating impairments in the regulation of extracellular volume distribution. If the cycle is not interrupted, anasarca will develop, which is usually associated with an extremely poor prognosis. In summary, the sick neonate has limited capacity to maintain appropriate intravascular volume and to regulate the volume and composition of the interstitium, and thus high vigilance is required by the caretaker in appropriately managing intravascular volume, including avoiding routine use of albumin in the critically ill neonate (Uhing, 2004).

Regulation of the Extracellular Solute and Water Compartment

The osmolality of the extracellular compartment is tightly maintained within 2% of the osmolar set point, which lies between 275 and 290 milliosmole (mOsm) (Robertson and Berl, 1986). Blood pressure and serum sodium concentration (i.e., the main

contributor to osmolality under homeostatic conditions) are monitored by baroreceptors and osmoreceptors respectively. The effector limb of the regulatory system consists of the heart, vascular bed, kidneys, and intake of fluid in response to thirst. The inability of critically ill term and preterm neonates to maintain fluid balance by responding to thirst places increased importance on caregiver management of fluid administration. By regulating the function of the effector organs, several hormones play a role in the control of the extracellular compartment, including the renin–angiotensin–aldosterone system, vasopressin, ANP, brain (B-type) natriuretic peptide (BNP), bradykinin, prostaglandins, and catecholamines. Effective regulation of the extracellular compartment and intravascular volume also depends on intact cardiovascular function and capillary endothelium integrity (Robertson and Berl, 1986). For example, under physiologic conditions, an increase in the extracellular volume is reflected by an increase in the circulating plasma volume, leading to increased blood pressure and renal blood flow. The ensuing increase in glomerular filtration and urine output returns the extracellular volume to normal. In critically ill neonates, however, the capillary leak and reduced myocardial responsiveness resulting from immaturity and underlying pathologic conditions limit the increase in the circulating blood volume when extracellular volume expands. Thus especially in sick preterm neonates, blood pressure may rise only transiently (Lundström et al., 2000), and renal blood flow may remain low after volume boluses as fluid rapidly leaks into the interstitium. Inappropriate central regulation of vascular tone results in vasodilatation, further decreasing effective circulating blood volume and compromising tissue perfusion; this leads to impaired gas exchange in the lungs, resulting in hypoxia with further increases in capillary leak. Unless it is interrupted by appropriate therapeutic measures, a vicious cycle with further deterioration readily occurs in the sick neonate.

Maturation of Organs Regulating Body Composition and Fluid Compartments

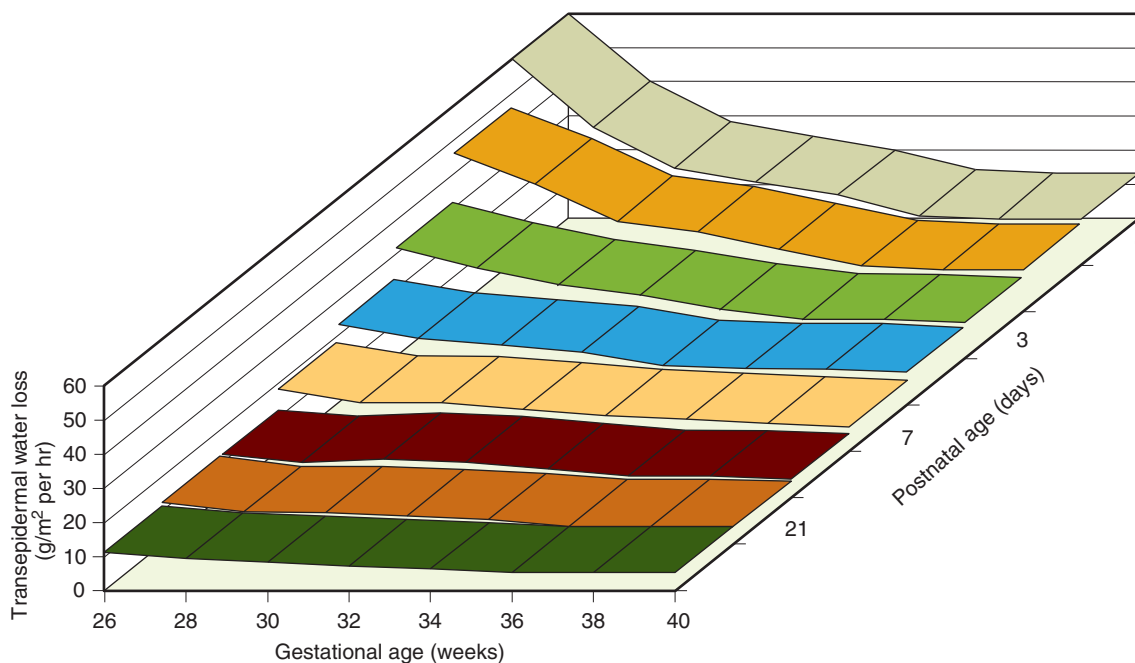
The heart, kidneys, skin, and endocrine system play the most important roles in the regulation of ECF (and thus intracellular fluid) and electrolyte balance in the neonate. Immaturity of these organ systems, especially in infants with very low birth weight (VLBW), results in a compromised regulatory capacity, which must be noted when one is estimating daily fluid and electrolyte requirements in these patients.

Maturation of the Cardiovascular System

There is a direct relationship between gestational maturity and the ability of the neonatal heart to respond to acute volume loading (Baylen et al., 1986). The blunted Starling response of the immature myocardium results from its lower content of contractile elements and incomplete sympathetic innervations (Mahony, 1995). Because central vasoregulation and endothelial integrity are also developmentally regulated (Gold and Brace, 1988; Bauer, 2011), an appropriate effective intravascular volume is seldom maintained in the critically ill preterm neonate. Since regulation of the extracellular volume requires the maintenance of an adequate effective circulating blood volume, the immaturity of the cardiovascular system contributes to the limited capacity of sick preterm neonates to effectively regulate the total volume of their extracellular compartment.

Maturation of Renal Function

The kidney has a crucial role in the physiologic control of fluid and electrolyte balance. It regulates extracellular volume and



• **Fig. 30.4** Transepidermal water loss in relation to gestational age during the first 28 postnatal days in newborns who are appropriate for their gestational age. There is an exponential relationship between transepidermal water loss and gestational age, the water loss being higher in preterm newborns than in term newborns. Transepidermal water loss is also significantly affected by postnatal age, especially in the immature preterm newborn. The measurements were performed at ambient air humidity of 50% and with the newborns calm and quiet. (From Hammarlund K, Sedin G, Stromberg B. Transepidermal water loss in newborn infants. VIII: relation to gestational age and post-natal age in appropriate and small for gestational age infants. *Acta Paediatr Scand.* 1983;72:721–728.)

osmolality through the selective reabsorption of sodium and water respectively. Immaturity of renal function renders preterm neonates susceptible to excessive sodium and bicarbonate losses (Modi, 2004; Brewer, 2011). In addition, the inability of the preterm neonate to respond promptly to a sodium or volume load results in a tendency toward extracellular volume expansion with edema formation. Because prenatal steroid administration accelerates maturation of renal function (van den Anker et al., 1994), preterm neonates exposed to steroids in utero have a better capacity to regulate their postnatal ECF contractions. During the first few weeks postnatally, hemodynamically stable but extremely immature infants produce dilute urine and may develop polyuria because of their renal tubular immaturity. As tubular functions mature, their concentrating capacity gradually increases from the second week to the fourth week of postnatal life. However, it takes years for the developing kidney to reach the concentrating capacity of the adult kidney (Linshaw, 2011).

Maturation of the Skin

Although the epidermis of term neonates is well developed and cornified, in extremely immature neonates it consists of only two or three cell layers (Chu and Loomis, 2011). The absence of an effective barrier to the diffusion of water increases TEWL in the immature neonate. TEWL through immature skin can result in early postnatal hypertonic dehydration, with rapid changes in intracellular volume and osmolality. In many organs, especially the brain, these abrupt changes can result in cellular dysfunction and ultimately cell death. Gestational age, postnatal age, the pattern of intrauterine growth, and environmental factors (e.g., humidity and temperature) affect transepidermal free water loss (Fig. 30.4).

Postnatal skin cornification occurs rapidly, but full maturation of the epidermis does not occur until 2–3 weeks of age (Cartledge, 2000). Chronic intrauterine stress (Hammarlund et al., 1983) and prenatal steroid treatment (Aszterbaum et al., 1993) also enhance maturation of the skin.

Maturation of End-Organ Responsiveness to Hormones Involved in the Regulation of Fluid and Electrolyte Balance

Several hormones directly regulate the volume and composition of the extracellular compartment by altering renal sodium and water excretion and by inducing changes in systemic vascular resistance and myocardial contractility. These include the renin–angiotensin–aldosterone system, vasopressin, ANP, and BNP. Other hormones, including the prostaglandins, bradykinin, and prolactin, modulate the actions of many of the regulatory hormones.

Renin–Angiotensin–Aldosterone System. Decreases in renal capillary blood flow stimulate renin secretion from the juxtaglomerular cells of the kidney, which in turn catalyzes the conversion of angiotensinogen to angiotensin I. Angiotensin-converting enzyme hydrolyzes angiotensin I to angiotensin II, which can then bind to the cell membrane-bound receptors AT_1 and AT_2 (Sequeira et al., 2011). Angiotensin induces vasoconstriction, increased tubular sodium and water reabsorption, and the release of aldosterone (Sequeira et al., 2011). Aldosterone increases potassium secretion and further enhances sodium reabsorption in the distal tubule; therefore the primary function of this system is to protect the volume of the extracellular compartment and maintain adequate tissue perfusion (Bailie, 1992). However, its effectiveness in the neonate is somewhat limited by the decreased responsiveness of the immature kidney to the sodium-retaining and water-retaining

effects of these hormones (Sulyok et al., 1985; Feld et al., 2011). Vasodilatory and natriuretic prostaglandins generated in the kidney (Gleason, 1987) are the main counterregulatory hormones balancing the renal actions of the renin–angiotensin–aldosterone system. Therefore when prostaglandin production is inhibited by indomethacin, the unopposed vasoconstrictive and sodium-retentive actions of the activated renin–angiotensin–aldosterone system contribute to the development of the drug-induced renal failure in the preterm neonate (Gleason, 1987; Seri, 1995; Seri et al., 2002).

Vasopressin. Vasopressin (antidiuretic hormone) regulates the osmolality of the extracellular compartment and directly effects vascular tone through the V_{1a} and V_2 receptors. Vasopressin selectively raises free water reabsorption through the upregulation of aquaporin-2 water channels in the collecting duct, resulting in blood pressure elevation (Elliot et al., 1996; Linshaw 2011). Although it appears that the developing kidney is less sensitive to circulating vasopressin, plasma levels of vasopressin are markedly elevated in the neonate, especially after vaginal delivery, and its cardiovascular actions facilitate neonatal adaptation (Pohjavuori et al., 1985; Linshaw, 2011). The high vasopressin levels are in part also responsible for the diminished urine output of the healthy term neonate during the first day after birth. Under certain pathologic conditions, the dysregulated release of, or the end-organ unresponsiveness to, vasopressin significantly affects renal and cardiovascular functions and electrolyte and fluid status in the sick preterm and term neonate (Svenningsen et al., 1974). In the syndrome of inappropriate secretion of antidiuretic hormone (SIADH), an uncontrolled release of vasopressin occurs in sick preterm and term neonates, with resulting water retention, hyponatremia, and oliguria. In the syndrome of diabetes insipidus, the lack of pituitary production of vasopressin or renal unresponsiveness to vasopressin results in polyuria and hypernatremia.

Atrial Natriuretic Peptide. Via its direct vasodilatory and renal natriuretic actions, the hormone ANP regulates the volume of the extracellular compartment in the fetus and neonate in a fashion opposite to that of the renin–angiotensin–aldosterone system (Seymour, 1985; Needleman et al., 1986; Solhaug and Jose, 2011). ANP has a direct inhibitory effect on renin production and aldosterone release (Christensen, 1993).

The stretch of the atrial wall caused by an increase in the circulating blood volume is the most potent stimulus for ANP release. Plasma levels are high in the fetus (Claycomb, 1988) and along with BNP, ANP likely plays a role in cardiac development (Das et al., 2009). There are a few specific conditions in which the actions of ANP are directly relevant for the neonatologist. For example, the hormone is involved in the regulation of both the fluid shifts during labor (Bauer, 2011) and the extracellular volume contraction during postnatal transition (Kojima et al., 1987; Tulassay et al., 1987; Rozycki and Baumgart, 1991; Ronconi et al., 1995). Furthermore, the oliguric effects of positive end-expiratory pressure ventilation are due in part to a decrease in ANP secretion (Christensen, 1993) along with the enhanced release of vasopressin (El-Dahr and Chevalier, 1990).

Brain (or B-Type) Natriuretic Peptide. BNP is released from the ventricular myocardium in response to increases in wall tension. Similarly to ANP, BNP causes natriuresis, diuresis, and vasodilatation, while inhibiting the renin–angiotensin–aldosterone system (Kojima et al., 1989; Gemelli et al., 1991; Holmes et al., 1993). Compared with ANP, BNP and the inactive N-terminal fragment of BNP (NT-proBNP) demonstrate longer half-lives and thus may be more useful clinical biomarkers as their levels are relatively more

stable over time (Vijlbrieff, 2012). NT-proBNP is renally excreted, and renal function should be considered when one is interpreting levels (Breuer, 2007).

BNP levels increase rapidly after birth, with the levels by the end of the first day up to 20-fold higher than those at birth (Yoshiyoshi et al., 1995; Mir et al., 2003), and they correlate with the downward trend in pulmonary arterial pressure, diuresis, and renal maturation in the days after birth, unlike ANP levels (Ikemoto et al., 1996). By causing vasodilatation and diuresis, high levels of BNP play a critical role in the hemodynamic transition of the fetus. BNP levels continue to fall during the first week after birth (Koch et al., 2003; Mir et al., 2003). The usefulness of measuring BNP levels is limited by the variability of levels in the first few days after birth as well as the variety of assays available to measure BNP levels. Its utility may ultimately lie in repeated measurements in the same patient over time with the same assay in order to follow trends.

Prostaglandins. Prostaglandins have a well-documented, counterregulatory role for the renal vascular and tubular effects of renin–angiotensin–aldosterone and vasopressin (Bonvalet et al., 1987). The inhibition of prostaglandin synthesis by indomethacin results in clinically important and sometimes detrimental renal vascular and tubular effects in the preterm neonate (Mercanti et al., 2009). Importantly, ibuprofen demonstrates fewer side effects than indomethacin on renal and mesenteric blood flow (Mercanti et al., 2009). How prostaglandins modulate the effects of the other regulatory hormones of neonatal fluid and electrolyte homeostasis is less well studied.

Prolactin. Prolactin plays a permissive role in the regulation of fetal and neonatal water homeostasis (Coulter, 1983; Pullano et al., 1989). High fetal plasma prolactin levels contribute to the increased tissue water content of the fetus. Postnatal prolactin levels remain high in the preterm neonate until approximately the 40th postconceptional week (Perlman et al., 1978). Low levels have been associated with increased risk of developing respiratory distress syndrome (RDS) (Gluckman et al., 1978; Hauth et al., 1978; Smith et al., 1980).

Management of Fluid and Electrolyte Homeostasis

General Principles of Fluid and Electrolyte Management

Fluid and electrolyte management is the cornerstone of neonatal intensive care, and appropriate management requires an understanding of the previously outlined physiologic principles and careful monitoring of key clinical data. Requirements vary substantially from infant to infant and in the same infant over time; therefore fluid prescription must be individualized and frequently reassessed. The primary goals are to maintain the appropriate ECF volume, ECF and intracellular fluid osmolality, and ionic concentrations.

Assessment of Fluid and Electrolyte Status

Maternal conditions during pregnancy, drugs and fluids administered to the mother during labor and delivery, and specific fetal and neonatal conditions all affect early fluid and electrolyte balance. Excessive administration of free water or oxytocin use in the mother can result in hyponatremia in the neonate. Maternal therapy with indomethacin, angiotensin-converting enzyme inhibitors, furosemide, and aminoglycosides can all adversely affect neonatal renal function. A newborn's history of oligohydramnios or birth asphyxia may also alert the clinician to the possibility of abnormal renal function. In young neonates, altered skin turgor, sunken anterior

fontanel, and dry mucous membrane are not sensitive indicators of dehydration, but tachycardia, hypotension, metabolic acidosis, and oliguria may be seen when intravascular volume is moderately to severely affected. In addition, edema usually occurs early when there is volume overload or illness. Serial measurements of body weight, intake and output, and serum electrolyte levels will usually provide the most precise and accurate information regarding overall fluid status.

Normally, both term and preterm neonates will void within the first 24 hours after birth (Clark, 1977). In most neonates without hemodynamic compromise, urine output increases from 1–2 mL/kg per hour on the first postnatal day to 3–5 mL/kg per hour by the third to fifth postnatal day and is associated with a weight loss of 5%–10% in term infants and 10%–15% in preterm neonates (Bidiwala et al., 1988). Frequently, onset of diuresis heralds resolution of RDS (Engle et al., 1983). In critically ill newborns and in situations of altered homeostasis, additional clinical data that may help in diagnosis and management include blood urea nitrogen (BUN) levels, serum and urine osmolality or specific gravity, and urine electrolyte and serum bicarbonate levels, along with close monitoring of blood pressure and heart rate. The frequency of monitoring depends on the extent of immaturity, the underlying pathologic condition, and the severity of the fluid and electrolyte disturbance.

Water Homeostasis and Management

Water Losses

Free water loss can be categorized as either insensible (skin and respiratory track) or sensible (urine and feces). Urine output is the most important source of sensible water loss. Extremely preterm neonates without systemic hypotension or renal failure usually lose 30–40 mL of water per kilogram per day in the urine on the first postnatal day and approximately 120 mL/kg per day by the third day. In stable, more mature preterm neonates born after 28 weeks' gestation, urinary water loss is approximately 90 mL/kg per day on the first postnatal day and 150 mL/kg per day by the third day (Coulthard and Hey, 1985). Because of their renal immaturity, preterm neonates have a tendency to produce dilute urine, thereby increasing their obligatory free water losses. In term neonates, urinary water loss is considerably less, approximating 40–60 mL/kg per day by the third day.

Normal water losses in the stool are less significant, amounting to approximately 10 mL/kg per day in term neonates and 7 mL/kg per day in preterm neonates during the first postnatal week (Sedin, 1995). Water losses in the stool increase thereafter and are influenced by the type of feeding and the frequency of stooling, which is higher in breastfed neonates.

In the preterm neonate, consideration of daily insensible water losses (IWLs) through the skin is critical (see Fig. 30.4). During the first few postnatal days, in a nonhumidified environment, TEWLs may be 15-fold higher in extremely premature neonates born at 23–26 weeks' gestation than in term neonates (Sedin, 1995). Although the skin matures rapidly after birth, even in extremely immature neonates, insensible losses are still somewhat higher at the end of the first month than in the term counterparts. Prenatal steroid exposure is associated with substantially less IWL in preterm neonates (Aszterbaum et al., 1993; Sedin, 1995; Omar et al., 1999).

Appropriate management of the neonate's immediate environment most effectively counteracts the high degree of IWL through immature skin. Among environmental factors, ambient humidity has the greatest effect on TEWL. In extremely immature neonates,

a rise in the ambient humidity in the incubator from 20%–80% decreases the TEWL by approximately 75% (Sedin, 1995). However, the use of an open radiant warmer more than doubles TEWLs (Flenady and Woodgate, 2003), and it is now standard of care to maintain extremely immature neonates in humidified incubators. Other factors that increase transepidermal IWL include phototherapy, especially when the neonate is nursed under low humidity, activity, airflow, elevated body, and environmental temperature as well as skin breakdown and skin or mucosal defects (e.g., gastrochisis, epidermolysis bullosa).

IWLs from the respiratory tract depend mainly on the temperature and humidity of the inspired gas mixture and on the respiratory rate, tidal volume, and dead space ventilation. In a healthy term newborn, the water loss through the respiratory tract is approximately half the total IWL if the ambient air temperature is 32.5°C and the humidity is 50% (Sedin, 1995). However, in neonates undergoing mechanical ventilation, there will be no IWL through the respiratory tract if the ventilator gas mixture is humidified at body temperature.

Extraordinary water losses are also seen in the neonate requiring intensive care. The most commonly encountered extraordinary losses occur when a nasogastric tube is placed under continuous suction (discussed in Surgical Conditions). Large losses may also occur in association with phlebotomy, chest tubes, surgical drains, ostomies, and fistulas as well as with emesis or diarrhea.

Management of Water Requirements

When managing the fluid status of the neonate, the clinician must consider fluid requirement dictated by three broad categories. First, any existing deficits or surpluses must be estimated. Secondly, ongoing maintenance needs to replace usual sensible and insensible losses, and support growth must be calculated. Finally, additional needs as a result of extraordinary losses should be anticipated. Importantly, while administered together, the composition of each of these fluids is unique and must be considered individually. For example, fluids given to replace ongoing losses from a chest tube or ostomy will require a different electrolyte composition than simple maintenance fluids to maintain hydration, and these will differ again from fluids given to support growth. The neonate's prenatal history, birth weight, gestational age, and need for mechanical ventilation and the environment in which the neonate is to be cared for should be considered when initial fluid and electrolyte needs are being determined. Frequent reevaluations are necessary. The most useful parameter for monitoring fluid balance is the weight of the baby, as rapid changes in weight will reflect changes in water balance. Serial weights can be used to estimate the IWL with use of the following formulas:

$$\text{IWL} = \text{Fluid Intake} - \text{Urine Output} + \text{Weight Loss}$$

or

$$\text{IWL} = \text{Fluid Intake} - \text{Urine Output} - \text{Weight Gain.}$$

It is reasonable to initiate fluid volume on the basis of the sum of an allowance for sensible water loss of 30–60 mL/kg per day and the estimated IWL. Fig. 30.4 shows usual IWL ranges by gestational and postnatal age. Factors previously outlined that predictably affect IWL should be considered when fluids are being prescribed. Prevention of excessive IWL rather than replacement of increased IWL is associated with fewer complications in the preterm neonate and can usually be achieved by modification of the neonate's environment. See Table 30.1 for usual maintenance fluid administration based on birth weight. These numbers are guidelines for initial

TABLE 30.1 Estimated Maintenance Fluid Requirements

| Birth weight (g) | DAILY FLUID REQUIREMENTS (mL/kg) | | | |
|------------------|----------------------------------|---------|----------|---------|
| | Day 1 | Day 2 | Days 3–6 | Day 7+ |
| <750 | 100–140 | 120–160 | 140–200 | 140–160 |
| 750–1000 | 100–120 | 100–140 | 130–180 | 140–160 |
| 1000–1500 | 80–100 | 100–120 | 120–160 | 150 |
| >1500 | 60–80 | 80–120 | 120–160 | 150 |

management only; the approach must subsequently be individualized on the basis of laboratory values and other clinical data.

It is important to remember that the TBW excess and extracellular volume expansion of preterm neonates imply that their negative water and sodium balance during the first 5–10 postnatal days (see Fig. 30.2; Shaffer and Meade, 1989) represents an appropriate adaptation to extrauterine life and should not be compensated for by increased fluid administration and sodium supplementation. If this principle is not followed, and a positive fluid balance (i.e., weight gain) is achieved during the transitional period, preterm neonates have been shown to be at higher risk of a more severe course of RDS (Shaffer and Weismann, 1992) and a higher incidence of PDA (Bell et al., 1980), congestive heart failure (Bell et al., 1980), pulmonary edema (Shaffer and Weismann, 1992), necrotizing enterocolitis (Bell et al., 1979), and BPD (Van Marter et al., 1990; Oh et al., 2005). However, most of the published studies on outcomes related to fluid balance were performed in the presurfactant era and before the widespread use of corticosteroids prenatally.

Infants with ELBW and others with anticipated fluid problems should be weighed daily or twice daily. Serum sodium levels should be measured every 4–6 hours until they have stabilized, usually by 3–4 days after birth, and urine output should be recorded and reviewed every 6–8 hours. In ELBW infants, electrolyte levels should be checked by 12 hours postnatally to help guide fluid management. Once data are available, fluids should be increased if weight loss is greater than 1%–2% per day in term neonates and 2%–3% per day in preterm neonates, if urine output is inappropriately low, if urine specific gravity is rising, or if serum sodium concentration is rising. Overall, expected and appropriate weight loss during the first week postnatally is up to 10% in term neonates and up to 15% in preterm neonates. Conversely, fluids should be decreased if weight is not falling appropriately and serum sodium concentration is decreasing. The goal is to reach 140–160 mL of fluids per kilogram per day by 7–10 days to allow adequate caloric intake.

Treatment of Fluid Overload

Fluid overload commonly occurs in sick neonates, often because of the use of fluid bolus administration for hypotension. The diagnosis is based on weight gain, edema, and often hyponatremia. Overhydration can sometimes be prevented by the use of blood transfusions or dopamine instead of colloid or crystalloid, if appropriate, for blood pressure support. In addition to reducing the need for volume boluses, dopamine may facilitate the process of extracellular volume contraction via its renal and hormonal effects (Seri, 1995). Once overhydration has occurred, management

is usually effected by 10%–20% decrements of total daily fluid intake and with careful monitoring of clinical and laboratory signs to ensure maintenance of adequate intravascular volume as well as normal glucose and electrolyte status while the ECF contraction occurs. While administration of albumin followed by a diuretic (e.g., furosemide) is frequently practiced in an attempt to mobilize interstitial fluid, there is little evidence to support this practice, and it may be counterproductive because of albumin leaking into the interstitial space (Uhing, 2004).

Treatment of Dehydration

Dehydration in the neonate may be suspected on the basis of history or clinical signs and confirmed by laboratory studies. One may estimate the total water deficit by using weight changes, calculating total inputs and outputs, and following serial sodium levels. Appropriate treatment requires consideration of sodium status. For additional details of fluid correction and sodium management, see the discussion in Treatment of Hyponatremia.

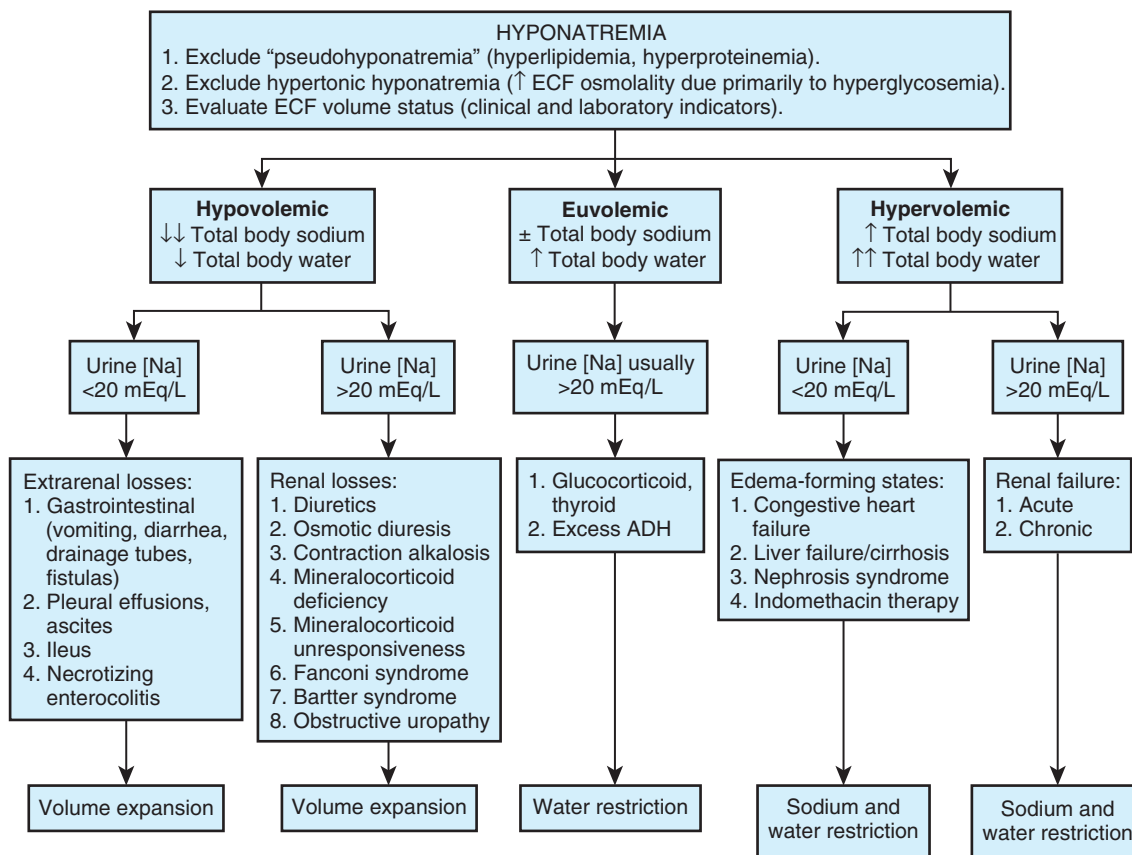
Sodium and Potassium Homeostasis and Management

Serum sodium values should generally be kept between 135 and 145 milliequivalent (mEq)/L. Sodium chloride supplementation of 1–2 mEq/kg per day should be started in preterm and sick term neonates only after completion of the postnatal extracellular volume contraction, usually after the first few days of age or after more than 5% of birth weight has been lost (Hartnoll et al., 2001). In general, as long as the neonate's fluid balance is stable, maintenance sodium requirements do not exceed 3–4 mEq/kg per day, and providing this amount usually ensures the positive sodium balance necessary for adequate growth. However, extreme prematurity and pathologic conditions associated with delayed transition or disturbance of fluid and electrolyte balance may significantly alter the neonate's daily sodium requirement. For example, although preterm neonates have a limited ability to excrete a sodium load (Hartnoll, 2003), they also may waste large amounts of sodium in their urine. In addition, neonates recovering from an acute renal insult and preterm neonates with immature proximal tubule functions who are in a state of extracellular volume expansion (Ramiro-Tolentino et al., 1996) may need daily sodium bicarbonate (NaHCO_3) supplementation to compensate for their greater renal bicarbonate losses.

Hyponatremia

Hyponatremia (serum sodium concentration <130 mEq/L) represents a deficit of sodium in relation to body water content and may be caused by either total body sodium (TBS) deficit or free water excess. In either situation, TBW may be decreased (hyponatremia with volume contraction), normal, or increased (hyponatremia with volume expansion). Chronic hyponatremia has been associated with adverse long-term outcomes, especially in preterm neonates, including increased incidence of sensorineural hearing loss and cerebral palsy (Murphy et al., 1997; Ertl et al., 2001). Although hyponatremia may primarily be a marker of illness severity, there is also evidence that sodium supplementation in preterm neonates is associated with better long-term outcomes, thus underscoring the importance of appropriate sodium management (Al-Dahhan et al., 2002).

Prevention of hyponatremia is the best approach in management; however, when hyponatremia occurs, to initiate effective treatment, it is important to attempt to determine the primary cause and duration of the hyponatremia and whether there is associated volume expansion or contraction. The most common cause of



• **Fig. 30.5** Flow diagram for the clinical evaluation of and therapy for neonates with hyponatremia. ADH, Antidiuretic hormone; ECF, extracellular fluid. (Modified from Avner ED. Clinical disorders of water metabolism: hyponatremia and hypernatremia. *Pediatr Ann.* 1995;24:23–30.)

hyponatremia in the sick neonate is excessive administration or retention of free water. In these situations the TBS content is normal, and the appropriate treatment is restriction of free water intake and not administration of sodium. In situations of true sodium deficit, one can estimate the deficits by assuming 70% of total body weight as the distribution space of sodium. The formula for calculating sodium (Na^+) deficit is:

$$\text{Na}^+ \text{ Deficit (or Excess) (mEq)} \approx 0.7 \times \text{Body Weight (kg)} \times ([\text{Na}^+]_{\text{desired}} - [\text{Na}^+]_{\text{current}}).$$

In most situations of depletion hyponatremia (i.e., true sodium deficit), two-thirds of the replacement sodium should be provided in the first 24 hours, and the remainder should be provided in the next 24 hours. Frequent measurements of serum levels of electrolytes are needed to ensure that the correction is occurring appropriately. With severe hyponatremia (serum sodium concentration <120 mEq/L), regardless of whether the hyponatremia is due to free water overload or TBS deficit, correction of the serum sodium concentration up to 120 mEq/L is recommended with administration of 3% saline solution (513 mEq of sodium per liter). This recommendation is due to concern of increased risk of neurologic complications associated with severe hyponatremia, although this relationship has not been explicitly demonstrated in large cohorts of neonatal patients (Riggs, 2002). This correction should be done over 4–6 hours, depending on the severity of hyponatremia (Avner, 1995), with use of the preceding formula and with close monitoring of serum sodium changes. Although

rapid IV bolus administration of 4–6 mL of 3% saline solution per kilogram has been effective in children with seizures or coma (Sarnaik et al., 1991), rapid and complete correction of low serum sodium concentration in adults with chronic hyponatremia have been shown to be associated with pontine and extrapontine myelinolysis (Nardone et al., 2016). Although this association has not been demonstrated in neonates, the potential risk of neurologic injury necessitates caution with rapid correction of long-standing hyponatremia. Once the serum sodium concentration has reached 120 mEq/L, complete correction of hyponatremia should be performed more slowly over the next 48 hours. In patients with asymptomatic hyponatremia whose serum sodium concentration exceeds 120 mEq/L, hypertonic infusions are not indicated. Additional therapy should be directed toward fluid restriction if the hyponatremia is dilutional or sodium repletion if the hyponatremia is depletional. For depletion hyponatremia, 5% dextrose in water with 0.45%–0.9% saline is a reasonable replacement fluid once the sodium is above 120 mEq/L. Chronic sodium losses in more stable neonates can also be corrected with enteral sodium chloride administration. Fig. 30.5 summarizes the clinical evaluation of and therapy for neonates with hyponatremia.

Hypernatremia

Hypernatremia (serum sodium concentration >150 mEq/L) reflects a deficiency of water relative to TBS and is most often a disorder of water rather than sodium homeostasis. The presence of hypernatremia does not reflect the TBS content, which can be high, normal, or low depending on the cause of the condition.

• BOX 30.2 Conditions Causing Hypernatremia**Hypovolemic Hypernatremia**

Inadequate breast milk intake
Diarrhea
Radiant warmers
Excessive sweating
Renal dysplasia
Osmotic diuresis

Euvolemic Hypernatremia**Decreased Production of Antidiuretic Hormone**

Central diabetes insipidus, head trauma, central nervous system tumors (craniopharyngioma), meningitis, or encephalitis

Decreased or Absence of Renal Responsiveness

Nephrogenic diabetes insipidus, extreme immaturity, renal insult, and medications such as amphotericin, hydantoin, and aminoglycosides

Hypervolemic Hypernatremia

Improperly mixed formula
Sodium bicarbonate administration
Sodium chloride administration
Primary hyperaldosteronism

Hypernatremia can also be associated with hypovolemia, normovolemia, or hypervolemia (Box 30.2). If hypernatremia is primarily due to changes in sodium balance, it can result from pure sodium gain or, more commonly, sodium gain coupled with a lesser degree of water accumulation or, rarely, water loss. It is important to recognize that neonates with hypernatremic dehydration often do not demonstrate overt clinical signs of intravascular depletion and dehydration until late in the course of the condition. The hypernatremia-induced hypertonicity causes water to shift from the intracellular to the extracellular compartment, resulting in intracellular dehydration but with relative preservation of the extracellular compartment. Compared with other organs, the CNS has a unique and more effective adaptive capacity to respond to the hypernatremia-induced hypertonicity, leading to a relative preservation of neuronal cell volume. The shrinkage of the brain stimulates the uptake of electrolytes such as sodium, potassium, and chloride (immediate effect). However, these electrolytes, at higher than normal intracellular concentrations, have severe adverse effects on intracellular enzyme functions. The concomitant, hypernatremia-induced and hyperosmolality-induced synthesis of osmoprotective amino acids and organic solutes (delayed response, starting perhaps around 4–6 hours into the process) thus serves as a defensive mechanism to protect cellular functions. These idiogenic osmols, such as taurine, glycine, glutamine, sorbitol, and inositol aid in maintaining normal brain cell volume during longer periods of hyperosmolar stress and limit the accumulation of intracellular sodium and chloride (Trachtman, 1991). As long as hypernatremia develops rapidly (within hours), as in accidental sodium loading, a relatively rapid correction of the condition is usually safe. Intracellular fluid accumulation does not occur because the accumulated electrolytes (sodium, potassium, and chloride) are rapidly extruded from the brain cells, and the development of cerebral edema is unlikely. In these cases, reducing serum sodium concentration by 1 mEq/L per hour (24 mEq/L per day) is appropriate (Adrogué and Madias, 2000).

In contrast to rapidly developing hypernatremia, in cases of chronic hypernatremia, the dissipation of idiogenic osmols in

response to correction of the hypernatremia occurs slowly over several days (Adrogué and Madias, 2000). Thus in these chronic cases, or in cases in which the time frame over which hypernatremia developed is unknown, the hypernatremia should be corrected more slowly, at a maximum rate of 0.5 mEq/L per hour (12 mEq/L per day). If correction is performed more rapidly in these cases, the abrupt fall in the extracellular tonicity results in the movement of water into the brain cells, which have a relatively fixed hypertonicity because of the presence of the osmoprotective molecules. The result is the development of brain edema with potentially deleterious consequences (Molteni, 1994; Adrogué and Madias, 2000). More recently, a large population-based study showed that neonates admitted to hospital with dehydration (weight loss >12% of birth weight) and hypernatremia (serum sodium concentration ≥ 150 mEq/L), but without shock, respiratory failure, infarct, or gangrene and in a managed care setting, did not have increased rates of adverse neurodevelopmental outcome at 5 years (Escobar et al., 2007). The authors noted that these favorable outcomes may not be generalizable to neonates presenting with more severe symptoms.

In the breastfed term neonate, hypernatremia most commonly develops in association with dehydration secondary to inadequate breast milk intake (Molteni, 1994) but has also been associated with high sodium levels in maternal breast milk, especially from mothers of neonates not successfully lactating (Peters, 1989; Abu-Salah, 2001; Karthikeyan and Modi, 2003; Scott et al., 2003). Reduction in breastfeeding frequency has been shown to be associated with a marked rise in the sodium concentration of breast milk (Neville et al., 1991). Thus the initial clinical presentation of neonates with breastfeeding-associated hypernatremia is a bodyweight loss of 10% or more, poor hydration state, lethargy, and poor feeding. Recognition may be delayed because these neonates may appear quiet and content initially, and, because of the slow development of the process, signs of extracellular volume contraction may be less prominent until the development of the full clinical presentation consisting of lethargy, irritability, hypotonia, and in some instances seizures, and cardiovascular collapse with renal failure. This presentation can be associated with serious CNS morbidity from both the hypertonicity (sagittal or other venous sinus thrombosis) and inappropriately rapid rehydration therapy (brain edema) (Lohr et al., 1989; van Amerongen et al., 2001). Although thorough follow-up studies of neonates with breastfeeding-associated severe hypernatremia are not available, observational studies suggest that up to 5% of these neonates experience brain damage (cerebral hemorrhage, edema, thrombosis or infarction) (Lavagno et al., 2016).

In the extremely immature neonate, early hypernatremia most commonly occurs from excessive transepidermal free water losses. The condition usually develops rapidly, within 24–72 hours after birth. The diagnosis is based on the attendant decrease in body weight, an increase in serum sodium concentration, and the clinical signs of extracellular volume contraction. Prevention of this condition can usually be accomplished by frequent monitoring of serum electrolyte levels, appropriate adjustments of free water intake, and the early use of humidified incubators (Modi, 2004). Application of ointments reduces TEWL but is associated with increased rates of sepsis and is thus not recommended (Edwards et al., 2004).

The central and nephrogenic forms of diabetes insipidus are much less common causes of hypernatremia and occur because of the lack of production of and renal responsiveness to vasopressin. Central diabetes insipidus can be congenital or can be acquired

secondary to neurologic insult (Chaudhary et al., 2011). Hypernatremia can also develop in response to excessive sodium supplementation, mainly in the sick neonate receiving repeated volume boluses for cardiovascular support or NaHCO_3 for metabolic acidosis. In these cases, clinical signs of edema, increased body weight, and the history of volume boluses help to establish the diagnosis.

Treatment of Hypernatremia. Thorough analysis of the medical history and the changes in clinical signs, laboratory findings, and body weight are necessary to determine the major etiologic factor in hypernatremia and thus the appropriate treatment. In the critically ill neonate, the cause of the serum sodium abnormality may be multifactorial, making the treatment strategy less straightforward. Although some cases of hypernatremia are a result of sodium excess with normal or high TBW, most cases in neonates are due to hypernatremic dehydration. Treatment of this condition is generally divided into two phases: the emergent phase, where the intravascular volume is restored, usually by administration of 10–20 mL of isotonic saline per kilogram, and the rehydration phase, where the sum of the remaining free water deficit and usual maintenance needs is administered evenly over at least 48 hours.

The free water deficit can be calculated as follows:

$$\text{H}_2\text{O Deficit (or Excess) (L)} \approx 0.7 \times \text{Body Weight (kg)} \times \left(\frac{[\text{Na}^+]_{\text{current}} (\text{mEq/L})}{[\text{Na}^+]_{\text{desired}} (\text{mEq/L})} - 1 \right).$$

In this formula, $(0.7 \times \text{body weight})$ is the estimation of TBW. When dehydration is diagnosed, correction should generally occur over 24 hours, with half of the correction occurring over the first 8 hours and the remainder over the next 16 hours. Longer correction times are indicated when dehydration is accompanied by moderate (serum sodium concentration >160 mEq/L) to severe (serum sodium concentration ≥ 175 mEq/L) hypernatremia, particularly when it is chronic as discussed earlier.

Alternatively, one can consider the amount of free water required to decrease the serum sodium concentration by a desired amount. The amount of free water required to decrease the serum sodium concentration by 1 mEq/L is 4 mL/kg with moderate hypernatremia but only 3 mL/kg when the serum sodium concentration is as high as 195 mEq/L (Molteni, 1994). Therefore the amount of free water required to decrease the serum sodium concentration by 12 mEq/L over a 24-hour period when hypernatremia is moderate (serum sodium concentration >160 mEq/L) is calculated as follows:

$$\text{Free Water Required} = \text{Current Weight (kg)} \times 4 \text{ mL/kg} \times 12 \text{ mEq/L}$$

or

$$\text{Free Water Required} = \text{Current Weight (kg)} \times 48 \text{ mL/kg per Day.}$$

The amount of free water required to decrease the serum sodium concentration by 12 mEq/L over a 24-hour period when hypernatremia is severe (serum sodium concentration >175 mEq/L) is calculated as follows:

$$\text{Free Water Required} = \text{Current Weight (kg)} \times 36 \text{ mL/kg per Day.}$$

The free water contents of the common IV fluids are listed in Table 30.2. It is important to note that sodium must be delivered with the free water replacement to avoid the hypernatremia being corrected too rapidly. In most mild to moderate hypernatremic

TABLE 30.2 Free Water Content (as Volume Percent) of Common Intravenous Solutions at Normal and High Serum Sodium Concentrations^a

| Intravenous Fluid | SERUM SODIUM CONCENTRATION | | | |
|----------------------------|----------------------------|-----------|--------------|-----------|
| | 145 mEq/L | | 195 mEq/L | |
| | Isotonic (%) | Water (%) | Isotonic (%) | Water (%) |
| 5% dextrose in water | 0 | 100 | 0 | 100 |
| 0.2% saline | 22 | 78 | 17 | 83 |
| 0.45% saline | 50 | 50 | 39 | 61 |
| 0.9% saline | 100 | 0 | 79 | 21 |
| Lactated Ringer's solution | 86 | 14 | 68 | 32 |

^aIsotonic saline provides 21% free water when given to a patient with a serum sodium concentration of 195 mEq/L and therefore will induce undesirable decreases in serum sodium concentration when used for volume resuscitation in the severely dehydrated hypernatremic neonate. Modified from Molteni KH. Initial management of hypernatremic dehydration in the breastfed infant. *Clin Pediatr*. 1994;33:731–740.

states (serum sodium concentration 150–160 mEq/L), during the rehydration phase, replacement fluids of 5% dextrose in 0.2% normal saline (31 mEq/L) or 0.45% normal saline (77 mEq/L) are appropriate. Infants with serum sodium levels greater than 165 mEq/L should initially be given 0.9% saline to avoid sudden drops in serum sodium concentration. When the serum sodium concentration is greater than 175 mEq/L, however, even normal saline will be hypotonic compared with the patient's serum. In these instances of severe hypernatremia, an appropriate amount of 3% saline (513 mEq/L) should be added to the IV fluid so that the sodium concentration in the fluid is approximately 10–15 mEq/L less than the serum sodium level (Rand and Kolberg, 2001). The relative free water content of an IV solution for a specific patient with sodium perturbations can be calculated with the formula:

Percentage of Free Water

$$= 1 - (\text{Intravenous Fluid Sodium} / \text{Serum Sodium}).$$

Serum electrolyte levels should be monitored every 2–4 hours until the desired rate of decline in serum sodium concentration is established. At this point, the frequency of the laboratory measurements can be relaxed to every 4–6 hours until the serum sodium concentration is less than 150 mEq/L. The speed of correction of hypernatremia depends on the rate of its development. This approach provides a reasonable chance that the serum sodium concentration will gradually decrease to the normal range over 2–4 days. Except in cases of acute massive sodium overload, the goal should be to lower the serum sodium concentration at a rate no greater than 1 mEq/L per hour. A slower pace of correction of 0.5 mEq/L per hour is prudent in patients with hypernatremia of chronic or unknown duration to avoid iatrogenic CNS sequelae.

While free water deficits are being corrected, the usual maintenance fluids and electrolytes must also be provided. Ongoing urine losses should be replaced volume for volume every 4–6 hours with a solution tailored to the urine's electrolyte concentration (usually 0.225%–0.45% normal saline). Extraordinary losses caused by open wounds, tubes, drains, ostomies, emesis, and/or diarrhea

TABLE 30.3 Approximate Electrolyte Composition of Body Fluids (mEq/L)

| Body Fluid | Sodium | Potassium | Chloride |
|-----------------|---------|-----------|----------|
| Gastric | 20–80 | 5–20 | 100–150 |
| Small intestine | 100–140 | 5–15 | 90–130 |
| Bile | 120–140 | 5–15 | 80–120 |
| Ileostomy | 45–135 | 3–15 | 20–115 |
| Diarrhea | 10–90 | 10–80 | 10–110 |

should always be considered in the dehydrated or hypernatremic infant and also accounted for in fluid management. The composition of this latter replacement solution depends on the electrolyte concentration of the fluid loss. The most common extraordinary loss, gastric fluid, contains significant amounts of sodium and chloride. See [Table 30.3](#) for approximate electrolyte compositions of body fluids. Because of the association between hyponatremia and neurologic injury in hospitalized pediatric patients ([Moritz and Ayus, 2003](#)), thoughtful consideration of fluid tonicity must be considered when the replacement fluid composition is being determined in the treatment of hypernatremia. Some have advocated routine administration of isotonic (“normal” saline) fluids regardless of sodium requirement to avoid “hospital-acquired hyponatremia” caused by overadministration of free water ([Moritz and Ayus, 2003](#); [Powell, 2015](#)). This approach is not without risk, given the overdose of sodium that occurs with administration of normal saline ([Holliday et al., 2004, 2007](#)). A more reasonable approach may be to base the appropriate fluid prescription on accurately assessed fluid deficits and ongoing requirements, with thoughtful consideration of sodium requirements of each compartment, as well as frequent monitoring of serum sodium changes ([Holliday et al., 2004, 2007](#)).

Once serum sodium concentration, urine output, and renal function are normal, the patient should receive standard maintenance fluids, either intravenously or orally, depending on his or her condition. Potassium replacement (usually by addition of 20–40 mEq of potassium per liter of replacement fluid) should not begin until adequate urine output has been established. At this time, electrolyte status must still be monitored for an additional 24 hours to ensure that complete recovery has occurred. Hyperglycemia and hypocalcemia commonly accompany hypernatremia. The use of insulin to treat the hyperglycemia is not recommended, because it can increase brain idiogenic osmol content. Hypocalcemia should be corrected with appropriate calcium supplementation.

Potassium Homeostasis and Management

Serum potassium concentration should be kept between 3.5 and 5 mEq/L. In the early postnatal period, neonates, especially immature preterm neonates, have higher serum potassium concentrations than older persons. The cause of the relative hyperkalemia of the newborn is multifactorial and involves developmentally regulated differences in renal function, sodium potassium–adenosine triphosphatase activity ([Vasarhelyi et al., 2000](#)), and hormonal milieu. Exposure to steroids prenatally in premature neonates is associated with a decreased incidence of hyperkalemia, believed to be due to improved renal function ([Omar et al., 2000](#)).

In general, potassium supplementation should be started only after urine output has been well established, usually by the third

postnatal day. Supplementation should be started at 1–2 mEq/kg per day and increased over 1–2 days to the usual maintenance requirement of 2–3 mEq/kg per day. Some preterm neonates may need more potassium supplementation after the completion of their postnatal volume contraction because of their increased plasma aldosterone concentrations, prostaglandin excretion, and disproportionately high urine flow rates. Most neonates will require additional potassium supplementation if they are receiving diuretics.

Hypokalemia. Hypokalemia in the neonate is usually defined as a serum potassium level of less than 3.5 mEq/L. Hypokalemia can occur from potassium loss due to diuretics, diarrhea, renal dysfunction, or nasogastric drainage from inadequate potassium intake or from shift of potassium into the intracellular compartment in the presence of alkalosis. Electrocardiogram (ECG) manifestations of hypokalemia include flattened T waves, prolongation of the QT interval, or the appearance of U waves. Except in patients receiving digoxin, hypokalemia is rarely symptomatic until the serum potassium concentration is less than 2.5 mEq/L. This degree of hypokalemia can result in cardiac arrhythmias, ileus, and lethargy.

Treatment of Hypokalemia. Hypokalemia is treated by slow replacement of potassium either intravenously or orally, usually in the daily fluids. Rapid administration of potassium chloride is not recommended because it may be associated with life-threatening cardiac dysfunction. In extreme emergencies, potassium can be given as an infusion over 30–60 minutes of not more than 0.3 mEq of potassium chloride per kilogram. If hypokalemia is secondary to alkalosis, the total body potassium content is usually normal, and the alkalosis should be corrected before an increase in the potassium intake is considered.

Hyperkalemia. Hyperkalemia in the neonate is defined as a serum potassium level greater than 6 mEq/L in a nonhemolyzed specimen. It is important to understand that most of the body’s potassium is contained within cells; therefore serum potassium levels do not accurately reflect total body stores. However, a serum potassium level greater than 6.5 to 7 mEq/L can be life threatening, even if total body stores are normal or low, because of its effect on cardiac rhythm. ECG manifestations of hyperkalemia include peaked T waves (the earliest sign), a widened QRS configuration, bradycardia, tachycardia, supraventricular tachycardia, ventricular tachycardia, and ventricular fibrillation. Because pH affects the distribution of potassium between the intracellular and the extracellular space, serum potassium levels may rise acutely during acidosis. The clinician should be aware of the potential for life-threatening arrhythmias to occur in infants with chronic lung disease receiving diuretics and potassium supplements who develop a sudden respiratory deterioration with acidosis.

Hyperkalemia is very common in the very preterm neonate, occurring in more than 50% of neonates weighing less than 1000 g ([Mildenberger and Versmold, 2002](#)). Another common cause of hyperkalemia is renal dysfunction, of particular concern in very preterm neonates and in neonates whose course is complicated by asphyxia or hypotension. In addition, hyperkalemia secondary to release of potassium from dying cells often complicates IVH, tissue ischemia (i.e., volvulus or necrotizing enterocolitis), and intravascular hemolysis. Less commonly, hyperkalemia may be one of the earliest manifestations of congenital adrenal hyperplasia or may occur because of other causes of neonatal acute adrenal insufficiency.

Treatment of Hyperkalemia. When hyperkalemia is diagnosed, all potassium intake should be discontinued, and the ECG should

TABLE 30.4 Medications Used for Treatment of Hyperkalemia

| Medication | Dosage | Onset | Length of Effects | Mechanism of Action | Comments and Cautions |
|------------------------|---|-----------------------------|-------------------|--|--|
| Calcium gluconate | 100 mg/kg intravenously over 2–5 min | Immediate | 30 min | Protects the myocardium from toxic effects of potassium; no effect on total body potassium | Can worsen digoxin toxicity |
| Sodium bicarbonate | 1–2 mEq/kg | Immediate | Variable | Shifts potassium intracellularly; no effect on total body potassium | Maximum infusion: mEq/min in emergency situations |
| Tromethamine | 3–5 mL/kg | Immediate | Variable | Shifts potassium intracellularly; no effect on total body potassium | — |
| Insulin plus dextrose | Insulin 0.1–0.15 U/kg intravenously plus dextrose 0.5 g/kg intravenously | 15–30 min | 2–6 h | Shifts potassium intracellularly; no effect on total body potassium | Monitor for hypoglycemia |
| Albuterol ^a | 0.15 mg/kg every 20 min for three doses then 0.15–0.3 mg/kg | 15–30 min | 2–3 h | Shifts potassium intracellularly; no effect on total body potassium | Minimum dose 2.5 mg |
| Furosemide | Per os: 1–4 mg/kg per dose once or twice per day Intravenously: 1–2 mg/kg per dose given every 12–24 h | 15 min to 1 h | 4 h | Increases renal excretion of potassium | — |
| Sodium polystyrene | 1 g/kg rectally every 6 h | 1–2 h (rectal route faster) | 4–6 h | Removes potassium from the gut in exchange for sodium | Use with extreme caution in neonates, especially preterm neonates; contains sorbitol; may be associated with bowel necrosis and sodium retention |

^aFrom Singh BS, Sadiq HF, Noguchi A, Keenan WJ. Efficacy of albuterol inhalation in treatment of hyperkalemia in premature neonates. *J Pediatr*. 2002;141:16–20.

be monitored. [Table 30.4](#) presents medications used in management of significant hyperkalemia. Calcium gluconate stabilizes cardiac membranes, and alkali therapy (sodium bicarbonate), insulin/glucose, and inhaled albuterol ([Singh et al., 2002](#)) all rapidly enhance cellular uptake of potassium and can cause a sharp drop in serum potassium levels in life-threatening situations but will not decrease total body potassium content. Intravenously administered furosemide and rectally administered sodium polystyrene sulfonate (Kayexalate) enhance potassium excretion and will lower total body stores but require at least several hours to take effect. Furthermore, use of polystyrene sulfonate to treat hyperkalemia in preterm neonates (<29 weeks' gestational age or <1250 g birth weight) has been associated with intestinal complications, including hematochezia and necrotizing enterocolitis ([Milley and Jung, 1995](#); [Rugolotto et al., 2007](#)). Dialysis or exchange transfusion may be used when the hyperkalemia is life threatening and other measures do not result in relief.

Clinical Conditions Associated With Fluid and Electrolyte Disturbances

Extreme Prematurity

Infants born between 23 and 27 weeks' gestation, or with a birth weight of less than 1000 g (i.e., ELBW), are at particular risk of acute abnormalities of both fluid and electrolyte status in the immediate postnatal period. TEWL is much higher than in more

mature preterm neonates (see [Fig. 30.4](#)), and thus it is difficult for water balance to be maintained unless this water loss is prevented. Historically, when ELBW infants were cared for in an open warmer without the use of a plastic heat shield or a humidified incubator, up to 150–300 mL of free water per kilogram per day could be lost through the skin during the first 3–5 days of postnatal life. Neonates whose mothers received glucocorticoids prenatally often have fewer problems because prenatally administered glucocorticoids enhance maturation of the epidermis and cardiovascular and renal function, resulting in increases in urine output and fractional excretion of sodium ([Omar et al., 1999](#); [Ali et al., 2000](#)).

Because serum sodium concentration is a reliable clinical indicator of extracellular tonicity, monitoring of this parameter every 6–12 hours during the first 2–3 days postnatally coupled with daily (or twice daily) measurements of body weight provides valuable information and appropriate guidance for the fluid and electrolyte management of the extremely immature preterm neonate. Serum osmolality may be directly measured in patients in whom calculated serum osmolality is more than 300–320 mOsm/L.

[Table 30.1](#) shows suggested maintenance fluid requirements by birth weight and postnatal day of life. Because immature neonates in an incubator with an ambient air humidity of 50%–80% require significantly less free water and less frequent measurements of serum electrolyte and osmolality ([Sedin, 1995](#)), open radiant warmers should rarely be used for these patients. Their initial parenteral fluid should contain between 5%–10% dextrose, protein

concentrations of 2.5–3.5 g/kg, and maintenance calcium, with no additional sodium or potassium. Fluid intake is then increased by 10–30 mL/kg per day every 6–12 hours if the serum sodium concentration rises from the baseline, the goal being to keep the serum sodium concentration below 145–150 mEq/L. As skin integrity improves during the course of the second to third days, serum sodium concentration starts to fall. At this time, a stepwise reduction in total fluid intake is obligatory to allow a complete contraction of the extracellular volume to occur and to minimize the possibility of free water overload with its attendant enhanced risks for the development of ductal patency, pulmonary edema, and worsening lung disease.

Critically ill, extremely immature neonates often receive excess sodium with volume boluses, medications, and the maintenance infusion of their arterial lines. Therefore extra sodium supplementation should usually not be started during the first few postnatal days, to prevent a rise in TBS concentration and thus extracellular volume, which will hinder the appropriate postnatal diuresis. However, one must be careful as a positive sodium balance is a prerequisite for appropriate growth, and thus after the transitional period, it must be ensured. Potassium chloride supplementation may be started as soon as urine output has been established and the serum potassium concentration is less than 5 mEq/L. Extremely premature neonates are at risk of the development of both oliguric and nonoliguric hyperkalemia, so the serum potassium concentration should be monitored closely, and supplementation should be discontinued if warranted by changes in serum potassium values or in renal function.

Many critically ill preterm neonates retain their originally high extracellular volumes, even when sodium and water intakes are restricted, and such neonates also tend to lose more bicarbonate in the urine. Proximal tubular bicarbonate reabsorption may be gestation-age appropriate even in the VLBW neonate despite the immaturity of its renal function, as long as extracellular volume contraction occurs (Ramiro-Tolentino et al., 1996). Therefore the presence of the extracellular volume expansion appears to be an important factor in the renal bicarbonate wasting in these neonates. The diagnosis of functional proximal tubular acidosis in such cases should not rely solely on the finding of an alkaline urine pH, because the distal tubular function is usually mature enough to acidify the urine once the serum bicarbonate concentration has decreased to its new threshold. Provided that liver function is normal, daily supplementation of bicarbonate, in the form of sodium acetate, potassium acetate, or both, rapidly begins to normalize blood pH and serum bicarbonate concentration in these neonates and also increases urine pH, aiding in the diagnosis. Once extracellular volume contraction occurs, these neonates generally achieve a positive bicarbonate balance (Ramiro-Tolentino et al., 1996), and supplementation becomes unnecessary.

Other general guidelines in the fluid and electrolyte management of the immature preterm neonate during the first postnatal week are (1) daily calculation of fluid balance and estimation of sodium balance, (2) daily measurements of body weight, serum electrolyte levels, and plasma glucose levels, and (3) testing of urine samples for glucose and osmolality or specific gravity. The frequency of testing and the addition of other tests, including the measurement of serum albumin concentration and osmolality, depend on the clinical status, the severity of the underlying disease, and the fluid and electrolyte disturbance of the individual patient. Serum creatinine and especially BUN levels are not accurate measures of fluid status in the first postnatal weeks, but following their trend can be helpful. There is little to no association between BUN levels

and protein intake (Ridout et al., 2005), even when changes in renal function are taken into account (Weintraub et al., 2015).

Transient Tachypnea of the Newborn

Transient tachypnea of the newborn is a self-limited respiratory complication in term and late preterm neonates caused by delayed clearance of fetal lung fluid in the immediate postdelivery period. These neonates present with mild to moderate respiratory distress frequently requiring supplemental oxygen and distending pressure provided by a nasal cannula, a high-flow nasal cannula, or continuous positive end-expiratory pressure for a duration ranging from 24–96 hours.

Fluid management for these patients can impact the duration of their illness. In one study, for late preterm and term neonates requiring respiratory support for greater than 48 hours (“severe” transient tachypnea of the newborn), patients with restricted fluid intake (40 mL/kg per day in term neonates and 60 mL/kg per day in late preterm neonates) compared with standard fluid intake (60 mL/kg per day in term neonates and 80 mL/kg per day in late preterm neonates) had decreased time on respiratory support, decreased hospital costs, and a trend toward decreased length of stay. There was no increase in hypoglycemia (defined as a blood glucose concentration <40 mL/dL) or other factors potentially affecting the safety of care (Stroustrup et al., 2012).

Respiratory Distress Syndrome

There is a well-established relationship between fluid and electrolyte imbalance and RDS. Surfactant deficiency results in pulmonary atelectasis, elevated pulmonary vascular resistance, poor lung compliance, and decreased lymphatic drainage. In addition, preterm neonates have low plasma oncotic and critical pulmonary capillary pressures and experience pulmonary capillary endothelial injury from mechanical ventilation, oxygen administration, and perinatal hypoxia (Sola and Gregory, 1981; Dudek and Garcia, 2001). These abnormalities alter the balance of the Starling forces in the pulmonary microcirculation, leading to interstitial edema formation, with further impairment in pulmonary functions.

In the presurfactant era, an improvement in pulmonary function occurred only during the third to fourth postnatal day. This improvement was usually preceded by a period of brisk diuresis characterized by small increases in glomerular filtration rate and sodium clearance and a larger rise in free water clearance (Costarino and Baumgart, 1991). Although the exact mechanism for this diuresis is not known, it is likely that improving endogenous surfactant production and capillary integrity promoted the recovery of the pulmonary capillary endothelium and lymphatic drainage. The ensuing changes in Starling forces then favored reabsorption of the hypotonic interstitial lung fluid into the circulation, increasing intravascular volume and organ perfusion, followed by the delayed “physiologic” diuresis. Prenatal administration of steroids and postnatal use of surfactants have clearly altered the course and clinical presentation of RDS (Kari et al., 1994; Ballard and Ballard, 1995; Carlton et al., 1995).

Bronchopulmonary Dysplasia

BPD is a multifactorial disease. Many risk factors have been associated with the development of BPD, including a high fluid and salt intake during the first few days to weeks of postnatal life. Specifically, higher fluid intake and lack of appropriate weight loss during the first 10 postnatal days are associated with significantly higher risk of BPD, even after other known risk factors have been controlled for (Oh et al., 2005). Furthermore, a recent study

found an association between a neonate's cumulative fluid intake in neonates with an intake of more than 345 mL/kg in the second through fourth postnatal days and an increased incidence of severe BPD (Guo et al., 2015). Therefore careful fluid and electrolyte management during the first few weeks of life, allowing the appropriate degree of weight loss, are of great importance in decreasing the incidence and severity of this condition (see Chapter 48).

Patent Ductus Arteriosus and Treatment With Indomethacin/Ibuprofen

Fluid, electrolyte, and acid–base management can affect the PDA, as increased fluid administration, increases in extracellular volume, and metabolic acidosis prolong patency of the ductus arteriosus (Hammerman, 1995; see Chapter 54). Accordingly, clinical management aimed at preventing the occurrence of ductal patency involves thoughtful management of fluid and electrolyte balance (Clyman, 1996).

In the preterm neonate, indomethacin administration has been shown to have clinically significant, although mostly transient, renal side effects because of decreased prostaglandin production through inhibition of cyclooxygenase. In the indomethacin-treated neonate, the unopposed renal vasoconstriction and sodium and water reabsorption lead to decreases in renal blood flow and glomerular filtration rate and to increases in sodium and free water reabsorption. These side effects occur despite the diminishing left-to-right shunt through the closing ductus. Characteristic clinical findings include a rise in serum creatinine level, oliguria, and hyponatremia (Cifuentes et al., 1979). Hyponatremia occurs because the free water retention caused by the unopposed renal actions of high plasma vasopressin levels is out of proportion to the sodium retention induced by angiotensin and noradrenaline. Fluid management of the preterm neonate receiving indomethacin must focus on maintaining an appropriately restricted fluid intake and avoiding extra sodium supplementation until urine output increases and renal function recovers. As the prostaglandin inhibitory effects of indomethacin diminish following the last dose, renal prostaglandin production returns to normal, and the retained sodium and excess free water are usually rapidly excreted, especially with the improvement in cardiovascular status as the ductal shunt decreases.

Ibuprofen is an alternative inhibitor of cyclooxygenase for the treatment of PDA and appears to have equivalent efficacy in closing the symptomatic PDA, with fewer adverse effects (Ohlsson et al., 2015). Specifically, ibuprofen administration is associated with less renal and gastrointestinal (GI) dysfunction (Van Overmeire et al., 2000) and no apparent effect on cerebral perfusion (Mosca et al., 1997; Patel et al., 2000). The lack of an effect on cerebral perfusion likely explains why prophylactic ibuprofen does not reduce the incidence of severe IVH (Ohlsson and Shah, 2015). For more details, see Chapter 61.

Syndrome of Inappropriate Antidiuretic Hormone Secretion

In the preterm and term newborn, SIADH may be associated with birth asphyxia, intracerebral hemorrhage, RDS, pneumothorax, and the use of continuous positive pressure ventilation (El-Dahr and Chevalier, 1990; Hidalgo-Mora et al., 2011). It has also been described in relation to infectious diseases, including meningitis, bronchiolitis, and pneumonia. The syndrome is characterized by oliguria, free water retention, decreased serum sodium concentration and serum osmolality, increased urine concentration, and weight gain caused by edema formation. However, because the urinary concentrating capacity of the newborn is limited, a less than

maximally diluted urine satisfies the diagnosis of SIADH in the presence of the other symptoms. The treatment is based on fluid and sodium restriction despite the oliguria and hyponatremia, as well as on appropriate circulatory and ventilatory support. The clinician must remember that TBS content is normal, but TBW content is elevated in such a neonate, and that it is particularly dangerous to treat the hyponatremia caused by free water retention with large amounts of sodium. Because of their more immature renal function, ELBW neonates during the first few weeks of postnatal life usually do not exhibit the full-blown syndrome despite their sometimes excessively high plasma vasopressin levels (Aperia et al., 1983).

Diminished vasopressin secretion or complete unresponsiveness of the renal tubules to vasopressin results in polyuria, dilute urine production, and increased serum osmolality (Werny et al., 2015), otherwise known as *diabetes insipidus*. This condition is not common in neonates but can occur in association with CNS injury or disease, such as in meningitis, in cerebral hemorrhage affecting the pituitary gland (central diabetes insipidus), or in an inherited form (nephrogenic diabetes insipidus). In addition, diabetes insipidus is one of the features of congenital developmental abnormalities also affecting the pituitary gland, such as septo-optic dysplasia (de Morsier syndrome). The treatment of neonates with this condition consists of facilitating adequate free water intake and the use of desmopressin with or without chlorothiazide (Nofal and Lteif, 2015).

Surgical Conditions

Surgery has a major effect on metabolism, fluid balance, and electrolyte balance in the newborn. Preterm neonates with acute or chronic lung disease are especially sensitive and respond to the procedure with significant catabolic responses, increases in capillary permeability, with the attendant shift of fluid into the interstitial space, and retention of sodium and free water (John et al., 1989). The retention of sodium and free water is secondary to the decrease in effective circulating blood volume and to the increased plasma levels of sodium-retaining and water-retaining hormones, including catecholamines, renin–angiotensin–aldosterone, and vasopressin.

Preoperative management has a significant effect on outcome and should aim at maintaining adequate effective circulating blood volume as well as cardiovascular and renal function. In preterm neonates who have evidence of absolute or relative adrenal insufficiency (Watterberg, 2002), as well as those who have received prolonged steroid therapy for hypotension or lung disease, the provision of stress doses of steroids may be necessary. In the postoperative period, appropriate close monitoring and maintenance of the integrity of the cardiovascular system through the judicious use of volume expanders and vasopressor–inotropic support, if required, meticulous replacement of ongoing surgical and nonsurgical fluid and electrolyte losses, close monitoring, and intense and effective communication between the neonatal team and the surgical team are essential to ensure a successful outcome. As capillary integrity improves, reabsorption and excretion of the expanded interstitial fluid volume occurs, with normalization in the secretion of hormones regulating fluid and electrolyte balance. At this time the provision of maximized nutritional support becomes essential to restore the anabolic state and growth of the neonate.

The most commonly encountered surgical water losses occur when a nasogastric tube is placed under continuous suction to provide relief for the GI tract in conditions such as necrotizing enterocolitis and postoperative management after abdominal surgery. Because these losses may be substantial, they should be monitored

and a portion of the losses should be replaced every 6–12 hours to maintain appropriate water and electrolyte balance. However, free water retention often develops after surgery; therefore full replacement of the nasogastric free water loss is not usually recommended. One of the approaches to the fluid–electrolyte management of postoperative neonates may be to replace half of the removed/lost volume or increase the total fluid limit for a period to account for these losses. The composition of the replacement solution depends on the electrolyte concentration of the fluid loss. Gastric fluid usually contains 50–60 mEq of sodium chloride per liter, and therefore 0.45% sodium chloride with potassium is normally used as the fluid of choice for replacement. See Table 30.3 for estimated electrolyte compositions of body fluids.

Acid–Base Balance

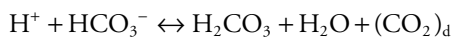
Physiology of Acid–Base Balance Regulation

Like adults, newborns must maintain their extracellular pH, or hydrogen ion concentration, within a narrow range. A normal pH is essential for intact functioning of all enzymatic processes and therefore the intact functioning of all organ systems of the body. Newborns are subjected to many stresses that can affect their acid–base balance. In addition, neonates, especially if they are premature, have a limited ability to compensate for acid–base alterations; therefore acid–base disturbances are common in the neonatal period. An understanding of the principles of acid–base regulation is essential for proper diagnosis and treatment of these disturbances.

In healthy humans, the normal range of ECF hydrogen ion (H^+) concentration is 35–45 mEq/L. Because pH is defined as the negative logarithm of hydrogen ion concentration ($pH = -\log[H^+]$), these hydrogen ion concentrations correspond to a pH range of 7.35–7.45. Acidosis is a downward shift in pH to less than 7.35, and alkalosis is an upward shift in pH to more than 7.45. Alterations in normal pH are resisted by complex physiologic regulatory mechanisms. The main systems that maintain pH are the body's buffer systems, the respiratory system, and the kidneys. Some of these systems respond immediately to sudden alterations in hydrogen ion concentration, whereas others respond more slowly to changes but maintain the overall balance between acid and base production, intake, metabolism, and excretion over the long term.

The physiologic regulatory systems that respond immediately to changes in acid–base balance include the various intracellular and extracellular buffers as well as the lungs. A buffer is a substance that can minimize changes in pH when acid or base is added to the system. The extracellular buffers, which include the bicarbonate–carbonic acid system, phosphates, and plasma proteins, act rapidly to return the extracellular pH toward normal. The intracellular buffers, which include hemoglobin, organic phosphates, and bone apatite, act more slowly and require several hours to reach maximal capacity.

The most important extracellular buffer is the plasma bicarbonate–carbonic acid buffer system, in which the acid component (carbonic acid [H_2CO_3]) is regulated by the lungs, and the base component (bicarbonate [HCO_3^-]) is regulated by the kidneys. The buffer equation is as follows:



where $(CO_2)_d$ represents the dissolved carbon dioxide. At equilibrium, the amount of dissolved CO_2 exceeds that of H_2CO_3 by a factor of 800:1; therefore for practical purposes, dissolved CO_2

and H_2CO_3 can be treated interchangeably. The fact that CO_2 excretion can be controlled by the respiratory system markedly increases the efficiency of this buffer system at physiologic pH. The enzyme carbonic anhydrase allows rapid interconversion of H_2CO_3 to H_2O and CO_2 . If the hydrogen ion (H^+) concentration increases for any reason, hydrogen combines with HCO_3^- , driving the buffer reaction toward greater production of H_2CO_3 and CO_2 . CO_2 crosses the blood–brain barrier and stimulates CNS chemoreceptors, leading to increased alveolar ventilation and decreased concentration of extracellular CO_2 . This respiratory compensation begins within minutes after a pH change and is complete within 12–24 hours. A similar compensation occurs in response to a decrease in H^+ concentration, leading to decreased alveolar ventilation and a resultant increase in extracellular CO_2 concentration.

The relationship between the two components of the bicarbonate–carbonic acid buffer system and pH is expressed by the Henderson–Hasselbalch equation:

$$pH = pK_a + \log \left(\frac{[HCO_3^-]}{[H_2CO_3]} \right).$$

Because H_2CO_3 is in equilibrium with the dissolved CO_2 in the plasma, and because the amount of dissolved CO_2 depends on the partial pressure of CO_2 , the equation can be modified as follows:

$$pH = pK_a + \log \left(\frac{[HCO_3^-]}{0.03} \right) \times PaCO_2.$$

Both the original equation and the modified equation are clinically difficult to use; therefore the modified Henderson–Hasselbalch equation can be rewritten as the Henderson equation without logarithms for easier clinical use:

$$[H^+] = 24 \times \frac{PaCO_2}{[HCO_3^-]}.$$

This last equation clearly describes the clinically most important aspect of acid–base regulation by the bicarbonate–carbonic acid buffer system, that the change in the ratio of $PaCO_2$ to HCO_3^- concentration, and not in their absolute values, determines the direction of change in H^+ concentration and thus in pH. The status of the plasma bicarbonate–carbonic acid buffer system can be monitored easily by serial blood gas measurements, making understanding of this buffer system important in clinical care.

The physiologic regulation system that responds more slowly to changes in acid–base balance is the renal system. There must be a long-term balance between net acid increase caused by intake and production and net acid decrease caused by excretion and metabolism. Although infant formula and protein-containing IV fluids have small amounts of preformed acid, most of the daily acid load is derived from metabolism. A large amount of the acid produced is in the form of the volatile H_2CO_3 , which can be excreted in the lungs. Nonvolatile or fixed acids are also produced and must be excreted through the kidneys. The nonvolatile acids are normally sulfuric acid produced in the metabolism of the amino acids methionine and cysteine and, to a lesser extent, phosphoric acid, lactic acid, hydrochloric acid, and incompletely oxidized organic acids. In addition to the excretion of nonvolatile acids, however, the kidneys have a role in long-term acid–base regulation by controlling renal HCO_3^- excretion.

Two regions of the kidney act to achieve urinary acidification: the proximal tubule and the collecting tubule. The proximal tubule acidifies the urine by two mechanisms. The first mechanism is by the reabsorption of any HCO_3^- already present in the blood that

is being constantly filtered through the glomeruli. The proximal tubule reabsorbs 60%–80% of all filtered HCO_3^- and performs this role through the exchange of Na^+ for H^+ across the luminal membrane of the proximal tubular cells via the Na^+/H^+ exchanger. The excreted H^+ combines with filtered HCO_3^- , producing H_2CO_3 through the activity of carbonic anhydrase in the cellular brush border. The H_2CO_3 is then quickly converted to CO_2 , which crosses into the tubular cell, where HCO_3^- is regenerated and reabsorbed back into the bloodstream, mostly in exchange for chloride (Cl^-). The regenerated H^+ ion reenters the cycle at the Na^+/H^+ exchanger.

The second mechanism by which the proximal tubule acidifies urine is by the production of ammonia (NH_3). Inside the tubular cell, NH_3 is produced by the deamination of glutamine. The NH_3 is secreted into the tubular lumen, where it combines with and traps free H^+ to form ammonium (NH_4^+).

The remaining urinary acidification occurs mostly in the collecting tubule. H^+ secreted in this region of the kidney is sufficient to combine with or titrate any remaining filtered HCO_3^- or any filtered anions, such as phosphate and sulfate. Hydrogenated phosphate and sulfate anions produce the titratable acid of the urine. The collecting tubule also takes up NH_3 from the medullary interstitium and secretes it into the urine, where again it can combine with and trap H^+ as NH_4^+ . This urinary NH_4^+ can act as a cation and can be excreted with urinary anions such as Cl^- , phosphate (PO_4^-), and sulphate (SO_4^-), thereby preventing loss of cations such as Na^+ , Ca^{2+} , and K^+ . Total acid secretion in the kidney can be represented by

$$\text{Titratable Acid} + \text{NH}_4^+ - \text{HCO}_3^-$$

and under normal conditions should equal the net production of acid from the diet and metabolism that is not excreted in the form of CO_2 through the lungs.

In adults, the steady state for renal compensation for respiratory alkalosis is reached within 1–2 days and that for respiratory acidosis is reached within 3–5 days. Newborns are able to compensate for acidemia through the previously described renal mechanisms, although the renal response to acid loads is limited, especially in premature neonates born before 34 weeks' gestation. Reabsorption of HCO_3^- in the proximal tubule and distal tubular acidification are also decreased, with a fairly rapid gestational age-dependent maturation of these functions after birth (Jones and Chesney, 1992).

To accomplish the tight regulation of pH necessary for survival, H^+ ions generated in the form of the volatile acid H_2CO_3 are excreted by the lungs as CO_2 . H^+ ions generated in the form of nonvolatile acids are buffered rapidly by extracellular HCO_3^- and more slowly by intracellular buffers. HCO_3^- is then replenished by the kidneys via the reabsorption of much of the filtered HCO_3^- and by the excretion of H^+ in the urine as NH_4^+ and titratable acids.

Disturbances of Acid–Base Balance in the Newborn

General Principles

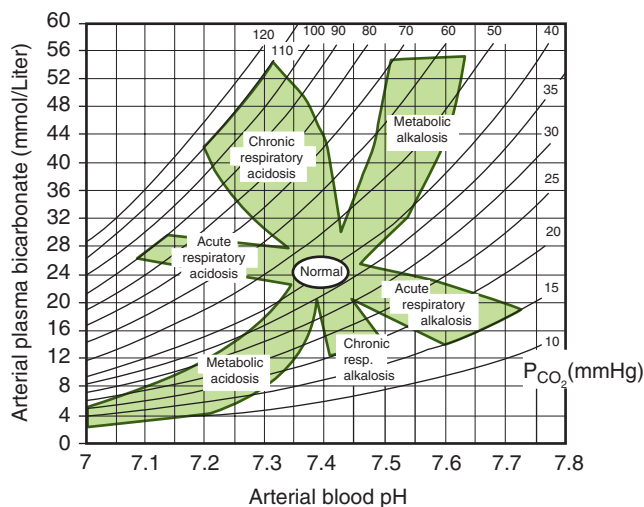
The evaluation of the acid–base status in a newborn is one of the most common laboratory assessments made in the neonatal intensive care unit. The status of this system can be monitored with blood gas measurements and should be the starting point for the evaluation of any acid–base disorder. In the blood gas measurement, the pH and PaCO_2 levels are directly measured; from these, the HCO_3^- level and base excess or deficit are calculated.

The whole blood buffer base, defined as the sum of the HCO_3^- and non- HCO_3^- buffer systems, is another important blood gas value used in evaluating acid–base disturbances. The difference between the observed whole blood buffer base of any blood gas sample and the expected normal buffer base of that sample is called the base excess or base deficit. The base excess and base deficit give an accurate measure of the amount of strong acid or base respectively that would be needed to titrate the pH back to normal once the respiratory contribution of the acid–base disturbance is also corrected. For example, a base excess of 10 mEq/L indicates that there is an additional 10 mEq of base per liter (or loss of 10 mEq of H^+ per liter) that is contributing to the acid–base abnormality. Conversely, a base deficit of 10 mEq/L indicates there is relatively more acid (or less base) in the ECF than expected after the effect of PaCO_2 on pH has been accounted for.

Acid–base disorders are classified according to their cause as being either metabolic or respiratory. Metabolic acidosis occurs as a result of the accumulation of increased amounts of nonvolatile acid or decreased amounts of HCO_3^- in the ECF. Metabolic alkalosis occurs as a result of increased amounts of HCO_3^- in the ECF. Respiratory acidosis is caused by hypoventilation and decreased excretion of volatile acid (CO_2), whereas respiratory alkalosis is caused by hyperventilation and increased excretion of CO_2 .

Acid–base disorders are also classified according to the number of conditions causing the disorder. When only one primary acid–base abnormality and its compensatory mechanisms occur, the disorder is classified as a *simple acid–base disorder*. When a combination of simple acid–base disturbances occurs, the patient has a mixed (or complex) acid–base disorder. Because secondary physiologic regulatory mechanisms often compensate for the alteration in pH caused by primary disturbances, it is sometimes difficult to differentiate simple from mixed disorders or even a simple disorder from its resulting compensation. One important principle that allows the determination of primary acid–base disturbance is that the compensatory regulatory mechanisms do not completely normalize the pH.

Nomograms, such as the one shown in Fig. 30.6, can help in the diagnosis of the primary disturbance. The nomogram describes the 95% confidence limits of the expected compensatory response



• Fig. 30.6 Acid–base nomogram illustrating the 95% confidence limits for compensatory responses to primary acid–base disorder. (From Cogan MG, Rector Jr FC. Acid-base disorders. In: Brenner BM, Rector Jr FC, eds. *The Kidney*. Philadelphia, PA: WB Saunders; 1986.)

TABLE 30.5**Expected Compensatory Mechanisms Operating in Primary Acid–Base Disorders**

| Acid–Base Disorder | Primary Event | Compensation | Rate of Compensation |
|------------------------------|--|--|--|
| Metabolic Acidosis | | | |
| Normal anion gap | Decreased HCO_3^- concentration | Decreased PCO_2 | For 1 mEq/L decrease in HCO_3^- concentration, PCO_2 decreases by 1–1.5 mmHg |
| Increased anion gap | Increased acid production Increased acid intake | Decreased PCO_2 | For 1 mEq/L decrease in HCO_3^- concentration, PCO_2 decreases by 1–1.5 mmHg |
| Metabolic Alkalosis | Increased HCO_3^- concentration | Increased PCO_2 | For 1 mEq/L increase in HCO_3^- concentration, PCO_2 increases by 0.5–1 mmHg |
| Respiratory Acidosis | | | |
| Acute (<12–24 h) | Increased PCO_2 | Increased HCO_3^- concentration | For 10 mmHg increase in PCO_2 , HCO_3^- concentration increases by 1 mEq/L |
| Chronic (3–5 days) | Increased PCO_2 | Increased HCO_3^- concentration | For 10 mmHg increase in PCO_2 , HCO_3^- concentration increases by 4 mEq/L |
| Respiratory Alkalosis | | | |
| Acute (<12 h) | Decreased PCO_2 | Decreased HCO_3^- concentration | For 10 mmHg increase in PCO_2 , HCO_3^- concentration increases by 1–3 mEq/L |
| Chronic (1–2 days) | Decreased PCO_2 | Decreased HCO_3^- concentration | For 10 mmHg decrease in PCO_2 , HCO_3^- concentration decreases by 2–5 mEq/L |

 HCO_3^- , Bicarbonate; PCO_2 Modified from Brewer ED. Disorders of acid-base balance. *Pediatr Clin North Am.* 1990;37:429–447.

to a primary abnormality in either PaCO_2 or HCO_3^- concentration. Table 30.5 summarizes the expected respiratory and metabolic compensatory mechanisms for primary acid–base disorders (Brewer, 1990). If the compensation in a given patient differs from that predicted in Fig. 30.6 or Table 30.5, the patient either has not had enough time to compensate for a simple acid–base disturbance or has a mixed acid–base disorder. Furthermore, the complete correction of an acid–base disturbance occurs only when the underlying process responsible for the abnormality has been treated effectively.

For identification of the primary disturbance, the analysis of blood gas values must be considered in light of the patient's history and physical findings and with an understanding of expected compensatory responses. Further laboratory evaluation is indicated if the problem is not immediately obvious or if the response to therapy is not as expected. The evaluation of the acid–base disturbance should always involve efforts to determine the underlying cause of the disturbance, because adequate treatment requires correction of the underlying disorder, if possible.

Transitional Physiology After Birth

As part of a discussion of normal physiology, it is important to understand the in utero environment just before delivery of the newborn and its effects on neonatal acid–base analysis shortly after birth. Hyperventilation of pregnancy is a known phenomenon with corresponding maternal PaCO_2 levels of approximately 31–34 mmHg (Thorp and Rushing, 1999). This relative respiratory alkalosis in the mother is compensated for by a corresponding metabolic acidosis in the mother and therefore in the fetus. As a result, umbilical arterial blood gases have a normal pH range of 7.20–7.28, with a corresponding base deficit ranging from 2.7 ± 2.8 mEq/L to 8.3 ± 4.0 mEq/L (Sykes et al., 1982; Riley and Johnson, 1993).

In other words, a mild metabolic acidosis in the newborn shortly after birth can be expected and explained by normal physiology.

Metabolic Acidosis

Metabolic acidosis is a common problem, particularly in the critically ill newborn. Metabolic acidosis occurs when the drop in pH is caused by the accumulation of acid other than H_2CO_3 in the ECF, resulting in loss of available HCO_3^- , or by the direct loss of HCO_3^- from body fluids. Patients who have metabolic acidosis are divided into those with an elevated anion gap and those with a normal anion gap.

The anion gap reflects the unaccounted acidic anions and certain cations in the ECF. The unmeasured anions normally include the serum proteins, phosphates, sulfates, and organic acids, whereas the unaccounted cations are the serum potassium, calcium, and magnesium ions. Thus in clinical practice, the anion gap is estimated with the formula:

$$\text{Anion Gap} = [\text{NH}_4^+]_{\text{serum}} - ([\text{Cl}^-]_{\text{serum}} + [\text{HCO}_3^-]_{\text{serum}}).$$

The normal range of the serum anion gap in newborns is 8–16 mEq/L, with slightly higher values in very premature newborns. Accumulation of strong acids because of increased intake, increased production, or decreased excretion results in an increased anion gap acidosis, whereas loss of HCO_3^- or accumulation of H^+ results in a normal anion gap acidosis. A decrease in serum potassium, calcium, and magnesium concentrations, an increase in serum protein concentration, or a falsely elevated serum sodium concentration can also result in an increased anion gap in the absence of metabolic acidosis. In clinical practice, although a serum anion gap value greater than 16 mEq/L is highly predictive of the presence of lactic acidosis and a value less than 8 mEq/L is highly predictive of the absence of lactic acidosis, an anion gap value between 8

and 16 mEq/L cannot be used to differentiate between lactic and nonlactic acidosis in the critically ill newborn (Lorenz et al., 1999). Therefore if the anion gap is within this high normal range and lactic acidosis is suggested, measurement of serum lactate is indicated. At present, with the routine availability of measurement of lactic acid from low-volume blood samples by blood gas machines, the determination of the origin of acidosis ("anion gap," i.e., net strong acid gain, or "non-anion gap," i.e., buffer loss–induced acidosis) has become simpler in most clinical presentations of metabolic acidosis.

An increased anion gap metabolic acidosis in the newborn is most commonly caused by lactic acidosis secondary to tissue hypoxia, as seen in asphyxia, hypothermia, severe respiratory distress, sepsis, necrotizing enterocolitis, and other severe neonatal illnesses. Other important but much less common causes of an increased anion gap metabolic acidosis in the neonatal period are inborn errors of metabolism, renal failure, and intake of toxins (Box 30.3). Box 30.4 lists inborn errors of metabolism that can manifest themselves as increased anion gap metabolic acidosis in the newborn period.

In the syndrome of late metabolic acidosis of prematurity, first described in the 1960s, otherwise healthy premature infants at several weeks of age demonstrated mild to moderate increased anion gap acidosis and decreased growth. All the infants were receiving high-protein cow's milk formula and presented with higher

net acid excretion compared with controls. However, this type of late metabolic acidosis is rarely seen nowadays, probably because of the use of special premature infant formulas and changes in regular formulas with decreased casein-to-whey ratios and lower fixed acid loads.

A normal anion gap metabolic acidosis most commonly occurs in the newborn as a result of HCO_3^- loss from the extracellular space through the kidneys or the GI tract. Hyperchloremia develops with the HCO_3^- loss because a proportionate rise in serum chloride concentration must occur to maintain the ionic balance or to correct the volume depletion in the extracellular compartment. The most common cause of normal anion gap metabolic acidosis in the preterm newborn is a mild, developmentally regulated, proximal renal tubular acidosis with renal HCO_3^- wasting. In newborns with this disorder, the serum HCO_3^- concentration usually stabilizes at 14–18 mEq/L in the early postnatal period. The urinary pH is normal once the serum HCO_3^- has stabilized at this level, because the impairment in proximal tubular HCO_3^- reabsorption is not associated with an impaired distal tubular acidification of similar magnitude (Jones and Chesney, 1992). The diagnosis of this temporary cause of acidosis can be established by the recurrence of a urinary alkaline pH when serum HCO_3^- concentration is raised above the threshold after HCO_3^- or acetate supplementation. Even term newborns have a lower renal threshold for HCO_3^- than adults with normal plasma HCO_3^- levels in the range of 17–21 mEq/L. In most infants, plasma HCO_3^- concentration increases to adult levels over the first year as the proximal tubule matures. Other common causes of normal anion gap metabolic acidosis seen in neonatal intensive care units are GI HCO_3^- losses, often caused by increased ileostomy drainage, diuretic treatment with carbonic anhydrase inhibitors, and dilutional acidosis, with rapid expansion of the extracellular space through the use of non- HCO_3^- solutions such as 0.9% sodium chloride in the hypovolemic newborn.

The presence of metabolic acidosis in the newborn may be suspected by the clinical presentation and the history of predisposing conditions, including perinatal depression, respiratory distress, blood or volume loss, sepsis, and congenital heart disease associated with poor systemic perfusion or cyanosis. Metabolic acidosis is confirmed by blood gas measurements. Specific laboratory evaluation of electrolytes, renal function, lactate, and serum and urine amino acids may be undertaken, depending on the diagnosis that is suggested clinically. Fig. 30.7 shows a simple flow diagram outlining an approach to diagnosis of metabolic acidosis in the newborn. It is important to emphasize that newborns might not manifest an increased anion gap in the setting of lactic acidosis (Lorenz et al., 1999). However, the availability of lactic acid measurement along with the blood gas measurements in today's intensive care units aids in the rapid determination of the type of the lactic acidosis.

The morbidity and mortality associated with metabolic acidosis depend on the underlying pathologic process, the severity of the acidosis, and the responsiveness of the process to clinical management. By far the most important intervention for a newborn with a metabolic acidosis is identification of the pathologic process responsible for the development of acidosis and the taking of measures to correct it. It is important to emphasize that the indiscriminate administration of a base, such as sodium bicarbonate, as supportive therapy for all types of metabolic acidosis is unproven in its efficacy (Aschner and Poland, 2008).

The question facing neonatal providers is how low does the pH need to fall before base administration might be associated with improvement in clinical condition for newborns with metabolic

• BOX 30.3 Common Causes of Metabolic Acidosis

Increased Anion Gap

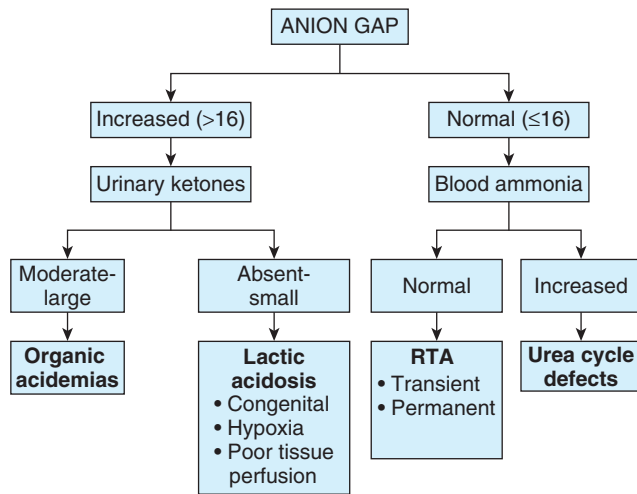
- Lactic acidosis caused by tissue hypoxia
 - Asphyxia, hypothermia, shock
 - Sepsis, respiratory distress syndrome
- Inborn errors of metabolism
 - Congenital lactic acidosis
 - Organic acidosis
- Renal failure
- Late metabolic acidosis
- Toxins (e.g., benzyl alcohol)

Normal Anion Gap

- Renal bicarbonate loss
 - Bicarbonate wasting caused by immaturity
 - Renal tubular acidosis
 - Carbonic anhydrase inhibitors
- Gastrointestinal bicarbonate loss
 - Small bowel drainage: ileostomy, fistula
 - Diarrhea
- Extracellular volume expansion with bicarbonate dilution
- Aldosterone deficiency
- Excessive chloride in intravenous fluids

• BOX 30.4 Inborn Errors of Metabolism Associated With Metabolic Acidosis

- Primary lactic acidosis
- Organic acidemias
- Pyruvate carboxylase deficiency
- Pyruvate hydroxylase deficiency
- Galactosemia
- Hereditary fructose intolerance
- Type I glycogen storage disease



• **Fig. 30.7** Diagnostic Approach of Increased Anion Gap and Normal Anion Gap Metabolic Acidosis in the Newborn. Newborns with lactic acidosis may not have an increased anion gap, and lactate concentration should be directly measured if this is suspected from the history and physical examination. RTA, Renal tubular acidosis. (Modified from Lorenz JM, Kleinman LI, Markarian K, Oliver M, Fernandez J. Serum anion gap in the differential diagnosis of metabolic acidosis in critically ill newborns. *J Pediatr.* 1999;135:751–755)

or severe mixed acidosis. An early study suggested that NaHCO_3 administration to mechanically ventilated preterm and term neonates receiving neuromuscular blockade with an arterial pH of less than 7.25 transiently improved systemic and organ blood flow (Fanconi et al., 1993). However, in a more recent study using more sophisticated hemodynamic evaluation in hemodynamically stable preterm neonates born at 30 weeks' gestation or earlier, myocardial contractility was found to be unaffected by acidosis even at pH values close to 7.00 during the first 2 weeks postnatally. However, and similar to that seen in adults, worsening acidosis in more than 3-day-old preterm neonates was associated with decreased systemic vascular resistance and increased left ventricular output. However, there was no effect of acidosis on vascular resistance and cardiac output during the immediate postnatal period (less than 4 days of postnatal life). It is clear that more data are needed to adequately address this question, especially because of the concern for harm associated with the administration of base. Indeed, a number of studies have shown an association between the use of base and increased mortality and incidence of IVH (Usher, 1967; Simmons et al., 1974; Papile et al., 1978), increased cerebral blood volume regardless of the rate of administration (van Alfen-van der Velden et al., 2006), and decreased intracellular pH with cellular injury (Lipshultz et al., 2003).

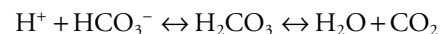
If a decision has been made to administer base, the clinician has traditionally had three options: sodium bicarbonate, sodium (or potassium) acetate, and tromethamine. NaHCO_3 is the most widely used buffer in the treatment of metabolic acidosis in the neonatal period. Bicarbonate should not be given if ventilation is inadequate, because its administration results in an increase in PaCO_2 with little to no increase in pH and an increase in intracellular acidosis. Therefore NaHCO_3 should be administered slowly and in diluted form only to newborns with documented metabolic acidosis and adequate alveolar ventilation. Once a blood gas measurement has been obtained, the dose of NaHCO_3 required to fully correct the pH can be estimated with the use of the following formula:

$$\text{Dose of NaHCO}_3 \text{ (mEq)} = \text{Base Deficit (mEq/L)} \times \text{Body Weight (kg)} \times 0.3.$$

NaHCO_3 is confined mostly to the ECF compartment. Although there are controversies regarding the actual bicarbonate space in humans, body weight (kg) \times 0.3 (or 30% of the infant's total body weight) represents its estimated volume of distribution in the neonate. Most clinicians would use half of the calculated total correction dose for initial therapy to avoid overcorrection of metabolic acidosis. Subsequent doses of NaHCO_3 are then based on the results of additional blood gas measurements.

When clinicians are faced with a chronic non-anion gap metabolic acidosis caused by a prematurity-related proximal renal tubular acidosis with bicarbonate wasting, many choose to replace these losses over time. In this instance, either sodium acetate or potassium acetate can be used as an alternative to NaHCO_3 . Sodium acetate is a conjugate base of a weak acid (acetic acid) with a pK_b of 9.25; pK_b is a measure of the strength of a base, which depends on its base dissociation constant. Sodium acetate has been shown in one study to be an effective alternative to NaHCO_3 in correcting this type of acid–base abnormality when added to parenteral nutrition (Peters et al., 1997). The median dosages of acetate used in this randomized controlled trial were 2.6 mmol/kg per day on postnatal day 4 of life and 4.1 mmol/kg per day on postnatal day 8. Neonates randomized to receive acetate had an increased base excess, pH, and PaCO_2 , and they received less bicarbonate boluses compared with control neonates.

Historically, tromethamine has been used as an alternative buffer to sodium bicarbonate, but in recent times its commercial availability has been intermittently limited. The theoretical advantages of tromethamine over NaHCO_3 in the treatment of metabolic acidosis include its more rapid intracellular buffering capability, its ability to lower PaCO_2 levels directly, and the lack of an increase in the sodium load (Schneiderman et al., 1993). Tromethamine lowers PaCO_2 by covalently binding H^+ and thus shifting the equilibrium of the reaction



to the left, resulting in a decrease in the amount of CO_2 and an increase in the amount of HCO_3^- . Tromethamine administration has been associated with the development of acute respiratory depression, most likely secondary to an abrupt decrease in PaCO_2 levels as well as from rapid intracellular correction of acidosis in the cells of the respiratory center (Robertson, 1970). In addition, when large doses of tromethamine are administered, hyponatremia (Seri et al., 1998), hypoglycemia, hyperkalemia, an increase in hemoglobin oxygen affinity, and diuresis followed by oliguria can occur. As tromethamine is hyperosmolar, a slow infusion rate is recommended at a dose calculated from the formula:

$$\text{Dose of Tromethamine (mL)} = \text{Base Deficit (mEq/L)} \times \text{Body Weight (kg)}.$$

Finally, during the correction of metabolic acidosis, regardless of the method chosen, particular attention should be paid to ensuring an appropriate potassium balance. Because potassium moves from the intracellular to the extracellular space in exchange for H^+ when acidosis occurs, the presence of a total body potassium deficit might not be appreciated during metabolic acidosis. Hypokalemia may become evident only as the pH increases and potassium returns to the intracellular space. Furthermore, intracellular acidosis cannot be completely corrected until the potassium stores are restored. Therefore close monitoring of serum electrolyte levels and careful

potassium supplementation are important during the correction of metabolic acidosis in the sick newborn.

Respiratory Acidosis

Respiratory acidosis occurs when a primary increase in PaCO_2 develops secondary to impairments in alveolar ventilation that result in an arterial pH of less than 7.35. Primary respiratory acidosis is a common problem in newborns and causes include hyaline membrane disease, pneumonia owing to infection or aspiration, PDA with pulmonary edema, chronic lung disease, pleural effusion, pneumothorax, and pulmonary hypoplasia. The initial increase in PaCO_2 is buffered by the non- HCO_3^- intracellular buffers without noticeable renal compensation for at least 12–24 hours (see Table 30.5). Renal metabolic compensation reaches its maximum levels within 3–5 days, and its effectiveness in the newborn is influenced mainly by the functional maturity of proximal tubular HCO_3^- transport. Management of respiratory acidosis is directed toward improving alveolar ventilation and treating the underlying disorder. This may include escalation of noninvasive respiratory support, intubation and mechanical ventilation, alternative modes of mechanical ventilation, and/or administration of surfactant.

Metabolic Alkalosis

Metabolic alkalosis is characterized by a primary increase in the extracellular HCO_3^- concentration sufficient to raise the arterial pH above 7.45. In the newborn, metabolic alkalosis occurs when there is a loss of H^+ , a gain of HCO_3^- , or a depletion of the extracellular volume with the loss of more chloride than HCO_3^- . It is important to understand that metabolic alkalosis generated by any of these mechanisms can be maintained only when factors limiting the renal excretion of HCO_3^- are also present.

Metabolic alkalosis can result from a loss of H^+ from the body, from either the GI tract or the kidneys, that induces an equivalent rise in the extracellular HCO_3^- concentration. The most common causes of this type of metabolic alkalosis in the newborn period are continuous nasogastric aspiration, persistent vomiting, and diuretic treatment. Less common causes of H^+ losses are congenital chloride-wasting diarrhea, certain forms of congenital adrenal hyperplasia, hyperaldosteronism, posthypercapnia, and Bartter syndrome.

Metabolic alkalosis can also result from a gain of HCO_3^- , such as occurs during the administration of buffer solutions to the newborn. In the past a metabolic alkalosis was intentionally created when NaHCO_3 or tromethamine was used to maintain an alkaline pH to decrease pulmonary vasoreactivity in infants with persistent pulmonary hypertension, a practice not recommended anymore. Currently, iatrogenic metabolic alkalosis is primarily due to long-term excessive administration of HCO_3^- , lactate, citrate, or acetate in IV fluids and blood products. Because excretion of HCO_3^- is normally not limited in the newborn, metabolic alkalosis resulting from HCO_3^- gain alone should rapidly resolve after administration of HCO_3^- is discontinued. However, if the alkalosis is severe and urine output is limited, inhibition of the enzyme carbonic anhydrase by the administration of acetazolamide may enhance elimination of HCO_3^- .

Metabolic alkalosis can also result from a loss of ECF containing disproportionately more chloride than HCO_3^- , the so-called contraction alkalosis. During the diuretic phase of normal postnatal adaptation, preterm and term newborns retain relatively more HCO_3^- than chloride (Ramiro-Tolentino et al., 1996). The obvious clinical benefits of allowing this physiologic extracellular volume

contraction to occur, especially in the critically ill newborn, clearly outweigh the clinical importance of a mild contraction alkalosis that develops after recovery. No specific treatment is needed in such cases, because with the stabilization of the extracellular volume and renal function after recovery, acid–base balance rapidly returns to normal. Contraction alkalosis due to other causes, however, may require treatment.

As mentioned previously, for metabolic alkalosis to persist, factors limiting the renal excretion of HCO_3^- must be present. The kidneys are usually effective in excreting excess HCO_3^- , but this ability can be limited under certain conditions, such as decreased glomerular filtration rate, increased aldosterone production, and the more common clinical situation of volume contraction–triggered metabolic alkalosis with potassium deficiency. In the last condition, there is a direct stimulation of Na^+ reabsorption coupled with H^+ loss in the proximal tubule and an indirect stimulation of H^+ loss in the distal nephron by the increased activity of the renin–angiotensin–aldosterone system. Contraction alkalosis responds to administration of saline to replace the intravascular volume in conjunction with additional potassium supplementation to account for renal potassium wasting. In the other disorders, however, the primary problem of reduced glomerular filtration rate or elevated aldosterone concentration must be treated for the alkalosis to resolve.

One of the most commonly encountered clinical scenarios of chronic metabolic alkalosis actually occurs in the form of a mixed acid–base disorder in a preterm neonate with chronic lung disease receiving long-term diuretic treatment. Such a newborn initially has a chronic respiratory acidosis that is partially compensated for by renal HCO_3^- retention. Prolonged or aggressive use of diuretics can lead to total body chloride and potassium depletion and contraction of the extracellular volume, thus exacerbating the metabolic alkalosis. By stimulating proximal tubular Na^+ reabsorption and thus H^+ loss, distal tubular H^+ secretion, and renal ammonium production, the diuretic-induced hypokalemia contributes to the severity and maintenance of the metabolic alkalosis. Furthermore, metabolic alkalosis per se worsens hypokalemia, because potassium moves intracellularly to replace hydrogen as the latter shifts into the extracellular space. Although the serum potassium concentration may be decreased, the serum levels in the newborn do not accurately reflect the extent of total body potassium deficit because potassium is primarily an intracellular ion, with approximately 98% of the total body potassium being in the intracellular compartment. In addition, the condition is accompanied by marked hypochloremia and hyponatremia. Hyponatremia occurs in part because sodium shifts into the intracellular space to compensate for the depleted intracellular potassium. If the alkalosis is severe, alkalemia (pH >7.45) can supervene and result in hypoventilation. In this situation, potassium chloride supplementation, and not sodium chloride supplementation, reverses hyponatremia and hypochloremia, corrects hypokalemia and metabolic alkalosis, and increases the effectiveness of diuretic therapy. Because chloride deficiency is the predominant cause of the increased pH, ammonium chloride or arginine chloride also corrects the alkalosis. These agents do not affect the other electrolyte imbalances such as the hypokalemia, so they should not be the only therapy given.

It is important to keep ahead of the potassium losses in infants receiving long-term diuretic therapy rather than to attempt to replace potassium after intracellular depletion has occurred. Because the rate of potassium repletion is limited by the rate at which potassium moves intracellularly, correction of total body potassium deficits can require days to weeks. In addition, there is also a risk

of acute hyperkalemia if serum potassium levels are driven too high during repletion, particularly in newborns in whom an acute respiratory deterioration may occur. Indeed, with worsened respiratory acidosis, potassium will move from the intracellular to the extracellular space. The routine use of potassium chloride supplementation and close monitoring of serum sodium, chloride, and potassium levels are therefore recommended during long-term diuretic therapy to prevent these common iatrogenic problems.

Respiratory Alkalosis

When a primary decrease in PaCO_2 results in an increase in the arterial pH beyond 7.45, respiratory alkalosis develops. The initial hypocapnia is acutely titrated by the intracellular buffers, and metabolic compensation by the kidneys returns the pH toward normal within 1–2 days (see Table 30.5). Respiratory alkalosis is the only simple acid–base disorder in which, at least in adults, the pH can be completely normalized by the compensatory mechanisms (Brewer, 1990). The cause of respiratory alkalosis is hyperventilation, which in the spontaneously breathing newborn is most often caused by fever, sepsis, retained fetal lung fluid, mild aspiration pneumonia, CNS disorders, or urea cycle defects.

In the neonatal intensive care unit, the most common cause of respiratory alkalosis is iatrogenic secondary to hyperventilation of the intubated newborn. Because findings suggest an association between hypocapnia and the development of periventricular leukomalacia (PVL) (Wiswell et al., 1996; Okumura et al., 2001) and chronic lung disease (Garland et al., 1995) in ventilated preterm neonates, avoidance of hyperventilation during resuscitation and mechanical ventilation is of utmost importance in the management of sick preterm newborns. It appears that later onset of hypocarbia is more deleterious in terms of the development of PVL (at 26 hours of age vs. 15 hours of age), with 88% of these patients having poor neurodevelopmental outcome at a median age of 46 months. The current trend of managing preterm neonates with permissive hypercapnia has made the incidence of hypocapnia and respiratory alkalosis much less common. However, it is of note

that *hypercapnia in the transitional period* is associated with increased incidence of periventricular hemorrhage/IVH in very preterm neonates (Kaiser et al., 2006; Fabres et al., 2007). In one study of ventilated preterm neonates, the incidence of hypocapnia was 4%, and hypocapnia was more common in the first 3 days of life (van Kaam et al., 2013). The treatment of neonatal respiratory alkalosis consists in the specific management of the underlying process causing hyperventilation.

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Extremely Low-Birth-Weight Infants

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KEY POINTS

- Although the incidence of preterm birth in the United States has recently fallen, the incidence of extremely low birth weight (ELBW) births remains static at approximately 1% of all births.
- A significant proportion of ELBW survivors continue to suffer from both short-term and long-term complications.
- Current data suggest that many spontaneously breathing, extremely premature infants can be managed successfully with continuous positive airway pressure (CPAP) therapy started in the delivery room.
- Metaanalyses of prospective randomized trials of early CPAP therapy versus intubation and early surfactant treatment show that initial CPAP therapy is associated with a decreased risk of death or bronchopulmonary dysplasia.
- Future research in the care of ELBW infants should focus on identifying best practices so as to narrow the variability in approach to care and with the goal being to prevent long-term disability.

In the past few decades the field of neonatology has experienced significant progress in medical care and improvement in overall patient survival. Advancement in technology, greater use of glucocorticoids prenatally, regionalization of perinatal and high-risk neonatal care, and a more comprehensive understanding of the physiology of the immature infant have all contributed to dramatic increases in survival of very preterm infants. Care of premature infants with birth weights between 1000 and 1500 g has become almost routine in most neonatal intensive care units (NICUs) in the United States.

One of the most significant remaining challenges in neonatology is the care of extremely low birth weight (ELBW) infants (birth weight <1000 g). These infants present one of the greatest medical and ethical challenges to the field. Although they represent a small percentage of overall births and NICU admissions, ELBW infants are often the most critically ill and at the highest risk of death and long-term morbidity of any NICU patient. They also contribute disproportionately to overall hospital days and consume a large percentage of NICU personnel time, effort, and costs of care. Care of these infants is in constant evolution, as a result of new discoveries in both basic and clinical research as well as growing clinical experience. This chapter will review some of the special challenges in and practical aspects of the management of the ELBW infants.

Epidemiology

The percentage of babies born preterm (<37 weeks' gestation) steadily rose in the United States in the early years of the first decade of this century (Behrman and Stith-Butler, 2007), reaching a peak in 2006 at 12.8% of all births. However, more recent data suggest that the overall preterm birth rate has started to decline (Martin et al., 2017). This decline has been driven primarily by a decrease in the birth rate of late preterm infants. The rate of extreme prematurity has remained static, with approximately 1% of births occurring before 28 weeks' gestation. There remains a significant racial disparity in the incidence of extreme preterm birth, with the African-American ELBW birth rate being nearly double that of the Hispanic and non-Hispanic white populations.

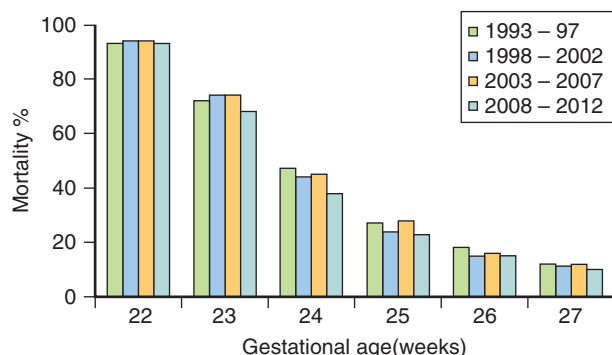
Preterm births are associated with the greater availability of assisted reproductive technologies (ARTs) in the United States. Among infants conceived with ARTs in 2012, 34.9% were born preterm (Sunderam, 2015). These technologies result in a higher incidence of preterm infants, in part because of the higher frequency of multiple gestations (Schieve et al., 2002; Sunderam, 2015). In 2012, infants conceived with ART accounted for 19.6% of all multiple-birth infants, representing 19.2% of all twin infants and 29.6% of all triplet or higher-order infants (Sunderam, 2015). Multiple gestations add to the potential morbidity of extremely premature birth because of a higher frequency of intrauterine growth restriction and other medical complications of pregnancy.

Several studies have shown increased survival in the smallest and most premature infants in the past 20 years (Fig. 31.1) (Fanaroff et al., 2007; Stoll et al., 2010, 2015). Improved survival has not been accompanied by a change in the incidence of several major morbidities among survivors at the lowest gestational ages (<26 weeks' gestation) (Stoll et al., 2015) (Table 31.1). However, for infants born at a gestational age (GA) between 26 and 28 weeks, the incidence of severe intracranial hemorrhage, severe retinopathy of prematurity, and periventricular leukomalacia decreased from 2000 to 2012 in the Eunice Kennedy Shriver National Institute of Child Health and Human Development cohort of infants born in institutions participating in the Neonatal Research Network (Stoll et al., 2015). Trends for survival without major in-hospital morbidity in this same cohort suggest an overall improvement, albeit small, for infants born between 25 and 28 weeks' gestation. However, the rates of bronchopulmonary dysplasia (BPD) increased,

likely due to overall improved survival. With the current trends in survival and in-hospital morbidity, the absolute number of extremely premature infants who survive to NICU discharge and in whom a major morbidity is diagnosed in the neonatal period remains high. A significant percentage of these infants continue to have neurodevelopmental and neurosensory disability into childhood (Hack et al., 2005; Marlow et al., 2005; Moore et al., 2012; Adams-Chapman et al., 2015; Linsell et al., 2015).

Perinatal Management

Extremely premature infants born in high-risk perinatal referral centers, especially those with a high volume of such infants, have better short-term outcomes than infants transferred to such centers after birth (Arad et al., 1999; Towers et al., 2000; Chien et al., 2001; Cifuentes et al., 2002; Shah et al., 2005; Bartels et al., 2006; Phibbs et al., 2007; Jensen et al., 2015). Therefore if clinically feasible, the pregnant woman who seems likely to deliver an extremely premature infant should be transferred to a high-risk perinatal center for the expertise in both obstetric and neonatal management. On arrival, the expectant mother should be evaluated for factors that may have predisposed her to preterm labor and should be assessed for the status of the fetal membranes and the presence or absence of chorioamnionitis. In addition, the best



• **Fig. 31.1** Mortality by Gestational Age at Birth in the National Institute of Child Health and Human Development Neonatal Research Network, 1993 to 2012. (From Stoll BJ, Hansen NI, Bell EF, et al. Trends in care practices, morbidity, and mortality of extremely preterm neonates, 1993–2012. *JAMA*. 2015;314:1039–1051.)

obstetric estimate of GA (by the date of the last menstrual period and early ultrasonographic dating, if available), ultrasonographic assessment of fetal size and position, and the presence of other medical or obstetric complications (preeclampsia, placenta previa, abruptio placentae) should be documented. Specimens for recto-vaginal cultures to detect the presence of group B streptococci should also be obtained on admission (Schrag et al., 2002), and treatment with penicillin or ampicillin (or vancomycin for the patient with a severe penicillin allergy) should be initiated until culture results are available. Although unlikely to arrest labor for an extended period, tocolytic agents should be considered for women with preterm uterine contractions without evidence of chorioamnionitis. There is widespread agreement that glucocorticoids should be offered to any woman in whom delivery at 24 to 34 weeks' gestation seems likely. However, a recent systematic review of 17 observational studies concluded that treatment with glucocorticoids at gestational ages less than 24 weeks (22 to 23 weeks) improved survival and should be offered to women who desire active postnatal resuscitation of their newborn (Park et al., 2016).

Premature rupture of the fetal membranes (PROM) occurs in 30%–40% of women who deliver prematurely (Mazor et al., 1998). If PROM is diagnosed without evidence of intrauterine inflammation and/or infection, consideration should be given to the use of prophylactic antibiotic therapy (ampicillin or erythromycin) for the mother. Several studies have shown that such therapy prolongs the latency period, reduces the incidence of chorioamnionitis and endometritis, and improves neonatal outcome (Gibbs and Eschenbach, 1997; Mercer et al., 1997; Mazor et al., 1998; Phupong and Kulmala, 2012). Tocolytic and antibiotic therapy in the setting of PROM may prolong latency by 48 to 72 hours in many extremely preterm pregnancies in which delivery seems likely, allowing the administration of a complete course of glucocorticoids to the mother (Phupong and Kulmala, 2012).

Prenatal Consultation

If possible, all parents who are at risk of delivery of an extremely premature newborn should have a consultation with a neonatologist before the newborn's birth, preferably jointly with a perinatologist caring for the mother (Finer and Barrington, 1998; Cummings et al., 2015). There are several goals of this consultation. First, the neonatologist and perinatologist should inform the parents about

TABLE 31.1 Survival (Greater Than 12 Hours After Birth) and Selected Morbidities for 22-Week to 28 Week Gestational Age Infants Born in National Institute of Child Health and Human Development Neonatal Research Network Sites

| | SURVIVAL (%) | | | |
|---|--------------|--------------|--------------|--------------|
| | 1993 to 1997 | 1998 to 2002 | 2003 to 2007 | 2008 to 2012 |
| Survived to discharge without major morbidity | 18 | 24 | 25 | 29 |
| Necrotizing enterocolitis | 9 | 9 | 11 | 10 |
| Bronchopulmonary dysplasia | 36 | 43 | 42 | 45 |
| Severe intraventricular hemorrhage | 13 | 17 | 14 | 15 |
| Severe retinopathy of prematurity | 14 | 17 | 16 | 12 |
| Late-onset sepsis | 35 | 35 | 36 | 24 |

Data from Stoll BJ, Hansen NI, Bell EF, et al. Trends in care practices, morbidity, and mortality of extremely preterm neonates, 1993–2012. *JAMA*. 2015;314:1039–1051.

TABLE 31.2 Major Problems of Extremely Low Birth Weight Infants

| System | Short-Term Problems | Long-Term Problems |
|-------------------------------|--|---|
| Respiratory | Respiratory distress syndrome Air leaks Bronchopulmonary dysplasia Apnea of prematurity | Chronic lung disease Reactive airway disease |
| Gastrointestinal, nutritional | Feeding intolerance Necrotizing enterocolitis Growth failure | Growth failure Failure to thrive Inguinal hernias |
| Immunologic, infection | Immune deficiency Perinatal infection Nosocomial infection | Respiratory syncytial virus |
| Central nervous system | Intraventricular hemorrhage Periventricular white matter disease | Cerebral palsy Neurodevelopmental delay Hearing loss |
| Ophthalmologic | Retinopathy of prematurity | Blindness, retinal detachment Myopia Strabismus |
| Cardiovascular | Hypotension Patent ductus arteriosus | Hypertension in adulthood (?) Pulmonary hypertension |
| Renal | Water, electrolyte imbalance Acid–base disturbances | |
| Hematologic | Iatrogenic anemia Frequent transfusions Anemia of prematurity | |
| Endocrine | Transient hypothyroxinemia Cortisol deficiency | Impaired glucose regulation Increased insulin resistance |

the proposed management of the pregnancy and delivery of the newborn, including a discussion of the advantages of administration of glucocorticoids to the mother, the possible need for cesarean delivery, and delivery room care and resuscitation of the baby. Second, the parents should be informed about the potential risks of both the extent of prematurity and the proposed therapeutic interventions (Tables 31.2–31.3). Third, the neonatologist should investigate the parents' beliefs and attitudes about delivery of an extremely premature newborn and the potential for long-term morbidity. This outline for prenatal consultation is especially important if delivery is expected at the borderline of viability (23 to 24 weeks' gestation) (Cummings et al., 2015).

Data are sparse about the process and results of prenatal consultation with the parents of an extremely preterm newborn. Some studies suggest that significant incongruity exists among the attitudes of obstetricians, nurses, neonatologists, and parents about active delivery room resuscitation and treatment of extremely premature newborns (Streiner et al., 2001; Zupancic et al., 2002). Reuss and Gordon (1995) have shown that the obstetric judgment of fetal

viability was associated with an 18-fold increase in survival for infants with a birth weight less than 750 g, indicating that obstetric attitudes and decisions influence outcome. Obstetricians and nurses tend to overestimate, and parents tend to underestimate, mortality and morbidity in premature infants (Haywood et al., 1994; Doron et al., 1998). Parents report being more in favor of intervening regardless of GA or condition at birth compared with health care professionals (Streiner et al., 2001). In a survey by Ballard et al. (2002), most neonatologists responded that they would respect parents' wishes about resuscitation of a borderline viable newborn. In practice, when parental preferences about active resuscitation are known before delivery of an extremely preterm newborn, neonatologists report that they would alter their management of the newborn in the delivery room accordingly after assessment of the baby after birth (Doron et al., 1998; Arzuaga et al., 2014), indicating the important role for prenatal consultation. However, parental attitudes are likely strongly influenced by physician recommendations about aggressive resuscitation conveyed during the prenatal consultation. This consultation is further complicated by significant variability in physician's opinions about the benefits and burdens of providing intensive care at the borders of viability (De Leeuw et al., 2000; Cummings et al., 2015). Physician attitudes may be influenced by fear of litigation for not actively resuscitating an extremely premature newborn even if parental choices are made clear, as well as local, regional, and national norms (Partridge et al., 2009).

The earliest GA at which resuscitation should be initiated remains controversial among neonatologists (Raju et al., 2014), making firm recommendations for the actual content of prenatal consultation difficult (Kaempf et al., 2006; Cummings et al., 2015). Most studies that describe the outcome of premature infants are based on birth weight rather than GA and thus confound the effects of extremely preterm birth with those of intrauterine growth restriction, but both parents and physicians are usually faced with making decisions on the basis of the anticipated GA at delivery. However, the likelihood of survival without serious sequelae may be influenced by factors other than GA. Using prospectively collected data from the Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network on infants born at 22 to 25 weeks' gestation between 1998 and 2003, Tyson et al. (2008) presented an analysis of some of these factors. In a multivariate analysis, prenatal exposure to glucocorticoids, female sex, singleton gestation, and higher birth weight (in 100-g increments) were each associated with a decrease in the risk of death or of survival with neurodevelopmental impairment. The reduced risk was similar to the risk for infants with an additional week of gestational age. Other data from several studies suggest that in terms of survival and potential for long-term disability, encouragement of active management is appropriate for women expected to deliver at 25 weeks' gestation or later (Piecuch et al., 1997; Wood et al., 2000; Rysavy et al., 2015). More recent studies suggest that proactive perinatal and neonatal management may increase the rate of survival as well as survival without disability in infants born at gestational ages as low as 22 to 23 weeks (Rysavy et al., 2015; Serenius et al., 2015). Even with proactive management, however, the risk of death and severe morbidity is significantly higher before 25 weeks' gestation (El-Metwally et al., 2002; Rysavy et al., 2015; Stoll et al., 2015). Mortality approaches 70%–80% for infants delivered at 23 weeks' gestation, with most survivors having neurodevelopmental sequelae (Vohr et al., 2000; Wood et al., 2000; Rysavy et al., 2015; Stoll et al., 2015). These data should be shared with parents during prenatal consultation, and

TABLE 31.3 Treatment Guidelines for Initial Management of Extremely Low Birth Weight Infants

| Time After Birth | Guideline | Time After Birth | Guideline |
|------------------|--|-------------------------|---|
| Delivery room | Ensure good thermoregulation. “Gentle” ventilation as required. Avoid hyperventilation and hyperoxia. Administer surfactant (if prophylaxis approach). Initiate NCPAP (if early CPAP approach). | First 24 to 48 h | |
| NICU admission | Obtain weight measurement. Administer surfactant within first hour (if rescue approach). Establish vascular access: Peripheral intravenous catheter. Umbilical arterial catheter. Umbilical venous catheter (central, double lumen). Start administration of intravenous fluids as soon as possible with dextrose and amino acid solution. Limit evaporative water losses (humidified incubator). Minimize stimulation. Avoid hyperventilation and hyperoxia. Maintain target oxygen saturations between 88% and 93%. Obtain specimens for complete blood count with differential, blood culture, blood glucose measurement. Give antibiotics as indicated. Give parents information about their child. | Cardiovascular | Monitor blood pressure, give vasopressors as required. Assess newborn for hemodynamically significant patent ductus arteriosus. Obtain echocardiogram as indicated. |
| | | Respiratory | Give additional surfactant doses as indicated. Maintain low tidal volume ventilation. Avoid hyperventilation and hyperoxia. Extubate infant and start use of CPAP as soon as possible. |
| | | Fluid management | Obtain weight every 12 to 24 h. Monitor serum electrolyte, blood glucose, and calcium concentrations every 4 to 8 h. Limit evaporative water losses. Administer skin care. |
| | | Hematologic | Obtain second blood count. Administer transfusion support as indicated. Monitor bilirubin level, give phototherapy as indicated. |
| | | Infection | Consider discontinuing use of antibiotics if blood culture results are negative at 48 h. |
| | | Nutrition | Start administration of amino acid solution, parenteral nutrition. |
| | | Neurologic | Minimize stimulation. Perform screening head ultrasonography. |
| | | Social | Arrange to meet family. |

CPAP, Continuous positive airway pressure; NCPAP, nasal continuous positive airway pressure; NICU, neonatal intensive care unit.

appropriate guidance should be given for decision making about the pregnancy, delivery, and resuscitation of the infant. Some have argued that, because of the risk of long-term disability associated with extreme preterm birth and the difficulty of making an informed decision immediately before delivery, parents should be given the opportunity to discuss these issues during routine prenatal care (Harrison, 2008).

General Principles of Care Specific to Extremely Low Birth Weight Infants

First Hours

The first few hours after admission to the NICU are critical for the ELBW infant, although there are significant variations in practice among hospitals and practitioners. Careful adherence to details in the delivery room and during the first few hours after birth is essential to help avoid some of the immediate and long-term complications often experienced by the ELBW infant. All NICUs should have a consistent approach to the initial care of these fragile newborns in the delivery room and on admission to the NICU. A suggested treatment guideline for the first few hours after birth is presented in Table 31.3, and screening guidelines for common complications are shown in Table 31.4.

Delivery Room

At delivery, strict attention to maintenance of body temperature by means of rapid, gentle drying of the newborn and the use of adequate heat sources is paramount to avoid cold stress. A polyethylene occlusive skin wrap or bag should also be used immediately after delivery to prevent initial evaporative heat losses (Vohra et al., 1999; Reilly et al., 2015). Many ELBW newborns need some form of assisted ventilation immediately after birth because of no or poor respiratory effort. If positive pressure ventilation is required, it should be provided with low inspiratory pressure to prevent overdistention of the lungs, which can result in air leak and other lung injury, and adequate positive end-expiratory pressure (PEEP) to maintain lung volume by use of a flow-inflating bag, or optimally a T-piece resuscitator that allows more consistent delivery of inspiratory pressure and PEEP. Hyperventilation and hyperoxia should also be avoided; oxygen delivery should be titrated over the first few minutes after birth with use of pulse oximetry and time-specific oxygen saturation targets, with a saturation target of 85%–95% at 10 minutes after birth (Wyckoff et al., 2015). The use of room air for resuscitation of these newborns has been proposed to protect them from hyperoxia and damage to the lungs by oxygen free radicals. However, studies have not shown a benefit for short-term outcomes in the use of low initial oxygen concentration (21%–30%)

**TABLE
31.4****Recommended Screening for Common Complications of Extremely Low Birth Weight Infants**

| Complication | Screening |
|--|--|
| IVH | HUS on days 1 to 3; repeat on days 7 to 10. |
| Germinal matrix hemorrhage | Repeat HUS at 30 days. |
| Intraventricular Hemorrhage (IVH) | Repeat HUS every 3 to 7 days until stable or resolved. |
| IVH with ventricular dilation or intraparenchymal bleeding | Repeat HUS every 3 to 7 days until stable or resolved. Consider measurement of resistive indices for progressive ventricular dilatation. |
| Periventricular white matter disease | HUS at day 30; repeat at 36 weeks' postmenstrual age or at discharge. Consider magnetic resonance imaging if HUS findings are equivocal. |
| ROP | Perform ophthalmologic examination at 4 to 6 weeks' postnatal age. Repeat every 2 weeks if no ROP. Repeat weekly if ROP present. Repeat twice weekly for prethreshold disease or rapidly progressive ROP. |
| Audiology screening | Hearing screen no earlier than 34 weeks' postmenstrual age, but before discharge home. |

HUS, Head ultrasonography; IVH, intraventricular hemorrhage; ROP, retinopathy of prematurity.

compared with high oxygen concentration (>65%) in initial resuscitation of preterm newborns (Wyckoff et al., 2015). Since, in most studies, resuscitated babies were in approximately 30% oxygen at the time of stabilization, the International Liaison Committee on Resuscitation currently recommends that initial resuscitation of preterm newborns start with 21%–30% oxygen.

Current data suggest that many spontaneously breathing, extremely premature newborns can be managed successfully with continuous positive airway pressure (CPAP) therapy started in the delivery room (Aly et al., 2005b; Lindner et al., 1999; Morley et al., 2008; SUPPORT Study Group of the Eunice Kennedy Shriver NICHD Neonatal Research Network et al., 2010a). Routine use of CPAP immediately after delivery may obviate the need for intubation in newborns with a GA of 24 weeks or more, and increasing experience with this approach has been shown to improve its success (Aly et al., 2005b; Finer, 2006). According to one report, mechanical ventilation was avoided in approximately one-third of newborns with a GA of 25 weeks or less and in nearly 80% of newborns with a GA of 28 weeks or more with use of this approach (Aly et al., 2005b). Metaanalyses of available randomized trials of CPAP versus prophylactic surfactant indicate that aggressive use of early CPAP therapy in the delivery room is associated with a reduced risk of death or BPD (Rojas-Reyes et al., 2012; Fischer et al., 2013; Schmolzer et al., 2014) and should be considered the preferred mode of initial respiratory support in the spontaneously breathing ELBW newborn after birth.

Some controversy remains as to the best approach for early exogenous surfactant treatment in extremely premature newborns who require intubation in the delivery room or in whom early CPAP therapy fails because of severe respiratory distress. Soll (2009) reported a metaanalysis of eight randomized trials of the use of natural surfactant as prophylaxis versus as a rescue strategy for established respiratory distress syndrome (RDS) in the ELBW newborn. Administration of exogenous surfactant before 15 minutes of age resulted in a reduction in the rates of neonatal death, air leak, and the combined outcome of BPD or death compared with a selective rescue approach. These results suggest that if newborns do require intubation and mechanical ventilation soon after birth,

exogenous surfactant therapy should be administered as soon as possible, preferably in the delivery room.

Admission to the Neonatal Intensive Care Unit

All newborns should be weighed on admission; frequent determination of subsequent weights is a valuable tool in managing fluid and electrolyte balance. In many centers, ELBW infants are initially placed under a radiant warmer for easier access (i.e., for catheter placement). Because of the high transepidermal fluid losses in these newborns, intravenous (IV) solutions containing 5%–10% dextrose should be administered as quickly as possible after admission, and efforts should be made to reduce evaporative water losses by an increase in the relative humidity surrounding the newborn. Fluid requirements increase at lower GAs, owing to both an increased surface area to body weight ratio and skin immaturity. Renal immaturity may also result in large losses of fluid and electrolytes that must be monitored and replaced. Generally, IV fluids administered at a rate of 80 to 100 mL/kg per day will be sufficient to maintain adequate hydration as long as evaporative water losses are minimized. Hydration status over the first few days after birth is assessed by changes in body weight. In addition, serum electrolyte concentrations should be measured every 6 to 8 hours depending on the baby's degree of skin immaturity; blood glucose levels should be monitored as well to assess the baby for glucose intolerance and hyperglycemia. Serum sodium level in the first 2 to 3 days after birth is a reasonable marker of free water status, but urine sodium losses as the glomerular filtration rate increases make these measurements less reliable thereafter.

A plastic heat shield used in concert with the radiant warmer may decrease transepidermal water losses; alternatively, a polyethylene tent with an infusion of warmed humidified air may be used. Several studies suggest that fluid management is improved by use of a humidified incubator instead of a radiant warmer, because of lower water losses (Gaylord et al., 2001; Meyer et al., 2001). Exposure to high humidity may raise the rate of skin colonization with gram-negative organisms, although no increase in the rate of nosocomial infections was observed in several randomized trials

comparing incubators with radiant warmers (Flenady and Woodgate, 2002). However, temperature regulation for the smallest newborns in the first few days may be more difficult in an incubator than in a servo-controlled radiant warmer, because of rapid drops in air temperature as the incubator doors are opened to care for the newborn (Meyer et al., 2001).

Vascular Access

Close monitoring of blood pressure, arterial blood gases, and serum chemistries during the first few days after birth is required in most sick ELBW newborns; therefore it is advantageous to insert an umbilical arterial catheter for reliable access in newborns who require assisted ventilation. Infusion of half-normal saline with 0.5 unit of heparin per milliliter at a low rate (0.5 to 1 mL/hour) is usually enough to maintain catheter patency. Although use of a saline solution rather than a dextrose–water solution in the umbilical arterial line may complicate fluid and electrolyte management, this disadvantage is offset by the advantage of reliable measurement of blood glucose levels, which are frequently required, without disturbing the newborn. Placement of a central umbilical venous catheter (tip at the inferior vena cava–right atrial junction) at the same time as the umbilical arterial catheter is placed also provides the clinician with reliable venous access for infusion of fluids, medications, and blood products. Use of a double-lumen umbilical venous catheter often obviates insertion of a peripheral IV line in the first few days after birth and helps to preserve IV sites and skin integrity.

The length of time that umbilical catheters are left in place differs by hospital. In most centers, use of umbilical lines is generally discontinued after 7 to 10 days because of the potential for catheter-related infection and vascular complications, although it is unusual for arterial access to be needed beyond a few days after birth. Before the umbilical venous catheter is removed, it is advisable to insert a percutaneous central venous catheter with its tip at the junction of the superior or inferior vena cava and the right atrium, dedicated to infusion of parenteral nutrition (see later section on [Nutritional Management](#)). This catheter helps to maintain IV access for nutritional purposes without raising the risk of catheter-related infections and reduces the need to establish and maintain peripheral IV lines (Parellada et al., 1999; Janes et al., 2000). The incidence of complications from percutaneous central catheters, including catheter-related infections, is lower if a limited number of NICU personnel insert and maintain the lines.

Skin Care

The skin of an infant born at 23 to 26 weeks' gestation is extremely immature and is ineffective as an epidermal barrier. Poor epidermal barrier function in the extremely preterm newborn leads to disturbances in temperature regulation and water balance as well as breakdown in skin integrity, which can increase the risk of infection. The stratum corneum, which is responsible for epidermal barrier function, does not become functionally mature in the fetus until approximately 32 weeks' gestation (Rutter, 2000). However, acceleration of the maturation process occurs after birth, so most extremely premature newborns have a mature epidermal barrier by approximately 2 weeks after birth. Until that time, full-thickness skin injury can occur in the ELBW infant from seemingly innocuous causes, such as local pressure from body positioning, removal of adhesives, and prolonged exposure to products containing alcohol or iodine. Such injury can lead to larger transepidermal water

losses, an even greater risk of nosocomial infection, and significant scarring.

Because of these risks, preservation of skin integrity should be incorporated into the care of the extremely preterm newborn (Table 31.5). Limited use of adhesives and extreme care on their removal, frequent repositioning of the newborn to avoid pressure points on the skin, and use of soft bedding or a water mattress are the minimum requirements. Hydrocolloids (e.g., DuoDerm, ConvaTech, Bridgewater, New Jersey, United States) applied to the baby in areas where adhesive tape may come in prolonged contact with the skin (i.e., umbilical catheter or endotracheal tube fixation) to prevent the direct application of tape to the baby may be useful, because it is more easily removed than standard adhesive tape. Polyurethane adhesive dressings (Tegaderm, 3M, St. Paul, Minnesota, United States) or hydrogels (Vigilon, Bard Medical, Covington, Georgia, United States) may also be used to protect areas of skin friction and superficial wounds.

Prophylactic application of preservative-free emollient ointments (Aquaphor, Beiersdorf, Hamburg, Germany) to protect the skin of the ELBW infant has been studied (Lane and Drost, 1993; Nopper et al., 1996; Edwards et al., 2004). Several small

TABLE 31.5 Practical Guidelines for Skin Care of Extremely Low Birth Weight Infants

| Interventions | Guidelines |
|-----------------------|--|
| Adhesive application | <p>Increase adhesive tack by application to dry, clean skin surface.</p> <p>Avoid use of alcohol for skin cleansing.</p> <p>Use smallest amount of tape possible.</p> <p>Use a hydrocolloid or pectin-based layer on the skin before application of heavy adhesive.</p> <p>Avoid use of adhesive over areas of skin breakdown.</p> <p>Avoid use of adhesive bonding agents (e.g., benzoin).</p> <p>Use hydrophilic gel or pectin-based adhesives preferentially.</p> |
| Adhesive removal | <p>Avoid use of adhesive removers and solvents.</p> <p>Use a warm, wet cotton ball to periodically saturate hydrogel adhesives (avoid overdrying and oversaturation).</p> <p>Facilitate removal of adhesive with mineral oil, petrolatum, and emollients if reapplication is not necessary.</p> |
| Emollient application | <p>Infants born at <27 weeks' gestation may benefit from emollient use.</p> <p>Avoid use of multidose containers (e.g., large jars).</p> <p>Use nonperfumed, nonirritating hydrophilic emollients.</p> <p>Recognize potential for emollients to interfere with adhesive and conductive properties of monitoring devices.</p> |
| Emollient removal | <p>Wipe off gently with a soft cloth or gauze if site is contaminated.</p> <p>Avoid repeated attempts to thoroughly cleanse the skin (undesired friction effect).</p> <p>Remove emollients before attaching thermistors or other monitoring devices.</p> |

Data from Hoath S, Narendran V. Adhesives and emollients in the preterm infant. *Semin Neonatol*. 2000;5:289–296.

studies demonstrated smaller transepidermal water losses, improved skin condition, and lower risk of suspected or proven nosocomial infection with prophylactic application of emollient ointments. However, one report documented an increase in the rate of systemic yeast infections in a single NICU coincident with a change to use of prophylactic emollients in ELBW infants, who returned to the baseline after such use was discontinued (Campbell et al., 2000). In the largest study to date, infants randomized to receive prophylactic emollient had better skin integrity during the first month after birth but had a higher rate of nosocomial bacterial infection compared with a control group with no emollient use (Edwards et al., 2004). Given these concerns, routine use of prophylactic emollients is not recommended; however, selective use in ELBW infants at risk of significant skin breakdown may be effective as an adjunct to other types of local skin care already described.

Mechanical Ventilation and Noninvasive Respiratory Support

A high percentage of ELBW infants require some level of assisted ventilation to survive. Data from animal models of RDS indicate that positive pressure ventilation with large tidal volumes damages pulmonary capillary endothelium, alveolar and airway epithelium, and basement membranes (Dreyfuss, 1998). This mechanical damage results in leakage of fluid, protein, and blood into the airways, alveoli, and interstitial spaces, leading to inhibition of surfactant activity and further damage to the lungs. As such, the use of CPAP as a first-line method of respiratory support to avoid mechanical ventilation for ELBW infants has increased in the past several years (Schmolzer, 2014). In infants who do require mechanical ventilation, data suggest that a ventilator strategy that avoids large changes in tidal volume may reduce ventilator-induced lung injury in ELBW infants, an important management goal. The optimal mode, timing, and application of ventilatory support used in the initial management of the ELBW infant to meet this goal remain controversial. However, the objectives of all strategies of assisted ventilation in the ELBW infant should be similar and are (1) to provide the lowest level of ventilatory support possible that will both support adequate oxygenation and ventilation and prevent atelectasis and (2) to try to reduce acute and chronic lung injury secondary to barotrauma, volutrauma, and oxygen toxicity (Clark et al., 2000; Berger et al., 2013; Committee on Fetus and Newborn, 2014).

Noninvasive Ventilation

In a classic study comparing the incidence of BPD between NICUs, Avery et al. (1987) reported that the NICU with the lowest incidence used CPAP more frequently and more aggressively than the other NICUs. Van Marter et al. (2000) confirmed these observations.

Theoretically, early CPAP protects the immature lung from injury caused by positive pressure tidal breaths, by preserving surfactant function, increasing alveolar volume and functional residual capacity, enhancing alveolar stability, and improving ventilation-perfusion matching. It may also stimulate the growth of the immature lung. Most NICUs now use CPAP (delivered via nasal prongs at 5 to 8 cmH₂O) as the initial mode of assisted ventilation for spontaneously breathing ELBW infants, starting in the delivery room (Lindner et al., 1999; Rojas-Reyes et al., 2012; Fischer et al., 2013). CPAP used this way has been reported

in retrospective studies to decrease the need for mechanical ventilation (Poets and Sens, 1996; Gittermann et al., 1997), the need for surfactant treatment, and the incidence of BPD (Lindner et al., 1999; Aly, 2001; de Klerk and de Klerk, 2001; Aly et al., 2005b). In one prospective study (Morley et al., 2008), infants with a GA of 25 to 28 weeks who were breathing spontaneously but required ventilatory assistance at 5 minutes after birth were randomly assigned to treatment with nasal CPAP or intubation and mechanical ventilation. In the infants assigned to receive CPAP, 56% did not require intubation, and surfactant use was halved. Although respiratory outcomes at 36 weeks' postmenstrual age were equivalent in the two study groups, a greater number of infants who were assigned to initial treatment with CPAP had pneumothorax (9% versus 3%). However, as noted already, metaanalyses of prospective randomized trials of early CPAP therapy versus intubation and early surfactant treatment show that initial CPAP therapy was associated with a decreased risk of death or BPD, with the number needed to treat to prevent a case of death or BPD ranging from 25 to 35 (Rojas-Reyes et al., 2012; Fischer et al., 2013). Alternatively, CPAP may be used after prophylactic or rescue surfactant therapy is given during a brief period of intubation and positive pressure ventilation (INSURE [intubate, surfactant, extubate] technique) (Verder et al., 1999; Booth et al., 2006; Dunn et al., 2011). Observational studies of this approach show that approximately one-fourth of infants born before 27 weeks' gestation do not require a subsequent course of mechanical ventilation and are less likely to develop BPD (Dani et al., 2004; Booth et al., 2006). In randomized trials of the INSURE technique versus more prolonged mechanical ventilation after surfactant administration in infants with RDS, the INSURE approach was associated with a reduced need for mechanical ventilation and reduced need for supplemental oxygen at 28 days (Polin et al., 2014). Alternative surfactant delivery methods, including instillation into the pharynx, aerosolized surfactant, or delivery through a thin endotracheal catheter to prevent the need for intubation with an endotracheal tube, are currently under investigation (Kribs and Hummler, 2016).

CPAP can be delivered by a conventional mechanical ventilator with continuous flow, a variable-flow device that adjusts flow through the respiratory cycle, or "bubble" CPAP, in which a tube is immersed in water to the desired depth to generate CPAP, with continuous gas flow bubbling through the immersed tube. Animal data suggest that bubble CPAP increases lung volume and improves gas exchange better than conventional CPAP (Pillow et al., 2007), perhaps secondary to effects of the higher intranasal pressures generated by the bubbling water (Kahn et al., 2008). Other data suggest that work of breathing and thoracoabdominal asynchrony may be lessened with variable-flow devices (Liptsen et al., 2005). Whether these differences are clinically relevant remains to be elucidated.

Many units have started to use a high-flow nasal cannula (HFNC) as an alternative to conventional CPAP devices. HFNC therapy usually refers to the delivery of blended, heated, and humidified oxygen at flows greater than 1 L per minute via small binasal prongs. Reported advantages of HFNC therapy include ease of use and a lower incidence of nasal breakdown compared with conventional CPAP therapy (Manley and Owen, 2016). Potential disadvantages include variable distending pressure delivery (both low and high), and some studies have shown a longer duration of respiratory support with HFNC therapy compared with CPAP therapy. Randomized trials to date comparing HFNC therapy with CPAP therapy as either initial or postextubation support in

extremely preterm infants are limited but suggest that HFNC therapy may be an acceptable alternative to CPAP therapy in some infants, particularly those with a GA of 26 weeks or more (Cummings and Polin, 2016; Owen and Manley, 2016).

Conventional Mechanical Ventilation

Many different modes of conventional mechanical ventilation are available to clinicians in the NICU for the respiratory management of the ELBW infant requiring mechanical ventilation. All advanced modes of mechanical ventilation for newborns include some version of synchronized intermittent mandatory ventilation (SIMV). In the premature infant, SIMV, in which the inspiratory cycle is synchronized with the patient's own effort, is better than conventional intermittent mandatory ventilation in terms of oxygenation, ventilation, work of breathing, and blood pressure variability (Cleary et al., 1995; Hummler et al., 1996; Jarreau et al., 1996). However, technologic limitations in the ability of some ventilators to synchronize breaths generated by very weak inspiratory efforts may prevent the use of SIMV in the smallest infants. With advancement in ventilator design, other modes of patient-triggered ventilation, including volume guarantee, assist control, and pressure support, have become available and have supplanted conventional SIMV as first-line ventilatory therapies (Reyes et al., 2006). Volume-targeted strategies generally allow ventilation at lower pressures and better controlled tidal volumes than conventional SIMV and, as such, may be protective to the surfactant-deficient lung. In metaanalyses, volume-targeted compared with pressure-limited ventilator strategies are associated with a lower risk of death or BPD, pneumothorax, hypocarbia, days of ventilation, and the combined outcome of periventricular leukomalacia and grade 3 or 4 intraventricular hemorrhage (IVH) (Wheeler et al., 2010; Peng et al., 2014). Whether these short-term benefits result in long-term improvement in respiratory or neurodevelopmental outcomes remains uncertain (Stefanescu et al., 2015). However, hyperventilation (partial pressure of carbon dioxide [PaCO_2] <35 mmHg) has been associated with a higher risk of the development of BPD (Garland et al., 1995; Van Marter et al., 2000) and neurodevelopmental sequelae in ELBW infants (Wiswell et al., 1996).

In response to this suggestion of worse pulmonary outcome in hyperventilated infants, a strategy of minimal ventilation, or permissive hypercapnia, has been proposed for conventional ventilation in ELBW infants (Ryu et al., 2012). In the largest study to date of this ventilatory strategy, Carlo et al. (2002) randomly assigned 220 infants with birth weights between 501 and 1000 g to receive either minimal ventilation (target $\text{PaCO}_2 >52$ mmHg) or routine ventilation (target $\text{PaCO}_2 <48$ mmHg). No difference in the rate of death, BPD, or other major short-term morbidities was seen between the two groups. Potential risks of a high PaCO_2 include increases in both cerebral perfusion and pulmonary vascular resistance and lower pH. Without clear data to support the benefit and safety of higher levels of hypercapnia, most centers target their conventional ventilatory strategy to maintain PaCO_2 between 45 and 55 mmHg in the first several days of mechanical ventilation in the ELBW infant.

In neonates whose lung disease prevents extubation in the first several days after birth, changes in lung dynamics in the first 1 to 2 weeks often necessitate a change in ventilatory strategy. In the early stages of chronic lung disease, increased airway resistance and decreased lung compliance may require higher mean airway pressures, peak inspiratory pressures, and PEEP and

longer inspiratory time than are usually used in initial ventilatory management. Once the need for more prolonged mechanical ventilation has been established, many centers tolerate higher target PaCO_2 values in an attempt to limit further ventilator-induced lung injury.

High-Frequency Ventilation

Considerable interest has been generated in the past 20 years in the application of high-frequency ventilation (HFV) in newborns who have respiratory failure, because this technique allows ventilation with small tidal volumes. The results of studies using HFV in animal models of RDS have been promising in the prevention of lung injury, but the results of clinical studies of this ventilatory technique have not. Despite many clinical trials, controversy continues to surround the indications for HFV in ELBW infants, whether HFV is more effective than other modes of ventilation for RDS, whether HFV reduces adverse outcomes (specifically BPD), and whether HFV is more likely to have significant long-term complications than conventional mechanical ventilation.

Early trials of HFV before surfactant replacement therapy demonstrated no pulmonary advantage of HFV over conventional mechanical ventilation and suggested an increase in rates of air leak and intracranial abnormalities in HFV-treated infants (The HIFI Study Group, 1989). Later trials in ELBW infants who were treated with surfactant also failed to demonstrate any reduction in the incidence of BPD in the HFV-treated infants (Rettitz-Volk et al., 1998; Thome et al., 1999; Johnson et al., 2002).

A rigorously controlled trial of HFV as the primary mode of assisted ventilation compared with conventional mechanical ventilation was the first to suggest a small advantage from the early use of HFV in reduction in the incidence of BPD or death without an increase in the incidence of short-term complications in ELBW infants. Courtney et al. (2002) compared high-frequency oscillatory ventilation (HFOV) with synchronized intermittent mandatory ventilation in a randomized trial that enrolled 500 ELBW infants. They found a small but significant decrease in the incidence of BPD in survivors, a requirement of fewer doses of exogenous surfactant, and shorter time to successful extubation in the HFOV-treated group. No differences in other complications of prematurity were observed between the two groups. In another study of HFOV versus conventional ventilation, while no differences were seen in the incidence of BPD in the original trial (Johnson et al., 2002), superior lung function at adolescence was observed in individuals who were assigned to receive HFOV (Zivanovic et al., 2014).

These results suggest that, when used in experienced hands according to strict protocol, HFV confers some protection from lung injury in ELBW infants. It remains unclear whether this same advantage is gained when HFV is used in usual clinical circumstances in less experienced centers and whether the incidence of other complications may be affected by the mode of ventilation. Some NICUs with the most experience with HFV use it routinely as the initial mode of ventilation for ELBW infants. Most centers continue to use volume-targeted ventilation with low tidal volumes and reasonable ventilation goals as the initial mode of ventilation for ELBW infants, reserving HFV for infants in whom conventional ventilation and surfactant therapy fail. This latter practice seems advisable, given the potential risks of HFV, including inadvertent lung overdistention, impaired cardiac output, and increased central venous pressure that may lead to intracranial hemorrhage.

Postextubation Continuous Positive Airway Pressure and High-Flow Nasal Cannula Therapy for Respiratory Distress Syndrome and Apnea

CPAP is commonly used in ELBW infants after extubation to stabilize functional residual capacity and reduce the frequency of apneic spells after the lung disease has improved. All ELBW infants should be extubated as soon as they have recovered from acute RDS and should be given a trial of CPAP to protect them from further ventilator-induced lung injury. When used in combination with methylxanthine therapy, CPAP decreases the need for reintubation because of progressive respiratory distress or apnea (Davis et al., 2003). HFNC therapy has been studied as a substitute for conventional CPAP therapy to prevent postextubation failure. Data from these studies suggest that HFNC therapy is similar to CPAP therapy in preventing extubation failure in infants born at more than 26 weeks' gestation and is associated with less nasal trauma (Cummings and Polin, 2016; Manley and Owen, 2016).

Caffeine treatment before extubation has also been shown to lower the incidence of apnea and the need for reintubation in premature infants. Many ELBW infants benefit from prolonged use of CPAP by nasal prongs after extubation, especially those with frequent or severe episodes of apnea and bradycardia resistant to methylxanthine. Some centers currently provide assisted ventilation through nasal prongs (nasal intermittent positive pressure ventilation) to avoid intubation (Kirpalan et al., 2013); this method may be more successful than conventional CPAP therapy in preventing postextubation failure in some infants (Bhandari et al., 2009). However, the smallest and least mature infants may need prolonged mechanical ventilation because of frequent, severe apneic spells that are unresponsive to other therapies.

Adjunctive Therapies to Prevent Bronchopulmonary Dysplasia

Vitamin A Supplementation

ELBW infants have low stores of vitamin A. Because of the role of vitamin A in promoting lung healing, its deficiency has been linked to a higher risk of development of BPD. In a large multicenter trial, vitamin A supplementation (5000 IU given intramuscularly three times per week for 4 weeks) reduced biochemical evidence of vitamin A deficiency and decreased the incidence of BPD by 12% without adverse effects in ELBW infants who required mechanical ventilation or supplemental oxygen at 24 hours of age (Tyson et al., 1999). Given these efficacy data and the apparent safety, many NICUs choose to administer vitamin A supplementation as described previously to all ELBW infants starting at 24 hours after birth. However, widespread acceptance of this therapy has been limited by concerns over the need for thrice weekly intramuscular injections and the associated pain and stress to the infant as well as by chronic medication shortages.

Caffeine

In a prospective, masked, randomized trial involving newborns with a birth weight of 500 to 1250 g, the initiation of treatment with caffeine citrate (20 mg/kg loading dose, followed by 5 mg/kg per day) in the first 10 days after birth decreased the rate of BPD (a secondary outcome) as compared with placebo (36% vs 47%) without adverse effects (Schmidt et al., 2006). The composite primary outcome of death, cerebral palsy, cognitive delay, deafness,

or blindness at 18 to 22 months of age was also reduced in the babies randomized to receive caffeine citrate (Schmidt et al., 2007), although these benefits did not persist at 5 years of age (Schmidt et al., 2012). Earlier (<3 days) compared with later (>3 days) caffeine administration appears to be associated with better outcomes in ELBW infants (Patel et al., 2013). The effect of caffeine on the incidence of BPD may be secondary to less ventilator-induced lung injury in the caffeine-treated infants, because shorter duration of positive pressure ventilation and supplemental oxygen therapy was associated with its use (Schmidt et al., 2006). Whether *prophylactic* use of caffeine for the primary prevention of BPD or to improve neurodevelopmental outcomes should be recommended in all ELBW infants regardless of the need for mechanical ventilation remains unclear and requires further study.

Inhaled Nitric Oxide

Inhaled nitric oxide (iNO) may improve the pulmonary outcome in some very low birth weight (VLBW) infants through mechanisms that are thought to involve decreased pulmonary vascular resistance or improved ventilation-perfusion matching, bronchodilatation, antiinflammatory effects, promotion of lung remodeling in response to injury, or normalized surfactant function. As a rescue therapy for ELBW infants with severe hypoxic respiratory failure, iNO therapy does not improve either pulmonary outcome or survival, and it may be associated with increased mortality or an increased incidence of IVH (Van Meurs et al., 2005). The results of trials of iNO therapy involving premature infants with less severe lung disease who were at risk of BPD are mixed. In the Nitric Oxide for the Prevention of Chronic Lung Disease study, a multicenter trial involving ventilator-dependent infants with a birth weight of 500 to 1250 g, treatment with iNO started at 7 to 14 days and continued for a total of 23 days and increased survival without BPD as compared with placebo (Ballard et al., 2006). In contrast, in a multicenter trial of iNO treatment started before 48 hours of age and continued for 21 days (20 parts per million [ppm] for 3 to 4 days, then weaned to 10 ppm, 5 ppm, and 2 ppm for 7 days each) in infants with a birth weight of 500 to 1250 g who continued to require mechanical ventilation, only iNO-treated infants with a birth weight greater than 1000 g had a pulmonary benefit (Kinsella et al., 2006). Treated infants in this latter study were also less likely to have ultrasonic evidence of brain injury than were control infants. Long-term follow-up from the latter two studies suggests that iNO therapy is safe and may improve long-term pulmonary outcomes (Hibbs et al., 2008; Watson et al., 2009). However, iNO use for the prevention of BPD is not approved by the US Food and Drug Administration, and routine use of iNO for the prevention of BPD should be discouraged until additional studies are available to define clinical benefit, if any, and the dosing and duration of therapy.

Systemic Corticosteroids

Inflammation also plays an important role in the pathogenesis of BPD; therefore pharmacologic doses of systemic corticosteroids have been widely used for prevention and treatment of BPD in ELBW infants. The use of systemic corticosteroids for the prevention or treatment of BPD is one of the best studied pharmacologic interventions in ELBW infants. Several studies have examined early (<7 days of age) administration of corticosteroids, usually dexamethasone, to prevent the development of BPD in infants at risk (Rastogi et al., 1996; Garland et al., 1999; Stark et al., 2001). A metaanalysis of studies in which corticosteroids were used to prevent rather than treat established BPD suggested that early

corticosteroid treatment in ELBW infants results in more rapid extubation and a lower incidence of BPD (Halliday et al., 2002; Doyle et al., 2014a), although no effect on mortality was observed. However, a higher incidence of short-term complications, including hyperglycemia, hypertension, poor growth, and intestinal perforations and bleeding, was observed in the corticosteroid-treated infants (Garland et al., 1999; Stark et al., 2001; Watterberg et al., 2004; Doyle et al., 2014a). More importantly, long-term follow-up data suggest that exposure to corticosteroid for prevention or treatment of BPD raises the risk of neurologic sequelae in treated infants, including poor head growth, cerebral palsy, and developmental impairment (Watterberg et al., 2010; Doyle et al., 2014b).

The apparently higher risk of long-term sequelae without an effect on overall mortality has tempered enthusiasm for systemic corticosteroid treatment to prevent or treat BPD in ELBW infants. A possible role for postnatal administration of corticosteroids in the prevention of BPD in selected infants, such as those exposed to chorioamnionitis, was suggested by a trial of early treatment with hydrocortisone (Watterberg et al., 2004). The risk of impaired neurodevelopment may be lower with hydrocortisone than dexamethasone (Rademaker et al., 2007; Watterberg et al., 2007), and some centers are substituting hydrocortisone for dexamethasone if corticosteroids are used postnatally.

Nutritional Management

Provision of adequate nutrition is central to effective care of ELBW infants. These infants are born with limited nutrient reserves, immature pathways for nutrient absorption and metabolism, and higher nutrient demands. In addition, medical conditions associated with extreme prematurity both alter requirements for and complicate the adequate delivery of nutrients. The goals of nutritional management of the ELBW infant are preservation of endogenous body stores, achievement of postnatal growth similar to intrauterine weight accretion and body composition, and maintenance of normal physiologic and metabolic processes concomitant with minimization of complications and side effects. However, few ELBW infants are able to meet these goals despite the use of central parenteral nutrition and caloric supplementation of enteral feedings. As a result, significant growth failure is commonplace (Berry et al., 1997; Ehrenkranz et al., 1999; Martin et al., 2009), although more recent data suggest that the incidence of severe growth failure may be decreasing with improved nutritional strategies (Griffin et al., 2016).

Enteral Nutrition

Medical problems of ELBW infants sometimes preclude initiation of enteral feedings for several days to weeks. However, the structural and functional integrity of the gastrointestinal tract depends on the provision of enteral feedings. Withholding enteral feedings at birth imposes risks of all the complications of luminal starvation, including mucosal thinning, flattening of the villi, and bacterial translocation. Early initiation (within the first few hours to days after birth) of low volumes of milk (10 to 20 mL/kg per day, preferably with expressed breast milk; trophic feedings, or “gut priming”) has been studied in several small trials in premature newborns (Schanler et al., 1999a; McClure and Newell, 2000). Trophic feedings are not meant to give the newborn significant nutrition but rather are meant to promote continued functional maturation of the gastrointestinal tract. Documented benefits of trophic feedings include higher plasma concentrations of gastrointestinal hormones, a more mature gut motility pattern, lower incidence of cholestasis, increased calcium and phosphorus

absorption, and improved and earlier tolerance of enteral feedings. Trophic feedings have not been associated with a higher risk of necrotizing enterocolitis or other adverse outcomes; therefore there is no clinical advantage to delaying initiation of feedings in the medically stable ELBW infant.

Feeding intolerance, indicated by gastric residuals that exceed 25%–50% of the volume fed, abdominal distention, or microscopic blood in the stool, is common in ELBW infants and may be difficult to differentiate from early stages of necrotizing enterocolitis. Feeding intolerance may preclude the advance of enteral nutrition for days to weeks, complicating nutritional management and prolonging the need for parenteral nutrition. Numerous feeding strategies that are designed to avoid episodes of feeding intolerance have been used in ELBW infants, including slow increase in enteral volume (~10 to 20 mL/kg per day), use of dilute rather than full-strength milk, continuous versus bolus tube feeding, and use of prokinetic agents. None of these feeding strategies have been found to be clearly superior, although bolus feedings may decrease episodes of gastric residuals compared with continuous tube feedings and may allow a more rapid advance to full enteral volumes as well as promote better growth.

Episodes of feeding intolerance also are reduced in ELBW infants fed human milk rather than specialized formulas for premature infants (Schanler et al., 1999b). Other benefits of giving human milk in ELBW infants include a more rapid advance to full enteral volumes and its positive immunologic effects, with an associated reduction in the risk of necrotizing enterocolitis and late-onset sepsis. Data also suggest that neurodevelopmental outcome may be improved in ELBW infants fed expressed breast milk (Vohr et al., 2006). Many NICUs use pasteurized donor human milk to supplement expressed human milk in mothers who are unable to provide enough breast milk to fully feed their infant or have medical or personal reasons for not providing expressed milk. It is unclear whether donor human milk provides benefits similar to those of maternally expressed milk since heat pasteurization of human milk eliminates many of its immunologic properties. Whether it is maternally expressed or provided by donors, human milk must be fortified with protein, calcium, phosphorus, sodium, and other minerals to provide adequate nutrition in the ELBW infant. In addition to commercially available human milk fortifiers, which increase the caloric density to approximately 24 calories per ounce, human milk can be fortified further to higher caloric densities with medium-chain triglycerides, glucose polymers, and added protein.

Premature infants who are fed fortified human milk may grow more slowly than infants fed premature formulas (Schanler et al., 1999b; Quigley et al., 2014), perhaps because of the variability in fat and caloric content of pumped breast milk or changes in the nutrient composition of human milk with fortification that affects fat absorption. Despite the potential for slower growth in infants fed human milk, its use should be strongly encouraged in ELBW infants because of the immunologic and other nutritional benefits. Further research on how best to fortify human milk is necessary to promote the best rate of growth in ELBW infants. In addition, even after recommended enteral dietary intakes are reached, many ELBW infants continue to have a cumulative energy and protein deficit, which in part explains their later growth failure (Ehrenkranz et al., 1999; Embleton et al., 2001; Martin et al., 2009).

Early Parenteral Nutrition

Protein losses in ELBW infants receiving a glucose infusion alone begin immediately after birth and can approach 1.5 g/kg per day

in the first 24 to 72 hours. Fortunately, these losses can be offset by early administration of an amino acid solution, even at low caloric intakes. Several studies have demonstrated the safety of early administration (within 24 hours after birth) of an amino acid solution, with no abnormal elevations of ammonia or blood urea nitrogen levels even in the most immature newborns (Rivera et al., 1993; Van Goudoever et al., 1995). To prevent early protein deficit, ELBW infants should be given a source of parenteral protein as soon as possible after birth. In one study, ELBW infants who received 3 g or more of protein per day at 5 days of age or less were less likely to have a weight below the 10th percentile at 36 weeks' postmenstrual age and suboptimal head growth at 18 months of age when compared with ELBW infants who received less protein supplementation after birth (Poindexter et al., 2006).

In most centers, parenteral nutrition is used exclusively during the first few days after birth and then is gradually reduced as enteral feedings are introduced. Longer duration of parenteral nutrition is associated with the development of a number of complications, including cholestasis, osteopenia, and sepsis. For example, the risk of an episode of late-onset sepsis in premature infants is 22-fold higher if parenteral nutrition is continued for more than 3 weeks compared with 1 week or less (Stoll et al., 2002b).

Management and Prevention of Infection

Bacterial and fungal infections are an important cause of illness and death among ELBW infants. In addition to the immediate morbidity and death, local and systemic inflammation caused by infections may increase the risk of development of other complications of prematurity, including BPD and brain injury. ELBW infants are frequently exposed to perinatal and delivery complications that raise their risk of early-onset infections (<72 hours). The need for prolonged IV access, exposure to parenteral nutrition, and mechanical ventilation also subject the ELBW infant to a high risk of late-onset nosocomial infections (>72 hours). The frequent infections seen in the ELBW population are related to immaturity of both humoral and cellular immunity. In addition to the judicious use of antimicrobial therapy, environmental controls, nursery surveillance, and modulation of the immature immune response have been proposed as possible interventions to prevent infections in extremely premature infant.

Early-Onset Infections

The incidence of early-onset bacterial infections in VLBW infants is approximately 1%–2%, with a mortality of approximately 30%–40% (Stoll et al., 2002a, 2011). The major risk factor for the development of perinatally acquired bacterial infections is PROM with chorioamnionitis, which frequently complicates premature deliveries (Higgins, 2016). However, many extremely premature births may be associated with intrauterine infection before membrane rupture (Goldenberg et al., 2000). In one study, 41% of premature infants with early-onset sepsis were born less than 6 hours after membrane rupture (Stoll et al., 2002a).

Because the clinical signs of perinatally acquired infection are nonspecific, the index of suspicion and the concern about the possibility of intrauterine infection should always be high in the case of premature birth. All ELBW infants, except those delivered for maternal indications with no labor, should be evaluated for infection at birth by means of a complete blood count with differential and blood culture, and empiric antibiotic therapy with ampicillin and an aminoglycoside should be initiated. A white

blood cell count of less than 5000 cells/ μ L, a ratio of immature to total neutrophils greater than 0.2 to 0.3, and neutropenia (absolute neutrophil count less than 1000 cells/ μ L) are all suggestive of infection but may also be seen in infants with other conditions, including maternal preeclampsia and hypertension. The duration of initial antibiotic therapy depends on the results of the blood culture, blood counts, the clinical course, and the perinatal history. If the blood culture is negative for bacterial growth at 48 hours and the newborn has improved clinically, consideration should be given to discontinuation of antibiotic therapy. Prolonged exposure to antibiotic therapy increases the likelihood of colonization with multiple antibiotic-resistant organisms, the development of fungemia, and necrotizing enterocolitis (Cotten et al., 2009); therefore antibiotic therapy should be reserved for infants with documented infection or a very high index of suspicion of infection based on clinical or historical factors.

The distribution of pathogens causing early-onset sepsis in VLBW infants has changed, likely because of increased use of antibiotics in the intrapartum period for prevention of group B streptococcal infections and treatment of preterm PROM (Puopolo and Eichwald, 2010). As the rate of infections from group B streptococci has diminished with intrapartum antibiotic prophylaxis, the proportion of documented infections from gram-negative organisms has risen (Table 31.6; Stoll et al., 2002a, 2011). An additional change in the epidemiologic characteristics of early-onset infections in premature infants, possibly related to increased use of antibiotics in the intrapartum period, is the increased frequency of infections

TABLE 31.6 Distribution of Pathogens Among 84 Cases of Early-Onset Sepsis Occurring in 5447 Infants Born Between September 1, 1998, and August 31, 2000

| Organism | Number of Cases | Percentage of Cases (%) |
|---------------------------------------|-----------------|-------------------------|
| Gram-negative bacteria | 51 | 60.7 |
| <i>Escherichia coli</i> | 37 | 44.0 |
| <i>Haemophilus influenzae</i> | 7 | 8.3 |
| <i>Citrobacter</i> | 2 | 2.4 |
| Other | 5 | 6.0 |
| Gram-positive bacteria | 31 | 36.9 |
| Group B streptococci | 9 | 10.7 |
| Viridans streptococci | 3 | 3.6 |
| Coagulase-negative staphylococci | 9 | 10.7 |
| Other streptococci | 4 | 4.8 |
| <i>Listeria monocytogenes</i> | 2 | 2.4 |
| Other | 4 | 4.8 |
| Fungi: <i>Candida albicans</i> | 2 | 2.4 |
| Total | 84 | 100 |

Data from Stoll B, Hansen N, Fanaroff A, et al. Changes in pathogens causing early onset sepsis in very low birth weight infants. *N Engl J Med.* 2002;347:240–247.

owing to ampicillin-resistant *Escherichia coli* strains in some centers (Joseph et al., 1998; Stoll et al., 2002a; Bizzarro et al., 2008; Stoll et al., 2011). Current recommendations for empiric antibiotic therapy for ELBW infants at risk of early-onset sepsis have not changed, but continued surveillance of the epidemiologic factors and antibiotic resistance patterns of isolates within individual units is warranted. In infants with severe illness that may be caused by sepsis, broadening initial antibiotic coverage to include a third-generation cephalosporin should be considered.

Late-Onset Infections

Nosocomial infection is a common though preventable complication of intensive care of the ELBW infant. The incidence of late-onset sepsis in ELBW infants who survive beyond 3 days of age is 25%–50%, depending on GA and birth weight, with the median age at onset of the first episode approximately 2 weeks (Fanaroff et al., 1998; Stoll et al., 2002b). The overall mortality rate is approximately 20% but may be as high as 80%, depending on the organism causing sepsis. The risk of neurodevelopmental impairment is increased by approximately 1.5-fold in ELBW survivors of nosocomial bloodstream infections (Stoll et al., 2004). Risk factors for the development of late-onset sepsis include prolonged parenteral nutrition and lipid use, the presence of a central venous catheter, longer duration of mechanical ventilation, and delay in initiation of enteral feedings (Stoll et al., 2002b). These practices and procedures are common events that may be unavoidable in the care of ELBW infants. However, large variations have been observed among NICUs in the rate of late-onset infections in premature infants (Brodie et al., 2000; Stoll et al., 2002b), suggesting that individual NICU practices may affect the incidence of nosocomial infections.

The most common cause of late-onset infections in ELBW infants in most US NICUs remains coagulase-negative *Staphylococcus* (CoNS). However, with recent attention to strategies to prevent late-onset infection (see later), the incidence of CoNS infection has fallen, as has the incidence of all-cause late-onset infections in ELBW infants, although significant intrainstitutional variation exists (Bizzarro, 2015). Infection with CoNS is almost never fatal but is associated with significant morbidity, such as prolonged ventilator use and hospital stay (Gray et al., 1995). Other organisms that cause late-onset infections in ELBW infants are associated with a much higher morbidity and mortality. The distribution of pathogens associated with the first episode of late-onset sepsis among 1313 infants in a cohort of VLBW babies over a 2-year period is shown in Table 31.7.

Presenting features of late-onset sepsis include increased apnea, feeding intolerance, abdominal distention, guaiac-positive stools, increased respiratory support, and lethargy and hypotonia (Fanaroff et al., 1998). Because these symptoms are nonspecific, ELBW infants are frequently evaluated for infection and treated with empiric antibiotic therapy. In one study, use of both vancomycin and antifungal therapy was inversely related to birth weight; approximately three-fourths of infants with a birth weight less than 750 g were treated with vancomycin during their hospital stay, and approximately one-third were treated with antifungals (Stoll et al., 2002b). Central catheters should be removed immediately to ensure adequate treatment of infants in whom sepsis is diagnosed, except for that caused by CoNS (Benjamin et al., 2001).

Endotracheal tube colonization with multiple organisms is common in infants who require prolonged mechanical ventilation. In general, such colonization should not be treated with antibiotics unless there is evidence of pneumonia or significant inflammation

TABLE 31.7

Distribution of Pathogens Associated With the First Episode of Late-Onset Sepsis in National Institute of Child Health and Development Neonatal Research Network Institutions, September 1, 1998, Through August 31, 2000

| Organism | Number of Cases | Percentage of Cases |
|----------------------------------|-----------------|---------------------|
| Gram-positive bacteria | 922 | 70.2 |
| Coagulase-negative staphylococci | 629 | 47.9 |
| <i>Staphylococcus aureus</i> | 103 | 7.8 |
| <i>Enterococcus</i> spp. | 43 | 3.3 |
| Group B streptococci | 30 | 2.3 |
| Other | 117 | 8.9 |
| Gram-negative bacteria | 231 | 17.6 |
| <i>Escherichia coli</i> | 64 | 4.9 |
| <i>Klebsiella</i> spp. | 52 | 4.0 |
| <i>Pseudomonas</i> spp. | 35 | 2.7 |
| <i>Enterobacter</i> spp. | 33 | 2.5 |
| <i>Serratia</i> spp. | 29 | 2.2 |
| Other | 18 | 1.4 |
| Fungi | 160 | 12.2 |
| <i>Candida albicans</i> | 76 | 5.8 |
| <i>Candida parapsilosis</i> | 54 | 4.1 |
| Other | 30 | 2.3 |
| Total | 1313 | 100 |

Data from Stoll B, Hansen N, Fanaroff A, et al. Late-onset sepsis in very low birth weight neonates: experience of the NICHD Neonatal Research Network. *Pediatrics*. 2002;110:285–291.

indicative of tracheitis (Klompas et al., 2014). Prospective surveillance of common isolates and antimicrobial resistance patterns within individual NICUs can help to guide empiric antibiotic therapy in ELBW infants being evaluated and treated for presumed sepsis. However, indiscriminate use of broad-spectrum antibiotics in the absence of true infection can alter antimicrobial resistance patterns (Goldmann et al., 1996), raising the risk of late-onset infections and complicating therapy.

Prevention of Nosocomial Infection

Because of the frequency and potential severity of late-onset sepsis in ELBW infants, several strategies to prevent infection have been proposed. Using these practices as a guideline, Horbar et al. (2001) observed a decrease in the CoNS infection rate from 22% to 16.6% over a 2-year period in VLBW infants in six study NICUs. Many other investigators have confirmed that changes in practice, primarily surrounding the use and care of central venous catheters, can reduce the overall burden of late-onset infections in individual NICUs, as well as in multicenter quality collaboratives (Kilbride et al., 2003; Aly et al., 2005a; Erdei et al., 2015; Piazza et al., 2016). Some NICUs routinely screen ELBW infants by stool or

respiratory secretion cultures for the presence of multiple antibiotic-resistant organisms, which would necessitate isolation (Gregory et al., 2009). Clusters of infections with unusual organisms should prompt surveillance cultures of infants, potential NICU environmental sources, and NICU staff (Foca et al., 2000). Restriction of broad-spectrum antibiotic use by hospital policy or treatment guidelines may limit the local spread of resistant organisms (Goldmann et al., 1996). Strict adherence to hand hygiene before and after every patient contact and avoidance of overcrowding within NICUs also help to decrease the incidence of infection. The use of alcohol-based hand gels at the bedside may improve compliance with hand hygiene (Harbarth et al., 2002; Helder et al., 2012).

In addition to practice and environmental controls, prophylactic use of antibiotics and modulation of the immune response of ELBW infants have been studied as methods to reduce the incidence of late-onset sepsis. Low-dose vancomycin given continuously via hyperalimentation solutions (Spafford et al., 1994; Baier et al., 1998), intermittently via a peripheral vein (Cooke et al., 1997), or vancomycin lock of the central venous catheter (Garland et al., 2005) has been shown to reduce the incidence of CoNS in premature infants at risk. Concern about the emergence of vancomycin-resistant organisms and the low mortality associated with CoNS infections have prevented widespread use of this approach. Prophylactic fluconazole given for 6 weeks lowered the incidence of fungal colonization and invasive disease in ELBW infants without associated complications or the emergence of resistant organisms (Kaufman et al., 2001, 2005; Manzoni et al., 2007). However, in a randomized study of prophylactic fluconazole compared with placebo in infants with a birth weight less than 750 g, while treatment decreased the incidence of invasive candidiasis, the composite outcome of death or invasive candidiasis was not different between the fluconazole-treated group and the placebo-treated group (Benjamin et al., 2014). These results suggest that routine universal fluconazole prophylaxis for ELBW infants is not warranted; however, such an approach might be advisable for NICUs with a high incidence of fungal infections in their ELBW population.

Prophylactic administration of polyclonal IV immunoglobulin (IVIG) to prevent late-onset sepsis has been studied extensively in premature infants. Several trials have shown a decrease in the incidence of documented sepsis by a small but significant amount in premature infants treated with prophylactic IVIG and no effect on mortality or other complications of prematurity (Lacy, 1995; Ohlsson, 2013). The costs associated with this therapy to achieve the small decrease in infection rates, as well as the increased exposure to blood products, have limited its use; it is unclear whether selective prophylactic IVIG treatment of ELBW infants at the highest risk of sepsis is warranted. Development of more targeted polyclonal or monoclonal γ -globulin preparations for specific organisms that cause sepsis in premature newborns may alter the use of IVIG in the future (Lamari et al., 2000; Weisman et al., 2009).

One strategy that has been under investigation for modulation of the immature immune response to help prevent infections in premature infants is treatment with hemopoietic colony-stimulating factors, including granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor (Modi and Carr, 2000). Most studies of these factors have been conducted in neutropenic, small-for-gestational-age infants or infants delivered by women with preeclampsia. Treatment with granulocyte colony-stimulating factor in neutropenic infants resulted in an increase in neutrophil counts and reduced the incidence of sepsis in one small trial (Kocherlakota and La Gamma, 1998); however,

a more recent randomized placebo-controlled trial in this same population did not show benefit after the first 2 weeks of therapy (Kuhn et al., 2009). Prophylactic granulocyte-macrophage colony-stimulating factor in small-for-gestational-age preterm infants increased neutrophil counts but did not affect the incidence of late-onset sepsis or improve survival compared with placebo. Taken together, these results suggest that modulation of cellular immune deficiency is likely not beneficial in ELBW infants (Carr et al., 2009).

One recent promising strategy for prevention of late-onset infections in preterm infants is supplementation with bovine lactoferrin, a mammalian milk glycoprotein involved in innate immune host defenses. In a randomized trial, bovine lactoferrin-treated VLBW infants had a lower incidence of first-episode late-onset sepsis compared with placebo-treated VLBW infants (Manzoni et al., 2009). Further study is required before routine use of this therapy can be recommended.

Neurosensory Complications

The major neurosensory complications associated with extremely premature birth are IVH, periventricular white matter injury, and retinopathy of prematurity. Although the incidence of severe IVH has fallen, thanks to the increased use of steroids prenatally and to improvements in NICU management, it remains a major cause of brain injury, with consequent increased risk of abnormal neurodevelopment. Pharmacologic approaches to its prevention after birth have generally been unsuccessful. Prophylactic indomethacin reduces the incidence of severe IVH but does not improve long-term neurodevelopment (Schmidt et al., 2001).

Periventricular white matter injury is the predominant form of brain injury in extremely preterm infants and correlates strongly with the development of cerebral palsy. Its pathogenesis is poorly understood, and no specific neuroprotective strategy is known. In some infants, cerebral blood flow and oxygen delivery measured with near-infrared spectroscopy vary during variations of blood pressure considered to be in the normal range, and this lack of autoregulation of cerebral blood flow may lead to ischemic white matter injury (Evans, 2006). Whether aggressive treatment of hypotension in ELBW infants prevents or may lead to subsequent brain injury is uncertain, probably because blood pressure, which is easily measured, does not correlate well with systemic or cerebral blood flow (Fanaroff et al., 2006; Limperopoulos et al., 2007). A higher rate of white matter injury occurs in the setting of maternal or neonatal infection or with elevated levels of proinflammatory cytokines in amniotic fluid or umbilical cord blood, suggesting that inflammation has a role in the pathogenesis (Viscardi et al., 2004). Advanced magnetic resonance imaging techniques in infants with white matter injury show disturbances in cerebral growth, with reduced volume of both gray and white matter (Inder et al., 2005). These observations might serve to explain the motor and cognitive dysfunction often seen in infants with white matter injury.

Retinopathy of prematurity, a vascular proliferative disorder that affects the incompletely vascularized retina of preterm infants, is a major cause of blindness in these children. Severe retinopathy is 18-fold more likely to develop in infants delivered at less than 25 weeks' gestation compared with 28 weeks' gestation (Fanaroff et al., 2007). Periods of hyperoxia owing to exposure to excessive inspired oxygen concentration contribute to the development of retinopathy (Saugstad, 2006); however, the optimal target range of oxygen saturation is not known. Because fetal hemoglobin shifts

the hemoglobin oxygen saturation curve to the left, oxygen saturations greater than 95% may be associated with arterial oxygen tension greater than 80 mmHg, possibly excessive for the ELBW infant. Conversely, oxygen saturation that is too low can increase the risk of injury to the brain or other end organs (Deulofriet et al., 2006).

Adjusting inspired oxygen concentration to target lower oxygen saturations in extremely preterm infants may decrease the rate of severe retinopathy, although it may be associated with other complications, including a higher risk of death. In a prospective observational study, ELBW infants treated in centers with a restrictive approach to oxygen delivery (i.e., saturation alarm limits of 70%–90%) had less retinopathy requiring cryotherapy (6.3% vs 27.7%) than did those in units with a liberal approach (i.e., alarm limits of 88%–98%), and neurodevelopmental outcome at 1 year of age was similar (Tin et al., 2001). In two other studies with historical controls, the incidence of severe retinopathy decreased after oxygen saturation alarm limits were lowered from 87%–97% to 85%–93% for infants born at 28 weeks' gestation and less until they reached 32 weeks' postmenstrual age (Chow et al., 2003; Vanderveen et al., 2006). Recently, different oxygen saturation targets have been tested in masked randomized trials (Askie et al., 2011). These trials compared two different target oxygen saturation ranges (85%–89% compared with 91%–95%) in infants born between 24 and 27 weeks' gestation. In two trials, retinopathy of prematurity occurred less frequently in survivors assigned to the lower oxygen saturation group. However, death before discharge occurred more frequently in the lower oxygen saturation group (SUPPORT Study Group of the Eunice Kennedy Shriver NICHD Neonatal Research Network et al., 2010; BOOST II United Kingdom Collaborative Group; BOOST II Australia Collaborative Group; BOOST II New Zealand Collaborative Group, 2013). In another similarly designed trial, no differences were found in the rates of death or retinopathy of prematurity by assigned oxygen saturation targets, suggesting that the best target range for oxygen saturation remains unclear (Schmidt et al., 2013; Manja, 2015).

Developmental and Parental Care

ELBW infants are particularly vulnerable to the potentially noxious stimuli of the NICU environment, including light, noise, frequent disturbances, and painful procedures. The ELBW infant reacts to the noisy and well-lit environment of many NICUs with greater variability of blood pressure, ventilatory requirements, and oxygen saturation as well as behavioral disorganization, which may have both short-term and long-term effects on outcome (Jacobs et al., 2002). Modification of the NICU environment to limit exposure of ELBW infants to such stresses—by lowering ambient light and reducing noise, clustering caregiving periods and procedures to allow periods of uninterrupted sleep, and using positioning aids to promote containment—is an intuitive part of their care. Newer NICU designs, transitioning from open common rooms to private room settings, may also facilitate a better environment for vulnerable infants and enhance parental involvement. These environmental and developmental interventions in the NICU can improve physiologic stability and some short-term outcomes in preterm infants, including decreased severity of BPD and shorter length of hospital stay. It remains unclear whether individualized, developmentally supportive care or other developmental interventions started in the NICU improve long-term outcome in ELBW infants (Symington et al., 2009).

In addition to environmental modifications, NICUs should promote parental involvement with their infants, even when they are critically ill. Open family presence guidelines and encouragement of parental caregiving when appropriate may help parents to bond with their baby. Many NICUs have embraced a philosophy of family-centered care, in which a stronger bond is forged with the families of NICU patients by encouraging collaboration among family members and care providers in policy and program development, professional education, and aspects of the delivery of care (Dunn et al., 2006). Skin-to-skin (kangaroo) care, in which the infant is placed unclothed on the mother's or father's bare chest, was originally developed in nonindustrialized countries to maintain temperature regulation in premature infants. It is now used in many NICUs to promote parental attachment. Skin-to-skin care may have a positive effect on infant state organization and respiratory patterns, increase the rate of infant weight gain, improve maternal milk production, and have long-term benefits in infant development and parents' perceptions of their babies (Feldman et al., 2002; Cho et al., 2016). Skin-to-skin care can be initiated in ELBW newborns within the first 2 weeks after birth, when they are medically more stable.

Future Directions

This chapter has presented some of the special needs of the ELBW infant. The medical care of the ELBW infant is a complex combination of knowledge of developmental physiology, evidenced-based interventions, and clinical experience. Wide variability in approaches to care of these infants exists among practitioners and NICUs, as does variability in outcomes. Nevertheless, NICUs involved in treating ELBW infants should develop a coherent approach to the medical and ethical aspects of their care. Future research should focus on identifying best practices to narrow the variability in the approach to care and with the goal to prevent long-term disability. In addition, as we gain a greater understanding of genetic influences on drug metabolism, individualized responses, and the risk of complications of prematurity, it is likely that specific therapies will be able to be targeted to the infants at the highest risk. As more of these tiny infants survive, it is the responsibility of neonatologists to stay abreast of clinical improvements and the short-term and long-term consequences of established and newly proposed medical interventions to provide the best care for these vulnerable infants and to keep parents informed and involved.

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32

Late Preterm Infants

SOWMYA S. MOHAN AND LUCKY JAIN

Had he been alive today, Patrick Bouvier Kennedy would have been hailed as a triumph of neonatal care—after all, he was the son of the former United States President, John F. Kennedy and former First Lady Jacqueline B. Kennedy. Born prematurely at 34 weeks gestation, he would have been aptly labeled as a late preterm neonate; however, based on his birth weight (2.1 kg) and gestational age, few would have predicted the outcome he had then, were he born in 2009. But then, in 1963, little was available to the clinician for the management of hyaline membrane disease—no routine use of neonatal ventilators, no device to provide airway positive pressure, no surfactant, and no antenatal steroids. He died two days after his birth; the New York Times obituary said that “the battle for the Kennedy baby was lost because medical science has not advanced far enough.”

(Lucky Jain and David Carlton, Personal Communication, 2009)

KEY POINTS

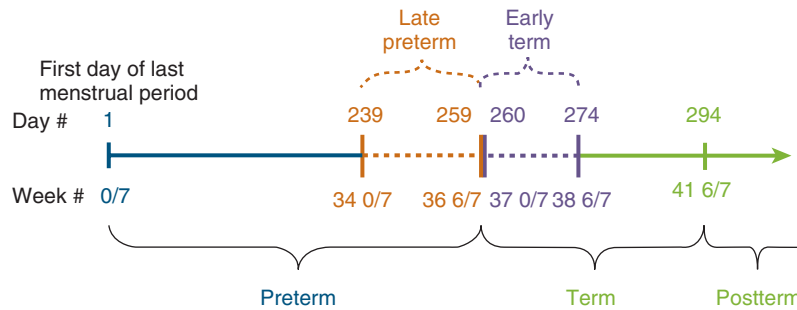
- Late preterm infants (34 to 37 weeks' gestation) are at risk of complications associated with prematurity. Because of their more mature appearance, there has historically been a lapse in early recognition and management of these issues.
- Mortality rises with the loss of each gestational birth week before 39 weeks.
- Late preterm infants have higher respiratory morbidity and mortality compared with full-term infants.
- Late preterm infants require close monitoring for feeding issues, hypoglycemia, hyperbilirubinemia, infection, and thermoregulation in the postnatal period and until discharge.
- Birth at earlier gestational ages has an impact on health and mortality beyond the neonatal period.

With nearly 4 million live births per year (Hamilton et al., 2015), the United States has one of the highest birth rates among industrialized countries; it also has the stigma of having a disproportionately high prematurity rate. Decades of efforts to reduce preterm births have not affected this formidable problem. In recent years the problem has been highlighted by the rise in births between 34 and 36½ weeks' gestation, a group referred to as *late preterm infants* (Fig. 32.1). Late preterm infants have a checkered history, having been passed off as nothing more than “near-term” infants, yet being feared as the “quick to spiral down” group when they develop respiratory distress or other neonatal complications. As the number of late preterm infants has grown, so has the awareness of their unique set of problems, such

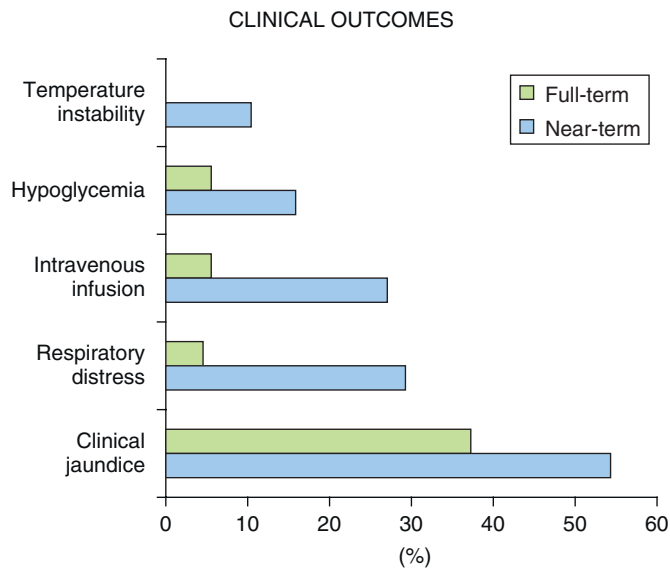
as delayed neonatal transition, wet lung syndrome, hypothermia, hypoglycemia, and hyperbilirubinemia (Fig. 32.2). Although not unique to this population, these complications have sufficient differences in their manifestations and care that this book devotes an entire chapter to the special considerations applicable to the clinical course and management of late preterm infants.

There has been a shift in the distribution of births away from full-term and postterm and toward earlier gestational ages (Davidoff et al., 2006). This shift has resulted in a disproportionately high rate of premature births, with estimates of up to 12.7% of live births being premature (Hamilton et al., 2015)—defined as less than 37 completed weeks' gestation or less than 260 days, counting from the first day of the last menstrual period (Raju, 2006). Within this group of premature babies, up to 75% are classified as late preterm infants (Adamkin, 2009). Although the reasons for such a high number are multifactorial, higher rates of induced deliveries, cesarean births, and efforts to reduce stillbirths may have contributed to the rise in late preterm births.

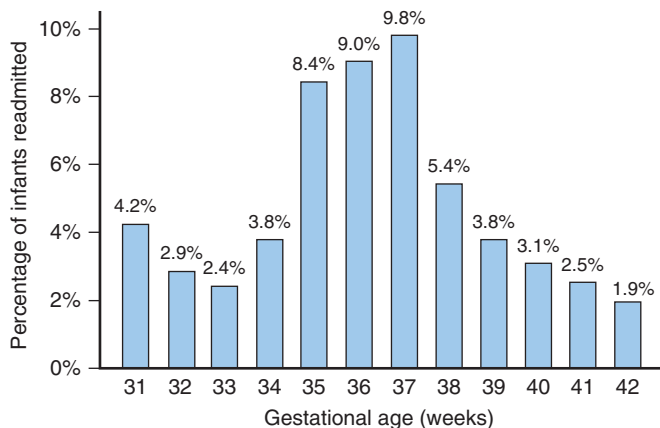
Late preterm newborns currently account for up to one-third of all neonatal intensive care unit (NICU) admissions (Ananth et al., 2013; Jacob et al., 2016), adding strain to the overburdened system of healthcare delivery, particularly in community hospitals and rural areas. These admissions range from short stays, for problems such as transient tachypnea of the newborn, to more complicated or extended NICU stays for problems such as persistent pulmonary hypertension of the newborn (PPHN). With the average NICU stay costing up to \$3500 per day, the economic impact of caring for the late preterm baby can be significant. For example, in 1996 the state of California alone could have saved \$49.9 million in healthcare costs by preventing nonmedically indicated deliveries between 34 and 37 weeks' gestation (Gilbert et al., 2003). In addition to the expense of the initial hospitalization, the cost of caring for a late preterm baby can also be compounded by the increased incidence of hospital readmissions as shown in Fig. 32.3 (Kuzniewicz et al., 2013) and the long-term care issues related to persistent problems. The effects of the increasing number of late preterm births create a societal burden in lost productivity, as parents take extended leave from work to be with their fragile newborns. More importantly, there may be lasting effects with neurodevelopmental delays extending into early school age. Because a significant proportion of brain growth occurs during the last 6 weeks of gestation (Adams-Chapman, 2006), late preterm infants are vulnerable to neuronal injury and disruption of normal brain development. Whereas more longitudinal studies are needed, preliminary studies show that late preterm infants are more likely to have a diagnosis of developmental delay within the first 3 years of life, require special needs preschool resources, and have more



• **Fig. 32.1** Definitions of Late Preterm and Early Term. (Modified from Engle WA, Kominiarek MA. Late preterm infants, early term infants, and timing of elective deliveries. *Clin Perinatol.* 2008;35:325–341.)



• **Fig. 32.2** Clinical outcomes in near-term infants (35 to 36 $\frac{6}{7}$ weeks) and full-term infants as a percentage of the patients studied. (Modified from Wang ML, Dorer DJ, Fleming MP, Catlin EA. Clinical outcomes of near-term infants. *Pediatrics.* 2004;114:372–376.)



• **Fig. 32.3** Readmission rate by gestational age within 30 days after discharge 2003 to 2012 (Kaiser Permanente Northern California). (Modified from Kuzniewicz MW, Parker SJ, Schnake-Mahl A, Escobar GJ. Hospital readmissions and emergency department visits in moderate preterm, late preterm, and early term infants. *Clin Perinatol.* 2013;40:753–775.)

• BOX 32.1 Characteristics of Late Preterm Infants

- Late preterm infants—defined as born at 34 to 36 $\frac{6}{7}$ weeks' gestation
- Physiologically immature with limited compensatory responses to extrauterine environment compared with full-term infants
- Greater risk than full-term infants of death and morbidities such as:
 - Temperature instability
 - Hypoglycemia
 - Respiratory distress
 - Apnea
 - Jaundice
 - Feeding difficulties
 - Dehydration
 - Suspected sepsis

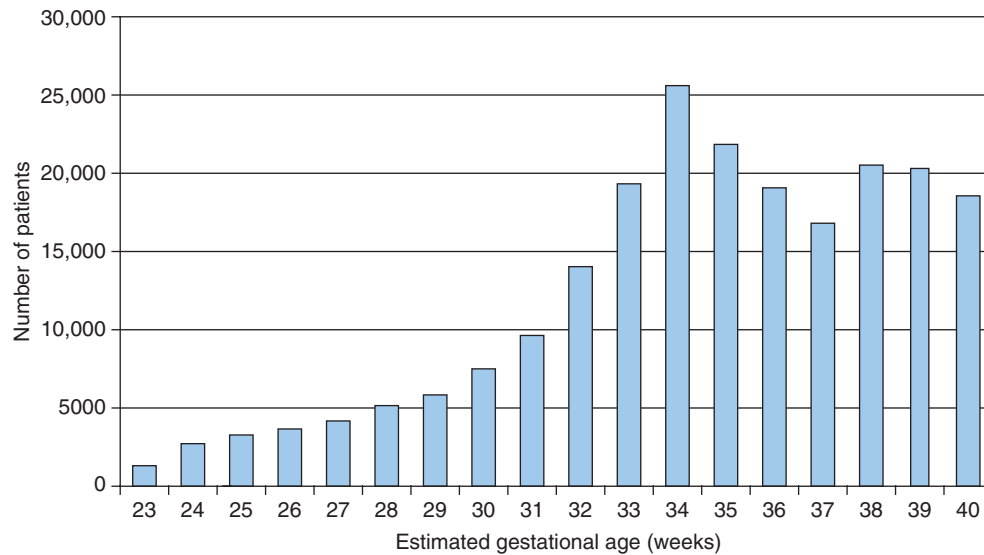
problems with school readiness (Williams et al., 2013; Shah et al., 2016).

Given their large numbers, the overall socioeconomic effects of the late preterm births can be significant. Strategies are required that can reduce the preventable fraction of late preterm births and work toward reducing the morbidity in situations where continuation of the pregnancy is deemed harmful to the fetus or the mother. This chapter explores the pathophysiology of the major morbidities that affect late preterm infants, the unique challenges faced by clinicians in the management of these conditions, and some recent research findings that may help reduce the disease burden and improve outcomes for this unique population.

Definition

Late preterm birth is an accepted term used for infants born between 34 and 36 $\frac{6}{7}$ weeks' gestation (see Fig. 32.1; Raju et al., 2006). This group of infants was initially referred to as *near-term infants*, but the misleading implication of maturity prompted the name change to *late preterm infants* (Box 32.1). This notion is further validated by recent studies showing that full-term infants born at 37 to 38 weeks' gestation have higher morbidity and mortality than those born at 39 weeks' gestation (Madar et al., 1999; Hansen et al., 2008; McIntire and Leveno, 2008; Shapiro-Mendoza et al., 2008); this has prompted the use of *early term* to describe births at 37 to 38 weeks' gestation.

The term *late preterm infant*, and the gestational age limits, were established by a panel of experts convened by the National Institutes of Health and the National Institute of Child Health and Human Development in 2005. While developing these criteria,



• **Fig. 32.4** Distribution of neonatal intensive care unit admissions by gestational age, highlighting the contribution made by late preterm and early preterm infants. Data were obtained from a large consortium of neonatal intensive care units under a common management. (Modified from Clark RH. The epidemiology of respiratory failure in neonates born at an estimated gestational age of 34 weeks or more. *J Perinatol*. 2005;25:251–257.)

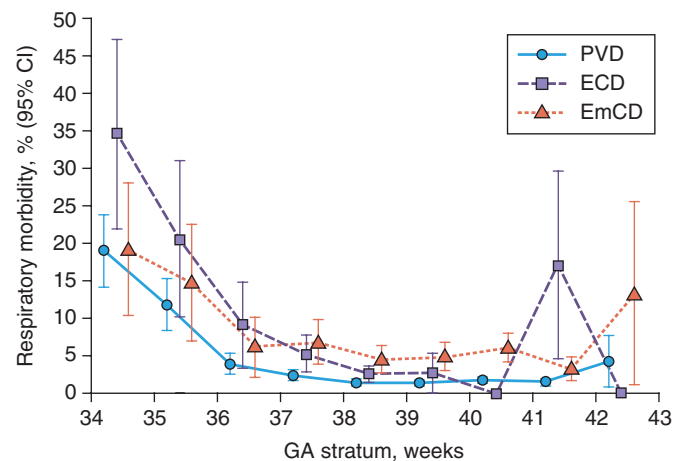
the group considered many factors, including the obstetric guidelines that considered 34 weeks to be a maturational milestone. Beyond 34 weeks' gestation, the level of surfactant is generally considered to be adequate, and so antenatal administration of steroids was not routinely offered to mothers with anticipated delivery (Raju et al., 2006) until recent findings from the Antenatal Late Preterm Steroids (ALPS) study were published showing significant decreases in respiratory complications in late preterm infants born to mothers who received betamethasone antenatally (Gyamfi-Bannerman et al., 2016). Unlike the smaller, more typical premature infant, late preterm infants appear mature because they are larger but have a higher incidence of transient tachypnea of the newborn (TTNB) (Wang et al., 2004; McIntire and Leveno, 2008), respiratory distress syndrome (RDS) (Wang et al., 2004; Clark, 2005), PPHN (Roth-Kleiner et al., 2003), respiratory failure, prolonged physiologic jaundice, late neonatal sepsis (Raju, 2006), thermoregulation issues, hypoglycemia, feeding difficulties (Dudell and Jain, 2006; Escobar et al., 2006; Fuchs and Wapner, 2006), and risk of injury to the developing brain, which can lead to neurodevelopmental problems. Altogether, these problems account for a substantially higher number of NICU admissions for late preterm newborns compared with their full-term counterparts (see Figs. 32.2 and 32.4).

Pathophysiology and Clinical Course

Although many of the diseases discussed in this section are not specific or unique to late preterm infants and are covered in other chapters in this book, it is important to understand and recognize them as part of the unique challenge of caring for late preterm newborns.

Respiratory

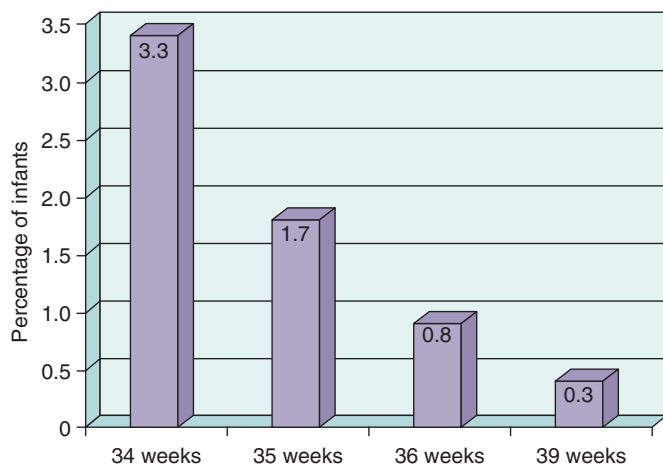
Several studies have consistently shown that late preterm infants have higher respiratory morbidity and mortality compared with full-term infants. Many late preterm infants develop respiratory



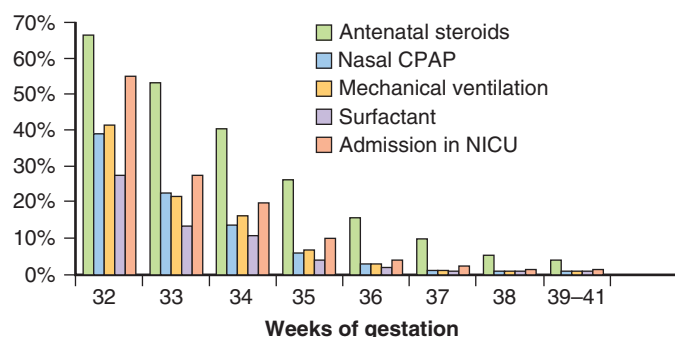
• **Fig. 32.5** Respiratory morbidity in late preterm and early term infants and the impact of the mode of delivery. *CI*, Confidence interval; *ECD*, elective cesarean delivery; *EmCD*, emergency cesarean delivery; *GA*, gestational age; *PVD*, planned vaginal delivery. (Modified from De Luca R, Boulvain M, Irion O, Berner M, Pfister RE. Incidence of early neonatal mortality and morbidity after late-preterm and term cesarean section. *Pediatrics*. 2009;123:e1064–e1071.)

distress soon after birth (defined as sustained distress for more than 2 hours after birth accompanied by grunting, flaring, tachypnea, retractions, or supplemental oxygen requirement), which occurs more often in late preterm infants than in full-term newborns (28.9% vs 4.2%, respectively) (Wang et al., 2004). In addition, within the early term and late preterm groups, infants born at 37 weeks' gestation are fivefold more likely and babies born at 35 weeks' gestation are ninefold more likely to have respiratory distress compared with babies born at 38 to 40 weeks' gestation (Escobar et al., 2006). For each gestational week of age, infants born by elective cesarean delivery tend to do worse (Fig. 32.5). Madar et al.

(1999) found that the incidence of respiratory distress was significantly increased with every week of gestation less than 39 weeks: 30 in 1000 infants born at 34 weeks' gestation, 14 in 1000 infants born at 35 weeks' gestation, and 7.1 in 1000 infants born at 36 weeks' gestation developed respiratory distress. The cause of respiratory distress is diverse and includes transient tachypnea of the newborn, RDS, persistent pulmonary hypertension, and apnea. Not surprisingly, of the affected babies, the incidence of respiratory distress requiring mechanical ventilation corresponded to the degree of prematurity: 3.3% of late preterm infants born at 34 weeks' gestation, 1.7% of late preterm infants born at 35 weeks' gestation, and 0.8% of late preterm infants born at 36 weeks' gestation (Fig. 32.6; McIntire and Leveno, 2008). The gestational age was also correlated with the need for any kind of respiratory support and admission to the NICU, as shown in Fig. 32.7. Late preterm infants can often present with delayed respiratory transition and transient tachypnea, but the course of respiratory distress in this patient population can be unpredictable (Mahoney and Jain 2013). The problem remains that because of their more mature appearance, their initial presentation with respiratory distress may be misleading



• **Fig. 32.6** Percentage of infants born at late preterm gestation who require mechanical ventilation. (Modified from McIntire DD, Leveno KJ. Neonatal mortality and morbidity rates in late preterm births compared with births at term. *Obstet Gynecol.* 2008;111:35–41.)



• **Fig. 32.7** Gestational age and rates of respiratory treatments and admission in a neonatal intensive care unit in a population of 173,058 liveborn infants (2000 to 2009). CPAP, Continuous positive airway pressure; NICU, neonatal intensive care unit. (Modified from Guoyon JB, Iacobelli S, Ferdunus C, Bonsante F. Neonatal problems of late and moderate preterm infants. *Semin Fetal Neonatal Med.* 2012;17:147–152.)

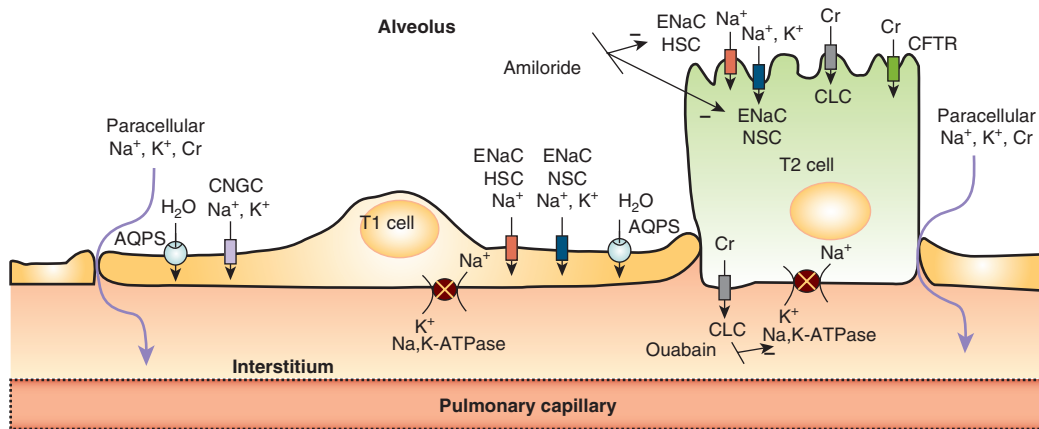
or not recognized, leading to delay in diagnosis and treatment of potentially more serious conditions such as RDS or PPHN.

Whereas respiratory issues often tend to be transient in a vast majority of these neonates, some spiral into severe PPHN and/or hypoxic respiratory failure requiring additional therapies such as extracorporeal membrane oxygenation (ECMO), inhaled nitric oxide therapy, or high-frequency ventilation (Ramachandrapa et al., 2011). Pulmonary hypertension is more likely in preterm infants (born at 34 to 37 weeks' gestation) who develop RDS than in similar infants born at 32 weeks' gestation. Such predisposition is attributed to a developmental increase in the amount of smooth muscle in the walls of pulmonary blood vessels. PPHN is associated with increased pulmonary vascular resistance (PVR) that eventually leads to right-to-left shunting by means of fetal vascular pathways and ventilation-perfusion mismatching (Dudell and Jain, 2006). Management of neonates who develop significant pulmonary hypertension can be challenging, given the self-propagated nature of hypoxia-induced pulmonary vasoconstriction.

A review of the Extracorporeal Life Support Organization Neonatal Registry from 1986 to 2006 by Ramachandrapa and Jain (2011) found that 14.7% of the ECMO patients during that period were late preterm infants and had a mean gestational age of 35.4 weeks. These infants were more likely to require ECMO secondary to hypoxic respiratory failure or RDS instead of aspiration syndromes, which is the primary insult for full-term infants requiring ECMO (Ramachandrapa et al., 2011). In addition, late preterm infants were older at cannulation (likely because most of these infants are asymptomatic at birth (but gradually develop an increasing oxygen requirement with subsequent development of PPHN), had a longer duration of ECMO support, and were more likely to have intraventricular hemorrhage and other neurologic complications than full-term infants. The overall survival rate was significantly lower (74%) for late preterm infants compared with full-term infants (89%) (Ramachandrapa et al., 2011).

Why is it that even in situations in which amniotic fluid testing shows a mature surfactant profile late preterm infants are nevertheless at risk of developing respiratory distress? Part of the answer lies in the delay in clearing fetal lung fluid. Throughout much of gestation, fetal lungs actively secrete fluid into alveolar spaces via a chloride secretory mechanism. This process can be blocked by inhibitors of Na–K–2Cl cotransport. The fluid that accumulates in the developing lung plays a critical role by providing a structural template that prevents the collapse of the developing lung and promotes its growth. At the time of delivery the lung epithelium becomes integral in the process of switching from placental to pulmonary gas exchange (Jain, 1999; Bland, 2001). For effective gas exchange to occur in the lungs, alveolar spaces must be cleared of excess fluid, and pulmonary blood flow must be increased to match ventilation with the perfusion that is occurring. If either the ventilation or the perfusion is inadequate, the infant will have a difficult time transitioning and will develop respiratory distress. In addition, during fetal development, many abnormalities can occur and interfere with the normal production of this lung fluid. Some problems during development include pulmonary artery occlusion, diaphragmatic hernia, and uterine compression of the fetal thorax from chronic leak of amniotic fluid. All these conditions inhibit normal lung development and growth (Jain and Eaton, 2006).

Although a small role in the clearance of this fluid can be attributed to Starling forces and “vaginal squeeze” (Jain, 1999; Bland, 2001), amiloride-sensitive sodium transport by lung epithelial cells through epithelial sodium channels (ENaCs) has emerged as



• **Fig. 32.8** Epithelial sodium (Na) absorption in the fetal lung near birth. Na enters the cell through the apical surface of both alveolar type I (ATI) and alveolar type II (ATII) cells via amiloride-sensitive epithelial Na channels (ENaC), both highly selective channels (HSCs) and nonselective channels (NSCs), and via cyclic nucleotide-gated channels (CNGCs; seen only in ATI cells). Electroneutrality is conserved with chloride movement through cystic fibrosis transmembrane conductance regulator (CFTR) or through chloride channels (CLCs) in ATI and ATII cells, and/or paracellularly through tight junctions. The increase in cell Na concentration stimulates Na-K-ATPase activity on the basolateral aspect of the cell membrane, which drives out three Na⁺ ions in exchange for two K⁺ ions, a process that can be blocked by the cardiac glycoside ouabain. If the net ion movement is from the apical surface to the interstitium, an osmotic gradient is created, which would in turn direct water transport in the same direction, either through aquaporins (AQPS) or by diffusion. (From Mahoney A, Jain L. Respiratory disorders in moderately preterm, late preterm, and early term infants. *Clin Perinatol*. 2013;40:665–678.) Na-K-Pase, sodium-potassium adenosine triphosphatase.

a key event in the transepithelial movement of alveolar fluid (Fig. 32.8; Bland, 2001; Jain et al., 2001). These ENaCs orchestrate the clearing of fluid from the fetal lungs, and disruption of their function has been implicated in several disease processes affecting the newborn, including TTNB and hyaline membrane disease. The late preterm infant is more susceptible to these problems, in part because ENaC expression is developmentally regulated and peak expression in the alveolar epithelium is achieved only at term gestation, which leaves the preterm infant with lower expression of these channels, thus reducing its ability to clear fetal lung fluid after birth (Smith et al., 2000).

High doses of glucocorticoids have been shown to stimulate transcription of ENaCs in several sodium-transporting epithelia and in the lung (Tomashek et al., 2007). In the alveolar epithelia, glucocorticoids were found to induce lung sodium reabsorption in the late-gestation fetal lung (Tomashek et al., 2007). In addition to increasing transcription of sodium channel subunits, steroids increase the number of available channels, by decreasing the rate at which membrane-associated channels are degraded, and increase the activity of existing channels. Glucocorticoids have also been shown to enhance the responsiveness of the lungs to β -adrenergic agents and thyroid hormones (Venkatesh and Katzberg, 1997).

In addition to problems with lung fluid clearance, several other factors may contribute to the overall burden of respiratory morbidity (Morrison et al., 1995; Levine et al., 2001; Roth-Kleiner et al., 2003; Kolas et al., 2006; Villar et al., 2007; Hansen et al., 2008). Given the shortcomings clinicians face in accurate estimation of gestational age, elective induction and cesarean delivery may have increased the burden of iatrogenic prematurity. In an attempt to minimize the occurrence of iatrogenic RDS in light of the increasing frequency of elective cesarean deliveries—commonly performed between 37 and 40 weeks' gestation (Hales et al., 1993)—fetal lung maturity testing was recommended before elective cesarean

deliveries. Because of the risks and complications associated with amniocentesis, this testing is done infrequently (Dudell and Jain, 2006), especially in light of recent studies showing that even late preterm infants and some early term infants born by cesarean delivery before the onset of labor have respiratory distress despite having mature surfactant profiles. This finding prompted the American College of Obstetrics and Gynecologists (ACOG) (2013) to recommend scheduling elective cesarean deliveries at 39 weeks' gestation or later or waiting for the onset of spontaneous labor. The notable exceptions to this recommendation were the medically indicated early deliveries. In 2013, in an effort to balance the well-established risks of late preterm births with the maternal, fetal, and placental complications associated with some pregnancies (i.e., placenta previa, multiple gestations, preeclampsia, etc.), the ACOG Committee on Obstetric Practice published a committee opinion on the suggested timing for delivery of these complicated pregnancies. For all these medically indicated late preterm deliveries, the ACOG Committee on Obstetric Practice recognized the need to individualize the timing of the delivery to balance maternal and newborn risks of late preterm birth versus continuation of the pregnancy (American College of Obstetricians and Gynecologists Committee on Obstetric Practice and Society for Maternal-Fetal Medicine, 2013). Unfortunately, factors related to the convenience of scheduled elective cesarean deliveries for both families and providers will continue to influence the timing of some elective cesarean deliveries (Dudell and Jain, 2006).

Some studies have shown that the overall morbidities in late preterm infants increase significantly for each week of in utero development lost before 38 weeks' gestation (Shapiro-Mendoza et al., 2008). Since one of the most significant morbidities is the higher risk of respiratory distress in infants born before 37 weeks' gestation, the findings of the recent multicenter randomized trial using antenatal betamethasone antenatally for women at risk of

late preterm delivery (ALPS study) warrant potential changes in the antenatal management of pregnancy during the late preterm gestation if delivery is imminent. This study showed that antenatal administration of betamethasone to women at risk of late preterm delivery (Table 32.1) decreased the need for substantial respiratory

TABLE 32.1 Characteristics at the Baseline of Women Enrolled in the Antenatal Late Preterm Steroid (ALPS) Study

| Characteristic | Betamethasone (n = 1429) | Placebo (n = 1402) |
|--|-----------------------------|-----------------------|
| Indication for Trial Entry | | |
| Preterm labor with intact membranes | 400 (28.0%) | 392 (28.0%) |
| Ruptured membranes | 316 (22.1%) | 304 (21.7%) |
| Expected delivery for gestational hypertension or preeclampsia | 370 (25.9%) | 385 (27.5%) |
| Expected delivery for fetal growth restriction | 46 (3.2%) | 48 (3.4%) |
| Expected delivery for oligohydramnios | 50 (3.5%) | 42 (3.0%) |
| Expected delivery for other indication | 247 (17.3%) | 231 (16.5%) |
| Gestational Age at Trial Entry | | |
| ≤34½ weeks | 369 (25.8%) | 399 (28.5%) |
| 35 to 35½ weeks | 571 (40.0%) | 532 (37.9%) |
| ≥36 weeks | 489 (34.2%) | 471 (33.6%) |
| Mean (±SD) maternal age (years) | 28.6±6.3 | 27.8±6.1 |
| Race or Ethnic Group^a | | |
| Black | 376 (26.3%) | 381 (27.2%) |
| White | 828 (57.9%) | 800 (57.1%) |
| Asian | 57 (4.0%) | 39 (2.8%) |
| Other, unknown, or more than one race | 168 (11.8%) | 182 (13.0%) |
| Hispanic | 405 (28.3%) | 448 (32.0%) |
| Other | | |
| Nulliparous | 457 (32.0%) | 448 (32.0%) |
| Smoking during current pregnancy | 204 (14.3%) | 186 (13.3%) |
| Preeclampsia or gestational hypertension | 433 (30.3%) | 440 (31.4%) |
| Gestational diabetes | 153 (10.7%) | 153 (10.9%) |
| Major congenital anomaly in infant ^b | 11 (0.8%) | 21 (1.5%) |

There were no significant differences between the two groups except for maternal age ($P = .001$) and Hispanic ethnic group ($P = .03$).

^aRace or ethnic group was self-reported. Patients of any race could report Hispanic background.

^bAlthough the presence of a major congenital anomaly was an exclusion criterion, these disorders were not discovered until birth.

SD, Standard deviation.

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support during the first 72 hours after birth, reduced rates of respiratory complications (such as TTNB and bronchopulmonary dysplasia; Table 32.2), and reduced the length of stay in the NICU for late preterm infants delivered by these women (Gyamfi-Bannerman et al., 2016). This study showed a significant reduction (from 14.4% in the placebo group to 11.6% in the betamethasone group; $P = .02$) in the rate of neonatal respiratory complications in late preterm infants delivered to women who had received betamethasone before delivery (Gyamfi-Bannerman et al., 2016).

Gastrointestinal

Nutrition

Feeding problems are one of the primary reasons for delay in the discharge of late preterm infants (Adamkin, 2006). Late preterm infants often have poor coordination of sucking and swallowing because of neuronal immaturity, decreased oromotor tone, and inability to generate adequate intraoral pressures during sucking (Polin et al., 2003; Kinney, 2006; Raju et al., 2006; Engle et al., 2007). Breastfeeding has also been shown to be more difficult for early term or late preterm infants compared with full-term infants (Raju, 2006). These problems can lead to poor caloric intake and dehydration.

These problems are compounded by the variations in practice and nutritional management of these infants, given the paucity of published guidelines. Studies have shown that issues such as hypoglycemia and poor feeding contributed to 27% of all late preterm babies requiring intravenous (IV) fluids, compared with only 5% of their full-term counterparts (Wang et al., 2004). In the face of poor enteral intake, parenteral nutrition may be indicated and can become an important therapy in the care of the late preterm infant but is often delayed in anticipation of a quick recovery (Adamkin, 2006). The challenge then becomes providing adequate nutrition to support growth and also equating the energy expenditure that can occur when the infant faces issues such as hypothermia, sepsis, and respiratory distress, which are often seen in late preterm infants. The energy expenditure of nongrowing low birth weight infants (birth weight less than 2500 g) is 45 to 55 cal/kg per day (Adamkin, 2006). These calories come from several sources in parenteral nutrition, including amino acids and lipids.

Late preterm infants are more adept at handling amino acids, allowing the protein content in parenteral nutrition to be started at 2 g/kg per day. With a protein intake of 2.5 to 3 g/kg per day (with adequate caloric intake), a late preterm infant can achieve weight gain similar to that of a full-term infant fed human milk (Adamkin, 2006). More controversial is the use of IV lipids in late preterm infants. Of the late preterm infants with respiratory distress or disease, there are two subgroups: (1) infants with parenchymal lung disease without increased PVR and (2) those with signs of PPHN or increased PVR (Adamkin, 2006). The concern over the use of lipids in the late preterm infant with lung disease stems from adult studies showing that failure to clear infused lipids has an adverse effect on gas exchange in the lungs (Greene et al., 1976). Contrary to those findings, preterm neonates randomized to different lipid infusion rates did not demonstrate any effect on alveolar–arterial oxygen gradient, arterial blood pH, or oxygenation when randomly assigned to modest doses of lipids (0.6 to 1.4 g/kg per day) in the first week of life (Adamkin, 2006). The other argument for restricted use of lipids in late preterm infants specifically addresses the infants with increased PVR and respiratory disease. The concern is that the high polyunsaturated fatty acid content of lipid emulsions (with excess omega-6 linoleic

TABLE 32.2 Neonatal Respiratory Outcomes in Late Preterm Infants Whose Mothers Received Placebo Versus Betamethasone Before Delivery in the Antenatal Late Preterm Steroid (ALPS) Study

| Outcome | Betamethasone (<i>n</i> = 1427) | Placebo (<i>n</i> = 1400) | Relative Risk (95% CI) | <i>P</i> |
|--|----------------------------------|----------------------------|-------------------------------|----------|
| Primary outcome ^a | 165 (11.6%) | 202 (14.4%) | 0.80 (0.66–0.97) | .02 |
| CPAP or high-flow nasal cannula therapy for a continuous period of ≥2 h | 145 (10.2%) | 184 (13.1%) | 0.77 (0.63–0.95) | .01 |
| Fraction of inspired oxygen of ≥0.30 for a continuous period of ≥4 h | 48 (3.4%) | 61 (4.4%) | 0.77 (0.53–1.12) | .17 |
| Mechanical ventilation | 34 (2.4%) | 43 (3.1%) | 0.78 (0.50–1.21) | .26 |
| ECMO | 0 | 0 | NA | NA |
| Stillbirth or neonatal death ≤72 h after birth | 0 | 0 | NA | NA |
| Severe respiratory complication ^b | 115 (8.1%) | 169 (12.1%) | 0.67 (0.53–0.84) | <.001 |
| CPAP or high-flow nasal cannula therapy for a continuous period of ≥12 h | 93 (6.5%) | 147 (10.5%) | 0.62 (0.48–0.80) | <.001 |
| Fraction of inspired oxygen of ≥0.30 for a continuous period of ≥24 h | 20 (1.4%) | 34 (2.4%) | 0.58 (0.33–1.00) | .05 |
| Need for resuscitation at birth ^c | 206 (14.5%) | 260 (18.7%) | 0.78 (0.66–0.92) | .003 |
| Respiratory distress syndrome | 79 (5.5%) | 89 (6.4%) | 0.87 (0.65–1.17) | .36 |
| Transient tachypnea of the newborn | 95 (6.7%) | 138 (9.9%) | 0.68 (0.53–0.87) | .002 |
| Apnea | 33 (2.3%) | 37 (2.6%) | 0.88 (0.55–1.39) | .57 |
| Bronchopulmonary dysplasia | 2 (0.1%) | 9 (0.6%) | 0.22 (0.02–0.92) ^d | .04 |
| Pneumonia | 6 (0.4%) | 13 (0.9%) | 0.45 (0.17–1.19) | .10 |
| Surfactant use | 26 (1.8%) | 43 (3.1%) | 0.59 (0.37–0.96) | .03 |
| Composite of respiratory distress syndrome, transient tachypnea of the newborn, or apnea | 198 (13.9%) | 249 (17.8%) | 0.78 (0.66–0.93) | .004 |
| Pulmonary air leak | 5 (0.4%) | 6 (0.4%) | 0.82 (0.25–2.68) | .74 |

Two participants in each group were lost to follow-up for the analysis of neonatal respiratory outcomes.

^aThe primary outcome was defined as any of the following occurrences within 72 hours after birth: CPAP or high-flow nasal cannula therapy for a continuous period of at least 2 hours, supplemental oxygen with a fraction of inspired oxygen of 0.30 or more for a continuous period of at least 4 hours, mechanical ventilation, stillbirth or neonatal death, or the need for ECMO.

^bA severe respiratory complication was defined as any of the following occurrences within 72 hours after birth: CPAP or high-flow nasal cannula therapy for at least 12 hours, supplemental oxygen with a fraction of inspired oxygen of 0.30 or more for at least 24 hours, mechanical ventilation, stillbirth or neonatal death, or the need for ECMO. Except for the duration of CPAP or high-flow nasal cannula therapy and the duration of a fraction of inspired oxygen of 0.30 or more, the criteria for a severe respiratory complication overlap with those of the primary outcome.

^cThe need for resuscitation at birth was evaluated in 1422 infants in the betamethasone group and 1390 in the placebo group.

^dExact confidence limits are provided for the difference between two binomial proportions.¹⁵

CI, Confidence interval; CPAP, continuous positive airway pressure; ECMO, extracorporeal membrane oxygenation; NA, not applicable.

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acid) feeds into the arachidonic acid pathways, leading to synthesis of prostaglandins and leukotrienes, which can increase vasomotor tone and result in hypoxemia (Adamkin, 2006). Despite the lack of firm evidence for the effects of lipid emulsions in infants with severe respiratory failure with or without pulmonary hypertension, the recommendation is that infants with respiratory disease, but not increased PVR, should receive adequate amounts of lipids to prevent essential fatty acid deficiency; in infants with elements of PPHN, administration of lipids should be avoided during the critical stages of their illness (Adamkin, 2006). Because of these issues and other concerns about parenteral nutrition (e.g., difficulty in optimizing nutrition, the need for IV access and the potential for infiltrates or infection, risk of cholestatic jaundice with prolonged use), enteral feeds should be started as soon as clinically possible while the infant is slowly weaned from parenteral nutrition.

In general, nutritional experts recommend that 34- and 35-week late preterm infants receive nutrient-enriched (22 kcal/oz) milk, whereas older 36- and 37-week late preterm infants with an uncomplicated neonatal course should be fed unfortified milk after discharge (Adamkin, 2006). These nutrient-enriched formulas have a higher protein content (1.9 g/dL versus 1.4 g/dL), increased energy (22 kcal/oz versus 20 kcal/oz), and additional calcium, phosphorous, zinc, trace elements, and vitamins compared with standard formulas (Adamkin, 2006). This enrichment becomes essential to the late preterm infant born at 34 to 35 weeks' gestation or the older group of late preterm infants who had a difficult NICU course, where the goal is to compensate for earlier deprivation of adequate nutrition and allow for somatic and brain growth during the first year. These issues notwithstanding, these nutritional guidelines are not always followed, leading to variability in

nutritional practices by providers. One study showed that although nearly 46% of late preterm infants were discharged home with recommendations for use of formula that contained more than 20 kcal/oz, this practice recommendation had a broad range of followers (4% to 72%) (Adamkin, 2006). The issue becomes more pressing in the late preterm infant with chronic conditions, such as bronchopulmonary dysplasia, that are often associated with growth failure caused by inadequate nutrient intake.

For mothers who choose to breastfeed their late preterm infant, this can often be more challenging compared with nursing a full-term infant. The challenge often lies in initiating and establishing breastfeeding because these infants are sleepier, have less stamina, have more difficulty maintaining body temperature, have problems with latching, sucking, and swallowing, and have more respiratory instability than full-term infants (Adamkin, 2006). Despite these obstacles, mothers should still be encouraged to provide breast milk, given the numerous proven benefits of breast milk. Recent studies have shown that the advantages of breast milk feeding for premature infants might be even greater than those for full-term infants (Adamkin, 2006).

Hypoglycemia

Hypoglycemia is defined as low circulating blood glucose concentrations, but the actual neonatal threshold value is still debated. A physiologic definition (blood glucose concentration <45 mg/dL) was established decades ago, on the basis of abnormal electroencephalograms at glucose levels that were lower than that value (Koh et al., 1988). Hypoglycemia is often missed in late preterm infants, mainly because of the early admission of these infants to the well-baby nursery or the mother's room in an effort to triage the limited number of acute care beds in the NICU and to allow the mother to bond with her new baby (Garg and Devaskar, 2006). However, developmental immaturity is associated with multiple problems, including decreased glycogen stores and feeding difficulties, both of which can lead to hypoglycemia (Box 32.2). Another potential factor in the incidence of hypoglycemia in the late preterm population is the use of steroids antenatally in mothers at risk of late preterm delivery. The ALPS study found that there was an increased incidence of hypoglycemia in the newborns whose mothers had received steroids antenatally compared with the placebo group. Neonatal hypoglycemia was found in 24% of the betamethasone group versus 15% of the placebo group, probably secondary to maternal hyperglycemia because of the steroids (Gyamfi-Bannerman, 2016). Therefore because of the multiple factors previously mentioned, it is not surprising that the incidence of hypoglycemia in preterm infants is threefold greater than that in full-term infants (Wang et al., 2004).

In addition, severe hypoglycemia is a well-known risk factor for neuronal cell death and adverse neurodevelopmental outcomes (Garg and Devaskar, 2006). If hypoglycemia is not recognized and treated in a timely manner with IV fluids or feedings, the infant can develop neurodevelopmental abnormalities because the compensatory mechanisms for protecting the brain from hypoglycemia are not fully developed (Cornblath and Ichord, 2000; Cornblath et al., 2000; Vannucci and Vannucci, 2001; Rozance and Hay, 2006). Early recognition, diagnosis, and treatment of hypoglycemia are therefore crucial to the late preterm infant's long-term outcome. We recommend that institutions develop protocols for routine testing of blood glucose levels in late preterm infants. One can use existing serum glucose screening protocols for infants at high risk of hypoglycemia (i.e., small for gestational age, large for gestational age, infant of a diabetic mother). If none

• BOX 32.2 Causes of Hypoglycemia in the Late Preterm Infant

Transient Hypoglycemia in the Late Preterm Infant

Maternal Conditions

- Glucose infusion in the mother
- Preeclampsia
- Drugs: tocolytic therapy, sympathomimetics
- Infant of diabetic mother

Neonatal Conditions

- Prematurity
- Respiratory distress syndrome
- Twin gestation
- Neonatal sepsis
- Perinatal hypoxia–ischemia
- Temperature instability: hypothermia
- Polycythemia
- Specific glucose transporter deficiency
- Isoimmune thrombocytopenia, Rh incompatibility

Persistent Hypoglycemia in the Late Preterm Infant

Endocrine Disorders

- Pituitary insufficiency
- Cortisol deficiency
- Congenital glucagon deficiency

Inborn Errors of Metabolism

- Carbohydrate metabolism: glycogen storage disease, galactosemia, fructose 1,6-diphosphatase deficiency
- Amino acid metabolism: maple syrup urine disease, propionic acidemia, methylmalonic acidemia hereditary tyrosinemia
- Fatty acid metabolism: acyl coenzyme A dehydrogenase defect, defects in carnitine metabolism, β -oxidation defects
- Defective glucose transport

are available, the following is recommended: glucose checks between 1 and 2 hours after birth, followed by testing before the next three consecutive feeds and then before alternate feedings for the remainder of the first 24 hours. If the blood glucose level is less than 40 to 45 mg/dL, then the hypoglycemia management protocol should be followed.

Hypoglycemia is not a problem in utero, because the fetus receives a steady supply of glucose primarily by maternal transfer through the placenta. Once the baby has been delivered, this constant supply of glucose is abruptly stopped, and the infant has to rely on glucose production primarily via hepatic glycogenolysis and gluconeogenesis (Halamek et al., 1997). After birth, the baby experiences a surge in the levels of catecholamines, glucagon, and corticosteroids, which play a key role in maintaining a euglycemic state. The increase in the levels of catecholamines leads to a surge in glucagon concentration and a decline in circulating insulin concentrations, which both contribute to maintaining a normal serum glucose level. Glucose levels are also affected by the unregulated insulin production by the immature pancreatic β cells (Garg and Devaskar, 2006). As a result, the late preterm newborn can experience significant hypoglycemia secondary to developmentally immature hepatic enzyme systems for gluconeogenesis, glycogenolysis, and hormonal dysregulation (Garg and Devaskar, 2006; Raju et al., 2006; Engle et al., 2007).

The neonatal glucose requirement is 6 to 8 mg/kg per minute, which is higher than that observed in adults (3 mg/kg per minute)

(Bier et al., 1977). This demand for glucose increases if the late preterm infant has coexisting conditions such as sepsis, birth asphyxia, or cold stress (Greisen and Pryds, 1989; Halamek et al., 1997; Halamek and Stevenson, 1998). Treatment options for hypoglycemia in the late preterm infant include establishing early feeds (supplementing them with formula if the quantity of breast milk is insufficient), glucose infusion through IV fluids, hydrocortisone, and glucagon (Garg and Devaskar, 2006). The treatment choice for the late preterm infant is based on the underlying cause of the hypoglycemia. Regardless of the cause of the hypoglycemia, it is important to constantly monitor the glucose levels until they stabilize and the baby is tolerating adequate nutrition.

Hyperbilirubinemia

Hyperbilirubinemia is the most common clinical condition requiring evaluation and treatment in the late preterm newborn and the most common cause for readmission during the first postnatal week of life (Maisels and Kring, 1998; Brown et al., 1999; Bhutani et al., 2004; Escobar et al., 2005). Late preterm infants are more likely than full-term infants to be rehospitalized for jaundice (4.5% vs 1.2% in full-term infants) (Escobar et al., 2005).

In general, neonatal hyperbilirubinemia in late preterm infants is more prevalent, more pronounced, and more protracted than in their full-term counterparts. Newman et al. (1999) showed that infants born at 36 weeks' gestation have an eightfold increase in the risk of developing a total serum bilirubin concentration greater than 20 mg/dL (343 μ mol/L) when compared with those born at 41 weeks' gestation or later. Part of the reason for this increased risk is the immature hepatic metabolic pathways for bilirubin and the overall immaturity of gastrointestinal function and motility. The decreased ability for hepatic uptake and conjugation puts the late preterm infant at increased risk of elevated serum bilirubin levels, and the jaundice then becomes more prolonged, more prevalent, and more severe (Bhutani and Johnson, 2006). In addition, late preterm infants are at increased risk of kernicterus at bilirubin levels equal to or lower than those of full-term infants (Bhutani and Johnson, 2006). Kernicterus is a devastating, chronic, and disabling condition characterized by the tetrad of choreoathetoid cerebral palsy, neural hearing loss, palsy of vertical gaze, and dental enamel hypoplasia (Watchko, 2006).

Whereas the need for universal predischarge bilirubin testing in neonates is debated, it is generally accepted that late preterm infants are at higher risk and should not be included in the same management guidelines as full-term infants. The recommendation from the American Academy of Pediatrics from 2004 for the management of hyperbilirubinemia recommends that all newborns be assessed for their risk of developing hyperbilirubinemia by use of predischarge total serum bilirubin or transcutaneous bilirubin measurements (Kuzniewicz et al., 2009). The effectiveness of this policy was studied by Kuzniewicz et al. (2009), and they concluded that universal bilirubin screening, whether using transcutaneous bilirubin or total serum bilirubin for measurements, was associated with increased identification of newborns needing phototherapy and a significantly lower incidence of severe hyperbilirubinemia.

This finding underscores the need for close monitoring of late preterm infants, particularly breastfed infants whose mothers may not have a proper milk supply before being discharged home. Early discharges should be avoided until proper feeding has been established, and early follow-up should be arranged (Wallenstein and Bhutani, 2013).

Infectious Diseases

Late preterm infants are also more susceptible to infections because of their immunologic immaturity. The timing of the infection categorizes them as congenital (acquired before delivery), early onset (usually acquired during delivery and presenting within the first 72 hours), or late onset (often acquired in the hospital and presents after 72 hours of life). Congenital infections are commonly attributed to rubella virus, cytomegalovirus, herpes simplex virus, and human immunodeficiency virus (HIV) (Benjamin and Stoll, 2006). The severity of the infection and its effect on the late preterm infant depend on the stage of pregnancy at which the maternal infection occurred. With both herpes simplex virus and HIV, maternal–infant transmission is more frequent if a mother has a primary infection at the time of delivery, whereas maternal viral load is the main risk factor for HIV transmission from the mother to the newborn (Benjamin and Stoll, 2006).

Early-onset sepsis is almost always caused by perinatally acquired infections. In most cases the late preterm infant is initially colonized by exposure to various organisms in the maternal genital tract, including group B streptococcus, *Escherichia coli*, and *Candida* species (spp.). Additional risk factors for developing sepsis are prolonged rupture of membranes (more than 18 hours), maternal fever, and chorioamnionitis (Puopolo et al., 2011; Polin and Committee on the Fetus and Newborn, 2012).

The third group—late-onset sepsis—may be caused by perinatally or postnatally acquired organisms but is usually a consequence of nosocomial transmission. The most common organisms are gram-negative rods, but sepsis could be caused by *Staphylococcus aureus*, *Enterobacter* spp., or *Candida* spp. Although the mortality rate is low for late preterm infants, infections increase the risk of complications and often involve longer hospital stays (Benjamin and Stoll, 2006).

In addition, late preterm infants undergo testing for sepsis more often than full-term infants (36.7% vs 12.6%; odds ratio 3.97; 95% confidence interval 1.82–9.21; $P = .00015$) and receive antibiotics more often and for a longer duration (30% receive a 7-day course vs 17% of full-term infants; Wang et al., 2004). Other studies show that the need for a sepsis evaluation increases with decreasing gestational age; 33% were evaluated for possible sepsis at 34 weeks' gestation compared with 12% at 39 weeks' gestation ($P < .01$), of which only 0.4% of infants had culture-proven sepsis (McIntire and Leveno, 2008). This higher frequency of sepsis screening of late preterm newborns (compared with full-term newborns) may be multifactorial. First, records show that one-third of all preterm deliveries occur after prolonged premature rupture of membranes, which can put the newborn at a significantly higher risk of infection. Second, the higher rate of sepsis work-ups in the late preterm baby may be a reflection of a standard protocol used for admissions to the NICU or may be due to their clinical presentation (e.g., respiratory distress, hypothermia, hypoglycemia), which could either be a sign of sepsis or a reflection of the infants' immaturity.

Thermoregulation

Because they are smaller, late preterm infants are susceptible to periods of hypothermia or cold stress. Unfortunately, as for other problems discussed earlier, this may be difficult to assess if the late preterm newborn has been sent to the mother's room or is not closely observed. Usually cold stress will manifest itself as tachypnea or apnea, poor feeding, poor color caused by peripheral vasoconstriction, and metabolic acidosis. Hypothermia and its related consequences can

delay the respiratory transition and exacerbate hypoglycemia; these signs and symptoms may also be misinterpreted as possible sepsis, which then leads to unnecessary interventions and work-ups.

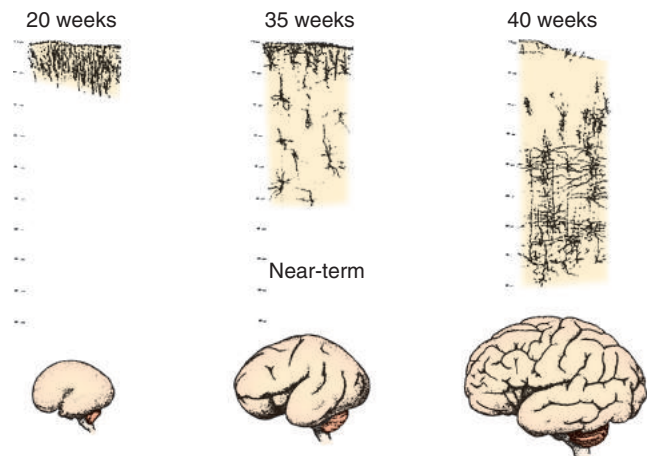
The reason that late preterm infants are more susceptible to temperature instability is because of their physiologic immaturity of thermoregulation, which in turn is dependent on the amount of brown and white adipose tissue and on body surface area (see Chapter 29). Nonshivering thermogenesis is controlled by the hypothalamic ventromedial nucleus through the sympathetic nervous system, which releases the neurotransmitter norepinephrine. The norepinephrine then causes the brown adipose tissue to liberate free fatty acids, which are eventually oxidized and produce heat (see Chapter 29). Late preterm infants have decreased stores of brown adipose tissue and the hormones responsible for brown fat metabolism (i.e., prolactin, norepinephrine, triiodothyronine, and cortisol). The stores of these hormones normally peak at term gestation, and the late preterm infant misses those last few weeks of in utero development. In addition to the decreased stores of hormones leading to thermogenesis, late preterm infants also have problems with hypothermia because of a decreased amount of white adipose tissue, which leads to less insulation and their smaller size. The late preterm infant's relatively smaller size, compared with full-term infants, leads to an increased ratio of surface area to body weight, which results in greater heat loss to the environment (see Chapter 29). Appropriate monitoring and triaging of the late preterm infant who is susceptible to temperature instability can avoid unnecessary morbidity, work-ups, interventions, and prolonged hospitalizations.

Neurodevelopment

During pregnancy, fetal lungs and brain are among the last organs to mature and are therefore more prone to injury. Not surprisingly, even healthy near-term or late preterm infants are at risk of developmental delays through the first 5 years of life (Raju, 2006). During the final few weeks of gestation, many aspects of brain maturity are still in progress. These aspects include maturing oligodendroglia, increasing neuronal arborization and connectivity, maturation of neurotransmitter systems, and continued brain growth that accounts for a 30% increase in brain size during the last few weeks of gestation (Fig. 32.9; Jain and Raju, 2013). At 34 weeks' gestation the brain weighs only 65% of the weight of the brain of a 40-week full-term infant (Billiards et al., 2006; Kinney, 2006). The brain of a late preterm infant is still immature and continues to grow until 2 years of age, when it reaches 80% of adult brain volume. In addition, the cerebral cortex is still smooth, and the gyri and sulci are not fully formed, and myelination and inter-neuronal connectivity are still incomplete. Multiple insults during this critical phase of neuronal and glial maturation cause white and gray matter injury, especially in the thalamic region and the periventricular white matter (Kinney, 2006). These events can be correlated subsequently to delayed development and special education needs; therefore it is important to start early developmental follow-up, anticipatory guidance, and interventions for infants born at 32 to 36 weeks' gestation (Chyi et al., 2008).

Hospitalization of the Late Preterm Infant

On the basis of the need for close monitoring and management of the various medical problems identified in late preterm infants, they are more likely than a full-term infant to require admission to an NICU. Despite this, individual hospitals and nurseries follow



• **Fig. 32.9** The immaturity of the laminar position and dendritic arborization of neurons, as demonstrated by Golgi drawings, in the cerebral cortex in the late preterm infant at 35 weeks' gestation is striking in comparison with neurons at midgestation (20 weeks) and at term (40 weeks). (From Kinney HC, Armstrong DD. Perinatal neuropathology. In: Graham DI, Lantos PE, eds. *Greenfield's Neuropathology*. 7th ed, London, United Kingdom: Arnold; 2002:557–559.)

different criteria regarding which infants to admit to the NICU, an intermediate care unit, or an observation area. Some routinely admit all infants born at less than 35 weeks' gestation to the NICU, whereas others do so on an individual basis. The most common reasons for admission include temperature instability, jaundice, respiratory distress, dehydration, poor feeding, and hypoglycemia (Wang et al., 2004; Vachharajani and Dawson, 2009). Studies have shown that 88% of infants born at 34 weeks' gestation, 12% born at 37 weeks' gestation, and 2.6% born at 38 to 40 weeks' gestation were admitted to the NICU (Engle and Kominiarek, 2008). Other studies have shown similar rates, and the overall trend was that the late preterm infant had significantly higher rates of NICU admission than 39-week infants (McIntire and Leveno, 2008; Ananth et al., 2013). In addition, the duration of hospitalization for the late preterm infant is inversely proportional to the baby's gestational age, which means that late preterm infants require longer hospitalization after birth than their full-term counterparts. On average, infants born at 34 weeks' gestation are hospitalized for 6 to 11 days, those born at 35 weeks' gestation are hospitalized for 4 to 6 days, and those born at 36 weeks' gestation are hospitalized for 3 to 4 days (Gilbert et al., 2003; Escobar et al., 2005; Phibbs and Schmitt, 2006; McIntire and Leveno, 2008; Khashu et al., 2009; Vachharajani and Dawson, 2009).

Mortality

Preterm birth is consistently recognized as the most pressing public health problem in perinatology by both clinicians and researchers, given its overall contribution to infant mortality. It also contributes substantially to neurocognitive, pulmonary, and ophthalmologic morbidity (Kramer et al., 2000). In a review of infant birth and death files from 1995 to 2002 in the United States, Tomashek et al. (2007) compared the overall and cause-specific mortality rates between singleton late preterm infants and full-term infants. They found that, despite significant declines since 1995 in mortality rates for late preterm and full-term infants, the infant mortality rate in 2002 was threefold higher in late preterm infants than in

TABLE 32.3 Mortality (Rate Per 1000 Live Births) in Infants Born at Late Preterm and Early Term Gestation

| Gestational Age (Weeks) | EARLY NEONATAL MORTALITY (1–7 DAYS) | | INFANT MORTALITY (1–365 DAYS) | |
|-------------------------|-------------------------------------|------------|-------------------------------|------------|
| | Mortality Rate | Risk Ratio | Mortality Rate | Risk Ratio |
| 34 | 7.2 | 25.5 | 12.5 | 10.5 |
| 35 | 4.5 | 16.1 | 8.7 | 7.2 |
| 36 | 2.8 | 9.8 | 6.3 | 5.3 |
| 37 | 0.8 | 2.7 | 3.4 | 2.8 |
| 38 | 0.5 | 1.7 | 2.4 | 2.0 |
| 39 | 0.2 | 0.8 | 1.2 | 1.2 |

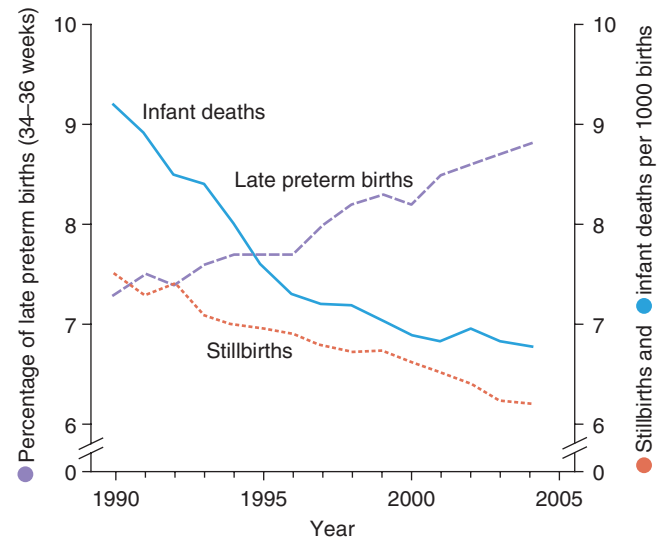
From Young PC, Glasgow TS, Li X, Guest-Warnick G, Stoddard G. Mortality of late-preterm (near-term) newborns in Utah. *Pediatrics*. 2007;119:e659–e665.

full-term infants (7.9 deaths vs 2.4 deaths per 1000 live births); early, late, and postneonatal mortality rates were sixfold, threefold, and twofold higher respectively. Young et al. (2007) found that in a large cohort from Utah, the relative risk of death increased for every decreasing week of gestational age less than 40 weeks (Table 32.3). In addition, a large study involving 133,022 infants born at 34 to 40 weeks' gestation found that neonatal mortality rates were significantly higher for late preterm infants (1.1, 1.5, and 0.5 per 1000 live births at 34, 35, and 36 weeks' gestation respectively compared with 0.2 per 1000 live births at 39 weeks' gestation, $P < .001$; McIntire and Leveno, 2008). In 2005, the US infant mortality rate for late preterm infants was 7.3 per 1000 live births versus 2.43 per 1000 live births in full-term neonates. Surprisingly, the mortality rate is 30% higher even for infants born between 37 and 39 weeks' gestation (early term) (Mathews and MacDorman, 2008). These studies and statistics again emphasize the fact that infants born just a few weeks early are at much greater risk of morbidity and death than those born at term gestation (Ananth et al., 2013; Brown et al., 2014). However, perinatal data collected over similar periods also reveal a remarkable decline in stillbirth rate (Fig. 32.10). Perinatologists argue that this reduction in fetal demise is directly related to close monitoring of the fetus and early intervention (delivery) when needed.

Recommendations

Admission Criteria

With more than 80% of all deliveries in the United States occurring in community hospitals (Jain and Raju, 2013), many of which have a relatively small number of deliveries and healthcare teams that might not always be equipped to assess and manage the needs of a late preterm infant, it becomes increasingly important to establish safeguards for the screening, identification, and appropriate triage of these patients. A subcommittee of the American Academy of Pediatrics Committee on the Fetus and Newborn (Engle et al., 2007) outlined recommendations for the care of the late preterm newborn. On the basis of these guidelines and the potential complications associated with late preterm infants, we recommend



• **Fig. 32.10** Trends in late preterm birth, stillbirth, and infant mortality in the United States, 1990 to 2004. The left axis shows trends in stillbirth and infant mortality rates; the right axis shows trends in late preterm births (34 to 36 weeks). Late preterm birth rates are shown per 100 live births, stillbirth rates are shown per 1000 total births, and infant death rates are shown per 1000 live births. (Modified from Ananth CV, Gyamfi C, Jain L. Characterizing risk profiles of infants who are delivered at late preterm gestations: does it matter? *Am J Obstet Gynecol*. 2008;199:329–331.)

that all infants born before 35 weeks' gestation and/or weighing less than 2300 g should be admitted to a transitional nursery where the infant can be monitored closely until there has been adequate time to assess the baby's vital signs, feeding abilities, and thermoregulation, among other issues, before the baby is sent to the mother's room. Sicker neonates who require intensive care obviously will need to receive higher levels of care. In addition, each nursery should establish guidelines for the frequency of monitoring vital signs, assessment for sepsis and use of antibiotics, and the use of supplemental oxygen. It is also important to determine a threshold (based on comfort level, staff training, and available resources) for transferring the newborn to a tertiary care center when the disease process associated with the late preterm infant continues to progress or worsen. Box 32.3 shows the recommendations for admission, management, and discharge of the late preterm infant. This list is not all-inclusive and was designed to be used as a guideline and not as a replacement for good clinical judgment.

Discharge Criteria

Because of the morbidities and risk factors associated with late preterm babies, they should not be discharged before 48 hours after birth. Before discharge, while the baby is still in the hospital, the following are recommended:

1. Vital signs should be within the normal range for at least 12 hours preceding discharge; this includes respiratory rate less than 60 breaths per minute, heart rate of 100 to 160 beats per minute, and axillary temperature of 36.5°C to 37.5°C in an open crib with appropriate clothing.
2. There should be documentation of passage of at least one stool spontaneously.
3. Adequate urine output should be accompanied by education of the parents about ways to assess the adequacy of output and

• BOX 32.3 Admission and Discharge Criteria and Management of Late Preterm Infants

Admission Criteria

- Admit all infants born before 35 weeks' gestation or weighing less than 2300 g at birth
- They should not be sent to their mother's rooms in the first 24 h until stable, unless arrangements can be made to provide transitional care and close monitoring in the mother's room

Hospital Management

- Physical examination on admission and discharge
- Determination of accurate gestational age on admission examination
- Vital signs and pulse oximetry check on admission, followed by check of vital signs every 3 to 4 h in the first 24 h, and every shift thereafter
- Caution against use of oxyhoods with high FiO_2 ; consider transfer to neonatal intensive care unit or tertiary care center if FiO_2 exceeds 0.4
- A feeding plan should be developed. Formal evaluation of breastfeeding and documentation in the record by caregivers trained in breastfeeding at least twice daily after birth
- Serum glucose screening per existing protocols for infants at high risk of hypoglycemia

Discharge Criteria

- Discharge should not be considered before 48 h after birth
- Vital signs should be within the normal range for the 12 h preceding discharge
- Respiratory rate less than 60 breaths per minute
- Heart rate of 100–160 beats per minute
- Axillary temperature of 36.5°C – 37.4°C measured in an open crib with appropriate clothing
- Passage of one stool spontaneously
- Adequate urine output
- Twenty-four hours of successful feeding: ability to coordinate sucking, swallowing, and breathing while feeding
- If weight loss is greater than 7% in 48 h, consider further assessment before discharge
- Risk assessment plan for jaundice for infants discharged within 72 h of birth
- No evidence of active bleeding at circumcision site for at least 2 h
- Initial hepatitis B vaccine has been given or an appointment has been scheduled for its administration
- Metabolic and genetic screening tests have been performed in accordance with local or hospital requirements
- The late preterm infant has passed a car seat safety test
- Hearing assessment has been performed and the results have been documented in the medical record; follow-up if necessary has been arranged
- Parents have been trained and demonstrate competency in caring for the infant
- Family, environmental, and social risk factors have been assessed; when risk factors are present, discharge should be delayed until a plan for future care has been generated
- Identification of a physician with a follow-up visit arranged for 24–48 h after discharge with a possibility of additional visits initially until the infant can demonstrate a consistent pattern of weight gain.

appropriate interventions if the urine output appears to decrease, with at least 24 hours of successful feeding with adequate coordination of sucking, swallowing, and breathing during feedings.

4. Weight loss should not exceed 7% of birth weight in the first 48 hours of life.

5. Serum or transcutaneous bilirubin level check—A transcutaneous bilirubin level higher than 12 mg/dL should warrant a serum bilirubin level check, which will then be stratified into a risk category by use of a bilirubin nomogram. Parents should also be educated on what to look for and what to do if their baby appears jaundiced.
6. Hearing screen, a car seat test, and metabolic and genetic screening tests should have been performed in accordance with state, local, and hospital protocols; if the baby is circumcised, there should be no bleeding at the site for at least 2 hours.
7. Hepatitis B vaccine should be given or an appointment should be made for its administration.
8. Parents should be educated about umbilical cord and skin care, identification of common signs and symptoms of illness, sleeping patterns and positions, use of the thermometer and the parameters for normal measurements, and responses to an emergency (i.e., CPR training before discharge).

If these guidelines (or other criteria outlined as standard of care) are not met, we recommend considering postponing discharge until the baby has been observed for a longer period and the issues have been resolved.

When additional risk factors are present (e.g., twin or multiple gestation, teenage mother), discharge should be delayed until an appropriate care plan has been generated. In addition, when indicated, it may be appropriate to arrange a nursing home health visit for closer monitoring, but this should not be used to replace the due diligence that must be done while the baby is in the hospital and the appropriate and timely follow-up with a pediatrician.

Follow-Up After Discharge

After discharge from the hospital, most of the medical care a newborn baby receives occurs in two main settings: the primary care physician's office and the emergency department. To avoid fragmented care by multiple emergency department visits and to allow an early assessment of the baby, it is recommended that the late preterm baby be brought for a checkup by its pediatrician within 24 to 48 hours after discharge from the hospital.

In addition, because of their increased risk of developmental delays, these infants should be monitored closely to ensure that all milestones are achieved appropriately and that early intervention (e.g., physical therapy, occupational therapy, speech therapy) is in place if needed. Early developmental testing can also be useful in determining any cognitive delays, which can then be addressed with individualized educational programs.

Readmission to the Hospital

The late preterm infant is susceptible to many of the problems of smaller preterm infants. Because of the multiple factors discussed throughout this chapter, the close observation and intensity of management provided to smaller premature infants are often lacking. In addition, there are often more lenient criteria for discharge, which sets them (and their parents) up for failure and eventual readmission to the hospital. [Tomashek et al. \(2007\)](#) looked at late preterm infants who were discharged early (<2 days after birth) from the hospital and found that 4.3% of late preterm infants and 2.7% of full-term infants were either readmitted or had an observational hospital stay.

Recent data suggest that the most frequent causes of emergency department visits by this subgroup of premature infants after being

discharged home are dehydration, feeding problems, respiratory distress, apnea, fever, infection, and jaundice (Jain and Cheng, 2006). Whether they are evaluated in the emergency department or in a pediatrician's office, it is important to have a lower threshold for readmitting these infants because of their vulnerability to serious complications. Their fragility is evidenced by their higher rate (4.4%) of rehospitalization than for full-term infants (2%; Escobar et al., 2005). In addition, 34- to 36-week infants who were never admitted to the NICU (or if admitted, their NICU stay lasted less than 24 hours) had nearly a threefold and 1.3-fold higher risk of readmission compared with full-term infants respectively (Escobar et al., 2005, 2006). Because late preterm infants are at a much higher risk of rehospitalization, they need close follow-up after discharge to assess breastfeeding and nutrition and to monitor them for jaundice.

Primary care physicians following up late preterm infants in an outpatient setting should also be cognizant that the higher risk of morbidities may persist beyond the neonatal period and into early childhood. One recent study showed that 30% of children younger than 2 years who were admitted to the pediatric intensive care unit for respiratory diseases were born prematurely. Of that group, 17% were former early preterm babies, and 12% were late preterm infants (Gunville et al., 2010).

Outcomes

As previously mentioned, late preterm babies are at a higher risk of long-term morbidities compared with their full-term counterparts. Although the highest risk of psychomotor, behavioral, cognitive, and other developmental disabilities still remains for the extremely low birth weight preterm infants, late preterm infants can also be affected. Late preterm infants often have a more subtle delay in their language skills and academic achievement. In addition, they have higher rates of attention-deficit and behavioral problems than their full-term counterparts (Mahoney and Jain, 2013). Since the distinction between full-term and late preterm infants has only recently been identified, the long-term data on outcomes are still being evaluated. A study on the role of prematurity and maternal factors in first-grade academic failure showed an increased chance of failure on the first-grade Criterion-Referenced Competency Test for former late preterm infants versus former full-term infants (Williams et al., 2013). In addition, two studies by Baron et al. (2009, 2010) looked at outcomes of late preterm infants and full-term infants at the age of 3 years. They found that the late preterm group had significantly lower scores on Differential Ability Scales-II visual spatial, visuomotor, executive function noun fluency, and verb fluency. In their follow-up study, they subdivided the group of late preterm infants into low-risk infants (not admitted to an NICU) and high-risk infants (admitted to an NICU) and found that the difference compared to the full-term group was mainly found in the high-risk group. Therefore they concluded that the increased risk of developmental delay of the high-risk late preterm infants (admitted to an NICU) is secondary to clinical instability, increased morbidities, sex differences, and lower birth weight—with male sex and neonatal morbidities contributing to early cognitive weaknesses. These studies add to the increasing evidence showing the vulnerability of late preterm infants, especially those who required NICU care, and the need for close neurodevelopmental follow-up (Vohr, 2013).

From a respiratory perspective, late preterm infants (even those without any significant respiratory disease in the neonatal period)

have compromised long-term lung function compared with their full-term counterparts. One study (Hoo et al., 2002) of airway function in healthy premature infants with a mean gestational age of 33.2 weeks (who did not have respiratory illness in the neonatal period) showed significantly diminished maximal expiratory flow at functional residual capacity at 1 year of age. Another study (Todisco et al., 1993) evaluated children who were former late preterm infants (without RDS or need for mechanical ventilation during their neonatal period) and compared them with their full-term siblings. They found that the former late preterm children had significantly increased mean residual volumes compared with their siblings. Some studies have also shown an increased risk of asthma in the late preterm population. A retrospective study by Goyal et al. (2011) revealed that compared with full-term babies, late preterm infants had an increased incidence (on the basis of diagnosis) of persistent asthma and increased use of inhaled corticosteroids.

Future Research

Since 1981 the number of premature births in the United States has increased by 30%. Most of this increase is attributable to the increased number of late preterm infants (Pulver et al., 2009)—a unique and high-risk subgroup of premature infants. The identification and care of this group and their unique challenges have only recently been recognized as a high-priority area of research in the field of neonatology. The awareness of the significance of morbidity and disease burden of the late preterm infant has sparked multiple initiatives in the practice of neonatology to address the need for early recognition and specialized care for this patient population. Many birthing hospitals now have late preterm protocols in their postpartum nurseries and NICUs addressing the inpatient care and discharge criteria for these babies as well as closer outpatient follow-up with primary care physicians in their postdischarge care. In addition, changes instituted in the care and timing of delivery of the late preterm gestation mother have also had a significant impact. One multicenter study showed that over a 1-year period, elective scheduled early term deliveries decreased from 27.8% in the first month of the study to 4.8% by the 12th month of the study. In addition, that study also showed that the rates of elective, scheduled, singleton, early term inductions, and cesarean deliveries decreased significantly in the same period (Oshiro et al., 2013). Another advance in the field of late preterm research is that modes of therapy considered standard of care for full-term babies are now being evaluated for their safety and efficacy in the late preterm population. There are several ongoing trials, including one by the Eunice Kennedy Shriver National Institute of Child Health and Human Development, to evaluate the use of therapeutic hypothermia in late preterm babies with hypoxic-ischemic encephalopathy.

There are many questions still to be answered regarding the care and outcomes associated with late preterm infants (Box 32.4). Topping this list of priorities is the need to know which babies are better off being delivered early and, for the rest, what can be done to take pregnancy as close to term as possible. Because answers to many issues related to preterm birth still remain elusive, clinicians have, rightly so, focused on ways to improve the postnatal management of these premature babies. New studies, albeit mostly retrospective, have added to the understanding of the spectrum of morbidities in late preterm infants; they have also formed the basis for current strategies to standardize management and optimize outcomes.

• BOX 32.4 Unanswered Questions and Future Directions

Epidemiology, Trends, Etiology, and Prevention of Late Preterm Births

- Is there a subset of preventable late preterm births?
- What is the effect of continuing such pregnancies (preterm rupture of membranes, preterm labor, medically indicated birth) on perinatal outcomes?
- Is there a role for antenatal administration of steroids in late preterm gestation pregnancies threatened with preterm delivery?
- Should singleton and multiple gestation late preterm deliveries be treated differently?

Clinical Management and Outcomes of Late Preterm Infants

- Can standardized “care paths” for late preterm infants improve outcomes?
- Optimal discharge strategies that minimize readmissions and other problems after discharge
- Strategies for management of rapidly progressive and/or severe morbidities in late preterm infants
- Standardized approach to central nervous system imaging and postdischarge follow-up

Long-Term Outcomes

- Prospective studies of long-term outcomes of symptomatic and asymptomatic late preterm births to determine which infants should have long-term follow-up

Suggested Readings

Definition, Epidemiology, and Background

Davidoff MJ, Dias T, Damus K, et al. Changes in the gestational age distribution among U.S. singleton births: impact on rates of late preterm birth, 1992 to 2002. *Semin Perinatol.* 2006;30:8-15.

Engle WA, Tomashek KM, Wallman C. “Late-preterm” infants: a population at risk. *Pediatrics.* 2007;120:1390-1401.

Raju TN, Higgins RD, Stark AR, et al. Optimizing care and outcome for late preterm (near-term) infants: a summary of the workshop sponsored by the National Institute of Child Health and Human Development. *Pediatrics.* 2006;118:1207-1214.

Pathophysiology and Clinical Course

Gyamfi-Bannerman C, Thom EA, Blackwell SC, et al. Antenatal betamethasone for women at risk for late preterm delivery. *N Eng J Med.* 2016;374:1311-1320.

Jain L. Alveolar fluid clearance in developing lungs and its role in neonatal transition. *Clin Perinatol.* 1999;26:585-599.

McIntire DD, Leveno KJ. Neonatal mortality and morbidity rates in late preterm births compared with births at term. *Obstet Gynecol.* 2008;111:35-41.

Wang ML, Dorer DJ, Fleming MP, Caitlin EA. Clinical outcomes of near-term infants. *Pediatrics.* 2004;114:372-376.

Mortality

Kramer MS, Demissie K, Yang H, Platt RW, Sauvé R, Liston R. The contribution of mild and moderate preterm birth to infant mortality. *JAMA.* 2000;284:843-849.

Tomashek KM, Shapiro-Mendoza CK, Davidoff MJ, Petrini JR. Differences in mortality between late-preterm and term singleton infants in the United States, 1995-2002. *J Pediatr.* 2007;151:450-456.

Long-Term Outcome

Chyi LJ, Lee HC, Hintz SR, Gould JB, Sutcliffe TL. School outcomes of late preterm infants: special needs and challenges for infants born at 32 to 36 weeks gestation. *J Pediatr.* 2008;153:25-31.

Shah PE, Kaciroti N, Richards B, Lumeng JC. Gestational age and kindergarten school readiness in national sample of preterm infants. *J Pediatr.* 2016;178:61-67.

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33

Neonatal Pharmacology

KAREL ALLEGAERT, ROBERT M. WARD, AND JOHN N. VAN DEN ANKER

KEY POINTS

- The key feature of neonatal physiology is fast maturation, resulting in extensive variability in pharmacokinetics (PK) and pharmacodynamics (PD), further aggravated by other covariates, including pharmacogenetics (PGx). Variability is the essence of both neonatal care and neonatal pharmacology.
- Thorough understanding of the factors, especially developmental changes, that affect pharmacokinetics (absorption, distribution, metabolism, elimination) in neonates helps to provide accurate dose adjustments to provide effective drug therapy.
- Adverse drug events are frequent and important aspects of drug therapy in neonatal care. Pharmacovigilance is based on prevention, detection, and assessment, but all these issues need to be adapted to the specific characteristics of this population. Minimization or prevention of these kinds of events can be achieved.

Dynamic changes related to growth and maturation in newborns create unique complexities in drug therapy that affect the variability in pharmacokinetics (PK) (absorption, distribution, metabolism, elimination) and pharmacodynamics (PD) (both desired efficacy and unwanted side effects). Pharmacologic studies during this period of rapid growth and physiologic maturation reveal diverse patterns of changes. These patterns also reflect the maturation of different drug-metabolizing enzymes or pathways of elimination throughout infancy. Therapeutic drug monitoring is applied to only a limited numbers of drugs (e.g., phenobarbital, gentamicin), because the concentrations need to correlate with both effects and possible side effects. Dose adjustments to reach the desired concentrations can be estimated at the bedside with use of simple calculations. Pharmacogenetics (PGx) or pharmacogenomics of drug-metabolizing enzymes, transporters, or receptors explains a large part of the observed variations among individuals in their responses to drugs. The immature, preterm, or critically ill newborn adds another level of complexity to this variability in drug disposition and effects.

Principles of Neonatal Therapeutics

In neonates a thorough understanding of factors (“covariates”) that influence drug concentrations will enhance accurate and effective drug therapy and may help identify the causes of treatment failure or therapy-related toxicity. Many relevant covariates are not

incorporated in a therapeutic plan, although the impact on the effectiveness and safety of the investigational drug as well as that of current drug therapy may be tremendous. Since PK (concentration–time profiles) and PD (concentration–effect profiles) in newborns follow the same general principles that govern drug actions in patients of all ages, the diagnosis, drug selection, and administration to achieve a therapeutic goal must consider the impact of *absorption*, *distribution*, *metabolism* and *excretion* on the dose–exposure relationship. In newborns these principles should include special consideration of the unique physiologic and pharmacologic features of these developmentally immature patients (Box 33.1).

Diagnosis

Effective treatment begins with an accurate diagnosis and assessment of symptoms. Although this applies to all areas of therapeutics, treatment in newborns presents specific challenges. Their small size and fragility may preclude useful, but inordinately invasive, diagnostic procedures. For example, newborns with chronic lung disease are treated for “bronchospasm” on the basis of the findings of decreased air entry, desaturation, and abnormal breath sounds. Relief of these symptoms with aerosolized bronchodilators may be interpreted as confirming this diagnosis. Although this may be correct, increased humidity or movement of the endotracheal tube bevel away from a pliable tracheal wall during aerosol treatment may also result in improvement. A similar argument can be made for suspected neonatal infection, the clinical diagnosis of a patent ductus arteriosus, or neonatal pain management. Therefore evaluation of an ineffective therapy should include reconsideration of the diagnosis, in the same way as conclusions about why a therapy succeeded should be made cautiously.

Absorption

Absorption is the movement of a drug into the systemic circulation. It generally requires the crossing of membranes or membrane barriers and is characterized by rate (time to peak) and extent (percentage of dose). The most commonly used route in neonates is *intravenous administration*. However, even this route of administration has challenges related to neonatal pathophysiology and limitations of intravenous infusion systems (e.g., slow flow, small volume, dead space volume, limited flush volume) (Sherwin et al., 2014; Linakis et al., 2016). Consequently, drugs should be infused into the patient as close as possible to the site of the venous

• BOX 33.1 Pharmacologic Principles and Pitfalls in the Management of Very Low Birth Weight Infants

1. Diagnosis:
 - a. Limited diagnostic procedures
2. Absorption:
 - a. Intravenous:
 - I. Drug injection away from patient
 - II. Uneven mixing of drugs and intravenous fluids
 - III. Delayed administration due to very low flow
 - IV. Part of the dose discarded with tubing changes
 - b. Intramuscular:
 - I. Poor perfusion limits absorption
 - II. Danger of sclerosis or abscess formation
 - III. Depot effect
 - c. Oral:
 - I. Poorly studied
 - II. Affected by delayed gastric emptying
 - III. Potentially affected by reflux
 - IV. Passive venous congestion may occur with chronic lung disease, decreasing absorption
3. Distribution (affected by):
 - a. Higher (85%) total body water (versus 65% in adults)
 - b. Lower body fat, i.e., about 1% body weight (versus 15% in term newborns)
 - c. Low protein concentration
 - d. Decreased protein affinity for drugs
4. Metabolism:
 - a. Half-life prolonged and unpredictable
 - b. Total body clearance decreased
 - c. Affected by nutrition, illness, and drug interaction
 - d. Affected by maturational changes
5. Excretion:
 - a. Decreased renal function, both glomerular filtration rate and tubular secretion/absorption

access. If a drug is injected further away from the infant and at a slow rate, the drug may reach the patient far too slowly to achieve effective concentrations. Infusion solution filters may further hamper drug delivery by blocking large molecules, by adsorption of the drug to the filter, or by allowing a heavier drug to settle in the filtration chamber and mix slowly (Sherwin et al., 2014; Linakis et al., 2016). For drugs in which the peak concentration matters (e.g., aminoglycosides) or when the driving force for tissue penetration is a concentration gradient between the circulation and the tissue (e.g., meningitis), these limitations may result in suboptimal therapy.

Intramuscular administration of drugs is used for slow release (e.g., vitamin K, palivizumab) but is a poor substitute for intravenous access and should be avoided for multiple doses. Absorption following intramuscular injection relates to muscle blood flow and depends on maturation and disease characteristics (e.g., hypothermia, shock). Furthermore, intramuscular administration may result in sclerosis of tissues, causing sterile abscesses, or create large intramuscular collections, which are subsequently absorbed slowly, producing a “depot effect” in which serum concentrations rise slowly over time.

Oral administration of drugs is preferred for treatment of chronic illnesses in newborns, but this route is not very well studied in acutely ill preterm neonates. Oral absorption depends on gastric emptying, gastric pH, intestinal motility, intestinal first-pass

metabolism, intestinal surface, and intestinal permeability. All these covariates display maturation and may be affected by disease characteristics (Mooij et al., 2012). Many newborns experience gastroesophageal reflux and delayed gastric emptying. This prolongs and delays absorption, which reduces the peak concentration and may also affect the total dose absorbed. Passive intestinal venous congestion caused by elevated right atrial pressure decreases drug absorption and may do so in premature infants with severe bronchopulmonary dysplasia (Peterson et al., 1980). Coadministration of medications to newborns with small volumes of milk or during continuous gastric or duodenal feedings may also alter absorption.

Buccal, lingual, or rectal administrations are additional enteral routes that are all associated with variability. For clinicians, this means that if enteral drug therapy fails, the impact of the route or feeding patterns on drug absorption must be considered. Finally, unanticipated absorption of drugs intended for topical effects (e.g., cutaneous, inhalation) should be considered but are also associated with relevant side effects (Kearns et al., 2003; Choonara, 2013).

Distribution

Distribution is the partitioning of drugs among various body fluids, organs, and tissues. The distribution of a drug within the body is determined by several factors, including organ blood flow, pH and composition of body fluids and tissues, physical and chemical properties of the drug (e.g., lipid solubility, molecular weight, and ionization constant), and drug transporter activity but also by the extent of drug binding to plasma proteins and other macromolecules (Kearns et al., 2003; Brouwer et al., 2015).

Physiologic differences between preterm neonates, children, and adults affect drug distribution. Total body water content ranges from 85% in premature newborns to 75% in term newborns to 65% in adults (Kearns et al., 2003; Allegaert and van den Anker, 2015a). Conversely, body fat content ranges from 0.7% or less in extremely premature newborns to 12% in term newborns. These differences affect the distribution of water-soluble (e.g., aminoglycosides, acetaminophen), lipophilic (e.g., propofol) drugs or nonpolar drugs (e.g., fentanyl). The protein-binding capacity of drugs in the circulation is lower in early infancy because of lower circulating protein concentrations (e.g., albumin, α_1 -acid glycoprotein) and lower binding affinity. Competitive binding with bilirubin is another specific issue to be considered in neonates (Kearns et al., 2003). With rare exceptions, only the free (unbound) drug crosses membranes, exerts pharmacologic actions, and undergoes metabolism and excretion. However, measurements of drug concentrations usually reflect total circulating drug concentrations, which consist of both free and protein-bound drug concentrations. Thus even when total circulating drug concentrations in the newborn may be low by adult standards, the free drug concentrations may still be equivalent or even higher than those in the adult because of the decreased protein binding.

Metabolism

Drug-metabolizing enzymes are crucial in the extent of drug biotransformation. Although the liver is considered the major organ responsible for drug biotransformation, other organs such as the intestines, lungs, and kidneys also contribute to drug metabolism. These metabolizing enzymes can be classified as participating in nonsynthetic phase I reactions (e.g., oxidation, reduction, hydrolysis) or synthetic phase II reactions (e.g., glucuronidation, sulfation,

acetylation). Their metabolites can subsequently be eliminated by renal, biliary, or other excretion routes and may also be therapeutically active or contribute to adverse effects.

The primary type of enzymes involved in phase I reactions are cytochromes P450 (CYPs). CYPs are microsomal, mixed-function oxidases that catalyze chemical changes within a molecule, such as hydroxylation, methylation, demethylation, addition of oxygen, and removal of hydrogen. There are thousands of different CYPs in plants and animals, on land and in water. CYPs with more than 36% of the same amino acid sequence are grouped as a numbered family (e.g., CYP3). Those with more than 77% polypeptide homology belong to the same subfamily (e.g., CYP3A), and specific genes are denoted with a number (e.g., CYP3A5). The amino acid sequence of these enzymes determines the tertiary structure that creates a hydrophobic pocket with selective binding for chemicals and drugs. This substrate specificity creates groups of drugs, often with similar structure and function, that are metabolized by the same CYP. CYPs mature at different rates and in different patterns. In addition, single nucleotide polymorphisms (SNPs) or substitutions in the DNA sequence for a CYP may reduce its metabolizing activity or completely eliminate it if the polypeptide cannot be formed (cf. PGx). Conversely, some individuals inherit multiple copies of a CYP, producing “supermetabolizers” (CYP2D6). The phase II conjugation enzymes, such as the uridine 5′-diphosphoglucuronosyltransferases (UGTs) have several forms with different substrate specificity (e.g., UGT1A1 for bilirubin, UGT2B7 for morphine) although they are not as absolutely selective as the CYPs.

All enzymes display isoenzyme specific maturation, and this affects drug metabolism throughout infancy. Consequently, the clearance of almost all drugs is decreased in neonates compared with older children and adults, but important variations occur among drug classes and among individuals, which prevents simple generalization. Maturation correlates best with postmenstrual age for some CYPs and with postnatal age for others. Some CYPs do not reach adult activity until the person is several years of age, while others develop activities twice those of the adult during childhood, which shows why generalizations about patterns of CYP maturation are seldom possible. Individual hepatic drug-metabolizing enzymes can be categorized into one of three classes on the basis of their developmental trajectories (*class I*, highest activity in fetal life; *class II*, low phenotypic activity in infancy, increasing at puberty; *class III*, substantial increase in the first months to years of life) (Hines, 2008). Although this classification is very helpful to explain and even predict maturational drug disposition, it should be used cautiously. We cannot simply miniaturize “major” and “minor” routes of elimination as initially documented in adults to (pre)term neonates. In the absence of an adult major route, a minor route, either metabolic or primary elimination, may be a more relevant route of clearance in neonates (e.g., caffeine elimination is through renal elimination in neonates and through metabolic elimination by CYP1A2 in adults). A similar argument can be made for acetaminophen. Glucuronide conjugation is usually low at birth. In contrast, conjugation through sulfation is usually active at birth. These different patterns are reflected in the developmental changes in acetaminophen metabolism in early life (Krekels et al., 2015).

Besides age-driven maturation, other factors such as nutrition, illness, PGx, or drug–drug interactions further affect the phenotypic activity of enzymes and organs responsible for drug metabolism in the newborn. Maturation changes in hepatic blood flow, drug transport into hepatocytes, synthesis of serum proteins, protein

binding of drugs, and biliary secretion further confound accurate predictions about drug metabolism after birth.

Excretion

Drug elimination from the body can occur through several mechanisms, including renal excretion, biliary excretion, transcutaneous loss, and pulmonary exhalation. Renal excretion is the most important pathway for elimination of unchanged drugs or metabolites, either by glomerular filtration or by renal tubular transport activity (reabsorption, secretion).

Glomerular function rises steadily after birth, whereas tubular function matures more slowly, causing a glomerular-to-tubular imbalance (Ligi et al., 2013). Neonatal renal function is diminished both in absolute terms and when normalized to body weight or surface area. The neonatal glomerular filtration rate averages about 30% of the adult rate per unit surface area. The creatinine clearance depends on both gestational and postnatal age and doubles by the end of the first month of life (Vieux et al., 2010). This postnatal increase in glomerular function relates to higher cardiac output, reduced renal vascular resistance, redistribution of intrarenal blood flow, and changes in intrinsic glomerular basement membrane permeability. These age-dependent dynamics of neonatal renal function markedly influence drug excretion. Similar to drug metabolism, however, the variability in renal elimination capacity and drug clearance is further affected by other covariates, such as hypoxemia, nephrotoxic drugs, underperfusion, hypothermia, and intercurrent renal diseases.

Some drugs, such as micafungin, nafcillin, and spironolactone metabolites, are eliminated primarily through biliary excretion. Drugs that are conjugated within the liver may also be excreted through bile only to enter the intestinal tract, where they may be deconjugated and undergo enterohepatic recirculation, similarly to bilirubin. Although biliary excretion is not well studied in newborns, clinical conditions such as parenteral nutrition–associated cholestasis suggest that it may be quite variable among specific patients and conditions.

Transporters play important roles in the uptake or removal of drugs. Compared with enzymes, the ontogeny is still poorly described. A recent review suggests that different developmental patterns for individual transporters are emerging (Brouwer et al., 2015). Organic anion transporter polypeptides provide facilitated transport of anions in many tissues, including the kidney and liver. P-glycoprotein (PGP), the permeability glycoprotein, is an efflux transporter that belongs to the adenosine triphosphate-binding cassette/multiple drug resistance family of transporters and prevents absorption of many compounds across the intestinal wall or into the brain. PGP expression has been described as limited at birth, reaching adult levels at 3–6 months of age (Lam et al., 2015). To put these findings into perspective, the limited PGP efflux activity increases opioid concentrations in the central nervous system of newborns and likely explains the higher incidence of apnea in neonates following opioid exposure.

Pharmacogenetics and Pharmacogenomics

The Human Genome Project has described the structures of many proteins (enzymes, transporters, receptors) that have a role in the PK and PD of many drugs currently used in pediatric and adult pharmacotherapy. Genetic variants that alter activity have been identified, many based on SNPs in these proteins. As mentioned earlier, genetic variation in drug-metabolizing enzymes

can have a significant influence on the relative activity within a particular individual. Large interindividual variation occurs for several isoenzymes and is often explained by inherited differences in activity. Changes in a single nucleotide in the DNA for one of these enzymes is designated with a star, such as CYP2C9*1, and these changes can alter the protein structure enough to decrease, completely inactivate, or increase its enzymatic activity. SNPs have been identified for many CYPs, and ethnic variations in these SNPs help predict when their activity is likely to be reduced or increased. A similar case can be built for drug transporters or receptors.

Knowledge of pharmacogenetic and pharmacogenomic factors that affect PK (metabolism, transport) or PD (receptor) has been important for understanding ways to avoid unanticipated drug effects in adults and to some extent in older children. The aim to individualize pharmacotherapy with the use of PGx reflects the fact that specific effects/side effects are not just randomly distributed but relate to genetic variation in the level of activity of transporters, drug-metabolizing enzymes, and/or receptors. Tailoring neonatal pharmacotherapy to individual patients with use of this knowledge holds great promise for the future and serves as a model of precision medicine in newborns (Allegaert and van den Anker, 2015a; Mooij et al., 2015).

The most commonly applied approach to evaluate PGx in neonates is to search for similar signals initially reported in adults or children (*from adult to newborn* approach). For drug-metabolizing enzymes such as CYP2C19 (pantoprazole dealkylation), CYP2D6 (tramadol O-demethylation), *N*-acetyl transferase 2 (isoniazide acetylation), or UGT2B7 (morphine glucuronidation), the impact of polymorphisms on neonatal drug metabolism has been documented (Allegaert and van den Anker, 2015a). However, these drug-metabolizing enzymes also mature during the first months or years of life. Consequently, it can be difficult to determine to what extent the variability in drug metabolism is explained by genetic expression, maturation, or other covariates. Drug metabolism is often reduced in neonates, and scaling of drug dose by simple body weight or allometrically with an exponential function will not fully compensate for differences in clearance that exist in this newborn population. Clearance often differs severalfold among adults, and the same degree of variation is emerging among neonates, whether due to maturation of the expression of these enzymes by gestational age (GA) or due to induction of protein synthesis after birth.

In addition to this approach, PGx should also be tailored to neonates and only mirror observations initially described in adults. Pharmacogenetic studies may also provide information on the ontogeny of processes that have not yet been well described, such as transporter or receptor ontogeny (pharmacogenetic concordance). A recent illustration of such an exploration is the impact of polymorphisms on neonatal abstinence syndrome following maternal opioid intake. Specific catechol-*O*-methyltransferase (*COMT*, 158 adenine [A] > guanine [G]) and μ -opioid receptor (*OPRM1*, 118A > G) polymorphisms were associated with the extent and the duration of neonatal abstinence syndrome (Wachman et al., 2013). Pharmacogenetic studies may focus on the concordance between genotype and phenotype within cohorts of newborns and young infants. A structured approach to assess the contribution of genetic variation in addition to maturation to pharmacokinetic/pharmacodynamic variability has been suggested and is based on five questions (Leeder, 2009):

1. What gene products (if known) are relevant for the disposition of a given compound?

2. What is the developmental trajectory (if known) of functional (e.g., transporter, enzyme, or receptor) activity?
3. Does allelic variation affect the function(s) of a given compound?
4. Does allelic variation affect the developmental drug disposition phenotype?
5. What is the developmental context of the relevant genes?

Pharmacokinetic Principles

Pharmacokinetics describes the time course of changes in drug concentrations within the body. Although rates of change are often described with differential equations, concepts useful at the bedside are emphasized here. More detailed mathematical discussions of PK can be found elsewhere (Buxton, 2006; Rowland and Tozer, 2010; Anderson, 2012).

Compartment

In PK, *compartment* refers to fluid and tissue spaces into which drugs penetrate. These compartments may or may not be equivalent to anatomic or physiologic volumes. In the simplest case the compartment may correspond to the vascular space and equal the volume of a real body fluid, blood. Large or quite polar molecules may be confined to this central compartment until they are eliminated by excretion or metabolism. Many drugs, however, diffuse reversibly out of the central compartment into tissues or other fluid spaces, referred to generically as *peripheral or tissue compartments*. Diffusion can be driven, in part, by differences in protein binding between compartments or can be affected by active transport (influx/efflux) processes. These kinds of compartments are seldom sampled directly, but their involvement in kinetic processes may be recognized from the graphical or mathematical description of the kinetics of a drug. The number of exponential terms necessary to adequately describe the kinetic profile of a drug designates the number of compartments involved, recognizing that many more compartments may exist. For appropriate clinical application, rarely more than three compartments are required to describe the PK of any drug.

Apparent Volume of Distribution

The apparent volume of distribution might be better termed the *volume of dilution* because it is a mathematical description of the volume (L or L/kg) that dilutes a dose (mg or mg/kg) to produce the observed circulating drug concentration (mg/L or $\mu\text{g/mL}$). To simplify cancellation of units, concentrations are expressed here as milligrams per liter (mg/L), which is the same as micrograms per milliliter ($\mu\text{g/mL}$), the more conventional unit for drug concentrations.

$$\text{Concentration (mg/L)} = \frac{\text{Dose (mg/kg)}}{\text{Apparent Volume of Distribution (L/kg)}}$$

For many drugs the volume of distribution does not correspond to a specific physiologic body fluid or tissue, hence the term *apparent*. In fact, the volume of distribution for drugs that are bound extensively in tissues may exceed 1.0 L/kg, a physiologic impossibility that emphasizes the arithmetic, nonphysiologic nature of the apparent volume of distribution. The determination of the volume of distribution is described later.

First-Order Kinetics

Removal of most drugs from the body can be described by first-order (exponential or proportional) kinetics, in which a constant *proportion or percentage* of a drug is removed per unit of time (e.g., 50% in one half-life interval), rather than a constant amount per unit of time. Consequently, for drugs exhibiting first-order kinetics, the higher the concentration, the greater the amount removed during a time interval. The following equations describe the concentration (C) of a drug whose first-order kinetics has a rate constant, k (per hour), at time t and an initial concentration of C_0 achieved after administration of a dose.

In differential equation form, the change in concentration with time is:

$$\frac{dC}{dt} = -kC$$

The solution to this differential equation gives the exponential form, which describes C at time t :

$$C_t = C_0 e^{-kt}$$

If this equation is transformed with use of the natural logarithm (\ln), it becomes:

$$\ln C_t = \ln C_0 - kt$$

The last equation is the equation of a straight line, so a graph that plots $\ln C_t$ versus t has an intercept of $\ln C_0$, the concentration at $t = 0$, and a slope of $-k$, the rate constant for the change in concentration. This can be used to calculate the half-life (see the next section) and to estimate appropriate dosages. Multiple rate constants in more complex equations are distinguished with the letter k and numbered subscripts or with Greek letters.

Half-Life

The elimination half-life ($t_{1/2}$) of a drug is the time required for its concentration to decrease by 50%. Consequently, half-life is linked to a first-order kinetic process because the same proportion, 50%, of the drug is removed during equal periods. Half-life can be determined mathematically from the elimination rate constant, k , as:

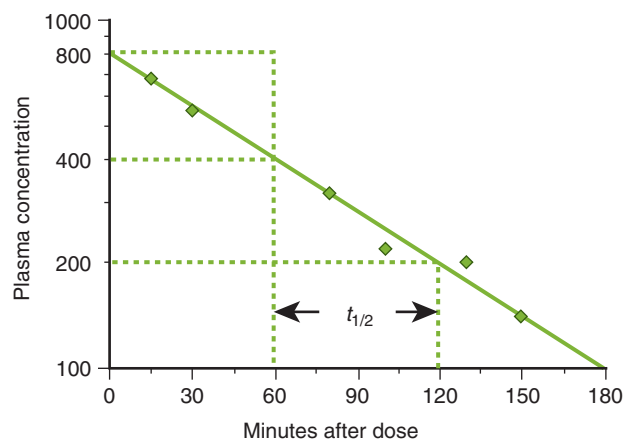
$$t_{1/2} = \frac{\text{Natural Logarithm } 2}{k} = \frac{0.693}{k}$$

Fig. 33.1 illustrates a graphical method for determination of half-life. Drug concentrations measured serially are graphed on semilogarithmic axes, and the best-fit line is determined either visually or by linear regression analysis. In this illustration of first-order kinetics, the concentration decreases by 50% (from 800–400 mg/L) during the first hour and decreases by another 50% (from 400–200 mg/L) during the second hour. Thus the half-life is 1 hour. More drug is removed during one half-life at higher concentrations, although the proportion removed remains constant. The exponential equation for this graph is:

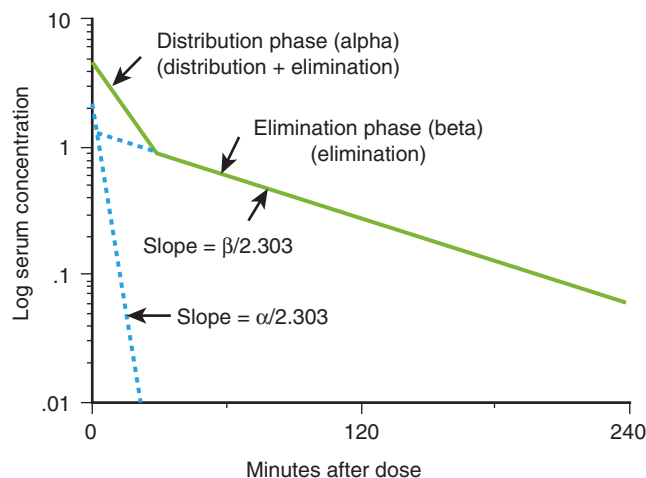
$$C = 800e^{-0.693t}$$

where $k = 0.693/\text{hour}$ and $C_0 = 800$, allowing a mathematical calculation of half-life with use of the equation previously described:

$$t_{1/2} = \frac{0.693}{k \text{ (/hour)}} = \frac{0.693}{0.693/\text{hour}} = 1 \text{ hour.}$$



• **Fig. 33.1** Apparent single-compartment first-order plasma drug disappearance curve illustrating graphical determination of half-life from the best-fit line of serial plasma concentrations.



• **Fig. 33.2** Multicompartment Serum Drug Disappearance Curve. α , Rate constant for distribution; β , rate constant for terminal elimination.

Multicompartment First-Order Kinetics

The rate of removal of many drugs from the circulation is often biphasic. An initial rapid decrease in concentration is referred to as the distribution (α) phase, often lasting 15–45 minutes, which is followed by a sustained slower rate of removal, the elimination (β) phase. Such biphasic processes are best visualized from semilogarithmic graphs of concentration versus time. When such semilogarithmic graphs show kinetics that best fit two straight lines, the kinetics are described as *biexponential*, or reflective of a drug that shows *two-compartment first-order pharmacokinetics* (Fig. 33.2). Two exponential terms are needed to describe the change in concentration over time, as:

$$C = Ae^{-\alpha t} + Be^{-\beta t}$$

In this equation the rate constant for distribution is designated α to discriminate it from the rate constant for terminal elimination (β), where A and B are the $t = 0$ intercepts for the lines describing distribution and elimination respectively. Division by 2.303 converts logarithms to natural logarithms.

After an intravenous dose, drug loss from the vascular space during the distribution phase occurs through both distribution

and elimination (see Fig. 33.2). The rate constant of distribution (α) can be determined by a plot of the difference between the total amount of drug lost initially and the amount of drug lost through elimination (Rowland and Tozer, 2010). This produces the line with the steeper slope (equal to $\alpha/2.303$) below the serum concentration graph in Fig. 33.2. The single slope of the distribution phase and of the terminal elimination phase does not imply that distribution or elimination occurs through a single process. The observed rates usually represent the sum of several simultaneous processes, each with differing rates, occurring in various tissues.

When the time course of drug elimination is observed for prolonged periods, a third rate of elimination, or γ phase, may also be observed and is usually attributed to the elimination of a drug that has reequilibrated from deep tissue compartments back into the plasma. Such kinetics are designated *three-compartment first-order pharmacokinetics*. The kinetics of a drug are expressed with the smallest number of compartments that accurately describes its concentration changes over time.

Apparent Single-Compartment First-Order Kinetics

When a semilogarithmic graph of concentration versus time reveals a single slope with no distribution phase, the kinetics are characterized as *apparent single-compartment first-order kinetics* (see Fig. 33.1). This kind of kinetics can occur when a drug remains entirely within the vascular space or central compartment or when a drug passes very rapidly back and forth between the blood and peripheral sites until it is metabolized or excreted by first-order kinetics. The adjective *apparent* is used because careful study (e.g., very early sampling) often shows that distribution occurs even though the kinetic curve has only a single slope. Single-compartment kinetics implies that the drug rapidly and completely distributes homogeneously throughout the body, which rarely occurs clinically.

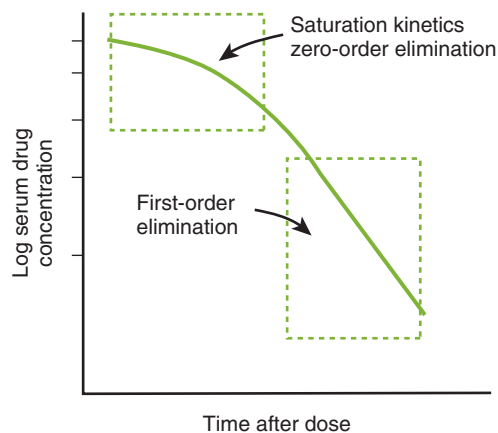
In many pharmacokinetic studies in newborns, blood samples are not obtained early enough to allow calculation of the distribution phase, and the kinetics are described as single-compartment kinetics. If sampling begins after the distribution phase, the concentration-time points may fit a single-compartment first-order model, which determines the elimination rate constant (β).

Zero-Order Kinetics

Some drugs demonstrate *zero-order kinetics*, in which a constant amount of drug, rather than a constant proportion or percentage, is removed per unit of time. This relationship can be expressed as:

$$\frac{dC}{dt} = -k$$

It is important to understand when zero-order kinetics occurs, how to recognize it, and how it affects drug concentrations. Zero-order kinetics is sometimes referred to as *saturation kinetics* because it may occur when excess amounts of drug completely saturate enzymes or transport systems such that they metabolize or transport only a constant amount of drug over time. Zero-order processes produce a curvilinear shape in a semilogarithmic graph of concentrations versus time (Fig. 33.3). When drug concentrations are high from a drug overdose or the pathway for elimination is impaired as in renal dysfunction, the kinetics may become zero order initially and be followed by first-order kinetics at lower concentrations. For drugs exhibiting zero-order kinetics, small increments in dose



• **Fig. 33.3** Saturation, or zero-order (serum concentration-dependent), and first-order (serum concentration-independent) pharmacokinetics.

• BOX 33.2 Drugs That Demonstrate Saturation Kinetics With Therapeutic Doses in Newborns

Caffeine
Chloramphenicol
Diazepam
Furosemide
Indomethacin
Phenytoin
Ethanol and other alcohols (excipient)

may cause disproportionately large increments in serum concentration. Certain drugs or excipients administered to newborns exhibit zero-order kinetics at concentrations observed in the clinical setting and must be recognized for potential accumulation (Box 33.2).

Noncompartmental Analysis

Noncompartmental analysis is based on describing drug exposure measured by the area under the concentration-time curve (AUC) without any assumptions about the pattern of elimination or the number of compartments (Buxton, 2006; Rowland and Tozer, 2010; Anderson, 2012). Central to this analysis is the determination of drug clearance from the dose and the AUC:

$$CL = \text{Dose}/\text{AUC}$$

where CL is clearance.

If the dose is administered intravenously, then noncompartmental analysis allows the direct determination of drug clearance with use of this relationship. Estimation of the elimination half-life is generally done with the slope of the log-transformed concentration measurements made during the end of a pharmacokinetic study. With an estimation of clearance and elimination rate, the apparent distribution volume (V) can also be estimated from:

$$CL = V \times k.$$

Thus noncompartmental PK provides a simple means to assess fundamental pharmacokinetic parameters that may be useful for dosing patients when detailed knowledge of the complete pharmacokinetic profile is not available or not needed (Gillespie, 1991).

Population Pharmacokinetics

Most pharmacokinetic studies in the newborn are limited by the amount of blood that can be safely removed for sampling. To determine the kinetics, population pharmacokinetic approaches are valuable, particularly because they can accommodate differences in dose, sampling time, and numbers (O'Hara et al., 2015). The population approach describes the concentration versus time profile for samples in all patients simultaneously, estimating population parameters that describe the average pharmacokinetic profile of the complete study group and patient-specific parameters that define the individual patients in the study. It can use fewer samples taken from each patient, if the samples are taken at different specified times over the complete time profile of interest for the study analysis. For example, one group of 28–32 weeks' gestation neonates might have samples drawn at 1, 4, and 12 hours, while another group of 28–32 weeks' gestation neonates might have blood sampled at 0.5, 2, and 8 hours. The concentrations from these two groups of similar patients are then analyzed in aggregate so as to provide information during both the distribution phase and the elimination phase, thus describing the kinetics with a limited volume of blood sampled from each patient.

Furthermore, the population approach allows the investigation of patient covariates of interest that might explain differences within the population of patients enrolled in the trial. Typical covariates such as postnatal age, GA at birth, weight, sex, and disease conditions can also be assessed for their contribution to differences seen between participants in a clinical study. These covariates can be very helpful for gaining a better understanding of factors that may alter the PK of infants that might otherwise be considered similar. Because of both conditions (fewer samples, exploration of covariates), the use of population pharmacokinetic studies in clinical studies is also strongly supported by the relevant regulatory agencies as an important opportunity to facilitate drug research in this population (Björkman, 2005; Meibohm et al., 2005).

Target Drug Concentration Strategy

Drug treatment of newborns commonly uses the *target drug concentration strategy* (Table 33.1), in which drug therapy corrects a specific problem by producing an effective concentration of free drug at a specific site of action (Anderson and Holford, 2013). The target site of drug action (e.g., central nervous system, subcutaneous tissue) is usually inaccessible for monitoring of concentrations. A specific concentration or range of circulating concentrations is correlated with the effective concentration at the site of action, which provides a “therapeutic” concentration range. However, we should be aware that such target drug concentrations are commonly extrapolated from other patient populations and are only rarely validated in newborns (Allegaert and van den Anker, 2015a).

The requirements for effective and accurate application of the target drug concentration treatment in adults have been discussed by Spector et al. (1988). When applied to newborns, these requirements highlight the special problems of drug therapy in these

patients and the special circumstances in which clinical drug concentration monitoring is appropriate. Some of these requirements are as follows:

- Availability of a reliable, valid analytic procedure for accurate measurement of drug concentrations in small volumes of blood
- A wide variation in PK among individuals with the knowledge that population-based kinetics do not accurately predict individual kinetics
- Drug effects proportional to plasma drug concentrations
- A narrow concentration range between efficacy and toxicity (narrow therapeutic index)
- Constant pharmacologic effect over time, in which tolerance does not develop
- Clinical studies that have determined the target and toxic drug concentration ranges

Therapeutic Drug Monitoring

Table 33.1 describes the basic assumptions of therapeutic drug monitoring: the total plasma drug concentrations correlate with dose but also with the circulating unbound drug concentrations and the unbound drug concentration at the site of action. Clinical measurements of drug concentrations are usually total drug concentrations, while the active portion is the unbound portion (see the discussion of distribution). Two broad indications for monitoring drug concentrations are to (1) attain effective concentrations and (2) avoid toxic concentrations. However, drug concentration ranges are not absolute reflections but only indirect markers of effective therapy. Patient response, not a specific drug concentration range, is the end point of therapy.

Although the concentrations of aminoglycoside or glycopeptide antibiotics are monitored frequently in newborns, the current perception is that toxicity is much more rare in newborns than in adults (Bhatt-Mehta et al., 1999; Kent et al., 2014). Because of the limited evidence of toxicity in newborns, it is more important to measure these concentrations to achieve effective concentrations for treatment of culture-proven infections than to avoid toxicity. In newborns with serious therapeutic problems, measurement of serum drug concentrations should be used to achieve effective concentrations as well as to avoid toxicity. When the desired concentration range and kinetic parameters are known, doses may be estimated to reach that concentration with single bolus doses or bolus doses followed by continuous infusions.

Pharmacokinetic-Based Dosing

The following equations can be used both to guide dosing and to derive kinetic parameters for individual patients.

$$\text{Dose} = \Delta C \cdot V_d = [C_{\text{desired}} - C_{\text{initial}}] \cdot V_d$$

$$(\text{mg/kg}) = (\text{mg/L}) (\text{L/kg}) = (\text{mg/L}) (\text{L/kg})$$

where C is the concentration and V_d is the volume of distribution.

TABLE 33.1 Target Drug Concentration Strategy

| | | | | | | | | |
|-----------|---|---------------------------------|---|-----------------------------------|---|--|---|------------------------------|
| Drug dose | ↔ | Plasma total drug concentration | ↔ | Plasma unbound drug concentration | ↔ | Target site unbound drug concentration | ↔ | Desired pharmacologic effect |
|-----------|---|---------------------------------|---|-----------------------------------|---|--|---|------------------------------|

This equation may be used to estimate dosage changes needed to increase or decrease concentration. For the first dose, the starting concentration is zero; for doses after the first, the calculation of the volume of distribution should use the change (Δ) in concentration from the preceding trough to the peak associated with that dose. To reach a desired concentration rapidly, a loading dose can be administered, followed by a sustaining infusion. The equation for calculation of infusion doses to maintain a constant concentration is as follows:

$$\begin{aligned}\text{infusion rate} &= k \cdot V_d \cdot C \\ (\text{mg/kg}) (\text{min}^{-1}) &= (\text{min}^{-1}) (\text{L/kg}) (\text{mg/L})\end{aligned}$$

where C is the concentration, V_d is the volume of distribution, and k is the rate constant of elimination.

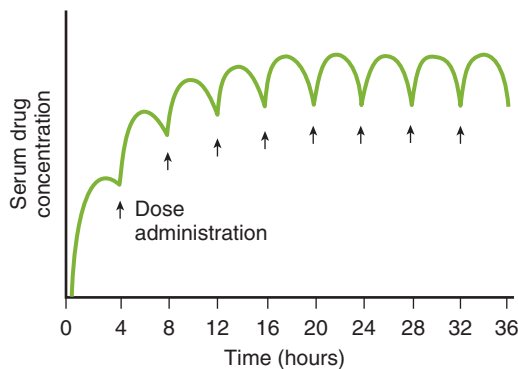
A steady state is reached when tissue concentrations are in equilibrium and the amount of drug removed equals the amount of drug infused. The time needed to reach a steady state depends on the elimination half-life. While the time needed is *not* shortened by the administration of a loading dose, a loading dose can allow the patient to achieve therapeutic concentrations rapidly, and these can be maintained with an infusion during the steady state.

It is important to consider that if drug clearance decreases, the steady-state concentration during an infusion will increase proportionally. Additionally, the half-life increases and the rate constant decreases. Since concentrations are not measured for most of the drugs administered by continuous infusion in the neonatal intensive care unit (NICU), it is important to adjust dosages for factors that reduce clearance, such as kidney or liver dysfunction or reduced kidney or liver blood flow, to avoid high and toxic concentrations.

Repetitive Dosing and the “Plateau Principle”

During the typical course of drug therapy, drug doses are administered before complete elimination of previous doses, and the drug accumulates in the body (Rowland and Tozer, 2010). As a consequence, during repeated administration, the peak and trough concentrations after each dose will increase until a steady-state situation has been reached. *Steady-state*, or *plateau*, concentrations are reached when the amount of drug eliminated equals the amount of drug administered during each dosing interval. During repetitive dosing, the steady-state concentrations achieved are related to the half-life, dose, and dosing interval relative to the elimination half-life (Rowland and Tozer, 2010; Buxton, 2006).

Fig. 33.4 illustrates a hypothetical concentration–time curve for a drug with a half-life of 4 hours administered orally every 4 hours, so the dosing interval corresponds to one half-life. Several important principles of PK are illustrated in Fig. 33.4, with the mathematics described in detail elsewhere (Buxton, 2006). Drug concentrations rise and fall with drug administration (absorption) and elimination. For dosing intervals of one half-life, accumulation is 88% complete after the third dose, 94% complete after the fourth dose, and 97% complete after the fifth dose. At steady state, the peak and trough concentrations between doses are the same after each dose. If a drug is administered with a dosing interval equal to one half-life, the steady-state peak and trough concentrations are two times those reached after the first dose. If the dosing interval is shortened to half of a half-life, the concentration decreases less before the next dose, more total drug is administered per day, and the steady-state peak and trough concentrations are considerably higher (3.4 times the peak and trough



• **Fig. 33.4** Multiple dosing with accumulation of serum drug levels to the steady-state concentration.

concentrations after the first dose). Therefore the shorter the dosing interval to half-life ratio, the higher the drug accumulation. As noted during infusions, the *length of time* required to reach steady-state concentrations depends primarily on the elimination half-life, not the dosing interval.

Clearance

Clearance of drugs, as for creatinine, describes the *volume of blood* from which all the drug is removed per unit of time. Clearance is proportional to organ blood flow and the intrinsic capacity of organs to metabolize or remove a drug from the circulation. In its simplest form, clearance is proportional to the flow to a single organ (Q) and to the arterial–venous difference in drug concentrations compared with the amount of drug in the circulation, expressed as follows:

$$CL = Q \cdot \frac{C_{\text{arterial}} - C_{\text{venous}}}{C_{\text{arterial}}}$$

Total body clearance usually reflects the combined clearance of multiple organs with different enzyme activities and different rates of blood flow. Clearance can be measured by the rate of appearance of a drug outside the body (such as urinary creatinine clearance) or by the rate of disappearance of a drug from the blood compared with the blood concentration. For calculations, *clearance* is defined as the dose divided by the area under the plasma AUC and by the rate of drug input per steady-state concentration (C_{ss}) average, where the rate of input is the dose/dosing interval (τ). For a drug administered by continuous infusion, this is simply the infusion rate (milligrams per kilogram per hour) divided by C_{ss} as follows:

$$CL = \frac{\text{Dose}}{\text{AUC}} = \frac{\text{Dose}/\tau}{C_{ss}} = \frac{\text{Infusion Rate}}{C_{ss}}$$

Once clearance is known, this equation can be rearranged to solve for the dose necessary to achieve any desired steady-state concentration:

$$\begin{aligned}\text{Infusion Rate} &= C_{ss} \cdot CL \\ (\text{mg/kg})\text{hr}^{-1} &= (\text{mg/L}) (\text{mL/kg})\text{hr}^{-1}\end{aligned}$$

Total body clearance changes significantly for several drugs during the period of fetal and infant development because renal clearance and/or the activity of drug-metabolizing enzymes increase with advancing gestational and/or postnatal age. If available, values for clearance and the volume of distribution at different stages of

development can be used to estimate the dosages needed to achieve and maintain “therapeutic” concentrations associated with desired clinical responses.

To illustrate this for renal clearance, a higher dose (mg/kg) and a further extended time interval are applied for aminoglycosides or vancomycin to compensate for the higher distribution volume (peak concentration) and the lower renal clearance (trough concentration) (Janssen et al., 2015; Valitalo et al., 2015). Studies of the analgesic fentanyl illustrate the developmental changes in metabolic clearance and, as a consequence, its kinetics and how they may be used to calculate dosages to reach and maintain concentrations associated with effective analgesia. Analgesia has been associated with a serum fentanyl concentration of 1–2 ng/mL (Santeiro et al., 1997). If analgesic treatment is initiated with a continuous infusion of fentanyl, five half-lives are needed to reach a steady state. The fentanyl half-life ranges from 3 hours in term newborns to 12.7 hours in preterm newborns (Koehntop et al., 1986; Santeiro et al., 1997). Because of this prolonged half-life, the patient may be inadequately treated for a long time unless a loading dose is administered to reach an effective concentration more rapidly. In general, initiation of analgesic treatment and increases in infusion dosages of analgesics should begin with a loading dose based on the estimated volume of distribution in the central compartment (circulation) and the desired concentration. Use of a loading dose shortens the time to reach higher effective analgesic concentrations but also increases the likelihood of toxicity, as has been reported with digoxin.

Limited data are available regarding the GA-related changes in fentanyl clearance, but two studies show that it increases with advancing GA (Koehntop et al., 1986; Santeiro et al., 1997) and postnatal age (Gauntlett et al., 1988; Santeiro et al., 1997). The linear graph of clearance versus gestational age from 38 neonates in whom treatment began within 47 hours after their birth was used to derive mean rates of clearance at different GAs as shown in Table 33.2.

Other investigators studied single-dose fentanyl kinetics during anesthesia and found an apparent central volume of distribution of fentanyl in neonates of 1.45 L/kg (Koehntop et al., 1986). This distribution volume is smaller than the steady-state volume of distribution of 5.1 L/kg also calculated after a single dose of fentanyl (Koehntop et al., 1986). In turn, the apparent steady-state volume of distribution after a single bolus dose of a lipophilic drug is usually smaller than that associated with continuous drug infusions, during which tissues throughout the body become saturated with drug. The steady-state distribution volume for fentanyl during continuous infusions was calculated as 17 L/kg (Santeiro et al.,

1997). Because fentanyl is a highly lipid-soluble drug, it distributes rapidly from the central compartment into the peripheral tissue compartment. This large distribution volume likely reflects the period during the infusion when drug is leaving the circulation to penetrate peripheral tissues, such as fat. Because it may take 15–60 hours to achieve a steady-state concentration (five half-lives) after a fentanyl infusion is begun or the infusion rate is increased, a patient may need repeated bolus doses to maintain effective plasma concentrations in the central compartment. The best approach is to repeat the calculated loading dose until the desired clinical effect is achieved. This also illustrates why, for sedation specifically, dosing should be adjusted to achieve the desired clinical effect. Clearance calculations, however, can guide the starting dosages to achieve effective sedation, as illustrated later.

The kinetic parameters for fentanyl in premature newborns reported by Koehntop et al. (1986) can be used to calculate a loading and infusion dose to reach a fentanyl concentration of 2 ng/mL, which is considered an analgesic concentration (Saarenmaa et al., 2000). This is estimated for a premature newborn at a GA of 33 weeks (note that nanograms per milliliter is equivalent to micrograms per liter) as:

$$C (\mu\text{g/L}) = \frac{\text{Load Dose } (\mu\text{g/kg})}{V_{d_{\text{central}}} (\text{L/kg})}$$

$$\begin{aligned}\text{Load Dose } (\mu\text{g/kg}) &= 2 (\mu\text{g/L}) \cdot 1.45 (\text{L/kg}) \\ &= 2.9 \mu\text{g/kg}\end{aligned}$$

$$\begin{aligned}\text{Infusion Rate } (\mu\text{g/kg} \cdot \text{h}) &= C (\mu\text{g/L}) \cdot \text{CL} (\text{mL/kg} \cdot \text{h}) \\ &= 2 \mu\text{g/L} \cdot 11.4 \text{ mL/kg/min} \\ &\quad \cdot 1 \text{ L/1000 mL} \cdot 60 \text{ min/h} \\ &= 1.4 \mu\text{g/kg} \cdot \text{h}\end{aligned}$$

Two studies have observed increases in fentanyl clearance with advancing postnatal age (Gauntlett et al., 1988; Santeiro et al., 1997). This postnatal increase in clearance of fentanyl likely relates either to maturation of CYP3A4 (the enzyme responsible for fentanyl metabolism) activity or to increased hepatic blood flow after birth, because fentanyl has a high hepatic extraction rate. For drugs such as fentanyl with a high hepatic extraction rate, the rate-limiting factor in clearance is the flow of blood to the liver (Saarenmaa et al., 2000). Some researchers have observed that increased intra-abdominal pressure reduces fentanyl clearance, which is likely caused by reduced hepatic blood flow (Koehntop et al., 1986; Gauntlett et al., 1988). Clinical changes known to increase or decrease fentanyl clearance should be used to adjust starting dosages, but dosing should be adjusted primarily for the desired clinical effect. Such an approach should also consider subsequent development of drug tolerance (i.e., that a higher concentration is needed to attain a similar effect).

Modeling and Simulations

Pharmacokinetic modeling and simulations can be used to estimate the impact of important developmental changes and disease processes on pharmacokinetic parameters for distribution volume, elimination rate, and total body clearance. These can identify clinical situations and conditions when dosages are likely to require modification (e.g., drug–drug interactions, genetic polymorphisms, renal impairment). Mathematical simulations create theoretical pharmacokinetic profiles for patients after a dose using the range of pharmacokinetic

TABLE 33.2 Developmental Pharmacokinetics of Fentanyl

| Gestational Age (Weeks) | Clearance at 0–47 h After Birth (mL/min kg) |
|-------------------------|---|
| 29 | 9.6 |
| 33 | 11.4 |
| 37 | 13.2 |
| 41 | 15.0 |

Data from Saarenmaa E, Neuvonen PJ, Fellman V. Gestational age and birth weight effects on plasma clearance of fentanyl in newborn infants. *J Pediatr*. 2000;136:767–770.

parameters determined from a patient population. These can then be calculated for 100–1000 hypothetical patients to define the expected range of concentrations that are likely after a dose. For drugs such as anti-infectives with which serum concentrations have been correlated with effectiveness, this provides estimates of how large a dose is needed to reach effective concentrations. A study on fluconazole kinetics in newborns illustrates this application (Wade et al., 2008). Modeling and simulation methods can also be used for clinical study design to support decisions about the number of participants, optimal times of sampling, covariates, phenotypic analyses, and population analyses.

Clinical Applications of Pharmacokinetics

How to Estimate Dose Adjustments

Gentamicin and phenobarbital will be used to illustrate the clinical application of the principles of PK and therapeutic drug monitoring discussed earlier. The calculations can be performed with standard arithmetic calculators and provide close enough estimates of the kinetics for drugs with a long half-life to adjust dosages at the bedside.

Gentamicin

Assume that the optimal gentamicin target concentrations are:

$$\begin{aligned}\text{Peak} &= 6\text{--}10\ \mu\text{g/mL} \\ \text{Trough} &= 0.5\text{--}2\ \mu\text{g/mL}\end{aligned}$$

Following the fourth conventional dose (4 mg/kg) of gentamicin to a hypotonic premature newborn, the peak concentration was 4.5 $\mu\text{g/mL}$; 18 hours after the peak was obtained, the trough was 2.25 $\mu\text{g/mL}$. It appears that the distribution volume is greater than anticipated, because the peak concentration is lower than expected, and the half-life is longer than anticipated, because the trough is higher than expected. The time of drug administration and that of blood sampling was confirmed (an important step), so the half-life is 18 hours, because the concentration decreases by 50% from 4.5–2.25 $\mu\text{g/mL}$ in 18 hours (assuming that the kinetics are exponential and first order).

$$\begin{aligned}\text{Vd (mL/kg)} &= \text{Dose (mg/kg)} \times (1000\ \mu\text{g/mg}) / \Delta C\ (\mu\text{g/mL}) \\ &= 4.0\ (\text{mg/kg}) \times 1000 / 4.5 - 2.25\ (\mu\text{g/mL}) \\ &= 4000\ \text{mL} / 2.25\ \text{kg} = 1.777\ \text{mL/kg}.\end{aligned}$$

To ensure a trough concentration of 2.0 $\mu\text{g/mL}$ or less, doses are administered every two half-lives, or every 36 hours. When two half-lives have passed after the fourth dose, the gentamicin concentration should be about 1.1 $\mu\text{g/mL}$ (50% of 2.25 $\mu\text{g/mL}$). Increasing the concentration from the 1.1 $\mu\text{g/mL}$ trough to more than 6 $\mu\text{g/mL}$ requires a concentration difference of 4.9 $\mu\text{g/mL}$ or more. With a distribution volume of 1.777 mL/kg, a dose of 8.7 mg/kg should raise the concentration from a trough of 1.1 $\mu\text{g/mL}$ to a peak of 6.00 $\mu\text{g/mL}$.

$$\begin{aligned}\text{Vd (mL/kg)} &= \text{Dose (mg/kg)} \times (1000\ \mu\text{g/mg}) / \Delta C\ (\mu\text{g/mL}) \\ 1.777\ \text{mL/kg} &= \text{Dose (mg/kg)} \times (1000\ \mu\text{g/mg}) / 4.9\ (\mu\text{g/mL})\end{aligned}$$

$$\text{Dose (mg/kg)} = (1.777/1000) / \text{kg} \times 4.9\ \text{mg} = 8.7\ \text{mg/kg}.$$

In one half-life, this concentration will decrease to 3.0 $\mu\text{g/mL}$, and in two half-lives, or 36 hours, it will decrease to 1.5 $\mu\text{g/mL}$.

Another 8.7 mg/kg dose will raise the peak concentration by 4.9 $\mu\text{g/mL}$ to 6.4 $\mu\text{g/mL}$, which will fall to 3.2 $\mu\text{g/mL}$ in one half-life and to 1.6 $\mu\text{g/mL}$ in two half-lives. The variation between the peak and trough concentrations after the last dose is within the desired range for the optimal gentamicin concentrations defined previously.

Phenobarbital

Seizures that were hard to control developed in a 3.6-kg asphyxiated newborn. Seizures continued after two 20 mg/kg phenobarbital doses until an additional 10 mg/kg dose was administered. A maintenance dose of 7 mg/kg per day was started 24 hours after the loading doses were administered. At 10 days, this child was increasingly somnolent. The phenobarbital level measured in a blood specimen drawn 2 hours after administration of the oral maintenance dose was 50 $\mu\text{g/mL}$. Additional doses were withheld, and the phenobarbital concentration was checked daily; the results were as follows:

- 24 hours: 40 $\mu\text{g/mL}$
- 48 hours: 31 $\mu\text{g/mL}$
- 72 hours: 25 $\mu\text{g/mL}$
- 96 hours: 21 $\mu\text{g/mL}$

The maintenance dose (7 mg/kg) was resumed immediately after the 21 $\mu\text{g/mL}$ concentration was measured and produced a peak concentration of 30 $\mu\text{g/mL}$ after administration of the dose. These concentrations and dosages can be used to calculate the volume of distribution and a dose to maintain the phenobarbital concentration between 20 and 30 $\mu\text{g/mL}$ as:

$$\begin{aligned}\text{Vd (L/kg)} &= \frac{\text{Dose (mg/kg)}}{\Delta C\ (\mu\text{g/mL} = \text{mg/L})} \\ &= \frac{7.0\ (\text{mg/kg})}{(30 - 21)\ (\text{mg/L})} \\ &= \frac{7\ (\text{mg/kg})}{9\ (\text{mg/L})} \\ &= 0.78\ \text{L/kg}\end{aligned}$$

Half-life can be estimated from inspection, because the concentration decreased from 50–25 $\mu\text{g/mL}$ in 72 hours. Thus it should take 72 hours for the concentration to decrease by one half-life from 30–15 $\mu\text{g/mL}$. Assuming that the elimination rate does not change, the concentration will decrease approximately 5 $\mu\text{g/mL}$ every 24 hours, or one-third of a half-life. Dividing the half-life into fractions is an approximation because it estimates the change in concentration as linear rather than exponential. To be more accurate, the concentration decreases by 59% in half of one half-life. Although this approximation violates certain principles of PK, it allows estimation of the change in concentration for each one-third of a half-life as one-third of the change during one half-life. Thus the concentration decreases by about 5 $\mu\text{g/mL}$ in 24 hours. The following approach can be used to estimate the daily phenobarbital dose needed to return the concentration to 30 $\mu\text{g/mL}$, a change in concentration of 5 $\mu\text{g/mL}$:

$$\begin{aligned}\Delta C\ (\text{mg/L}) &= \frac{\text{Dose (mg/kg)}}{\text{Vd (L/kg)}} \\ 5\ (\text{mg/L}) &= \frac{\text{Dose (mg/kg)}}{(0.78)\ (\text{L/kg})} \\ 3.6\ \text{mg/kg} &= \text{Dose (mg/kg)}\end{aligned}$$

Drug-Induced Illness

In a prospective study on the epidemiology of adverse drug reactions (ADRs) in 200 consecutively admitted neonates, 136 ADRs occurred in 60 neonates (30%), 20 were life threatening, and 24 were of moderate intensity (prolonged hospital stay) and about 50% of the neonates had multiple ADRs (Aranda et al., 1982). In a more recent study of a mixed population of 313 neonates admitted to the NICU or the intermediate care unit, 116 ADRs occurred in the neonates (17%), and 44% of these ADRs needed specific treatment (Belén Rivas et al., 2015). The reasons behind drug-related morbidity and mortality are diverse and complex. In addition to a lack of specific labeling for this special patient population, absence of age-appropriate neonate-friendly formulations and a high frequency of (poly)pharmacy, there is also the immature organ function and substantial comorbidities that will further increase the risks of ADRs in neonates (Allegaert and van den Anker, 2015b).

Legislative initiatives have resulted in a substantial increase in pharmacologic studies in children, with, as a consequence, a significant increase in label changes. Unfortunately, only a few included drug label changes for neonates (Laughon et al., 2014; Stiers and Ward, 2014). Neonatologists should realize that almost all compounds currently used in neonates were initially developed for other patient populations, with subsequent tailoring to neonates. Recently, the International Neonatal Consortium has been developed as part of the Critical Path Initiative to serve as a forum for the neonatal community to develop consensus statements (e.g., standardization of methods, standard-of-care consensus statements, population-specific biomarkers, modeling approaches, trial designs, clinical outcome assessment tools, formulation issues) with the ultimate goal to improve neonatal medicine development (International Neonatal Consortium, 2016; Davis and Turner, 2015).

Furthermore, drug-induced illness is rarely considered, and failure to recognize drug-induced illness in the newborn often results in continued or intensified pharmacologic interventions rather than discontinuation of treatment. To improve its detection, Du et al. (2013) suggested and validated an algorithm to detect ADR in neonatal intensive care. Prudent care of newborns must recognize and weigh the potential benefits of unstudied drug therapy against potential drug-induced morbidity and mortality. Some examples from the (recent) history of drug-induced mortality and morbidity should serve as a reminder of how more harm than good may accrue from uncontrolled, unstudied pharmacotherapy in neonates.

Illustrations of Drug-Induced Illnesses in Neonates

Chloramphenicol was released in the 1940s, and the recommended dosages were 50–100 mg/kg per day for patients weighing 15 kg or less. Before 1959, the year that Sutherland (1959) reported three cases of sudden death in newborns treated with high dosages of chloramphenicol (up to 230 mg/kg per day), the drug was considered “well tolerated and nontoxic” (Sutherland, 1959). Burns et al. (1959) reported the disturbing results of a controlled trial of the following four prophylactic treatment regimens for newborn sepsis: (1) no treatment, (2) chloramphenicol alone, (3) penicillin and streptomycin, and (4) penicillin, streptomycin, and chloramphenicol. The groups that received chloramphenicol (100–165 mg/kg per day) had higher mortality rates (60% and 68%), and the deaths of these newborns demonstrated the stereotyped sequence of symptoms and signs caused by chloramphenicol accumulation,

coined *grey baby syndrome* (Burns et al., 1959). This syndrome consisted of abdominal distention, poor peripheral perfusion and cyanosis, vasomotor collapse, irregular respirations, and death within hours of onset of these symptoms.

The discovery of the mechanism (glucuronidation deficiency) of chloramphenicol toxicity in newborns illustrates several important aspects of neonatal pharmacology. Because chloramphenicol was considered well tolerated in older children and adults, it was regarded as nontoxic to newborns. Higher doses were administered to newborns despite recognition that its clearance required glucuronide conjugation, which was known to be immature in newborns. The unexpected finding that chloramphenicol in dosages of 100–165 mg/kg per day could be lethal to newborns was demonstrated because the study conducted by Burns et al. (1959) included appropriate control groups. Similar case observations with the mechanism involved are summarized in Table 33.3 to illustrate that the same kind of observations can still be seen.

Reduction and Prevention of Medication Errors in Newborn Care

In two studies performed in NICUs, about one-third of the “iatrogenic” events were preventable, one-quarter related to drugs (Ligi et al., 2008; Kugelman et al., 2008). For drug errors, ordering and prescription as well as administration-related errors were observed. The top five preventable errors relate to incorrect doses, medications that are inappropriate for the medical condition, failure to monitor newborns for drug-related side effects, failure of communication, and failure to monitor drug levels. While many errors are inconsequential, others result in serious adverse effects. Medication errors incur significant costs, ranging from obvious ones such as direct patient injury, prolonged hospital stays, and additional corrective treatments to more subtle ones such as the costs associated with monitoring and regulation of medication use (Ligi et al., 2008; Kugelman et al., 2008).

The process for ordering, preparing, dispensing, and administering medications is often complicated and may contribute directly to errors. The frequency of those errors, however, may be reduced in almost every unit. A barcode medication administration system (verification of drug and patient), in combination with a double check when further manipulation (such as dilution) is needed, is required to effectively reduce this burden of targeted, preventable medication errors in the NICU setting (Morriss et al., 2009). Although these kinds of complex and expensive computerized systems may help reduce medication errors, caregivers can take steps that are completely within their control to reduce medication errors without waiting for changes in the entire pharmacy process within the hospital. Prescriptions and drug orders are a means of communicating, yet clinicians often devote too little attention to make these documents legible, clear, and unambiguous (ASHP guidelines on preventing medication errors in hospitals, 1993). Physicians should keep the following recommendations in mind to ensure that they communicate their medication orders more effectively:

- Write out instructions rather than use abbreviations.
- Avoid vague instructions (e.g., “take as directed”).
- Specify exact dosage strengths.
- Avoid abbreviations of drug names (e.g., MS could mean morphine sulfate or magnesium sulfate).
- Avoid “U” as an abbreviation for units as the “U” may be mistaken for a “0.”
- Avoid trailing zeroes (e.g., 5.0 mg).

TABLE 33.3 Illustrations of Formulation (Active Compound, Excipient)-Specific Drug-Induced Illnesses in Neonates and the Mechanism Involved

| Compound/Formulation | Clinical Syndrome | Mechanism Involved |
|---------------------------------|-------------------------|---|
| Sulfonamides (1956) | Kernicterus | Highly albumin bound antibiotic, competitive with endogenous compounds, including bilirubin. This results in higher free bilirubin level and subsequent kernicterus. |
| Benzyl alcohol (1982) | Gasping syndrome | Benzyl alcohol, coadministered as preservative in parenteral formulations results in accumulation in preterm neonates because of their limited metabolic (alcohol dehydrogenase) clearance capacity. Accumulation results in metabolic acidosis followed by seizures, bradycardia, gasping respirations, hypotension preceding cardiovascular collapse, and ultimately death. |
| Dexamethasone (2000) | Cerebral palsy | High-dose dexamethasone exposure in neonatal life results in an increased risk of displaying cerebral palsy during infancy, likely due to increased neuroapoptosis. |
| Lopinavir/ritonavir (2005) | Alcohol accumulation | Lopinavir/ritonavir (Kaletra) syrup contains both ethanol and propylene glycol. Impaired metabolism results in accumulation and subsequent hyperosmolality, lactic acidosis, renal toxicity, central nervous system impairment, cardiac arrhythmia, hemolysis, and collapse. |
| Ceftriaxone plus calcium (2009) | Cardiovascular collapse | Simultaneous administration of calcium-containing infusions and ceftriaxone results in intravascular precipitation, as observed during autopsy. |

- Use leading zeroes (e.g., 0.5 mg).
- Minimize the use of verbal orders.
- Ensure that prescriptions and signatures are legible, even if it means printing the prescriber's name that corresponds to the signature.

Changes in the pharmacy process can also be effective, as highlighted by [Campino et al. \(2016\)](#) who evaluated the impact of a care bundle intervention (protocol standardization, education) on the number of errors and documented significant reductions in both calculation errors (1.35%–0%) and accuracy error rates (from 54.7%–23% at the bedside and from 38.3%–14.6% in the hospital pharmacy) ([Campino et al., 2016](#)).

Drug Excretion in Breast Milk

The excretion of drugs in breast milk remains a source of confusion and concern for many physicians and families ([National Institutes of Health](#)). However, knowledge is increasing, and access to this knowledge through, for example, LactMed (<http://toxnet.nlm.nih.gov/newtoxnet/lactmed.htm>) is improved. The Pregnancy and Lactation Labeling Final Rule should also provide a tool to generate and collect more reliable information on maternal drug use during

breastfeeding. A common misconception about safety is “*when in doubt, do not provide breastfeeding*,” since breastfeeding itself provides benefits to the infant and the mother. On average, the nursing infant receives around 2%–3% of a maternal dose through milk, but drugs that are organic bases or are lipid soluble may reach higher concentrations in milk than in maternal serum, but this should not by definition affect the infant. To illustrate this more balanced approach, breastfeeding in infants of women taking antiepileptic drugs was associated with an improved neurodevelopment outcome compared with those who either did not breastfeed or breastfed for less than 6 months ([Meador et al., 2014](#)). Similarly, breastfeeding by opioid-taking women may help to control neonatal abstinence syndrome but may also be associated with neonatal oversedation ([Iseman et al., 2011](#); [Lefevre and Allegaert, 2015](#)). Overall, the available data regarding drug exposure of the newborn through human milk have been organized, in decreasing levels of concern, from drugs that are associated with adverse effects on the infant during nursing to those that are of concern pharmacologically to those that have not been associated with problems during nursing. The list of drugs that clearly cause problems during nursing is surprisingly short ([American Academy of Pediatrics Committee on Drugs, 2001](#)).

Summary

Drug therapy for newborns requires application of the basic principles of PK (drug absorption, distribution, metabolism, and excretion) and PD to estimate and individualize dosages. However, owing to a lack of GA-appropriate kinetic data in the rapidly changing fetus and newborn, drug therapy is still commonly based on empiric, off-label prescriptions. Combined with the extensive drug exposure of neonates in the NICU, this is hazardous and likely explains the high frequency of adverse, sometimes fatal, drug reactions. Methods appropriate for the study of therapeutics in

newborns present unique difficulties, but recent legislative and methodological progress provides additional assistance for investigators and helps advance this area of pharmacology ([Jacqz-Aigrain 2011](#); [US Congress, 2012](#); [Leong et al., 2012](#); [European Parliament and Council, 2014](#); [US Department of Health and Human Services, 2016](#)). Recent collaborative initiatives should further stimulate development of both new and already existing drugs in neonates ([Davis and Turner, 2015](#); [International Neonatal Consortium, 2016](#)).

Suggested Readings

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Neonatal Pain and Stress: Assessment and Management

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KEY POINTS

- Pain and discomfort in infants continue to be common occurrences in intensive care.
- Identification and management of neonatal pain remain challenging.
- Many neonatal pain scoring systems are available although most have not been validated in clinical practice.
- Untreated neonatal pain can result in long-term adverse outcomes such as alterations in brain structure and abnormal pain responses in childhood.
- Pharmacologic treatment of pain is widely used, but nonpharmacologic interventions may be as effective.
- Anesthesia for surgery remains concerning for potential adverse effects on the brain. New methods of neonatal pain recognition such as near-infrared spectroscopy and amplitude-integrated electroencephalography deserve further evaluation.

Relief of human suffering is one of the most important goals of all healthcare providers. Advances in neonatology have significantly reduced neonatal morbidity and mortality, but pain, discomfort, and stress remain sad realities for babies in the neonatal intensive care unit (NICU). Assessing, managing, and trying to limit these clinical realities, particularly while caring for critically ill neonates, remain challenging and increasingly controversial. Fortunately, considerable clinical and laboratory research and clinical dialogue continue to push neonatal providers toward best clinical practices in this problematic arena. This chapter describes the history, developmental biology, and public policies that have informed and shaped current clinical practices; it also summarizes relevant clinical and basic research regarding clinical assessment tools and both pharmacologic and nonpharmacologic management approaches. Finally, future directions in this field are suggested.

Historical Timeline

It is important to know the historical background of challenging medical issues so as to understand the imperatives for future directions. Management of neonatal pain and stress serves as an

excellent example of this philosophy. Thus a brief history of neonatal pain management follows:

- 1806: Morphine (named for Morpheus, Greek god of dreams) is isolated from the opium poppy by the pharmacist Friedrich Serturner.
- 1960s–1980s: Infants are believed to be too immature to feel pain; adverse effects of anesthetics are feared; the Liverpool method (pancuronium only, no anesthesia) is used widely for patent ductus arteriosus (PDA) ligation in premature infants.
- 1985: A landmark paper is published by [Anand et al. \(1985\)](#) describing adverse physiologic effects of the Liverpool method and improved outcomes with use of anesthesia for PDA ligation; anesthesia and postoperative pain medication begin to become more widely used in neonatal care.
- 1987: A joint statement is issued by the [American Academy of Pediatrics \(AAP\)](#) and the American Society of Anesthesiologists regarding the safety and efficacy of neonatal operative anesthesia and postoperative analgesia, regardless of age or maturity ([AAP, 1987](#)).
- 2000: The AAP and the Canadian Pediatric Society issue a joint statement regarding neonatal pain management directed at surgical anesthesia and postoperative and procedural pain assessment and relief ([Prevention and management of pain, 2000](#)).
- 2001: Neurodegeneration and behavioral deficits are reported in rodent pups following administration of general anesthetics, raising questions about their safety in human infants.
- 2003: The Joint Commission issues a mandate regarding pain assessment and management in all hospitalized patients, including neonates.
- 2004: The NEOPAIN trial (Neurologic Outcomes and Preemptive Analgesia in Neonates) results are published, concluding that preemptive morphine infusion did not improve outcomes in ventilated neonates and did not relieve procedural pain ([Anand et al., 2004](#)).
- 2006: Cerebral cortical activation is demonstrated following noxious stimuli in human infants despite lack of behavioral responses ([Slater et al., 2006](#)).
- 2009: More than 40 infant pain assessment tools are available around the world; most were developed for research and not validated for clinical use.

- 2010: Oral sucrose administration is found to decrease clinical pain scores in neonates but results in no changes in cortical activation (Slater et al., 2010).
- 2016: A well-controlled study demonstrates no difference in 2-year developmental outcomes after general versus regional anesthesia during neonatal surgery (Davidson et al., 2016).

Development of Public Policy

Since the late 1980s, in response to a public outcry regarding the recognition and management of pain in hospitalized patients, the US Department of Health and Human Services, the Joint Commission, and other professional organizations have promulgated mandates that dictate pain management practices for all patients, including neonates. The initial public policy statement regarding pain management for neonates undergoing surgical interventions was issued as a joint communication by the AAP Committee on Fetus and Newborn, Committee on Drugs, Section on Anesthesiology, and Section on Surgery and the American Society of Anesthesiologists (AAP, 1987). This statement strongly recommended that anesthesia and analgesia be given to neonates despite their age and cortical immaturity. The Acute Pain Management Guideline Panel of the US Agency for Health Care Policy and Research (Clinicians' quick reference guide, 1992) issued a second influential document unequivocally endorsing the need for pain management in neonates. These two documents helped initiate research studies addressing the recognition, prevention, and amelioration of neonatal pain.

The 2003 accreditation standards issued by the Joint Commission required healthcare providers to look across the continuum of life, including the neonatal period, at the complex nature of the pain experience so as to create new foundations for care without specifically providing instructions for assessing or managing neonatal pain. The subsequent conundrum was that neonatal caregivers were expected to assess and treat perceived neonatal pain and discomfort in the absence of evidence-based methods with which to base their assessment and therapy. Organizations commissioned interdisciplinary teams to incorporate regulatory directives and results of scientific investigation into institutional practice guidelines and standards for care (Anand and International Evidence-Based Group for Neonatal Pain, 2001; Howard et al., 2008a, 2008b; Lago et al., 2009). These guidelines include a patient's right to regular and systematic assessments of pain, interventions to relieve pain, evaluation of effectiveness of interventions, attention to long-term pain management needs, determination of the deleterious effects of unmanaged pain, and educational needs of families and staff members who provide care (Bell, 1994; Joint Commission, 2004; Howard et al., 2008a, 2008b; Lago et al., 2009).

As a result of these public policy initiatives and regulations, new tools for pain assessment and innovative methods to treat pain were developed and evaluated. Many questions remain unanswered, and new concerns have been raised. Neonates experience both acute and chronic pain. Treatment protocols for acute pain may not be appropriate for chronic pain, because the origin and resultant physiologic status can be quite different. A concrete result of these policies, guidelines, and interventions is the routine assessment and documentation of pain scores as a proxy for routine pain assessment for preterm and term neonates throughout their hospital stay. These scores, however, may not actually correlate with low pain exposure because of the prescribed way in which they are assigned (Rohan, 2014). Long-term use of narcotics and

other drugs leads to drug tolerance and the need for slow weaning to avoid drug withdrawal. The drugs themselves and drug tolerance and drug withdrawal may all contribute to adverse effects on brain development and neurodevelopmental outcomes.

Recent International Surveys of Clinical Practice

Nurses, physicians, and parents all play a front-line role in recognizing and ameliorating neonatal pain. The factors at play in determining the depth and breadth of pain assessment and treatment include nationality, level of education of bedside providers, use of clinical pain scales, time of day, and unit acuity. From surveys, nurses in Korea (Jeong et al., 2014) and Iran (Asadi-Noghabi et al., 2014) as well as physicians and nurses in southern India (Britto et al., 2014) routinely underestimate pain in neonates, do not acknowledge pain in neonates, and/or do not readily provide any type of analgesic measures for acute, procedural pain in neonates. When episodes of acute procedural pain were monitored in NICUs in Kenya, only 1 of 404 painful procedures had any form of analgesia, implying that more education is warranted to both recognize and treat neonatal procedural pain (Kyololo et al., 2014). In Spain, the types of routine pain assessments commonplace in US hospitals at the behest of the Joint Commission are not common. Many units did not routinely use clinical pain scales, and those that did used them inconsistently (Avila-Alvarez et al., 2015a). Despite the lack of pain scales, many intubated neonates in Spanish NICUs are receiving sedative infusions such as fentanyl and/or midazolam. The more regimented the unit in systematically assessing pain, the more likely it is that neonates receive these medications (Avila-Alvarez et al., 2015b). NICUs in France noted that painful procedures were more consistently ameliorated by analgesics during the morning hours than at other times of the day (Guedj et al., 2014). A survey of all physicians and nurses in seven UK NICUs reported that even though clinicians are knowledgeable about neonatal pain, there is still a large gap between knowledge and practice (Akuma and Jordan, 2012). Educational initiatives geared toward helping nurses understand and recognize pain can improve both bedside assessments and pain management (Aymar et al., 2014).

The EUROPEAN Pain Audit in Neonates (EUROPAIN) trial, completed in 2015, was a prospective trial involving a cohort of 6680 neonates enrolled in 243 NICUs in 18 European countries that cataloged demographics, methods of respiration, use of continuous or intermittent sedation, analgesia, or neuromuscular blockers, pain assessments, and drug withdrawal symptoms in the first 28 days of admission. Wide variations in practice were documented regarding documentation of pain scores, genres of respiratory support and their association with analgesia, and use of analgesia for any neonates receiving any modality of respiratory support. Surprisingly, despite awareness of the results of trials such as NEOPAIN (Anand et al., 2004), the median use of sedation or analgesia for intubated neonates was 89%, with 74% of these neonates receiving opioids, 25% receiving midazolam, and 25% receiving neuromuscular blockers as either a bolus (18%) or a continuous infusion (7%). The large, prospective nature of this study provides a compelling snapshot of how pain is assessed and how pharmacologic analgesia is prescribed to many neonates in the NICU (Carbajal et al., 2015).

Italy represents a unique example of the power of unified national guidelines and education. Surveys conducted in 2003 revealed that procedural pain in newborns was underestimated

or inadequately managed in Italian NICUs (Lago et al., 2005). Italian neonatologists accepted the remarkable challenge of drafting national pain guidelines, which were then implemented in 2005. Follow-up surveys conducted 5 years after adoption of these guidelines showed that most Italian NICUs provide some form of analgesia and sedation for invasive procedures, but their routine adherence to best practices for pain control and monitoring was not optimal (Lago et al., 2012, 2013). Despite tremendous efforts to increase awareness of and provide systematic approaches toward ameliorating pain, analgesia is not consistently reaching the bedside.

Knowledge translation and quality improvement activities are key research techniques available to adapt clinical practice to evidence-based recommendations. A focus group study (Stevens et al., 2011) conducted in three Canadian NICUs concluded that neonatal pain is managed most successfully when:

- There is a positive culture of interprofessional collaboration.
- Nurses are permitted to make decisions autonomously with regard to assessment and treatment of pain without interference from professional hierarchies.
- Patient conditions are less complicated.
- The NICUs are set up in a manner that facilitates analgesia by providing easy access to materials.
- NICUs have appropriate staffing, with time allowed for pain assessment and treatment.
- NICUs have a culture that emphasizes making time for analgesia.

An emphasis on uniform practice guidelines can also help. For example, implementing a neonatal pain and sedation protocol in two NICUs resulted in an increase in opiate prescription, an increase in pharmacologic interventions, and improved staff satisfaction without affecting the duration of mechanical ventilation, the length of intensive care stay, or adverse NICU (Deindl et al., 2013) or neurodevelopmental (Deindl et al., 2016) outcomes. Another example is a recent study demonstrating that a nursing-driven comfort protocol aimed at titrating morphine infusions on the basis of bedside pain scores decreased the use of morphine infusions solely for preemptive analgesia (Fleishman et al., 2015).

Ontogeny and Development of Pain and Stress Responses

The sensory system of the neonate, especially in the preterm neonate, is immature (Fig. 34.1). Afferent input from both noxious and nonnoxious stimuli terminates in the dorsal horn of the spinal cord in a diffuse manner on multiple cells, resulting in the infant's inability to distinguish between noxious and nonnoxious stimuli and limits the care provider's ability to correctly interpret the infant's behavioral response. In the neonatal rat, separation of sensory input is not completed until 3 to 4 weeks after birth (approximately 1 to 2 years in humans) (Beggs et al., 2002); this prevents the newborn from consistently differentiating touch from painful sensory input. The responses of the infant are therefore nonspecific. With repeated painful exposures, infants may lose any discriminatory ability and develop hypersensitive states for long periods. This hypersensitivity persists even if nonnoxious stimuli are introduced (Jennings and Fitzgerald, 1998). The responses are less synchronized in the immature central nervous system (CNS) because of underdeveloped myelination and slower synaptic transmission as manifested in longer and more variable latencies (Jennings and Fitzgerald, 1998; Fitzgerald, 2005).

Neuronal connections within the cortex appear to form at approximately 22 weeks' gestation (Kostovic et al., 2006), suggesting that higher cortical level pain processing may be limited despite the presence of a behavioral response (Fitzgerald and Walker, 2009). In addition, the neonate lacks sufficient descending modulatory control, thereby limiting its ability to benefit from endogenous control over noxious stimuli compared with adults (Fitzgerald and Koltzenburg, 1986; Hathway et al., 2006).

Neurophysiologic investigations have dramatically changed our understanding of nociception in the newborn. From use of research methods such as near-infrared spectroscopy (NIRS) and electroencephalography techniques such as amplitude-integrated electroencephalography, it is clear that cerebral cortical activation occurs with noxious stimulation, such as needle pokes, as early as 24 weeks' gestation. Moreover, cortical activation has been noted even in infants who manifest no behavioral response to a stimulus (Slater et al., 2006; Holsti and Grunau, 2007; Fitzgerald, 2015). The converse has also been noted in studies reporting that oral sucrose administration decreases clinical observational scores, with no changes noted in cortical activation from noxious stimulation (Slater et al., 2010; Stevens et al., 2013). Such findings raise the question that our patients may be perceiving pain at a cortical level despite our interventions (pharmacologic and nonpharmacologic) and that pain scoring tools may only provide limited information, possibly underestimating the pain response in neonates (Maxwell et al., 2013).

Noxious stimuli in adults result in the release of inflammatory and trophic factors that activate and sensitize nociceptors in the injured tissue. Such noxious stimuli lead to nociceptive afferent input to the CNS, exciting nociceptive circuits in the spinal cord, brainstem, thalamus, somatosensory cortex, cingulate cortex, and amygdale (Tracey and Mantyh, 2007; Woolf and Ma, 2007). However, noxious stimuli in infants do not evoke similar patterns of CNS activity. (Fitzgerald, 2005). The response to noxious stimuli is more diffuse and less spatially focused in infants. Studies in rats demonstrate major alterations in neuronal circuitry with maturation. These animal data may parallel developmental changes that have been noted in humans (Fitzgerald, 2005). Local tissue injury resulting from repeated heel sticks and invasive procedures triggers increased proliferation of nerve endings in surrounding tissues, particularly when this damage occurs early in gestation. As a result, scars (e.g., from heel sticks, old intravenous [IV] sites) and surrounding tissues can remain hypersensitive well beyond the neonatal period (Reynolds and Fitzgerald, 1995; Jennings and Fitzgerald, 1998).

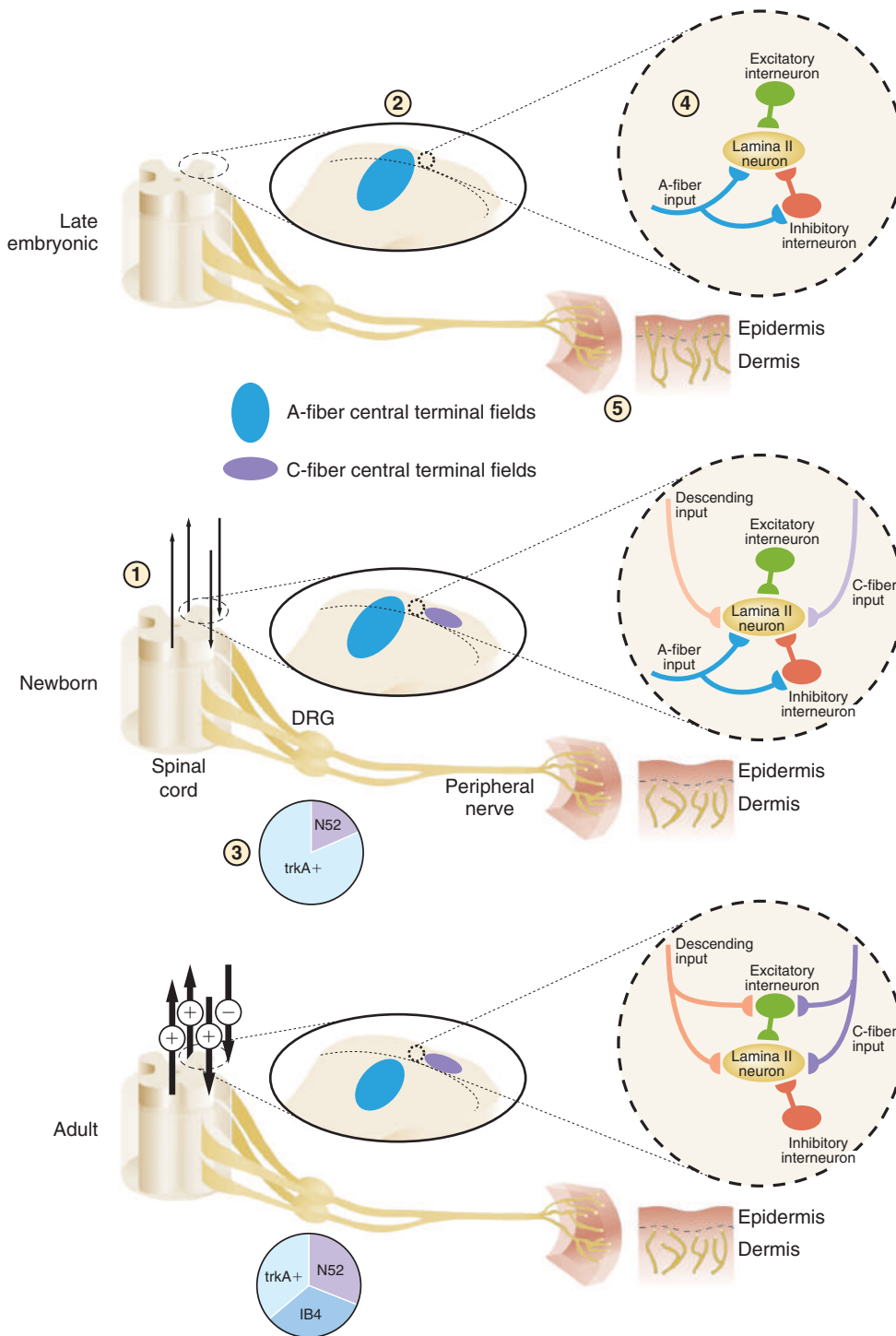
In summary, the immature infant nervous system:

- Lacks the ability to discriminate consistently between noxious and nonnoxious stimuli, often reacting with similar behavior to a variety of stimuli
- Lacks the ability to modulate pain responses
- Does not consistently manifest signs or symptoms that allow care providers to accurately assess the infant's level of pain and discomfort

Assessment of Neonatal Pain and Stress

Assessment of the Neonatal Behavioral Responses to Pain

Tools for assessment of neonatal pain and stress must be based on an understanding of the normal development of behavioral responses to pain and stress—an infant is not a small adult. Behavioral



• **Fig. 34.1** 1, In early postnatal life, descending fibers are present, but inhibitory and excitatory influences are weak or absent. The connections gradually strengthen, becoming fully functional at the end of the third postnatal week. 2, A-fibers are the first primary afferents to enter the dorsal horn gray matter and are present during the last few embryonic days. Their distribution is diffuse, with exuberant, more superficial projections gradually retracting over the first three postnatal weeks. C-fibers are present in the dorsal horn during late embryonic stages but only enter the gray matter 2 to 3 days before birth. Unlike A-fibers, they project to topographically appropriate regions in lamina II of the spinal cord as soon as they enter. C-fiber synaptic connectivity is present, although weak at the time of birth, with connections strengthening over the first two postnatal weeks. 3, At birth most (approximately 80%) of the dorsal root ganglion neurons express the nerve growth factor receptor *trkA*. Over the first postnatal week, this population reduces, with approximately half of these neurons losing their *trkA* expression and beginning to express receptors for glial cell line-derived neurotrophic factor (identifiable as the isolectin B4-binding population). 4, The balance of excitation and inhibition in the superficial dorsal horn develops postnatally, through changes in both local interneuron circuitry and descending fibers. A-fiber input is stronger in the neonate and weakens as the influence of C-fiber input increases. 5, Primary afferent innervations of the skin occur earlier than central projections. By late embryonic stages, primary afferents of all classes have reached the skin and innervate through the dermis into the epidermis. These projections die back during the immediate perinatal period to leave the full adult situation of dermal innervations present soon after birth. *DRG*, Dorsal root ganglion; *IB4*, isolectin B4; *N52*, *trkA*+, nerve growth factor receptor *trkA*. (From Beggs S, Fitzgerald M. Development of peripheral and spinal nociceptive systems. In Anand KJ, Stevens BJ, McGrath PJ, eds. *Pain in Neonates and Infants*. 3rd ed. Philadelphia, PA: Elsevier; 2007:15.)

responses to noxious stimuli in infants are not always predictable because of immaturity of the CNS; therefore assessment of pain and response to therapeutic intervention can be similarly unpredictable. Significant structural and functional changes occur in pain pathways during development, and these continue after birth.

The lack of verbal skills, immature behaviors in response to pain, and the nonspecific nature of physiologic indicators of pain in a critically ill patient all combine to make accurately assessing pain in term and preterm neonates quite challenging. The challenge of pain assessment is further compounded by typical NICU interventions such as mechanical ventilation, physical restraints,

and pharmacologic sedation, all of which mask distress behaviors (Maxwell et al., 2013). Because the gold standard of pain assessment (self-reporting using validated scales) is not applicable to neonatal patients, providers must rely on physiologic, behavioral, and biobehavioral indicators as a surrogate for self-reporting pain (Maxwell et al., 2013).

Infant Pain Scores

Multiple neonatal pain scoring tools integrate physiologic, behavioral, and biobehavioral indicators into a pain score. These tools

assess the response to noxious stimuli by categorizing the behavioral or physiologic reactions of the infant or a combination of both (Grunau and Craig, 1987). The behavioral responses can include limb movements, muscle tone, crying, and characteristic facial expressions. Crying is the least reliable indicator of neonatal pain (Grunau and Craig, 1987; Guinsburg et al., 1998; Stevens et al., 1993). The physiologic measures can include heart rate, oxygen saturation, and respiratory rate. Specific facial expression changes are believed to be the most reliable indicators of pain (brow bulge, eye squeeze, nasolabial furrow, taut lips, and open mouth); see Fig. 34.2 for examples of these characteristic facial changes. Use of facial expression changes can be challenging when the infant's face is partially covered with adhesives used to secure tubes and lines or with a phototherapy mask in place.

More than 40 discrete scoring tools exist to assess pain in neonates, many validated only for research studies (Ranger et al., 2007). Scoring tools that provide a multidimensional assessment of pain are preferred clinical tools; some tools lack sensitivity and specificity by relying, for example, only on changes in vital signs (Raeside, 2011). Some recent publications list currently available neonatal pain assessment tools (Maxwell et al., 2013; Committee on Fetus and Newborn and Section on Anesthesiology and Pain Medicine, 2016). Only five neonatal pain scales have undergone rigorous psychometric testing with patients serving as their own controls:

- Neonatal Facial Coding System
- Premature Infant Pain Profile (PIPP)
- Neonatal Pain and Sedation Scale
- Behavioral Infant Pain Profile
- Douleur Aiguë du Nouveau-né

The PIPP scale is one of the most commonly used and well-validated scales; a review of 62 studies of the PIPP score over 13 years reinforced this scoring measure as valid, reliable, and clinically feasible and useful at measuring acute procedural or postoperative pain (Stevens et al., 2010), with strength lying in a composite (measuring behavioral and physiologic indicators) approach. In 2014, researchers refined the scoring tool further, termed the PIPP-Revised score, to better encompass markers of pain and distress across the spectrum of gestational age (Stevens et al., 2014).

It would be ideal to use a neonatal pain scoring tool that best matches the anticipated type of pain that an infant may experience (acute procedural, postoperative, chronic pain). For example, a

prospective comparison of multiple pain scores in neonates after cardiac surgery demonstrated a scoring tool called the COMFORT (Maaskant et al., 2016) score most accurately correlated with pain and analgesic response in those patients (Franck et al., 2011b). Another specific example is noted with a validated scoring tool called the Echelle Doleur Inconfort Nouveau-Né (EDIN) developed specifically to assess prolonged pain in preterm infants (Debillon et al., 2001). That said, it is difficult to train and maintain provider skills in the use of multiple scoring tools. Determining which scoring tool best matches local patient composition allows care providers to become expert in using a single tool or two tools.

A common conundrum is that providers find themselves treating chronic pain related to complications of intensive care using tools designed and validated for acute, procedural, and/or postoperative pain (van Ganzewinkel et al., 2014). Few publications in the literature attempt to define chronic pain (and/or stress) in the newborn, leaving it to be measured with tools best validated and studied for measuring acute procedural pain. In 2014, researchers conducted an international survey of experts in neonatal pain, including physicians, nurses, researchers, and parents from around the world, and then used the Delphi method to propose a definition of chronic pain in the neonate. They put forth the following (van Ganzewinkel et al., 2014): "Chronic pain can often not be associated with a specific cause. It has no obvious endpoint in sight and is no longer proximate to an event or procedure. Chronic pain may alter perception causing nonnoxious events to be perceived as painful, leading to a chronic pain response. It depletes stress hormones, increases energy consumption, and therefore interferes with growth. As a consequence, chronic pain may likely prolong hospitalization and worsen or add to existing neonatal morbidities."

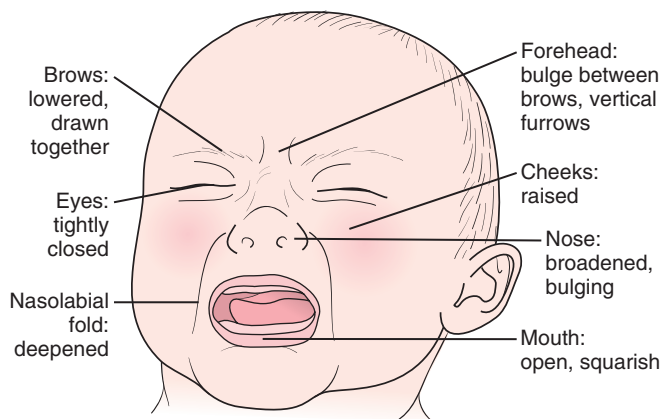
According to Allegaert et al. (2009), the optimal approach to neonatal pain management should include:

- Reducing the frequency of painful procedures
- Reducing environmental stressors
- Facilitating neurologic development
- Determining the best technique to minimize the pain and stress associated with procedures
- Delegating responsibility for pain assessment and treatment to the bedside nursing staff
- Using a balanced multimodal approach to pain control

The involvement of parents in managing their own child's pain is also important; parents randomized to receive a pain information booklet were better prepared to understand infant pain cues and comforting techniques and took more active roles in infant pain care while the infant was hospitalized (Franck et al., 2011a, 2012).

Bedside Noninvasive Neurophysiologic Measures to Evaluate Pain and Stress

Several additional modalities may help healthcare providers recognize pain in neonates. NIRS might be one of those. In a study of 29 infants of between 26 and 36 weeks' gestation at birth, cortical activation occurred over both somatosensory cortices during both unilateral tactile and painful stimulation (Bartocci et al., 2006). Cortical hemodynamic activity changes in response to noxious stimuli measured by NIRS were found to have an excellent correlation to the PIPP score (Slater et al., 2008). Amplitude-integrated electroencephalography also can be used to detect cortical activation in neonates (Toet and Lemmers, 2009). These and other new technologies will be needed to first recognize and then continually assess and manage pain and discomfort in our fragile patients.



• **Fig. 34.2** Facial Expressions of Physical Distress and Pain in the Infant. (Modified from Wong and Wilson's *Clinical Manual of Pediatric Nursing*, 5th ed.)

Long-Term Consequences of Neonatal Pain and Stress

Untreated pain and stress experienced during the neonatal period are linked to adverse long-term outcomes (Anand and Hickey, 1987; Abdulkader et al., 2008). The acute physiologic responses to pain (elevations of the levels of cortisol, catecholamines, and lactate, hypertension, tachycardia, respiratory instability, glucose instability, and changes in cerebral blood flow) affect developing organs, especially the brain. Exposure to pain and painful procedures is often directly related to the acuity of the hospitalization, so studies evaluating a direct relationship between pain and outcomes must be adjusted for multiple clinical confounders.

Data suggest that, with rigorous controls and statistics, structural changes on magnetic resonance imaging (MRI) at both term-corrected age (Zwicker et al., 2013) and 7 years of age (Ranger et al., 2015) may be related to increased exposure to pain. Untreated pain in the NICU is also associated with altered pain perception of subsequent immunizations after circumcision without anesthesia (Taddio et al., 1997; Grunau et al., 2007), abnormal cortisol responses to stress in later infancy (Grunau et al., 2007) and at school age (Brummelte et al., 2015), and altered pain responses in childhood (Grunau et al., 1998). Higher numbers of skin-breaking procedures from birth to term-corrected gestation predict lower cognitive and motor development indices of Bayley Scales of Infant Development at 8 and 18 months, after early illness severity, overall morphine exposure, and number of days of postnatal dexamethasone administration have been controlled for (Grunau et al., 2009). Greater neonatal pain exposure is also associated with lower body weight percentiles at 32 weeks' corrected gestation after adjustment for appropriate confounders (Vinall et al., 2012). There is concern that such hormonal changes might lead to the development of cardiovascular disease and type 2 diabetes in adulthood (Rosmond and Bjornorp, 2000; Kajantie et al., 2002).

Chronic pain can affect growth, immune function, recovery, and length of hospitalization. In addition, a growing body of evidence has drawn attention to the potential deleterious effects of repeated handling, stress, and pain on long-term memory, social and cognitive development, and neural plasticity (Anand and Hickey, 1987; Anand and Carr, 1989; Pokela, 1994; Taddio et al., 1995a; Anand, 1998; Jennings and Fitzgerald, 1998; Porter et al., 1999).

Clinical Pain and Stress Management Strategies

Surgical Anesthesia

The recognition that infants who undergo surgical interventions experience dramatic physiologic and metabolic changes, similar to those noted in adults, with significant pain has changed current practice. It is well recognized that infants perceive pain, and surgical intervention without anesthesia and analgesia leads to increased morbidity and excess mortality; however, experience obtained in older children and adults cannot be extrapolated to immature patients. Providing general anesthesia for neonates requires an intimate knowledge of the developmental status and function of each organ system. A complete review of this subject is beyond the scope of this chapter. Interested readers are referred to *A Practice of Anesthesia for Infants and Children* by Cote and Lerman (2013) or *Smith's Anesthesia for Infants and Children* by Davis et al. (2011).

Fetal Surgery

The fetus has increasingly become a candidate for surgical intervention to repair fetal anomalies such as congenital cystic adenomatoid malformation of the lung, sacroccygeal teratoma, congenital diaphragmatic hernia, and myelomeningocele and to treat fetal disorders such as severe anemia secondary to hemolytic diseases. Many fetal interventions are performed with general maternal anesthesia, which also provides fetal anesthesia, but certain procedures are attempted only with maternal analgesia and local anesthesia. Thus during those fetal surgical interventions, the fetus requires consideration for pain management to attenuate fetal physiologic and hormonal stress responses (Giannakouloupoulos et al., 1999; Fisk et al., 2001). Anand and Hickey (1987) summarized the available evidence regarding fetal and neonatal nociceptive activity and put all care providers on notice that late-gestation fetuses and newborns have intact cortical and subcortical centers necessary for pain perception and demonstrate physiologic responses to painful stimuli similar to those in adults. A review of the literature supports the contention that the fetus perceives pain (Lee et al., 2005; Brusseau, 2013). However, fetal anesthesia is not without risk. Fetal exposure to opioids may result in smaller brain volumes in the neonate (Yuan et al., 2014).

Neonatal Surgery

Selection of anesthetic agents for neonates must account for the developmental status and function of each organ system and the potential adverse and toxic effects of specific anesthetic agents. Animal models suggest that anesthetic agents can be both neurotoxic and neuroprotective in the immature brain. Frequently used anesthetics act by two principal mechanisms, either by decreasing excitation via *N*-methyl-D-aspartate receptors (e.g., ketamine, nitrous oxide) or by increasing inhibition via γ -aminobutyric acid receptors (e.g., benzodiazepines, barbiturates, propofol, etomidate, isoflurane, enflurane, halothane). In the immature rat brain, drugs that act by either of these two mechanisms can induce widespread neuronal apoptosis and abnormal synaptic development (Ikonomidou et al., 1999; Ishimaru et al., 1999).

The applicability of these findings to the human infant undergoing surgery has been questioned (Soriano and Anand, 2005). Some authors suggested that toxicity may occur in human infants (Wilder et al., 2009), but limitations in study design and data collection have precluded most investigations from proving causation. Moreover, the available data are conflicting (Kalkman et al., 2009; Hansen et al., 2011; Sprung et al., 2012). The US Food and Drug Administration (FDA) reviewed available information in 2007 and again in 2011 and concluded that no change in clinical practice was justified (Mellon et al., 2007; Rappaport et al., 2011). Recent work questions the association between anesthesia and abnormal neurodevelopmental outcomes in human infants (Davidson et al., 2016; Sun et al., 2016). Further answers should be forthcoming as the SmartTots initiative (joint effort of the International Anesthesia Research Society and the FDA) and the Pediatric Anesthesia and Neurodevelopment Assessment project publish results of their ongoing studies.

Regional anesthesia for neonatal surgery is commonly used for minor interventions. As more experience with regional anesthesia for major surgical procedures is gained, such practice is expanding. The advantages of regional anesthesia may include less postoperative apnea, less need for intubation, and better postoperative pain

control. [Bosenberg and Flick \(2013\)](#) reviewed the risks and benefits of regional anesthesia in the neonate in depth.

Postoperative Pain Management Strategies

Pain management after surgical intervention, like acute pain management, requires knowledge of the developmental status and function of end-organ systems and the potential adverse and toxic effects of specific analgesic agents. Unfortunately, the current body of neonatal pain literature focuses heavily on procedural pain management and lacks systematic data on acute perioperative pain management in the neonate ([Maitra et al., 2014](#)). The Association of Pediatric Anesthetists of Great Britain and Ireland commissioned guideline development for pain management after surgery and painful medical procedures with graded recommendations to allow better interpretation ([Howard et al., 2008a, 2008b](#)). These guidelines provide evidence-based recommendations and list best clinical practice points when published evidence is insufficient to make formal recommendations. In the developing world, if infants survive the challenges of both diagnosis and surgery for their congenital anomalies, postoperative pain is often not considered or is under-managed ([Mathew et al., 2011](#)).

[Taylor et al. \(2006\)](#) surveyed 10 NICUs regarding their postoperative pain assessment and management practices; they found that pain assessment documentation was extremely variable. Nursing documentation was performed for most infants, whereas few physicians documented any assessment. Most infants were treated with opioids, benzodiazepines, or both, and some infants (7%) received no analgesia despite recent major surgery. [Van der Marel et al. \(2007\)](#) evaluated the use of rectally administered acetaminophen as an adjuvant treatment with regard to continuous morphine infusion in postoperative neonates but could not demonstrate any additional analgesia effect. On the basis of the monitoring of pain scale data, neonates likely require significantly less morphine to control pain and discomfort than older infants do. [Bouwmeester et al. \(2003a, 2003b\)](#) determined that neonates required less morphine for postoperative pain control and that the dose requirement increased with age. Both studies found that morphine was equally effective whether given by bolus or given by continuous infusion.

Many neonates remain intubated for mechanical ventilation after major surgery. Analgesia can be provided via IV administration of an analgesic agent, either by intermittent bolus dosing or by continuous infusion. Epidurals are undoubtedly effective in treating postoperative pain and alleviating unwanted side effects of systemic opioids in adults and older children. While the literature does support the benefits of epidurals for perioperative pain control for term and preterm neonates ([Bosenberg and Flick, 2013](#)), there is concern that the risk of placing thoracic epidurals by physicians without sufficient experience exposes neonates to the possibility of catastrophic neurologic outcomes ([Bosenberg et al., 2011](#)). In a study of infants younger than 6 months who required thoracotomy for congenital pulmonary malformations, those randomized to receive epidural anesthesia had a shorter time to full feedings and reduced intensity of postoperative care when compared with those randomized to receive systemic analgesia ([Di Pede et al., 2014](#)). Regional anesthesia, when used alone, may also reduce the incidence of postoperative apnea in preterm infants ([Craven et al., 2003](#)). A study of parent-controlled and nurse-controlled analgesia as an alternative to continuous opioid infusion for pain management in postsurgical infants in the NICU provides promising data that parent-controlled and nurse-controlled analgesia may provide more

individualized care and may potentially reduce opioid consumption ([Czarnecki et al., 2014](#)).

Mechanical Ventilation

The use of mechanical ventilation in neonates with respiratory failure is a common practice, although less so than in the past 3 decades. In older children and adults who require mechanical ventilation, sedation is routinely provided—most often with opiates ([Gélinas et al., 2004](#)). Extrapolation of evidence from studies in adult patients is what initially led to the routine use of opiate sedation in neonates during mechanical ventilation ([Kahn et al., 1998](#)), despite limited information regarding safety and efficacy in neonates ([Simons et al., 2003](#); [Anand et al., 2004](#)). The ability to assess and treat neonatal discomfort and pain is limited, and preemptive use of pharmacologic sedation during mechanical ventilation in newborns, especially preterm infants, was—and still is—controversial ([Anand and Hall, 2007](#)).

Mechanical ventilation in neonates is associated with an increase in hormonal stress responses, including increased cortisol and catecholamine levels ([Guinsburg et al., 1998](#); [Quinn et al., 1998](#)). In the past, infants who appeared uncomfortable while ventilated demonstrated asynchronous respiratory effort (i.e., “fighting the ventilator”), compromised gas exchange, and altered stress responses ([Dyke et al., 1995](#)). Pain and stress in newborns receiving mechanical ventilation were associated with decreased pulmonary compliance, atelectasis, and intrapulmonary shunting ([Bolivar et al., 1995](#)). However, with the introduction and use of surfactant replacement therapy, noninvasive ventilatory support, and synchronized ventilation, many of these problems associated with fighting the ventilator have been eliminated ([Quinn et al., 1993](#); [Claure and Bancalari, 2009](#); [Keszler, 2009](#)).

A randomized double-blind, placebo-controlled clinical trial of almost 900 neonates (NEOPAIN) reported no beneficial effect of preemptive morphine infusions in ventilated preterm infants and an increased incidence of severe intraventricular hemorrhage in preterm infants born at 27 to 29 weeks' gestation receiving preemptive morphine ([Anand et al., 2004](#)). A parallel study conducted by [Simons et al. \(2003\)](#) randomized 150 ventilated neonates to lower-dose preemptive analgesia with morphine. This study also failed to find any benefit of empiric morphine analgesia and did not recommend this practice ([Simons et al., 2003](#)). Secondary analysis of the NEOPAIN trial found that morphine infusions were independently associated with increased risk of air leak and a longer requirement for ventilatory support ([Bhandari et al., 2005](#)) as well as longer time to reach full-volume enteral feedings ([Menon et al., 2008](#)). Neither this trial nor the smaller pilot trials that preceded it ([Orsini et al., 1996](#); [Anand et al., 1999](#)) provided evidence that routine narcotic sedation during mechanical ventilatory support in neonates is beneficial. A subsequent trial conducted in 2013 randomized premature neonates to receive continuous infusion of fentanyl or placebo. Similarly to prior trials of morphine, the results demonstrated increased duration of mechanical ventilation and no decrease in prolonged pain, as measured by the EDIN algometric scale, for the treatment group. Continuous fentanyl infusion did reduce acute pain scores, measured via PIPP scores, and had fewer side effects when compared with open-label fentanyl boluses. The authors concluded that, just like morphine, “there is no place for the routine use of continuous fentanyl infusion in ventilated preterm newborns because of a lack of continued pain score reduction and increased side effects of continuous infusion

compared with the bolus administration of fentanyl” (Ancora et al., 2013).

A Cochrane review evaluated the effects of opioid analgesics on pain, duration of mechanical ventilation, mortality, growth, and development in neonates requiring mechanical ventilation (Bellú et al., 2008). No differences in mortality, duration of mechanical ventilation, and short-term and long-term neurodevelopmental outcomes were found. If morphine sedation prolongs ventilatory support needs and the time to full enteral feeds, then an increase in the risk of complications related to the use of IV lines (bloodstream infections) and parenteral nutrition (cholestasis) should be expected (Menon and McIntosh, 2008). Hällström et al. (2003) studied risk factors for necrotizing enterocolitis in premature infants and found that the duration of morphine use was the strongest predictor for development of severe necrotizing enterocolitis. The Cochrane review concluded that “there is insufficient evidence to recommend routine use of opioids in mechanically ventilated newborns” (Bellú et al., 2008). Menon and McIntosh (2008) came to a similar conclusion in their review.

Procedures

Infants undergoing intensive care endure many painful procedures, often several times each day. Positive correlations between clinical pain scores and malondialdehyde, a marker for oxidative stress, suggest a significant relationship between procedural pain and oxidative stress in preterm neonates (Slater et al., 2012). The mode of delivery may alter acute pain response in term neonates, with those born vaginally having higher saliva cortisol levels and clinical pain response than those born by cesarean delivery (Schuller et al., 2012). Healthy term neonates who are sleeping or drowsy have decreased pain scores and a shorter duration of crying compared with awake term neonates when exposed to a painful stimulus (Mathai et al., 2011). Both pharmacologic and nonpharmacologic treatment strategies can decrease or completely relieve procedural pain and stress. Nonpharmacologic strategies including, but not limited to, sucrose administration, breastfeeding, nonnutritive sucking, sensorial saturation, and skin-to-skin care can be adapted for many procedures. These strategies are discussed later in Nonpharmacologic Analgesia.

Blood Sampling and Monitoring

Heel sticks are routinely performed to obtain blood samples from neonates. The most appropriate method for relieving pain from a heel stick has yet to be determined. The heel should be warmed to aid blood sampling. EMLA cream (eutectic mixture of local anesthetics) does not relieve the pain of a heel lance (Taddio et al., 1998; Stevens et al., 1999). Shah et al. (1997) and Larsson et al. (1998a, 1998b) demonstrated that neonates experiencing venipuncture had lower pain scores than those who underwent heel stick for blood sampling. In select neonates, venipuncture should be used preferentially over heel stick. Multiple studies have concluded that acetaminophen alone does not reduce the pain from heel lance (Ohlsson and Shah, 2015a).

Various nonpharmacologic interventions work well at decreasing pain associated with heel lance. A prospective study comparing the effect of fentanyl, facilitated tucking, and sensorial saturation on both clinical pain scores and cytokine markers of stress concluded that both fentanyl and sensorial saturation were efficacious at decreasing pain and stress from heel lance (Gitto et al., 2012). A Cochrane review of 19 studies including 1594 infants found

skin-to-skin care, often termed *kangaroo care*, to be effective at decreasing composite pain indicators for single painful procedures such as heel lance (Johnston et al., 2014).

Tracheal Intubation

Premedication before tracheal intubation facilitates the procedure, treats pain, and prevents bradycardia, tachycardia, and increased intracranial pressure in the newborn. In 2010 the AAP issued a policy statement with grade A evidence-based recommendations that all newborns should receive analgesic premedication for endotracheal intubation except for emergency intubations during resuscitation or newborns in whom instrumentation of the airway is likely to be extremely difficult (Kumar et al., 2010; Barrington, 2011). A key component of premedication is analgesia.

Premedication for intubation is not yet common practice in the NICU. International assessments of clinical practice continue to affirm nonuniform compliance with recommendations for periprocedural analgesia (Sarkar et al., 2006; Whyte et al., 2000; Wheeler et al., 2012). Concerns about rapid medication availability, ability to maintain the airway, and the ability to provide ongoing ventilatory support continue to cause controversy (Carbajal et al., 2007). The frequency with which neonatal providers use premedication for nonemergent intubation internationally is unknown. Debate about the best medication regimen is ongoing in the literature, particularly surrounding the INTubation - SURfactant - Extubation (INSURE) procedure, which involves intubation to administer surfactant, with subsequent rapid extubation to positive pressure (de Kort et al., 2013), the concern being that sedation for intubation may make rapid extubation more challenging.

Once the decision has been made to premedicate the patient, the next question is which medications to use. Atropine at 0.02 mg/kg IV or intramuscularly with no minimum dosage has a grade A evidence-based recommendation for its effects as a vagolytic (Kumar et al., 2010; Barrington, 2011). A rapid-acting analgesic agent should also be given; the current best choice is fentanyl, a fast-acting opioid, as morphine has been shown to be no better than placebo in randomized trials because of its slow onset of action (Kumar et al., 2010; Barrington, 2011). Remifentanyl, a short-acting opioid with extremely rapid onset and short half-life, is also an acceptable option for analgesia (Badiie et al., 2013; Avino et al., 2014). Rapid-onset muscle relaxants also carry grade A evidence-based recommendations for the ability to facilitate the procedure; however, many providers have concerns about removing spontaneous respiratory drive. Propofol, an anesthetic that provides no analgesia, may be an appropriate substitute for the combination of atropine and a muscle relaxant. The advantages of propofol may include continued spontaneous breathing during the intubation procedure. This tremendous advantage should be weighed against the uncertain pharmacokinetics and duration of action as well as minimal experience with this medication in neonates. In addition, there is the risk of pain when it is injected into small veins, with extreme pain if it extravasates (Barrington, 2011). Midazolam, a sedative, is contraindicated when preterm neonates are being intubated, as it has been associated with adverse events in a small prospective, randomized controlled trial (Kumar et al., 2010).

Circumcision

Neonatal male circumcision is the most common pediatric surgical procedure, with rates ranging from 42% to 80% of males depending on the country (Simpson et al., 2014). In 2012 the AAP

circumcision policy statement (AAP Task Force on Circumcision, 2012) not only affirmed the benefits of circumcision but unequivocally stated that analgesia must be provided during neonatal circumcision. Adequate analgesia means having a trained provider perform the procedure and provide pharmacologic analgesia (Task Force on Circumcision, 2012). Application of EMLA cream, a topical anesthetic, dorsal penile nerve block, which provides local analgesia, and subcutaneous ring block, which also provides local analgesia, are each evidence-based methods of pharmacologic analgesia. However, anesthesiologists argue that EMLA cream may be insufficient (Paix and Peterson, 2012). Subcutaneous ring block has been found to be more effective than EMLA cream or dorsal penile nerve block (Taddio, 2001). Nonpharmacologic techniques such as positioning or sucrose pacifiers, used alone, are not an acceptable alternative for pain control.

A randomized clinical trial assessing neonatal circumcision pain with a Gomco versus a Mogen clamp, performed in 251 infants, concluded that the Mogen clamp is associated with less physiologic signs of pain (lower heart rate, blood pressure, and salivary cortisol responses) but with no difference in Crying - Requires oxygen - Increased vital signs - Expression - Sleepless (CRIES) pain scores (Sinkey et al., 2015).

Other Invasive Procedures

Placement of a central venous catheter requires topical anesthesia with EMLA cream or infiltration of the skin with lidocaine. In addition, a parenteral opioid, such as morphine or fentanyl, is typically required (Taddio et al., 2006). Consideration should also be given to regional blocks, if technically feasible, for central line placement.

The pain of a lumbar puncture is compounded by both the needle puncture and the distress caused by the body position required for the procedure. EMLA cream has been shown to decrease the pain of lumbar puncture in children (Halperin et al., 1989). Fentanyl does decrease the pain of lumbar puncture, with the caveat that it can cause respiratory depression (Fallah et al., 2016).

Chest tube insertion requires an IV opioid, adequate local analgesia (lidocaine), or both.

Pharmacologic Analgesia

The severity of the pain, its cause, available administration routes, and potential side effects should all be considered during selection of an analgesic. Once medication administration has begun, careful monitoring for efficacy and side effects can decrease the risk of potential adverse events. A key component of effective pain management is continued reassessment after each intervention is introduced, although this is difficult to do with limited pain assessment tools.

Nonopioid Analgesics

Nonsteroidal Antiinflammatory Drugs (Indomethacin, Ibuprofen, Ketorolac)

Nonsteroidal antiinflammatory drugs (NSAIDs) inhibit prostaglandin synthesis by inhibiting the action of cyclooxygenases that are responsible for the breakdown of arachidonic acid to prostaglandins. Prostaglandins are part of the mechanism of local inflammatory response that results in pain; by inhibiting prostaglandin synthesis, NSAIDs are then able to inhibit pain. NSAIDs have many adverse physiologic and developmental effects, including sleep cycle disruption, increased risk of pulmonary hypertension,

cerebral blood flow alterations, decreased renal function by decreasing glomerular filtration rate (Allegaert et al., 2008), alteration in thermoregulatory control, and changes in platelet function. Development of the CNS, cardiovascular, and renal systems depends on prostaglandins, so these potential adverse effects are particularly worrisome for neonates and infants. Newborns younger than 21 days of age and less than 37 weeks' corrected gestational age were more likely to have bleeding events when given ketorolac for treatment of postoperative pain than older, maturer infants younger than 3 months of age in a retrospective review (Aldrink et al., 2011).

Acetaminophen

Acetaminophen is the most widely administered analgesic in patients of all ages. It inhibits the activity of cyclooxygenase in the CNS, decreasing the production of prostaglandins and peripherally blocking pain impulse generation (Arana et al., 2001). Neonates are able to form the metabolite that results in hepatocellular damage (Arana et al., 2001); however, it is not necessary to withhold acetaminophen therapy in newborns because of concerns of liver toxicity. In fact, the immaturity of the newborn's cytochrome P450 system may actually decrease the potential for toxicity by reducing production of toxic metabolites (Collins, 1981).

Pharmacodynamic and pharmacokinetic studies of acetaminophen in infants recommend less frequent enteral dosing intervals of acetaminophen (every 8 to 12 hours in preterm and term neonates) because of slower clearance times and higher rectal dosing because of decreased absorption (van Lingen et al., 1999; Arana et al., 2001; van der Marel, 2003). Rectally administered acetaminophen has a longer half-life, but absorption is highly variable because it depends on the individual infant and the placement of the suppository. It should also be noted that the suppository may contain the entire drug in its tip and should be divided lengthwise if a partial dose is desired. IV acetaminophen has become available in recent years, with dosing recommendations that lack validated pharmacokinetic or pharmacodynamic correlates (Allegaert et al., 2011). While rectally administered acetaminophen does not appear to decrease narcotic needs (van der Marel, 2007), IV paracetamol can decrease the need for morphine analgesia in preterm infants (Harma et al., 2016).

A 2015 Cochrane review emphasized that acetaminophen "does not significantly reduce pain associated with heel lance or eye examinations. [Acetaminophen] given after assisted vaginal birth may increase the response to later painful exposures. [Acetaminophen] should not be used for painful procedures given its lack of efficacy and its potential for adverse effects. [Acetaminophen] may reduce the total need for morphine following major surgery" (Ohlsson and Shah, 2015a).

Epidemiologic data suggest a link between perinatal acetaminophen exposure and an increased risk of developing asthma (Cheelo et al., 2015) and autism (Bauer and Kriebel, 2013; Ohlsson and Shah, 2015b; Andrade, 2016). While the association between acetaminophen and autism correlates with maternal use during pregnancy, the association with asthma is strongest with acetaminophen use in infancy. As with all epidemiology studies, causation is difficult to ascertain.

Opioid Analgesics

Opioids are believed to provide the most effective treatment for moderate to severe pain in patients of all ages, but there is a wide range of interpatient pharmacokinetic variability. Opioid dosing depends on the severity of the pain as well as the age and clinical

condition of the infant. One group of investigators suggested that opioids should be used in infants younger than 2 months only in a monitored setting such as an intensive or intermediate care unit (Yaster et al., 2003). Others have proposed a less conservative recommendation, restricting the use of opioids to monitored settings for any infant younger than 6 months.

Morphine

Morphine remains the gold standard opioid, used for pharmacologic pain treatment in neonates, although not necessarily because it has been shown to be the most effective. It is derived from the opium poppy and is metabolized in the liver by uridine diphosphate glucuronyltransferase into two active metabolites: (1) morphine 6-glucuronide (M6G), a potent opiate receptor agonist, and (2) morphine 3-glucuronide (M3G), a potent opiate receptor antagonist. Both metabolites and some unchanged morphine are excreted in the urine. The predominant metabolite in preterm and term neonates is M3G. Because of slow renal excretion, both metabolites can accumulate substantially over time, especially M3G (Saarenmaa et al., 2000; Bouwmeester et al., 2003a, 2003b). There is thus the potential for late respiratory depression because of a delayed release of morphine from less well-perfused tissues and the sedating properties of the active M6G metabolite.

Use of the lowest dose possible to achieve the needed analgesia is advised because the predominant metabolite of morphine in infants is M3G, a potent opiate receptor antagonist. Escalating morphine doses will also increase the levels of M3G in the infant, interfering with the goal of adequate analgesia. Dosages as low as 1 to 5 $\mu\text{g/kg}$ per hour can provide adequate analgesia, minimizing the risk of accumulation of high M3G levels, given that metabolite's prolonged half-life (Bouwmeester et al., 2003a, 2003b).

Clearance or elimination of morphine and other opioids is prolonged in infants, because of the immaturity of the cytochrome P450 system. The rate of elimination and clearance of morphine in infants 6 months or older approach that of adults. Chronologic age seems a better indicator than gestational age of how well an infant metabolizes opioids (Scott et al., 1999).

Infants are at greater risk of opioid-associated respiratory depression because of their immature respiratory control mechanisms. There is an increase in the levels of unbound or free morphine and M6G available to reach the brain as a result of the reduced concentration of albumin and α_1 -acid glycoproteins (Houck, 1998).

Hypotension, bradycardia, and flushing constitute the response to the histamine release associated with rapid IV administration of morphine. Histamine release may cause bronchospasm in infants with chronic lung disease, although this is not commonly seen. Morphine sedation may result in extended need for ventilatory support in neonates (Anand et al., 1999; Bhandari et al., 2005).

Dosing recommendations currently reflect the wide range of interpatient pharmacokinetic variability. In the past, an IV morphine dose of 0.03 mg/kg was suggested as a starting dose in infants not receiving mechanical ventilation, whereas an IV morphine dose of 0.05 to 0.1 mg/kg was recommended as an appropriate starting dose in infants on ventilators. Significantly lower doses are now recommended (Lynn et al., 2000; Saarenmaa et al., 2000; Bouwmeester et al., 2003a, 2003b, 2004; Anand et al., 2008). Titration to the desired clinical effect is done by adjustment of both the dose and the frequency of administration while the needs and responses are continually being assessed (Allegaert et al., 2009). As the use of morphine for analgesia and sedation in neonates is explored further, it is becoming clear that some of the risks may outweigh the potential benefits (Ng et al., 2003; Nandi et al.,

2004; Carbajal et al., 2005; Ranger et al., 2007; Anand et al., 2008; Black et al., 2008; Allegaert et al., 2009).

Fentanyl

Fentanyl is 80-fold to 100-fold more potent than morphine and causes less histamine release, making it potentially a better choice for infants with hypovolemia, hemodynamic instability, or congenital heart disease. Another clinical advantage of fentanyl is its ability to reduce pulmonary vascular resistance, which can be of benefit for infants who have undergone cardiac surgery, have persistent pulmonary hypertension, or need extracorporeal life support. Bolus doses of fentanyl must be administered over a minimum of 3 to 5 minutes to avoid chest wall rigidity, a serious side effect observed after rapid infusion. This adverse effect is treatable with naloxone or a muscle relaxant.

Fentanyl is highly lipophilic. It has a quick onset and relatively short duration of action, so it is typically used as a continuous infusion for treatment of postoperative pain. In infants aged 3 to 12 months, total body clearance of fentanyl is greater than that of older children, and the elimination half-life is longer because of its increased volume of distribution (Singleton et al., 1987). Fentanyl has an even more prolonged elimination half-life in infants with increased abdominal pressure (Koehntop et al., 1986; Gauntlett et al., 1988). Because of tachyphylaxis, continuous infusions of fentanyl are often increased to maintain constant levels of sedation and pain management. Infusion dosing can reach substantial levels, which then requires a prolonged withdrawal period.

A rebound transient increase in plasma fentanyl levels is a phenomenon known to occur after discontinuation of therapy in neonates. It is a result of the accumulation of fentanyl in fatty tissues, which can prolong its effects after continued use; therefore caution must be exercised in the use of repeated doses or continuous infusions.

Enterally Dosed Opioids

Methadone is used in infants, primarily in the context of withdrawal from either maternal narcotic use or long-term narcotic use in the NICU. The limited data regarding its efficacy and pharmacokinetics in this population suggest no dosing modifications are needed from those recommended for adults (Suresh and Anand, 1998; Chana and Anand, 2001; Ward et al., 2014). The respiratory depressant effect of methadone is longer than its analgesic effect; it is metabolized slowly and has a long half-life.

Codeine use in any pediatric patient is not recommended because of safety concerns (Andrzejowski and Carroll, 2016). No data are available regarding its effectiveness in neonates, and its use in this population is discouraged. Acetaminophen and codeine can be administered in a set formula, consisting of acetaminophen (120 mg) and codeine phosphate (12 mg per 5 mL) with alcohol (7%). The dose prescribed is limited by both the appropriate dose of codeine and the safe dose of acetaminophen. This combination is not recommended in neonates.

Oxycodone dosing at 0.05 to 0.15 mg/kg orally every 4 to 6 hours has been used in neonates, but no data are available to recommend this.

Mixed Opioid Agonist–Antagonists

Nalbuphine is a mixed agonist–antagonist opioid receptor drug; therefore its administration in infants of opioid-addicted mothers may precipitate withdrawal. This agent is equianalgesic with morphine and has an analgesic ceiling effect. Additional studies are needed regarding the safety and efficacy of nalbuphine use in

infants. It is not recommended for use in neonates, although it may be useful during opioid drug withdrawal treatment (Jang et al., 2006).

Long-Term Effects of Neonatal Opioid Exposure

Experimental Animal Studies

Perinatal and neonatal opioid exposure in experimental animals is associated with both short-term and long-term adverse neurologic effects that should make clinicians ask whether the use of such medications with questionable benefits is warranted. Opioid receptor-mediated signaling likely has a role in several aspects of early brain development (Durrmeyer et al., 2010). The developing cerebral circulation is extremely vulnerable to physiologic perturbations and the effects of drugs (Volpe, 1998). Cerebrovascular effects of drug exposure early in development can have lifelong consequences, including increased risk of stroke (Barker, 2000; Hanson et al., 2004; Craft et al., 2006). Data from previous studies suggest that perinatal narcotic exposure restricts brain growth, induces neuronal apoptosis, and alters behavioral pain responses later in life (Kirby et al., 1982; Handelsmann and Dow-Edwards, 1985; Seatriz and Hammer, 1993; Hu et al., 2002). The acute effects of exogenous narcotics, including morphine, on the developing cerebral circulation have been described in piglets and include modulation of prostaglandin-induced pial artery dilation during hypoxia, alteration in endothelin production, and increases in endothelin A receptor messenger ribonucleic acid expression (Armstead et al., 1990; Armstead, 1996; Van Woerkom et al., 2004). Endogenous opioids are important regulators of cerebrovascular tone and angiogenesis (Pasi et al., 1991; Blebea et al., 2000; Gupta et al., 2002; Poonawala et al., 2005). Permanent neurobehavioral and neuropathologic changes are demonstrable in a rodent model of neonatal stress and morphine exposure (McPherson et al., 2007; Boasen et al., 2009; Vien et al., 2009). Understanding the clinical relevance of these animal studies regarding the long-term effects of neonatal opioids is difficult because of species differences in the timing of brain development, the development of opiate receptors and major neurotransmitter systems, and the pharmacokinetics of administered opioids.

Clinical Studies

Clinical studies addressing the short-term and long-term effects of prolonged opiate use in neonates are limited. The few that exist are contradictory and confounded by illness severity. Bergman et al. (1991) described reversible encephalopathic changes in neonates receiving long-term sedative and narcotic infusions. Rože et al. (2008) presented 5-year neurodevelopmental outcomes in very low birth weight infants exposed to prolonged sedation or analgesia (defined as more than 7 days of sedative or opioid drugs). They found that exposed very low birth weight infants more frequently had severe or moderate disability at 5 years (42%) compared with those who were not exposed (26%), but after adjustment for gestational age and propensity score (as a way to ensure that treatment effects are compared only between infants who are equally likely to receive that treatment), the association was no longer significant.

Preterm infants (23 to 32 weeks' gestation at birth) evaluated at 36 weeks' postconceptual age in the NEOPAIN study demonstrated neurobehavioral abnormalities if exposed to morphine (Rao et al., 2007). A small, pilot follow-up of the NEOPAIN trial that included just 19 patients aged from 5 to 7 years showed an association between those given preemptive morphine infusions and smaller

head circumference, lower body weight, increased social problems, and altered response latencies (Ferguson et al., 2012).

A retrospective analysis of 100 extremely low birth weight premature infants found a correlation between worse cognitive scores on the Bayley Scales of Infant Development III test administered at 20 months of age with high levels of opioid exposure in the neonatal period after neonatal and social confounders had been controlled for (Koczek et al., 2016).

A prospective cohort of 188 infants born very preterm (mean 27.4 weeks) admitted to a single NICU were tracked for clinical course, painful procedures, and morphine exposure. When multiple clinical confounders were controlled for, brain MRI performed at term equivalent gestation in these neonates demonstrated a strong statistical association of morphine exposure with smaller cerebellar volumes and poor motor and cognitive outcomes (Zwicker et al., 2016). Possible confounders, including illness acuity, additional medications such as steroids and benzodiazepines, and morphine given preemptively make these results difficult to interpret (van den Anker et al., 2016).

Highlighting the conflicting reports in this area of research, MacGregor et al. (1998) demonstrated no adverse neurodevelopmental outcomes in a small group of newborns who received morphine for a median of 5 days. However, other investigators conducted a 5-year follow-up study on a subset of 60% of 150 neonates in the randomized controlled trial of preemptive analgesia for ventilated preterm neonates conducted by Simons et al. (2003). Their regression analysis, controlling for open-label morphine and clinically relevant variables, implied a trend toward more negative outcomes in individuals who received morphine with respect to intelligence, visual-motor integration, behavior, chronic pain, and health-related quality of life. Morphine use, both preemptive and open label, was also associated with a worse performance on the visual analysis subset of the intelligence quotient (IQ) test (de Graaf et al., 2011). When the same investigators conducted a follow-up at 8 to 9 years of age on a different subset of 80 of the original 150 patients, they obtained different results. Parents were asked to fill out the Dutch version of the Behavior Rating Inventory of Executive Functioning questionnaire, and the children underwent IQ testing. While the results of the IQ testing did not differ between the two groups, the questionnaire results implied that children aged 8 to 9 years who were exposed to morphine had significantly fewer problems on the subscales of inhibition, organization of materials, and monitoring as observed by parents and on the subscale of planning and organization as observed by their teachers (de Graaf et al., 2013). Eighty-nine of the original 150 were also assessed at 8 to 9 years of age for differences in thermal detection and pain thresholds, incidence of chronic pain, or overall neurologic functioning. No differences were found between those who received continuous morphine infusion in the NICU for preemptive analgesia and those who did not (Valkenburg et al., 2015).

The conflicting nature of these studies, multiple clinical confounders, and the fact that none of these studies were adequately powered to address the issue of long-term effects of neonatal morphine exposure make the question of long-term effects of morphine on the developing brain an area in need of robust research.

Topical and Local Anesthetics

Lidocaine reduces the pain and stress of venipuncture and IV catheter placement (Larsson et al., 1998, 1998b; Long et al., 2003). EMLA contains the active ingredients lidocaine and prilocaine.

The cream can be placed on the area where anesthesia is desired and then covered with an occlusive dressing for 1 hour before the procedure. Longer application times provide deeper local anesthetic penetration but can lead to toxicity. There is a slight risk of methemoglobinemia with the use of EMLA cream in patients who are glucose 6-phosphate dehydrogenase deficient and in infants younger than 12 months who are also receiving methemoglobinemia-inducing drugs such as acetaminophen, sulfonamides, nitrates, phenytoin, and class I antiarrhythmics. A rare occurrence, methemoglobinemia can happen when hemoglobin is oxidized by exposure to prilocaine. EMLA cream should not be used in patients with methemoglobinemia (Table 34.1 for recommended maximum doses of EMLA cream by age and weight). A study of 30 preterm infants found that a single 0.5-g dose of EMLA cream applied for 1 hour did not lead to a measurable change in methemoglobin levels (Taddio et al., 1995b). A systematic review concluded that EMLA cream diminishes the pain during circumcision. Limited efficacy was noted with pain from venipuncture, arterial puncture, and percutaneous venous line placement (de Oliveira Marcatto et al., 2011). EMLA cream was not found to diminish pain from heel lancing (Taddio et al., 1998). Oral sucrose or glucose administration may be as effective as EMLA cream for venipuncture (Abad et al., 2001; Gradin et al., 2002).

Sedatives

Benzodiazepines

Benzodiazepines such as lorazepam and midazolam are sedatives that activate γ -aminobutyric acid receptors and should not be used in place of an appropriate pain medication as this class of medication has no analgesic effect. For painful procedures an analgesic must be used in conjunction with the benzodiazepine. Benzodiazepines are administered to decrease irritability and agitation in infants, providing sedation.

In ventilated infants, benzodiazepines can help to avoid hypoxia and hypercarbia from breathing out of synchrony with the ventilator, although the common use of synchronized infant ventilators makes this clinical problem much less likely. When benzodiazepines are given as continuous infusions, dosing often escalates rapidly to maintain apparent sedation, creating a need for prolonged weaning. In three small clinical trials, midazolam provided more sedation to ventilated preterm neonates than placebo on the basis of sedation scales that had not been validated for preterm neonates. In the NOPAIN trial, midazolam was associated with a statistically significant higher incidence of adverse neurologic events such as death, severe intraventricular hemorrhage, and periventricular leukomalacia

(Anand et al., 1999). A metaanalysis concluded that there are “insufficient data to promote the use of intravenous midazolam infusion as a sedative for neonates undergoing intensive care ... [and there are] concerns about the safety of midazolam in neonates” (Ng et al., 2012).

Use of such medications has been associated with abnormal neurologic movements in both preterm infants (Lee et al., 1994) and term infants (Chess and D’Angio, 1998). In rats, prenatal exposure to diazepam results in long-term functional deficits and atypical behaviors (Kellogg et al., 1985); exposure of 7-day-old mice to diazepam induces widespread cortical and subcortical apoptosis (Bittigau et al., 2002); and midazolam potentiates pain behavior, sensitizes cutaneous reflexes, and has no sedative effect in newborn rats (Koch et al., 2008). Prolonged midazolam dosing in preterm infants has been correlated with abnormal hippocampal growth and poorer neurodevelopmental outcomes (Duerden et al., 2016). Whether these data can be extrapolated to human infants is unknown, but clinicians have reason to be concerned and should use these drugs with caution in the NICU.

Dexmedetomidine

Dexmedetomidine is a potent and relatively selective α_2 -adrenergic receptor agonist indicated for the short-term sedation of patients in intensive care settings, especially those receiving mechanical ventilatory support. The drug is administered by either bolus doses for short procedural sedation (1 to 3 $\mu\text{g/kg}$) or continuous IV infusion (0.25 to 0.6 $\mu\text{g/kg}$ per hour). Because dexmedetomidine does not produce significant respiratory depression, it has been used for procedural interventions in spontaneously breathing infants (Barton et al., 2008; Chrysostomou et al., 2009). As neonatologists have become more familiar with dexmedetomidine, its use has increased (O’Mara et al., 2009; Su and Hammer, 2011; O’Mara et al., 2012; Estkowski et al., 2015; McAdams et al., 2015; Plambech and Afshari, 2015); however, short-term and long-term safety and effectiveness information in neonates is limited. Animal studies have been reassuring, including several demonstrating that dexmedetomidine may be neuroprotective (Duerden et al., 2014; McAdams et al., 2015; Ren et al., 2016).

Nonpharmacologic Analgesia

Nonpharmacologic interventions for prevention or relief of neonatal pain and stress are numerous and widely publicized in the medical literature. These interventions have been used either as the sole method of pain control or in combination with pharmacologic interventions. Because opioid analgesia and sedation have not been proved to be efficacious and may possibly be harmful, alternative methods of pain and stress relief have been evaluated for efficacy and safety. A variety of approaches have been investigated. As stated clearly by Golianu et al. (2007), “these therapies may optimize the homeostatic mechanisms of the infant, thereby mitigating some of the adverse consequences of untreated pain, as well as facilitating healthy physiologic adaptations to stress.” However, widespread adoption of specific techniques is not consistent.

Orally administered sucrose is the most widely studied non-pharmacologic analgesic; it safely and effectively reduces the acute behavioral response to procedural pain (Stevens et al., 2013). Sucrose does not, however, reduce spinal reflex responses, cortical activity (Slater et al., 2010), or hyperalgesia (Taddio et al., 2009), raising philosophical and ethical questions about the use of sucrose for procedural analgesia. “Sugar may be better understood not as an analgesic, removing or relieving pain, but as a compensating

TABLE 34.1 EMLA Cream: Recommended Maximum Dose by Age and Weight

| Age and Body Weight Requirements | Maximum Total EMLA Dose (g) | Maximum Application Area (cm^2) | Maximum Application Time (h) |
|----------------------------------|-----------------------------|--|------------------------------|
| Birth to 3 months or <5 kg | 1 | 10 | 1 |
| 3 to 12 months and >5 kg | 2 | 20 | 4 |

Data from Taketomo CK, Hodding JH, Kraus DM, editors. *Pediatric & Neonatal Dosage Handbook*, 2014–2015. 21st ed. Hudson: Lexi-Comp; 1246–1248.

pleasure” (Wilkinson et al., 2012). Sucrose use in extremely preterm, unstable, and/or ventilated neonates is not adequately addressed in the literature nor is the effect of repeated sucrose administration (Stevens et al., 2013). When combined with other nonpharmacologic analgesic interventions, sucrose continues to effectively decrease neonatal behavioral responses to pain. Sucrose plus nonnutritive sucking, for example, provides improved pain relief (as assessed by behavioral scores) from heel lance when compared with placebo or either method alone (Thakkar et al., 2015).

Nonnutritive sucking with pacifiers reduces pain responses to heel prick, injections, venipuncture, and circumcision procedures (Shiao et al., 1997; South et al., 2005; Sexton and Natale, 2009). Infant massage has been demonstrated to decrease plasma cortisol and catecholamine levels in preterm infants (Kuhn et al., 1991; Acolet et al., 1993). Preterm neonates benefit from skin-to-skin contact (Ludington-Hoe, 2015) or facilitated tucking (Liaw et al., 2012; Lopez et al., 2015) to ameliorate acute procedural pain responses.

Skin-to-skin contact (also termed *kangaroo care*) is associated with greater physiologic stability and reduced responses to acute pain (Ludington-Hoe and Swinth, 1996; Fohe et al., 2000; Gray et al., 2000; Johnston et al., 2003; Bergman et al., 2004; Ludington-Hoe, 2015). Kangaroo care can decrease Neonatal Infant Pain Scale scores after vitamin K injections (Kashaninia et al., 2008). Maternal rocking has been shown to diminish neonatal distress (Jahromi et al., 2004). Breastfeeding during kangaroo care reduces the physiologic and behavioral responses to acute pain and stress

in neonates and has been recommended as the first line of treatment (Shah et al., 2006; Osinaike et al., 2007; Obeidar and Shuriquie, 2015), as does feeding of expressed breast milk (Sahoo et al., 2013).

The Neonatal Individualized Developmental Care and Assessment Program (NIDCAP) systematically changes a protocol-based model of nursing care to a relationship-based approach (Als et al., 1994). There is a significant body of empiric evidence that use of the NIDCAP approach improves the clinical and neurodevelopmental outcomes of preterm infants (Als et al., 1994; Als and Gilkerson, 1997; Brown and Heermann, 1997; Westrup et al., 2000; Kleberg et al., 2002; Wielenga et al., 2007), but metaanalyses of published studies could not demonstrate any improvement in long-term outcomes (Jacobs et al., 2002; Symington and Pinelli, 2006).

Another approach is multisensory stimulation (sensorial saturation) of preterm infants undergoing painful procedures. This approach entails simultaneous gentle massage, soothing vocalizations, eye contact, smelling a perfume, and sucking on a pacifier. This technique was associated with analgesia and calming of the infants in several reports from one unit (Bellieni et al., 2001, 2002, 2007, 2012).

Music therapy may reduce the behavioral and physiologic responses to acute procedural pain (Hartling et al., 2009). Warmth is also an analgesic for acute procedural pain in healthy term infants (Gray et al., 2012, 2015). Other interventions such as white noise (Karakoc and Turker, 2014) and flaxseed pillows (Diesel and Ercole, 2012) are under investigation for their effects as either an acute analgesic or a modulator of chronic pain.

Summary

Recognition and treatment of pain and discomfort in the neonate remain challenging issues. Despite significant progress in the understanding of human neurodevelopment, pharmacology, and more careful attention to the care of sick infants, there is still much to learn. While protecting and comforting these patients are important goals, available evidence remains limited, and yet external regulatory forces have required intervention to minimize distress. What is known about adult or older pediatric patients has frequently been applied to infants. Some good has come from these endeavors, but errors have been made along the way.

It is important to minimize pain and distress in these patients to avoid more aggressive interventions. Techniques such as grouping interventions and examinations with nursing care times and limiting skin breaking procedures by batching or decreasing blood draws can go a long way toward preventing unnecessary pain. Bedside providers should feel empowered to advocate for their patients by

making time for appropriate and effective pharmacologic and nonpharmacologic analgesia as a key step in pain prevention (Stevens et al., 2011). Such care will minimize the risks of adverse effects on neurodevelopment. Learning to provide good care without doing harm should be the goal.

Nonpharmacologic methods of pain and distress control should be explored further. When pharmacologic intervention is necessary for pain control, use the least amount of drug that controls the pain. Escalation of drug doses may, in fact, compound the problem.

As newer techniques (such as NIRS and amplitude-integrated electroencephalography) and medications (such as dexmedetomidine) are introduced to clinical practice, it must be demonstrated that such additions achieve the goal of pain control and are safe over the lives of our patients. Better tools are needed to help optimize the outcomes for our fragile infants.

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35

Palliative Care

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KEY POINTS

- Palliative care is the total care of a patient with a life-limiting illness regardless of the disease trajectory or treatment options chosen.
- There is a special focus on pain/symptom management, communication, quality of life, family support, and grief support.
- Roughly one-third of deaths in children's hospitals in the United States occur in neonatal intensive care units (NICUs). Many babies and families in the NICU would benefit from palliative care.
- While palliative care is essential to individuals in the NICU, it is often overlooked, and there are still many barriers to its being considered or implemented in suitable cases.
- Palliative care can readily be integrated into the management of neonates with life-limiting illnesses and can be provided concurrently with cure-oriented or life-extending care.

What Is Palliative Care?

The concept of palliative care has been acknowledged in medicine for centuries. The name is derived from the Latin word *palliare*, meaning to cloak, and has been more broadly interpreted to mean “to alleviate without the intent of curing.” Over the years the definition and scope of palliative care have evolved (Lutz, 2011). The field gained great momentum in the adult world during the 1960s with the hospice movement and the realization that modern medicine alone does not address all of the issues patients with serious and life-limiting illnesses experience. More recently, the incorporation of palliative care into the pediatric world has become critical (Feudtner et al., 2003; Knapp et al., 2009; Wolff et al., 2010; Bona et al., 2011; Jones, 2011; Rendón-Macías et al., 2011; Wolfe, 2011; Wolfe et al., 2011; Feudtner et al., 2013; Catlin et al., 2015). The World Health Organization defines pediatric palliative care as the total care of a child with a life-limiting illness. It involves caring for the mind, body, and spirit of the child and supporting the family through the process. It begins with diagnoses and continues regardless of the disease trajectory or treatment options chosen. There is a special focus on pain and symptom management and alleviating distress through a multidisciplinary approach (World Health Organization, 2015). Palliative care is not limited to EOL care. It can occur concurrently with other

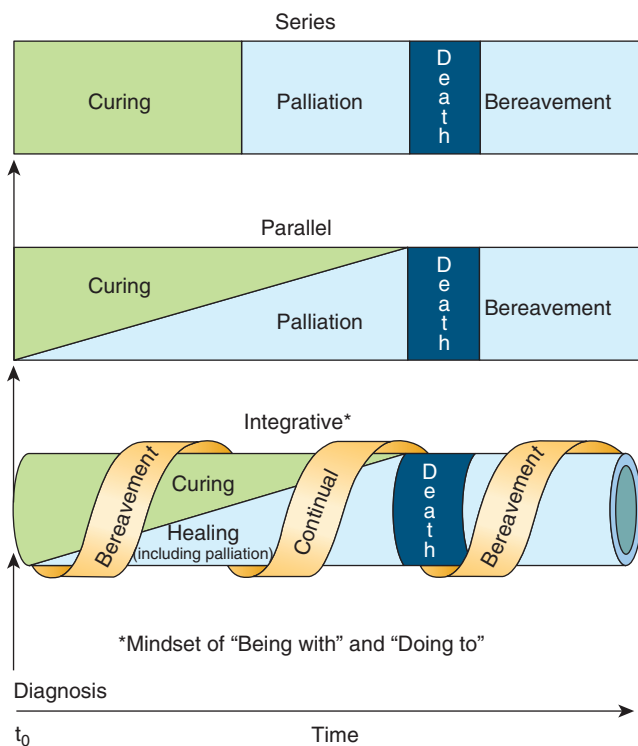
treatments in an attempt to cure a patient or prolong his or her life independently of when the transition to EOL care occurs. Hospice care is a small but important part of palliative care. Once it has been determined that there is no chance of meaningful survival, the focus of the care shifts to pain and symptom management, grief support, preparing for the dying process, and bereavement support (Davis et al., 2015).

In recent years, the American Academy of Pediatrics has acknowledged the importance of this growing field and recognizes that all large healthcare organizations providing care to children with life-limiting illnesses should have a dedicated pediatric palliative care team. The American Academy of Pediatrics has discussed in detail the principles for palliative care as well as the importance of educating trainees (American Academy of Pediatrics, Committee on Bioethics and Committee on Hospital Care, 2000; Section on Hospice and Palliative Medicine and Committee on Hospital Care, 2013). Governing academies and families alike have come to see the importance of the provision of palliative care in pediatrics and more specifically in taking care of the sickest babies. The US News and World Reports Best Children's Hospitals Rankings in Neonatology now include a section for neonatal intensive care unit (NICU)-specific palliative care programs.

Despite the recent increasing focus on neonatal palliative care, the idea has been around for decades. In the 1970s the difficulties of withholding therapies in a special care nursery were described for the first time (Duff and Campbell, 1973). In this landmark article, the authors describe a collaborative process in which parents and physicians through joint decision making determined the likelihood of a meaningful life as an outcome from the special care nursery was very low for 43 babies and opted to redirect care for them. They also acknowledge the difficulty of these decisions for parents and physicians, something that is still faced today. Several years later, the notion of adapting concepts from an adult hospice model to address the issues of caring for a neonate dying in various settings was described (Silverman, 1982; Whitfield et al., 1982; Butler, 1986; Landon-Malone et al., 1987).

Paradigms of Palliative Care

There are multiple paradigms in which palliative care can be provided (Fig. 35.1). In Fig. 35.1 the first model represents the serial approach. This is the earliest view of palliative care. Once a terminal



• **Fig. 35.1** Various models for providing palliative care. (Modified from Milstein J. A paradigm of integrative care: healing with curing throughout life, “being with” and “doing to.” *J Perinatol.* 2005;25:563–568.)

condition has been diagnosed, curative therapies continue until they are seen as futile. Then palliation and comfort measures are offered until the point of death. After death, bereavement support is offered. As the field of palliative care has evolved, the adult world has adapted the parallel model in which palliative care is introduced early in combination with curative therapies, and as the disease trajectory changes with time, the care model changes with it. Again, once death occurs, bereavement support is offered. The final model, the integrated model, is one that has been adopted most often by the growing field of pediatric palliative care and is most conducive to the unique environment of the NICU. When a life-limiting diagnosis is made, the concepts of palliation are introduced along with curative therapies and change with the disease trajectory. With recognition that the family is experiencing loss on multiple levels at the time of diagnosis, bereavement and grief support are offered in a multidisciplinary fashion from the time of diagnosis, through the entire illness course, and after death (Milstein, 2005).

Because the perinatal/neonatal period is so unique, it requires an approach to palliative care different from that in most other fields. The perinatal/neonatal period is usually a time of joy, anticipation of life, and hope for many memories to be made by growing as a family. When there is a diagnosis of a life-limiting condition that results in a fetal or neonatal death, every milestone and stage of the pregnancy, birth, life, and death are very different from what was hoped for and anticipated (Carter, 2004; Carter et al., 2006; Breeze et al., 2007; Munson and Leuthner, 2007; Romesberg, 2007; Balaguer et al., 2012; Catlin et al., 2015). Similarly, in the NICU environment, providers are trained to offer intensive and invasive therapies with the goal of saving lives. It

can be challenging to shift goals of care in the face of a life-limiting illness (Carter et al., 2006; Kain, 2006; Wright et al., 2011; Cortezzo et al., 2013).

Scope of the Problem

While neonatal–perinatal palliative care provides unique challenges for families and healthcare providers alike, it is something that most individuals who provide care for neonates will be faced with. Despite the many advances in medical therapies and improvements in neonatal survival and outcomes, newborns will still die. Often these babies will die regardless of the treatment options chosen by the family and medical care team. In 2013 in the United States, there were more than 1 million fetal deaths, with more than 20,000 occurring after 20 weeks’ gestational age. In addition, there were more than 15,000 neonatal deaths, accounting for nearly 70% of deaths within the first year of life (MacDorman and Gregory, 2015). Most of those who died within the first year experienced severe and chronic illnesses stemming from the time of birth. Roughly one-third of deaths in children’s hospitals in the United States occur in the NICU setting (Brandon et al., 2007). As a result, most medical care providers who care for neonates will be faced with the death of a patient and navigating the goals of care with a family.

Given the likelihood that neonatologists will be faced throughout their careers with patients having life-limiting illnesses, it is pertinent that they incorporate palliative care into daily practices to best care for patients and families. The uncertainty of prognosis, physical constraints of the NICU, time constraints, moral distress, and lack of education are known barriers to providing palliative care in the NICU (Lantos et al., 1994; McHaffie et al., 1999; Romesberg, 2007; Davies et al., 2008; Wright et al., 2011; Cortezzo et al., 2013). In an intensive care setting it can be challenging to shift from cure-directed aggressive and invasive interventions to palliation and comfort care. However, early initiation of palliative care has been associated with improved memory making, decreased pain, decreased rates of invasive procedures that will likely not change the outcome, and increased parental satisfaction (Eden and Callister 2010; Wolff et al., 2010; Cortezzo et al., 2015; Kenner et al., 2015). Consequently, it is imperative that healthcare providers view success for the unique subset of patients with life-limiting illnesses who will die despite intensive medical interventions as eliminating unnecessary suffering, supporting families through the dying process, and helping them find meaning in their baby’s life (Milstein, 2005; Carter et al., 2011). Failure in these situations is not the inability to sustain life but rather unnecessary pain and suffering and an undignified death.

Which Patients Benefit From Palliative Care in the Neonatal Intensive Care Unit?

Many neonates will respond to intensive care therapies, graduate from the NICU, and lead meaningful lives. Knowing this, which patients and families would benefit from palliative care in the NICU setting? There is a role for palliative care in caring for neonates with serious but possibly treatable conditions. Arguably, many aspects of palliative care should be incorporated into the multidisciplinary approach to the care of any baby in the NICU. Any family member of a newborn requiring resuscitation or intensive interventions, regardless of the ultimate outcome, experiences grief and loss. The family has to alter its expectations for a healthy and

uneventful pregnancy leading to a healthy baby. The family often requires spiritual, emotional, and psychosocial support to deal with this trauma both in the immediate period and moving forward (Sydor-Greenberg and Dokken, 2000).

However, there is a special and more pronounced role for palliative care when the likelihood of long-term survival is minimal. Often, when death is almost certain, continuing life-sustaining treatments may only prolong the suffering of the neonate and give the family false hope of meaningful survival. The care initiated and the treatments that follow may be aggressive and at the expense of the comfort of the baby. Palliative care focuses on the prevention and relief of physical pain and suffering of the periviable extremely premature infant, actively dying infants, or those severely compromised and possibly technology dependent, as well as on supporting the needs of the family (Lorenz, 2004). Everyone involved in the care of these neonates, as well as the neonates themselves, can strongly benefit from this type of care. Regardless of whether or not a neonate dies, lives with a permanent impairment, or goes on to live a normal life, there is a necessary role for palliative care. Beyond addressing symptoms such as pain, this approach offers support to families and addresses short-term and long-term measures to ensure that the infant has the best quality of life for as long as he or she may live (Carter and Levetown, 2004; Carter et al., 2011).

While every patient and family dynamic is different and goals need to be explored for each patient, there are several categories where it is appropriate to discuss transitioning from life-extending therapies to comfort measures (Carter and Bhatia, 2001; Carter, 2004; Carter and Levetown, 2004; Carter et al., 2006; Romesberg, 2007; Carter et al., 2011):

1. Any periviable, extremely premature newborns based on gestational age or birth weight, especially when they have significant complications
2. Neonates with multiple congenital anomalies incompatible with long-term survival. Under these circumstances, intensive care therapies may alter the time course and the baby's/family's life experience but will not, unfortunately, change the long-term outcome. These conditions may involve any single-organ or multiple-organ system and at times are the result of genetic abnormalities.
3. Newborns who have received intensive interventions and despite efforts are not responding to therapy, continue to decline, or have continued life-threatening events (severe hypoxic-ischemic encephalopathy, multiple-organ system failure, overwhelming sepsis, necrotizing enterocolitis totalis).

When these unfortunate situations arise, it is important to have a multidisciplinary approach to caring for the patient and family. The NICU provides a unique environment in which intensive life-sustaining therapies are provided that focus on comfort of the patient and involvement of the family in the care process (Carter, 2004). It is one of the few places in hospitals where a multidisciplinary team (physicians, nurses, social workers, chaplains, etc.) is focusing on multiple aspects of the total care of the baby-family unit. This lends itself nicely to the provision of palliative care because much of the framework already exists. Yet, while many care providers in NICU settings feel they are comfortable and competent in providing EOL care for neonates, there is a wide variety in the care patients receive and in the discussions of care with families (Leuthner, 2001; Carter, 2004; Cortezzo et al., 2015). Similarly, there may be attitudinal barriers to consulting palliative care clinicians. Evidence suggests, however, that once the need for and importance of palliative care in the NICU are realized, palliative

care clinicians can facilitate it being readily integrated into the management of neonates with life-limiting illnesses (Kang et al., 2014).

Components of Palliative Care in the Neonatal Intensive Care Unit

The major components of palliative care incorporated into the care of neonatal patients with a life-limiting illness are a focus on communication, quality of life, family support, and grief support (Catlin and Carter, 2002; Madden et al., 2015). Ideally, care providers who have become familiar with the family (including neonatologists, nurses, social workers, and chaplains) and have developed a rapport with them should initiate and be present for difficult conversations. These conversations with family members should be clear, direct, and honest (Janvier et al., 2014). They should happen in a quiet environment where the family has the care provider's undivided attention. Parents wish that difficult news be delivered in an honest and compassionate manner that lets them know their child matters (Widger and Picot, 2008; Kavanaugh et al., 2009; Armentrout and Cates, 2011; Janvier et al., 2014). After the diagnosis and the relative certainty of the prognosis have been discussed, the focus of these conversations should shift to exploring the values of the family, wherein the care team can better understand the family, how the family members make decisions, and what is important to them.

Key among goals in these conversations is helping to identify—and often redefine or redirect—their hopes. By being open and compassionate, providers can give family members a sense of realistic hope during this difficult time without misleading them toward a false sense of the likely survival (Widger and Picot, 2008; Eden and Callister, 2010; Armentrout and Cates, 2011). It is often helpful to focus the conversation on what is meaningful to the family members in the context of a poor prognosis (Carter et al., 2011). Their idea, or object, of hope may change over the course of the baby's life. With a better understanding of the family's values, the care team can participate with the family in shared decision making and ultimately define or redefine the goals of care. These conversations and the decisions made are unique to each family regardless of the diagnosis. It is not uncommon for parents and the care team to have different agendas and priorities during this time (Midson and Carter, 2010). It is critical to attend to the parent's priorities and ensure that they feel valued, listened to, and a member of the care team. It is the care provider's job to understand the family's values and goals and to make medical recommendations based on those goals. There are times when the choices of the family may be in opposition to the views of the care team (Kopelman, 2006; Back, 2009). It is the care team's obligation to continue conversations with the intent of better understanding the reasoning behind the family member's decisions/goals and supporting them through the process. The goal should not be to try to convince the family that an alternative approach is most appropriate. In partnership with the NICU team, palliative care clinicians may be instrumental in facilitating such ongoing conversations, especially as primary neonatal clinicians may change every week or every 2 weeks.

When the end of life (EOL) is approaching, it may also be important to have open conversations about organ or tissue donation, autopsy, and funeral, memorial, or burial services. Many NICUs have a team of social workers, chaplains, and psychologists to help attend to these needs. The addition of palliative care

clinicians can bring expertise in providing additional support during these very unique and difficult times. When there is an anticipated compassionate withdrawal of life-sustaining medical treatment, it may be beneficial to contact a liaison with a regional organ procurement organization to engage families in discussions about organ/tissue donation.

Regardless of the family's care goals or treatment options, the baby's quality of life should remain paramount. Care should be taken to ensure that painful procedures are minimized, unnecessary tests stopped, the physical environment is made comfortable and conducive to family bonding, and that pain and symptoms are addressed with nonpharmacologic and pharmacologic management when necessary.

Grief and Bereavement Support

Grief support should be initiated shortly after a life-limiting diagnosis. Initially such support may focus on grieving the loss of the healthy newborn and the life the family members anticipated in the context of still celebrating and enjoying their baby. Attention should be given to ensure the parents, siblings, grandparents, and extended family members are able to make memories with the baby. During this stressful time, especially if it is their first child, parents are often unable to think about what memories would be important for them to make. Some families wish to take pictures, have handprints/footprints or molds made, record heartbeats, bathe the child, sing/read to the child, take the child outside, have the child meet a family pet, taste foods, sleep in a bed, or participate in spiritual/religious ceremonies. Every effort should be made to help these families maximize the experiences they have with their baby during what may be a brief life span. Bereavement support for parents, siblings, and family members should start before the death of the baby and continue for a period afterward (Kenner et al., 2015). Parents appreciate bereavement support and follow-up phone calls from or meetings with physicians who provided care for their child and other forms of acknowledgment such as condolence cards (Kenner et al., 2015). It is also imperative to recognize that staff members benefit greatly from grief support (Cavaliere et al., 2010; Cortezzo et al., 2015). Often the NICU staff has been involved in the care of the baby for much of his or her life and has spent time becoming familiar with the family. News of a poor prognosis can be emotionally trying for such staff as well. Because the staff are attending to the immediate care needs of the baby, family, and other babies in the unit, they may be unable to process such difficult news. It is important that we support them as well.

Timing of the News

Early Prenatal Diagnosis

Families may learn of a life-limiting condition in the early prenatal period, late prenatal period, or early neonatal period (Wolfe et al., 2011). While the job of the healthcare team remains the same in supporting the family members and helping them find meaning in their baby's life, the timing can slightly alter the approach to care. If the news is given in the early prenatal period, parents often have time to begin grieving the loss of a healthy pregnancy and reframe their idea of hope. If palliative care is initiated early on, the family members may benefit from the involvement of palliative care providers who learn about the family and support them through the process. They can facilitate bonding and memory making

throughout the pregnancy. They also have time to navigate the goals of care and develop a care plan that includes a birth plan and advance care planning known by all providers at the time of delivery. Box 35.1 highlights important components of a perinatal palliative care birth plan. A delivery room resuscitation plan should be discussed ahead of time with the family (Catlin and Carter, 2002; Widger and Picot, 2008; Balaguer et al., 2012; Engelder et al., 2012; Kenner et al., 2015). But the staff still needs to remain ready to adapt such plans to the reality of the newborn's condition at birth. Families may change their minds, the newborn's condition may be better or worse than anticipated, and decision making remains rather fluid. Ethics and palliative care support may aid parents and facilitate their navigation through difficult decisions and how they choose to spend whatever amount of time they have with their baby.

Late Prenatal Diagnosis

If the news of a life-limiting illness is discovered late in the pregnancy or just before delivery, it often results in a more chaotic and less formulated approach. Teams are working rapidly to provide immediate care to the mother and fetus/neonate. There is often not time to have lengthy discussions or learn the parent's values. In the chaos, communication can be rushed and fragmented. It remains imperative to provide accurate information and support to the parents while making the environment as calm and private as possible, allowing the family members to express their emotions. In the time that follows, discussions can be continued.

Postnatal Diagnosis

If the news of a life-limited illness is not discovered until after delivery, parents are often shocked. Most assume that if a delivery

•BOX 35.1 Components of a Perinatal Palliative Birth Plan

Clarify Maternal Goals

- Site of delivery
 - Community or tertiary
- Fetal monitoring
 - E-FHR, auscultation, none
- Mode of delivery
 - Burdens, benefits, values
- Who will be in attendance?
 - Who matters?
- Maternal and neonatal medications
 - Anesthesia and otherwise

Clarify Neonatal Goals

- Specified components of resuscitation and care
 - Intubate, CPAP, O₂?
 - Medications and lines?
- Site of care of the baby
 - L & D, nursery, ICN, home
- Feeding plan
 - Breast, tube(s), cup, finger, no?
- Special events and spiritual care
 - Memories, mementoes, rituals
- Contingency postdischarge plan

CPAP, Continuous positive airway pressure; E-FHR, external fetal heart rate; ICN, Intensive Care Neonatal; L & D, labor and delivery.

goes well, their baby will be healthy. For them it is an abrupt loss of the healthy baby they were holding moments before they received the news. It is vital to continue to promote bonding with the baby and ease suffering. The care team should use the medical information along with the knowledge of the family's values to shape the goals of care and aid in decision making. Extra time is often necessary in this situation, with care to provide support for the family and staff in redefining and redirecting hope and the goals for the newborn's care.

Parents note that the delivery of the news and the discussions that follow shape their experience with the care team, their memories of time with their child, and their subsequent bereavement. Some parents report that the news about a diagnosis, prognosis, or treatment options were given in an insensitive manner. They wish to be included in the decision-making process and know that their input as parents is what matters the most. They also desire privacy, support, and time to come to terms with their loss (Contro et al., 2002; Davies and Connaughty, 2002; Berg, 2006; Dokken, 2006; Brosig et al., 2007; Henley and Schott, 2008; Eden and Callister, 2010; Armentrout and Cates, 2011).

End-of-Life Care

Palliative care is extremely important during EOL care, when a neonate is imminently dying. Every effort is made to ensure the family members can shape the experience of the death in a way that is most meaningful to them and that they are as prepared as they can be for the dying process. For some families, it is important that they understand the physiologic changes that they will likely see ahead of time. If family members wish to stay in the hospital for the death of their baby, every effort should be made to bring them to a private room within the NICU or to a different location within the hospital. The environment should be as peaceful and homelike as possible. Monitors and unnecessary equipment should be removed. Families should determine who they wish to have

present and if they would like to hold their baby or perform any rituals. Care should be taken to respect any cultural or spiritual desires of the family. If parents desire to take their baby home for EOL care, care providers should coordinate with local home hospice programs or home-health services to ensure that the family will have access to the care, support, equipment, and medication that will be needed during this time. Parents should be reassured that regardless of location, they will not be abandoned and that care will be taken to attend to pain and symptom management. The focus should be on what the team can do for the baby and family as opposed to what the team can no longer do (Catlin and Carter, 2002; Leuthner, 2004; Gale and Brooks, 2006). Symptom management is available with an array of medications (Carter and Jones, 2013), and dyspnea, pain, and agitation should be anticipated and appropriately assessed. Neonates feel pain and other symptoms of distress, and they should be addressed accordingly (Anand and International Evidence-Based Group for Neonatal Pain, 2001; American Academy of Pediatrics et al., 2007). Symptoms that may need to be treated at the EOL include pain, agitation, dyspnea, and secretions (Komatz and Carter, 2015). Nonpharmacologic interventions to address these issues are equally important, such as decreasing stimulation, a soothing environment, massage, elevating the head, fluid restriction, and gentle suctioning. At times, especially during the EOL, nonpharmacologic interventions will not be enough. It is important to anticipate this and alleviate the stress of the family. It is both clinically and ethically appropriate to provide pharmacologic symptom relief. While there may be a concern of hastening death with certain medications (e.g., opioids), at appropriate doses this generally does not happen. The intent to provide relief of pain and suffering, it can be argued, outweighs the small chance of respiratory depression. Tables 35.1–35.2 provide a guide to common pharmacologic symptom management at the EOL. It is important to note that these doses and the medication choices may need to be altered on the basis of the patient's previous exposure.

TABLE 35.1 Symptom Management for End-of-Life Care: Pharmacologic

| Symptom | Medication | Category | Starting Dose (per Kilogram) | Route and Interval |
|------------|----------------|--------------------------|--------------------------------------|------------------------------|
| Pain | Acetaminophen | Antipyretic Analgesic | 15 mg 6–8 mg | PO/PR q6 hour IV q8 hour |
| | Fentanyl | Opioid | 0.5–2 µg | IN/IV q2 |
| | Methadone | Opioid | 0.05–0.2 mg | IV/PO q12–24 |
| | Morphine | Opioid | 0.05–0.2 mg 0.2–0.5 mg | IV/IM q2 PO/sublingual q4 |
| Agitation | Lorazepam | Benzodiazepine | 0.05–0.1 mg | IV q4–6 |
| | Midazolam | Benzodiazepine | 0.05–0.1 mg 0.2–0.3 mg 0.25 mg | IV q2–4 Sublingual IN |
| Dyspnea | Morphine | Opioid | 0.15 mg 0.05–0.1 mg | PO/sublingual q2 IV q2 |
| | Lorazepam | Benzodiazepine | 0.05–0.1 mg | PO/IV q4 |
| Secretions | Atropine | Anticholinergic | 0.01–0.02 mg | PO q2 |
| | Glycopyrrolate | Anticholinergic | 0.01–0.02 mg | IV q4 |
| | | | 0.04–0.1 mg | PO q4 |

IV, Intravenous; IM, intramuscular; IV, intravenous; PO, per os; PR, per rectum; q2, every 2 hours; q2–4, every 2–4 hours; q4–6, every 4–6 hours; q6, every 6 hours; q8, every 8 hours; q12–24, every 12–24 hours.

TABLE 35.2 Symptom Management for End-of-Life Care: Nonpharmacologic

| Symptom | Action | Provider |
|-------------------|---|---------------------|
| Pain or agitation | Reduce ambient noise | Clinician |
| | Reduce procedural touching (handling, suctioning, laboratory tests, and imaging) | Clinician |
| | Reduce temperature swings | Clinician |
| | Swaddle; facilitated tuck | Clinician or parent |
| | Nonnutritive suckling | Clinician or parent |
| | Skin-to-skin contact | Parent |
| Dyspnea | Postural positioning (may be lateral, upright, or prone; may include neck, chest, or shoulder roll) | Clinician or parent |
| | Consider fan or humidified air | Clinician |
| Secretions | Oral and nasal suctioning | Clinician or parent |

Ethical Concerns

With advances in equipment and medicine, and more rigorously studied and implemented practices, increasingly neonates are benefiting from their stays in the NICU. The age of viability has dropped with continuously improving clinical capabilities (Rennie, 1996; MacDonald, 2002). As we become better at saving one group, the limits are pushed for another group of neonates (Singh, 2004). Yet with these advances, have come a variety of uncertainties and outcomes. Difficulty remains around the borderlines of viability with resuscitation, and when to forego life-sustaining therapies, or whether to redirect care. At 22 weeks of gestation some centers universally offer a treatment trial of intensive care whereas others do not, or do so on only an individual basis (Patel, Kandefer, et al., 2015; Rysavy, Li et al., 2015).

It is often difficult to predict a neonate's course or future complications. This uncertainty brings many questions and discussions among professionals and families about the neonate's response to intensive care, complications encountered, and what actions are in neonate's best interests (Whitelaw, 1986; Rhoden, 1989; Silverman, 1992; Sanders, Donohue et al., 1995; Rennie, 1996; MacDonald, 2002; Lorenz, 2004; Silverman, 2004). Families are often faced with decisions that are not seen elsewhere in medicine, and these decisions are complicated by both the urgency of need for action and the uncertainty of prognosis (Wolfe, Hinds et al., 2011). Many times both families and care teams feel that once care is initiated, everything possible must be done to save the neonate. However, there may come a point in time when certain life-sustaining interventions are considered acceptable by some families whereas others believe such continued intervention or technology dependence render a quality of life for their baby that they do not find acceptable.

Both ethically and legally withholding an intervention or withdrawing one already started are considered equivalent (Bell and Newborn, 2007). In other fields of medicine this seems to be more readily accepted. The thought of withdrawing LSMT from a neonate leads many parents to believe they are actively aiding in their baby's death. When based on notions or descriptions of futility or a perceived quality of life, a contest of whose perception of these terms matters most (clinicians or families) often results. Consequently, physicians may have difficulties addressing these issues, discussions may be limited, and if they occur it is late in

the treatment course (Lantos, Tyson et al., 1994; McHaffie, Cuttini et al., 1999).

Withdrawing/withholding LSMT, while a relatively infrequent phenomenon in a given NICU, is the most frequent mode of dying in both neonatal and pediatric ICUs (Walther, 2005; Fontana, Farrell et al., 2013; Meert, Keele et al., 2015). It is understandable that parents want to be sure that there is essentially no chance of survival before such decisions are made and it warrants open communication, transparency in prognostication, and attention to empathy (Singh 2004, Meyer, Brodsky et al., 2011).

Barriers to Palliative Care in the Neonatal Intensive Care Unit

While palliative care is essential to individuals in the NICU, it is often overlooked and there are many barriers. The NICU environment is often noisy and crowded, with little room for privacy (Carter and Bhatia, 2001). Also, with current advances in technology, death may be viewed as a failure as the technologic imperative is so operant that if a therapy is available, it must be used without full consideration of how burdensome it is or if it will ultimately benefit the neonate (Carter and Bhatia, 2001; Carter and Miller-Smith, 2016). Often families are overwhelmed by the technology and feel an incredible burden that the decisions are solely on their shoulders. If the outcome is certain and the treatment is a standard of care, then this is often not an issue. However, there is rarely such certainty.

Training in Neonatal–Perinatal Palliative Care

Clinicians' discomfort to address the limits of medicine and technology and redirect the prevailing care paradigm from one that is disease based and cure oriented to one that is palliative and comfort oriented is likely contributed to by a relative lack of palliative care training in neonatal and perinatal training programs. A study was published regarding training of neonatal fellows in discussions with families and helping them make decisions regarding critically ill neonates (Boss et al., 2009). Fellows from 83% of the accredited programs participated. For the most part, all of the fellows felt confident in their medical training. However, more than 40% stated that they had not had any formal communication training or clinical communication skills training. More than 90% felt that training in this area—specifically palliative care, spiritual needs, and managing conflicts of opinion—was lacking or nonexistent in their training program. It is imperative that neonatologists learn these skills because families who have lost a child often cite how important it is that the physician communicates with them in a way they can understand/handle and address all of their palliative care and spiritual needs so that collaboratively they can come up with a clear plan (Baker et al., 2007; Boss et al., 2009; Michelson et al., 2009; Orgel et al., 2010; Cortezzo et al., 2013, 2015).

Research Opportunities/Future Directions

While there have been many advances in the field of neonatal palliative care in recent years, there are still many exciting opportunities for research. Studies have yet to determine which interventions offer optimal pain and symptom management at the EOL. There are also many opportunities for research addressing communication

training and quality improvement investigations looking at the timing and delivery of palliative care, which paradigm of delivery is most effective, and how it will alter the course of living with neonatal loss for these families.

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36

Immunology of the Fetus and Newborn

JOERN-HENDRIK WEITKAMP, DAVID B. LEWIS, AND OFER LEVY

KEY POINTS

- The fetus and newborn express a distinct and evolving immune system that mediates transition from intrauterine life to the microbe- and antigen-rich world.
- Multiple mechanisms including regulatory T cells help ensure maternofetal immune compatibility.
- Newborns are highly reliant on soluble and cellular innate immune mechanisms whose ontogeny depends on gestational and postnatal age.
- Adaptive immunity in newborns features distinct ontogeny and functionality of T and B cells.
- Primary immunodeficiencies that present in early life include genetic defects in innate and adaptive immunity.
- Maternal and early-life immunization are the most effective biomedical interventions to prevent infection in the newborn and young infant.
- Some live vaccines such as bacille Calmette–Guérin, which is given to newborns in tuberculosis-endemic regions, may afford broad protection against infections with antigenically unrelated pathogens.

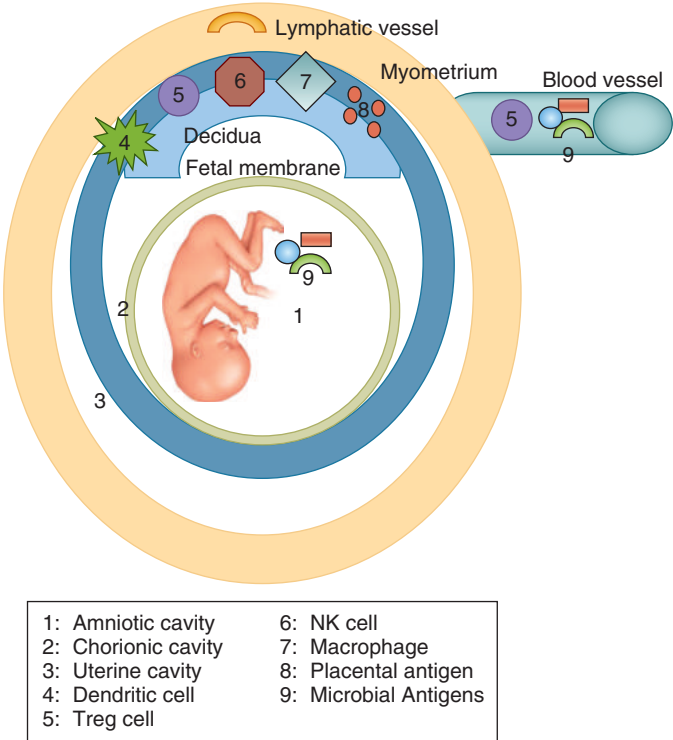
Of any age group, newborns and young infants are at the highest risk of infection-induced morbidity and death. Understanding the contribution of the newborn's distinct immune response to age-dependent susceptibility to infectious diseases has important implications for efforts to protect newborns from infection and entails review of the immunologic environment of pregnancy and the ontogeny of fetal and neonatal immunity (Kollmann et al., 2017). The unique functions of fetal, neonatal, and maternal immunity reflect adaptation to developmental challenges such as preservation of fetal well-being as an allogeneic graft versus adequate immunologic protection in the extrauterine environment. These immunologic transitions are regulated by a number of incompletely understood developmental and genetic mechanisms. The diversity and importance of these mechanisms are suggested by the heterogeneity and frequency of neonatal infections. Differences in immunologic responsiveness between newborns and adults are not defects or abnormalities but rather highly regulated ontogenic differences that facilitate transitions between distinct age-specific challenges. Just as the ductus arteriosus, a cardiopulmonary necessity in the intrauterine environment, closes at different rates in different infants, there is

variability in the pace at which developmentally and genetically programmed human fetal and newborn immunity changes from graft preservation to identification and destruction of invading pathogens.

Maternal and Placental Immunology

Immunologic tolerance to the growing fetus is a prerequisite for a successful pregnancy. The maternal–fetal interface is a dynamic site that encompasses multiple cellular interactions in an environment rich in cytokines and hormones. While immune mechanisms need to be in place to defend against microbial invasion (von Rango, 2008; Aagaard et al., 2014), the placenta is typically programmed to protect the fetus from rejection by the maternal immune system (Aluvihare et al., 2004; von Rango, 2008).

Several distinct but complementary innate and adaptive immune mechanisms contribute to the commensal immunologic relationship between the mother and the fetus throughout pregnancy (Fig. 36.1 and Table 36.1). Local (placental) and systemic (circulatory) factors mediate maternal tolerance to the fetus (Aluvihare et al., 2004). For example, human trophoblasts do not express conventional major histocompatibility complex (MHC) class I human leukocyte antigen (HLA)-A or HLA-B molecules, likely contributing to reduced alloantigenic recognition at the fetal–maternal interface. Human trophoblasts express HLA-C, principally during the first trimester of pregnancy (King et al., 1996a), and two nonclassical HLA molecules, HLA-E and HLA-G. HLA-G class Ib is expressed on extravillous cytotrophoblast and endothelial cells of fetal vessels in the chorionic villi as well as in amnion cells and amniotic fluid (Le Bouteiller et al., 1999; Rebmann et al., 2014). Unlike classical MHC molecules, HLA-G does not have a significant role in stimulating CD8⁺ T cells via the T-cell receptor (TCR) complex. Rather, the principal function of HLA-G molecules expressed by the trophoblast appears to be modulation of the activity of natural killer (NK) cells. HLA-G has additional immunomodulatory properties, including inhibition of activity of cytotoxic T cells, inhibition of alloproliferative responses by CD4⁺ T cells, and modulation of dendritic cell (DC) maturation and function (Carosella et al., 2008). These data reveal that the unique MHC class I molecule expression pattern on fetal trophoblast constitutes an intricate mechanism for orchestrating the activity of immune cells.



• **Fig. 36.1** Immunology of the Fetomaternal Interphase. While competent immune cells and bacteria can enter the fetomaternal interphase, multiple complex immune interactions are in place during healthy pregnancy to balance immune defense with immune tolerance between the mother and the allogeneic fetus. Several distinct but complementary innate and adaptive immune mechanisms contribute to the commensal immunologic relationship that exists between the mother and the fetus throughout pregnancy. In humans the decidua represents the uterine implantation site, where maternal blood flows into the intervillous space, “bathing” fetus-derived villous trees composed primarily of cytotrophoblasts. The surface of these villi consists of multinucleate syncytiotrophoblasts mediating nutrient and gas exchange between maternal and fetal tissues. The villi are directly exposed to circulating maternal immune cells. However, these cells do not recognize fetal tissue as “foreign” mainly because human trophoblasts lack classical class I and class II antigens and instead express human leukocyte antigen G (HLA-G). HLA-G and pregnancy hormones suppress natural killer (NK) cell and macrophage function and induce a bias of CD4⁺ T cells toward T_H2-type cytokine secretion. Dendritic cells are present in small numbers during pregnancy in decidual tissue but show impaired migration to draining uterine lymph nodes (entrapment). Regulatory T (Treg) cells specific to the fetus increase in number in the mother during gestation, and cells maintaining tolerance to fetal antigen can rapidly expand during a subsequent pregnancy. The role of exposure to microbial antigens in fetal immune priming seems important, but the exact mechanism is still being explored.

Other local factors contributing to maternal–fetal tolerance include selective degradation of tryptophan by the inducible enzyme indoleamine 2,3-dioxygenase inhibiting T-cell proliferation (Munn et al., 1998) and engagement of the proapoptotic molecule Fas on maternal lymphocytes by its ligand (FasL) on interstitial trophoblast cells (Hammer et al., 1999). FasL is expressed in both maternal and fetal components of the uteroplacental unit throughout gestation. Activated T cells express the Fas receptor, which delivers an apoptotic (death) signal when bound by FasL. Therefore expression of FasL limits the reciprocal migration of activated fetal and maternal T cells. Mice with a nonfunctional FasL demonstrate leukocyte infiltration and necrosis at the decidual–placental border,

TABLE 36.1 Selected Local and Systemic Factors Mediating Maternal Tolerance to the Fetus

| Factor | Function |
|--|---|
| Expression of nonclassical HLA molecules (e.g., HLA-G) | Inhibition of NK cells, CD4 ⁺ T cells, and cytotoxic T cells, and modulation of dendritic cell maturation and function |
| Indoleamine 2,3-dioxygenase | Depletes tryptophan and prevents T-cell proliferation |
| Fas ligand | Apoptosis of activated fetal and maternal T cells |
| Programmed death 1 and its ligand | Negative regulator of T-cell responses |
| Galectins | Apoptosis of activated fetal and maternal T cells |
| Decay-accelerating factor | Control of complement activation |
| Cytokines | T _H 2 bias prevents immune activation |
| Decidual macrophages | Suppressing immune activation |
| Decidual NK cells | Suppressing immune activation |
| FoxP3 ⁺ regulatory T cells | Suppressing immune effector cells (e.g., in response to paternal antigens) |

Microbiome Balanced immune response

FoxP3, Forkhead box P3; *HLA*, human leukocyte antigen, *NK*, natural killer.

with many resorption sites and small litters (Hunt et al., 1997). Progesterone-induced blocking factor is an immunomodulatory molecule released in response to progesterone by trophoblasts (Anderle et al., 2008). Its properties include indirect suppression of NK-cell function and inducement of bias of CD4⁺ T cells toward T_H2-type cytokine secretion (Szekeres-Bartho and Wegmann, 1996).

Galectins are expressed in human placenta primarily by the syncytiotrophoblast early in pregnancy (Than et al., 2009). On cell surface contact, galectins downregulate the cellular immune response, in part by inducing programmed cell death (apoptosis) of T lymphocytes (Liu and Rabinovich, 2010).

On a cellular level, DCs, an important type of antigen-presenting cell (APC) critical for cellular and humoral immune responses, play a prominent role in organ transplant rejection. The potential deleterious actions of DCs against the fetus may be curtailed by at least two factors: the progressive decline of decidual DC tissue densities shortly after implantation and impaired migration from decidual tissue to draining uterine lymph nodes. This entrapment of DCs during pregnancy may be a combined result of disappearing lymphatic vessels during decidualization (Volchek et al., 2010) and stromal cell–based processes limiting chemokine-directed cell migration (Collins et al., 2009). Other important cellular factors that may limit a potential immune reaction toward the fetus include the immunosuppressive phenotype of decidual macrophages (Gustafsson et al., 2008) and decidual NK cells.

Complement inhibition is essential for normal pregnancy in a murine model of antiphospholipid syndrome, an autoimmune condition characterized by thrombosis, thrombocytopenia, and recurrent fetal loss. In this model, fetal injury results from placental

inflammation initiated by local dysregulation of complement proteins. Both complement activation and fetal loss can be prevented by administration of anticoagulants with complement-inhibitory properties such as heparin but not by anticoagulants lacking complement-binding properties (Girardi et al., 2003, 2004). Some but not all human clinical interventional studies using anticoagulants with complement-binding properties to prevent fetal loss in antiphospholipid syndrome have suggested benefit (Di Nisio et al., 2005). Control of complement activation during human pregnancy is achieved by expression of decay-accelerating factor, membrane cofactor protein, and CD59 (protectin) on the trophoblast membrane (Denny et al., 2013), as well as high reproductive tract and systemic prostaglandin E_2 levels contributing to maternal immune tolerance to the fetus (Parhar et al., 1988).

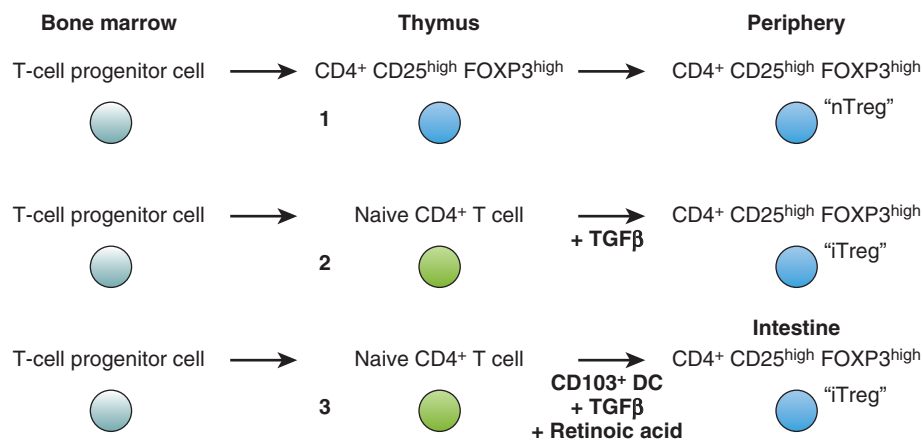
In addition to local components, systemic elements are in place to maintain immune tolerance at the maternal–fetal interface. Abatement of certain autoimmune diseases during pregnancy (de Man et al., 2008) and an increased risk of infections (Jamieson et al., 2006) provide evidence for a more generalized immunosuppressive state during pregnancy (Poole and Claman, 2004). Although the precise mechanisms underlying this phenomenon are incompletely characterized, several reproductive hormones may play critical roles. For example, a relatively large study demonstrated that 48% of patients with at least moderate disease activity of rheumatoid arthritis showed signs of remission in the first trimester of pregnancy, while approximately 40% had a disease flare-up in the postpartum period (de Man et al., 2008). The rate of relapse in multiple sclerosis declines during pregnancy (Confavreux et al., 1998), and treatment with pregnancy levels of estradiol significantly reduced enhancing lesions on brain imaging (Camras et al., 1992). In addition, estrogen (17β -estradiol) in concentrations typically expressed during normal pregnancy augments forkhead box P3 (FoxP3) expression and expansion of T regulatory (Treg) cells in vitro and in vivo (Polanczyk et al., 2004; Tai et al., 2008).

T cells and T-cell–derived cytokines play a central role in immune regulation and inflammation. T_H1 cells are involved in cellular

immunity and rejection and are characterized by production of interleukin (IL)-2, interferon gamma (IFN- γ), and tumor necrosis factor alpha (TNF- α). In contrast, T_H2 cells are mediators of humoral immunity and produce IL-4, IL-5, and IL-13. Traditional dogma holds that a bias in T-cell cytokine secretion toward T_H2 -type cytokines and away from T_H1 -type cytokines is an immunologic condition necessary for maintaining healthy pregnancy (Dealtry et al., 2000). For example, IL-10 is a pregnancy-compatible cytokine that plays a vital role in maintaining immune tolerance (Cheng and Sharma, 2015). Significant shifts in the T_H1/T_H2 balance have been associated with various immune-mediated pregnancy complications. However, the emerging role of NK cells and emerging cytokines such as IL-12, IL-15, IL-18, IL-19, and IL-20 question the T_H1/T_H2 paradigm as oversimplified (Chaouat et al., 2004; Menon et al., 2006). For example, a subset of CD4 T cells that produce IL-17A and IL-17F, called T_H17 cells, may be involved in rejection of the fetus (Wang et al., 2010).

Role of Regulatory T Cells in Pregnancy

The discovery of a distinct lineage of T lymphocytes with dominant immunosuppressive properties (Brunkow et al., 2001) enabled major breakthroughs in understanding the mechanisms, whereby adaptive allogeneic responses against paternal antigens are actively suppressed during pregnancy. This type of Treg cell is characterized by expression of a lineage-specific transcription factor, FoxP3 (Fig. 36.2). On activation via their antigen-specific TCR, Treg cells are capable of suppressing immune effector cells, including DCs and effector T cells through a variety of mechanisms (Tang and Bluestone, 2008), thereby preventing a fatal form of autoimmune disease throughout life (Kim et al., 2007). Treg cells and T_H17 cells are two distinct lymphocyte subsets with opposing actions and share a complex relationship. Treg cells have a role in suppressing autoimmune responses and preventing the rejection of the fetus, and a decrease in Treg cell number is associated with miscarriage. In contrast, T_H17 cells promote inflammation, transplant rejection, and



• **Fig. 36.2** Forkhead Box P3–Positive Regulatory T-Cell Subpopulations and Their Development. Most human regulatory T (Treg) cells in peripheral blood originate from the thymus and are typically called *natural Treg* (nTreg) or *thymus-derived Treg cells* (1). Treg cells can originate from naive T cells in the periphery and are induced to express the identifying transcription factor forkhead box P3 (FOXP3) under the influence of transforming growth factor beta (TGFβ), at least in vitro (2). The Treg cells are often called *induced Treg* (iTreg) cells. Most Treg cells in the intestinal mucosa are thought to be iTreg cells originating from naive T cells in the mucosal immune system under the influence of specific antiinflammatory dendritic cells (CD103⁺ dendritic cells), TGFβ, and retinoic acid (3). The iTreg cells play an important role in pregnancy maintenance. DC, Dendritic cell.

autoimmunity, and increases in T_H17 cell numbers and decreases in Treg cell numbers are associated with recurrent miscarriage (Wang et al., 2010a, 2010b; La Rocca et al., 2014). The numbers of Treg cells specific to the fetus increase in the mother during gestation, and cells maintaining tolerance to fetal antigen can rapidly expand during subsequent pregnancy (Rowe et al., 2012). Paternal antigens may induce expansion of Treg cells, likely contributing to maternal tolerance to the allogeneic fetus (Zenclussen et al., 2005). Maternal and fetal Treg cells are essential in promoting fetal survival by avoiding the recognition of paternal semiallogeneic tissues by the maternal immune system (Mold et al., 2008; La Rocca et al., 2014).

The interaction between the costimulatory molecule programmed death 1 (PD1) and its ligand (PDL1) plays important roles in maintaining tolerance at the fetomaternal interface. PDL1 is expressed on the trophoblasts of the placenta, and PD1 is expressed on the maternal effector T cells and Treg cells. PDL1 expression maintains Treg cell/effector T-cell ratios and suppresses increases in the number of T_H17 cells (D'Addio et al., 2011). Blockade of PDL1 signaling in animal models results in fetal rejection (Guleria et al., 2005). The role and ontogeny of fetal Treg cells is discussed more fully in the section entitled Adaptive Immunity.

Role of the Microbiome

The paradigm of a sterile uterus postulates that the fetus develops free of bacteria and antigenic agents (Mackie et al., 1999). However, bacteria can be cultured from amniotic fluid and fetal tissues in pregnancies complicated by preterm labor even without rupture of membranes (Goldenberg et al., 2000). Bacterial DNA can be detected in meconium and umbilical cord blood (UCB) of healthy neonates as well as amniotic fluid obtained by cesarean section and placentas following undisturbed, healthy pregnancies without histologic evidence for chorioamnionitis. The exact mechanisms by which bacteria pass from the mother to the fetus are being investigated. Increased bacterial translocation from the intestine to other organs can be found during pregnancy, and bacterial DNA signatures can be detected in the peripheral blood of pregnant women (Perez et al., 2007). Placental bacteria resemble most closely the human oral microbiome (Aagaard et al., 2014), suggesting hematogenous bacterial transfer. While not yet widely accepted in humans, maternal microbial transmission to the fetus is a universal phenomenon in animals, likely constituting an essential evolutionary act of symbiosis (Funkhouser and Bordenstein, 2013).

Globally, these studies imply a possibly critical role of maternal bacteria to inform normal immune development of the developing fetus. While the importance of fetal programming has been well described for cardiovascular and metabolic diseases, it is now also discussed in the context of environmentally influenced immune-mediated diseases. A possible reason for the initial exposure of bacterial molecular patterns to the fetus in utero is to prime the immune system and/or the epithelium to respond appropriately to pathogens and commensals after birth (Abrahamsson et al., 2015). For example, maternal exposure to farm animals during pregnancy was associated with greater Toll-like receptor (TLR) gene expression and lower risk of atopic sensitization in children (Ege et al., 2006). Similar protective effects against atopic sensitization were observed after dietary interventions during pregnancy, such as maternal supplementation with fish oil (Dunstan et al., 2003) or probiotics (Elazab et al., 2013). In contrast, antibiotic use in pregnancy was associated with asthma during the fifth year of life (Benn et al., 2002).

The exact mechanism of prenatal immune priming is unknown, but in an experimental asthma model, microbial exposure to pregnant mice resulted in epigenetic changes in promoter regions of cytokines associated with an allergic phenotype-increased expression of IFN- γ and reduced expression of IL-4, IL-5, and IL-13 (Furuya et al., 1990). This concept has been confirmed in human studies where exposure to farms during pregnancy has been associated with increased DNA demethylation of the FoxP3 locus and increased number and function of Treg cells in UCB cells (Schaub et al., 2009).

While exposure to commensal bacteria during pregnancy seems beneficial for a healthy developing immune system, inflammatory states during pregnancy can have long-lasting adverse effects on the offspring. For example, in murine models, maternal immune activation can trigger autism-like behavior and neuropathology in the offspring (Hsiao et al., 2013). Despite the importance of immune regulation for a healthy pregnancy and recent scientific advances, much remains to be learned regarding the molecular mechanisms used by the fetal immune system to promote tolerance or suppression.

Effect of Pregnancy Complications on the Developing Fetal Immune System

To enable initiation and maintenance of pregnancy, the intrauterine environment significantly shapes the developing immune system as is evident from the antiinflammatory cytokine profile and protection from atopic sensitization in offspring after maternal exposure to farming activities and farm dairy products during pregnancy (Ege et al., 2008; Pfefferle et al., 2010). This effect is at least in part mediated through an increase in the number of fetal Treg cells (Schaub et al., 2009).

In contrast, fetal exposure to inflammation during critical developmental windows can influence immune programming to augment inflammatory neonatal responses. Histologic chorioamnionitis (HCA) is a common complication of pregnancy, typically caused by intrauterine bacterial infection and defined by inflammation of the fetal membranes. Fetal exposure to HCA induces immune activation, resulting in fetal inflammatory response syndrome (FIRS), and shapes the neonatal transcriptomic immune response (Weitkamp et al., 2016). The clinical characteristics of FIRS consist of systemic inflammation and elevation of fetal plasma IL-6 and other proinflammatory cytokine levels (Gomez et al., 1998). Long-term sequelae of the sustained systemic inflammation precipitated by fetal exposure to HCA include blindness (Chen et al., 2011), cerebral palsy (Wu et al., 2000), impaired cardiac function (Romero et al., 2004), lung disease (Kramer et al., 2009), and disruption of normal fetal immune development (Leviton et al., 2011; O'Shea et al., 2012; Savasan et al., 2012; Bastek et al., 2014; Kallapur et al., 2014). In humans, placental infection, chorioamnionitis, or villitis together with a fetal inflammatory response appear to increase the risk of surgical necrotizing enterocolitis (NEC) (Moore et al., 2013).

Studies of fetal sheep and human UCB have demonstrated activation of the adaptive immune system following exposure to HCA. In a model of chorioamnionitis and FIRS caused by administration of intra-amniotic lipopolysaccharide (LPS) in rhesus monkeys at approximately 80% of gestation, fetal Treg cell generation in the thymus was inhibited, while the concentration of proinflammatory cells in the spleen increased (Rueda et al., 2016). The immunologic changes associated with endotoxin-induced systemic and organ-specific immune priming in the fetus can be

mimicked by administration of IL-1 α or IL-1 β , suggesting a possibly important role of IL-1 receptor signaling in FIRS. In a similar model using intra-amniotic injection of LPS 7 or 14 days before preterm delivery in fetal sheep, involution and activation of the fetal thymus with structural organ changes was observed (Kuypers et al., 2012). Furthermore, UCB derived from human neonates with clinical evidence of perinatal infection exhibited a higher proportion of T_H1 cells than UCB from uninfected neonates (Matsuoka et al., 2001).

Overall, epidemiologic and experimental data point to the central role of maternal immune activation and/or FIRS in the pathogenesis of many immune-mediated complications of prematurity such as chronic lung disease, brain damage, retinopathy of prematurity, gut injury, and behavior abnormalities. On the other hand, prenatal immune activation may improve vaccine responses and render the newborn more resistant to infectious challenges later in life (Strunk et al., 2012).

Developmental Fetal–Neonatal Immunology

Newborn and young infants, especially those born preterm, are at increased risk of developing a range of bacterial and viral opportunistic infections. This age-dependent susceptibility is in part based on immune ontogeny (Dowling and Levy, 2014). In the past few decades research has focused on the molecular, cellular, and functional bases for immunologic differences between newborns and older individuals, which we discuss as they relate to innate and adaptive immunity.

Innate Immunity

During fetal/newborn adaptation from the intrauterine environment to the colonization of skin and mucosal surfaces following birth, the innate immune system shields the newborn from infection while orchestrating the acquisition of protective adaptive immune responses (Levy, 2007). These innate mechanisms evolve across gestation and postnatal age (Fig. 36.3) and include protective barriers such as the vernix caseosa, which contains antimicrobial proteins and peptides (APPs) and microbicidal fatty acids (Tollin et al., 2005), developmentally controlled functional regulation of TLR signaling (Kollmann et al., 2012), expression of acute-phase reactants (Levy, 2007; Fig. 36.4) and complement proteins, and alterations in neutrophil and monocyte function (Forster-Waldl et al., 2005; Levy et al., 2006). Importantly, functional maturation of innate immunity enables colonization with commensal organisms while limiting potentially dangerous inflammatory responses (Kollmann et al., 2017). Herein we discuss key features of innate immunity in early life beginning with soluble-based defense systems that progress to leukocyte-based defense systems.

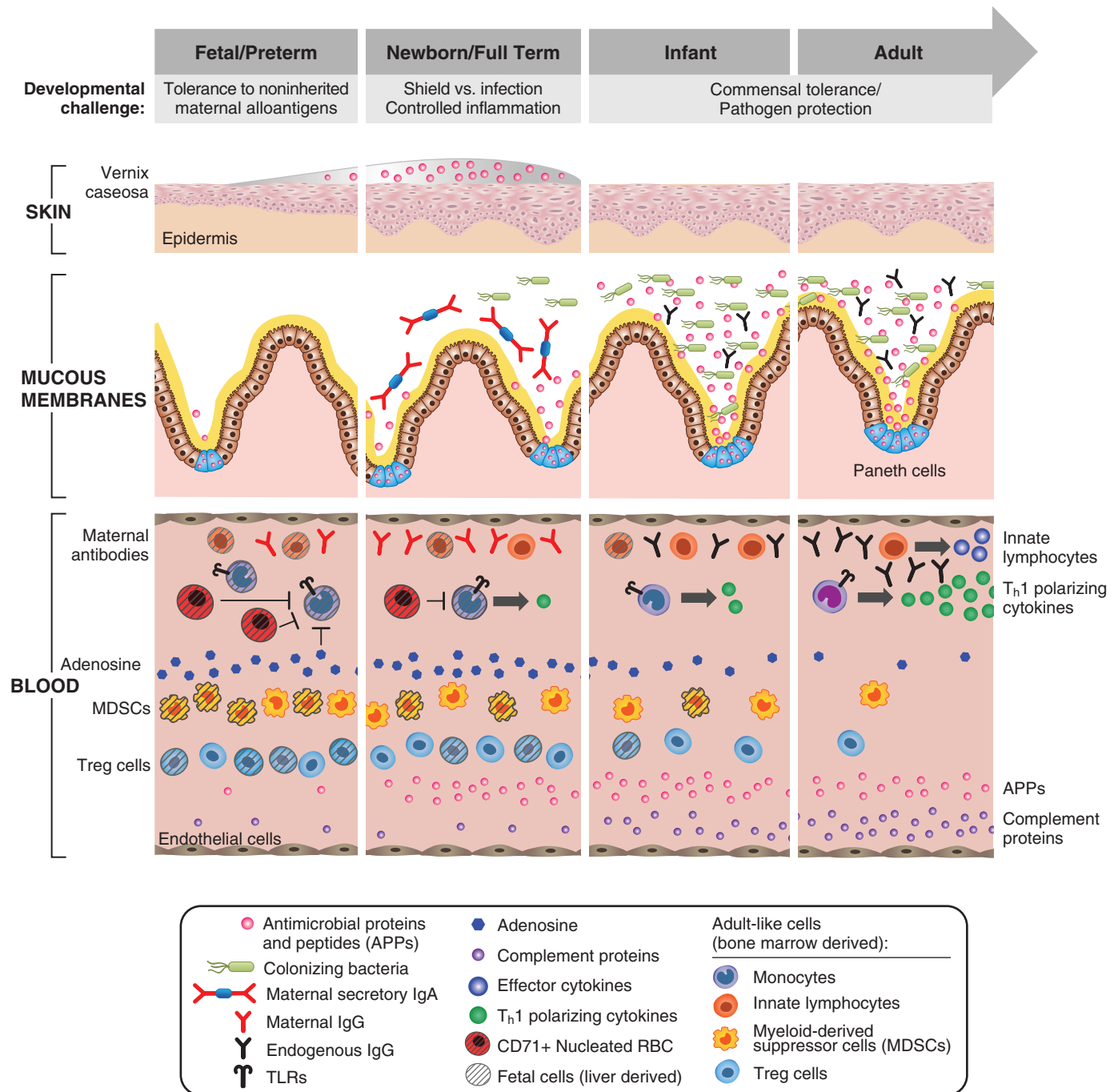
Complement

Central to the innate immune response is the complement system that consists of more than 40 plasma, cell surface, and regulatory proteins that interact to regulate multiple physiologic functions, including resistance to pyogenic infections, interaction between innate and adaptive immunity, and elimination of immune complexes, products of inflammatory injury, and apoptotic self cells (Zipfel and Skerka, 2009). Components of the complement system recognize and lyse bacteria, opsonize microorganisms, release anaphylatoxins, solubilize immune complexes, and induce B-cell proliferation and differentiation.

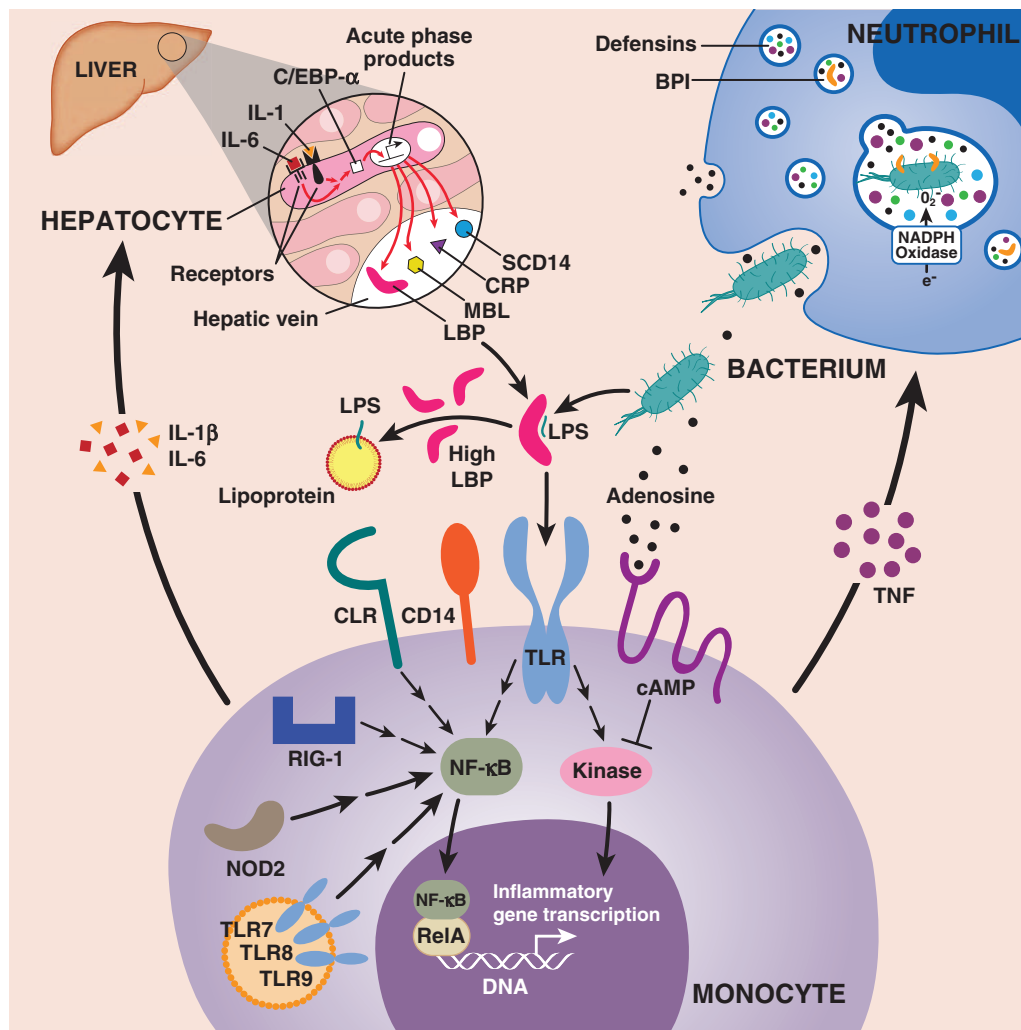
Activation of the complement cascade occurs via three pathways—classical, lectin, or alternative (Thiel, 2007; Zipfel and Skerka, 2009). Several characteristics of the complement cascade are important for fetal–neonatal immunity. First, the complement system features both antibody-dependent specificity via the classical pathway activation triggered by interaction of antigens with antibodies, and antibody-independent activation of the alternative and lectin pathways initiated by pathogen-associated structures such as endotoxin and polysaccharides. Thus for the fetus or infant who has not received from the mother or has not yet produced antigen-specific immunoglobulin (Ig) G for immunologic recognition, the alternative and lectin pathways may be critical for triggering the effector functions of the complement cascade (Kielgast et al., 2003; Simister, 2003). Second, the enzymatic activation of the complement cascade enables rapid functional amplification: deposition of a single Ig molecule or C3b fragment can generate enzymatic cleavage of thousands of later-acting components and thus multiple complement activities (Carroll, 2004; Zipfel and Skerka, 2009). In addition, the alternative pathway can be amplified via a positive feedback activation mechanism, because C3b, an activation product of the alternative pathway C3 convertase, is a component of this convertase (Janssen et al., 2006). As the fetus and newborn are particularly dependent on antibody-independent pathogen recognition for immunologic responsiveness, the positive amplification loop of the alternative pathway may be particularly critical for rapid generation of complement effector functions in early life in the absence of antibody-based recognition. Third, the continuous activation of the alternative pathway requires rigorous regulation in the fetus to avoid tissue damage during organ remodeling (Zipfel et al., 2007). Finally, the contributions of the lectin pathway to fetal–neonatal complement activation and fetal well-being are still under investigation.

Studies of fetal and neonatal complement have focused on quantification of serum concentrations of individual components, examining maternal–fetal transport of these proteins, assessing specific effector functions of the classical and alternative pathways, and investigating contributions of complement activation to common neonatal diseases. Detectable concentrations of C3 (1% of adult levels) and C1 inhibitor (20% of adult levels) can be measured as early as 5 to 6 weeks' gestation (Gitlin and Biasucci, 1969). By 26 to 28 weeks' gestation, both C3 and C1 inhibitor concentrations increased to 66% of adult levels. Functionally and immunochemically measured classical and alternative pathway protein concentrations in UCB increase with advancing gestational age, such that impairment in CH50 is particularly evident in the preterm (Grumach et al., 2014), and at full-term gestation the concentrations are only approximately 50%–75% of adult concentrations (Wolach et al., 1997; Sonntag et al., 1998). Although neonatal UCB lectin pathway component concentrations are lower than those in older children and adults, the correlation between mannose-binding lectin (MBL) and gestational age has not been consistently observed (Hilgendorff et al., 2005; Swierko et al., 2009). Of note, on the basis of studies of genetically determined, structurally distinct complement variants in maternal and umbilical cord serum, no transplacental passage from the mother to the fetus of C3, C4, factor B, or C6 has been observed (Colten et al., 1981).

Much remains to be learned regarding regulation of complement effector functions in the fetus and newborn. Activation of the alternative pathway or the lectin pathway enables opsonization of invading microorganisms without specific Ig recognition. Accordingly, for preterm infants or those without organism-specific maternal IgG, alternative or lectin pathway activation provides a



• **Fig. 36.3** Ontogeny of Skin, Soluble, and Cellular Innate Defense Systems. Host-protective barrier functions include physical, chemical, and functional components of the skin and mucous membrane epithelia of the fetus, neonate (birth to 28 days of age), and infant (1 month to 1 year of age). Skin: while physical and chemical barriers are impaired in early in life, especially in the preterm newborn, the vernix caseosa and skin epithelia of full-term newborns robustly express antimicrobial proteins and peptides (APPs). Mucous membranes: in parallel with and induced by an increasingly complex microbiota, the newborn intestinal mucosal epithelium rapidly changes structurally, with an increase in the population of crypts and crypt-based Paneth cells, as well as functionally with increasing APP expression. Blood: the composition of neonatal blood is distinct, with relatively low concentrations of complement components and APPs and high concentrations of the immunosuppressive purine metabolite adenosine. Plasma also contains maternal antibodies that are transferred beginning midgestation and supplemented by postnatal factors derived from breast milk. Innate immunity is detectable from the end of the first month of gestation, with changes driven largely by the increasing exposure to environmental microbes. Neonatal antigen-presenting cells such as blood monocytes express pattern recognition receptors (e.g., Toll-like receptors, *TLRs*) with distinct functional responses, including limited T_H1-polarizing cytokine production, to most stimuli. Adaptive immunity develops from 4 weeks of gestation onward, with changes driven by an evolving chimerism reflecting fetal (liver-derived, *shaded cells*) regulatory T (*Treg*)-cell-rich lymphocytes, and more adultlike (bone marrow derived, *unshaded cells*) lymphocytes with distinct epigenetically encoded functional programs. *Ig*, Immunoglobulin; *RBC*, red blood cell. (Modified from Kollmann TR, Kampmann B, Mazmanian SK, Marchant A, Levy O. Protecting the newborn and young infant from infectious diseases: lessons from immune ontogeny. *Immunity*. 2007;46:350–363.)



• **Fig. 36.4** Innate Detection, Signaling, and Effector Functions of Blood Phagocytic Leukocytes and Hepatocytes. Innate immune signals are detected via signaling loops. Monocytes express pattern recognition receptors, including C-type lectin receptors (CLRs), CD14, and Toll-like receptors (TLRs). TLR-mediated monocyte activation engages signaling pathways resulting in kinase and nuclear factor κ B (NF- κ B) pathway activation, culminating in inflammatory gene transcription, including generation of cytokines that amplify an antiinfective response. For example, interleukin (IL)-1 β and IL-6 engage cognate cytokine receptors on hepatocytes, inducing acute-phase response, including production and secretion of lipopolysaccharide (LPS)-binding protein (LBP), mannose-binding lectin (MBL), the pentraxin C-reactive protein (CRP), and soluble CD14 (SCD14). These molecules recognize and modulate inflammatory activity of microbial products. For example, LBP at low concentrations delivers LPS to TLR4, thereby enhancing signaling but at higher LBP concentrations detoxifies LPS by delivering it to plasma lipoproteins. Monocyte production of tumor necrosis factor (TNF) activates neutrophils that deploy antimicrobial systems that are oxygen dependent, such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, as well as those that are oxygen independent, such as membrane-active antimicrobial proteins and peptides, including defensins and bactericidal/permeability-increasing protein (BPI), an LBP homologue that binds to and neutralizes endotoxin. Soluble mediators, such as the antiinflammatory purine metabolite adenosine, can limit proinflammatory responses. cAMP, Cyclic adenosine monophosphate; C/EBP- α , CCAAT/enhancer binding protein; NOD2, nucleotide-binding oligomerization domain-containing protein 2; RelA, NF- κ B subfamily protein; RIG-1, retinoic acid-inducible gene 1.

critical mechanism for engaging complement effector functions (Super et al., 1989; Maruvada et al., 2008; Swierzko et al., 2009). The functional contribution of the classical pathway to effector functions has been assessed through the use of blood-mediated opsonophagocytosis by polymorphonuclear leukocytes of group B streptococci (GBS) type Ia (Edwards et al., 1983). This GBS serotype may be opsonized by classical pathway components in

the absence of specific antibodies and thus enables characterization of classical pathway function. In 8 of 20 neonatal serum samples examined, decreased bactericidal activity was detected and correlated with significantly lower functional activity of C1q and C4. These studies did not determine whether this decrease was mediated by an inhibitor of function or by an intrinsic change in functional activity of these components in neonatal sera. Studies of MBL

concentrations and pathway activity suggest a contribution of the lectin pathway to neonatal susceptibility to infection (Super et al., 1989; Sumiya et al., 1991; Thiel et al., 1995; Kilpatrick et al., 1996; Kielgast et al., 2003; Swierczko et al., 2009). Complement regulatory proteins (e.g., C4b-binding protein and factor H) also contribute to neonatal susceptibility as suggested by the failure of neonatal serum to reduce invasion by GBS and *Escherichia coli* into human brain microvascular endothelial cells (Maruvada et al., 2008). In vitro experiments in which killing of *E. coli* by neonatal serum samples was limited by C9, but not by other classical pathway components, suggest that this terminal complement component is apparently important for cytolysis of this pathogen (Lassiter et al., 1992, 1994). Although relatively lower concentrations of complement components likely contribute to poor control of bacterial replication, these complement concentrations are nevertheless sufficient, via C3- and factor B-dependent activity in the alternative pathway, to enhance GBS-induced production of TNF- α by monocytes in human newborn UCB tested in vitro (Levy et al., 2003).

In addition to relatively low serum concentrations of classical, alternative, and lectin pathway complement proteins, additional complement functions important for fetal and neonatal well-being can contribute to reduced capacity to activate the classical and alternative pathways. For example, fetal and neonatal serum demonstrates reduced concentration of C4b-binding protein, a critical regulator of classical pathway C3 convertase activity (Malm et al., 1988; Melissari et al., 1988; Moalic et al., 1988; Fernandez et al., 1989). Lower C4b-binding protein concentration increases the functional anticoagulant activity of protein S, with which it complexes and thereby contributes to decreased coagulation function of the fetus and newborn. Consideration of complement components that also express nonimmunologic functions will likely be important in characterizing developmental regulation of complement component production.

Complement activation contributes to tissue injury in several common neonatal diseases, including neonatal hypoxic-ischemic encephalopathy, NEC, meconium aspiration syndrome, and intrauterine growth restriction and fetal loss (Girardi and Salmon, 2003; Lassiter, 2004; Schultz et al., 2005; Girardi et al., 2006; Mollnes et al., 2008; Schlupbach et al., 2008). Unregulated complement activation may occur in selected infants undergoing extracorporeal membrane oxygenation therapy, raising concern for inflammatory injury on that basis (Johnson, 1994; Kozik and Tweddell, 2006). C5a is present in the cerebrospinal fluid of human newborns, at especially high concentrations in those born preterm (Pataky et al., 2016), raising the possibility that complement activation in the neonatal brain may contribute to preterm brain injury.

Overall, study of the complement system in early life, including characterization of the developmental and genetic regulation of this important group of plasma and cell surface proteins, promises to shed further light on immune ontogeny in relation to health and disease.

Antimicrobial Proteins and Peptides

The human body expresses natural antibiotics, including APPs that act alone and in combination with endogenous (e.g., complement) and exogenous (e.g., conventional antibiotic) systems to prevent infection and/or eliminate invading microorganisms (Levy, 2004). APPs are expressed by a range of cells, including epithelial cells and leukocytes, especially neutrophils (see Fig. 36.4), and are found associated both with cells and in plasma. Plasma levels of

APPs vary with gestational age such that preterm plasma is relatively deficient in multiple APPs, likely contributing to reduced microbicidal capacity (see Fig. 36.3; Battersby et al., 2016). Examples of APPs include (1) lactoferrin, an 80-kDa protein with iron-binding and direct membrane perturbing properties found in tear fluid, saliva, and neutrophil secondary granules, (2) the 5-kDa bactericidal/permeability-increasing protein, expressed on certain mucosal epithelia as well as neutrophil primary granules, with high affinity for LPS that enables it to neutralize the inflammatory activity of endotoxin and targets its microbicidal activity toward gram-negative bacteria, (3) 14-kDa phospholipase A₂, an acute-phase reactant expressed in liver with ability to enzymatically kill a range of gram-positive pathogens, and (4) 4-kDa disulfide-rich defensin peptides of neutrophil primary (azurophilic) granules with broad microbicidal activity (Levy, 2004). Ongoing efforts are aimed at developing congeners of APPs as novel antiinfective agents for individuals who are relatively APP deficient, including preterm infants and those undergoing chemoradiotherapy (Palmer et al., 2011). For example, oral administration of lactoferrin to human preterm newborns has shown promise in reducing the incidence of sepsis and NEC (Pammi and Abrams, 2015).

Innate Lymphoid Cells, Including Natural Killer Cells

Innate lymphoid cells (ILCs) are derived from a common lymphoid progenitor, are defined by the absence of antigen-specific B-cell receptors (BCRs) or TCRs, and do not express myeloid or DC markers (Klose and Artis, 2016). ILCs are divided into subgroups based in part on the cytokine profile they produce: (1) group 1 ILCs produce IFN- γ and are functionally dependent on the transcription factor T-bet; (2) group 2 ILCs produce type 2 cytokines (e.g., IL-4, IL-5, IL-9, and IL-13) in response to helminth infection and are dependent on ROR α and GATA3; and (3) group 3 ILCs produce IL-17A and/or IL-22 and are dependent on the transcription factor ROR γ t.

NK cells are the most studied of the ILCs. These group 1 ILCs constitute approximately 10%–15% of all peripheral blood lymphocytes. They are present in the spleen, lungs, and liver and are also rarely found in lymph nodes and thoracic duct lymph (Cerwenka and Lanier, 2001). NK cells represent up to 70% of all lymphocytes in the maternal decidual tissue (King et al., 1996b). They demonstrate distinct morphology, function, and surface molecule expression, including expression of CD16 (Fc gamma receptor [F γ R]III) and CD56 (nerve cell adhesion molecule 1). Mature NK cells appear larger and more granular than T or B cells (Cooper et al., 2001) and express both activating and inhibitory receptors that are used to selectively identify and kill virally infected cells and tumors (Biassoni et al., 2001). The presence of MHC class I molecules on potential target cells induces signals that suppress NK-cell function. MHC class I-deficient target cells activate NK-cell function, triggering release of lysosomal granules containing serine proteases, perforin, and transforming growth factor beta (TGF- β), thereby disrupting the target cell membrane and inducing an inflammatory response. Fetuses and neonates demonstrate reduced NK-cell activity compared with adults (Georgeson et al., 2001; Kadowaki et al., 2001).

NK cells are derived from a common hematopoietic progenitor that retains T-cell and B-cell developmental potential (Boos et al., 2008). NK cells first make their appearance in fetal liver as early as 6 weeks' gestation. Committed CD34⁺CD56⁺ NK cell progenitors have been identified in the fetal thymus, bone marrow, and liver. In the human neonate, the NK-cell population is immature: only half of all NK cells express CD56, and the NK-cell cytolytic activity

is lower (Dominguez et al., 1998). This functional reduction in NK-cell activity may contribute to the severity of neonatal herpes simplex virus (HSV) infections. Profound defects in NK-cell activity result in familial hemophagocytic lymphohistiocytosis (HLH), a disease characterized by fever, hepatosplenomegaly, cytopenia, hyperferritinemia, and hemophagocytosis. Familial HLH arises from mutations in genes that encode proteins involved in the granule-exocytosis pathway and can be fatal without bone marrow transplant (Orange, 2006; Jordan and Filipovich, 2008).

NK-cell receptors are fundamentally different from TCRs and BCRs. NK-cell receptor gene expression does not require gene segment rearrangement, and the receptors are not clonally distributed. Instead, NK cells use an array of stimulatory and inhibitory receptors to regulate their cytolytic functions (Lanier, 2008). A cluster of 10 or more genes encoding killer-cell Ig-like receptors (KIRs) is located on human chromosome band 19q13.4 (Biassoni et al., 2001). Each of these type I glycoproteins recognizes a different allelic group of HLA-A-, HLA-B-, HLA-C-, or HLA-G-encoded proteins, and each KIR is expressed by only a subset of NK cells. Another family of Ig-like receptor genes termed *ILT* is present near the KIR locus at 19q13.3. These receptors are not as restricted as the KIRs and bind multiple HLA class I molecules. A third inhibitory receptor gene locus is on chromosome band 12p12-p13, encoding a C-type lectin inhibitory heterodimeric receptor called *CD94/NGK2* that binds HLA-E. Those KIRs, ILT receptors, and *CD94/NGK2* molecules with long cytoplasmic tails and two immunoreceptor tyrosine-based inhibitory motifs (ITIMs) function as inhibitory receptors. On phosphorylation, the two ITIMs recruit and activate the Src homology domain 2 (SH2)-containing phosphatases, which turn off the kinase-driven activation cascade (Ravetch and Lanier, 2000). The KIR family member KIR2DL4 is distinct from other KIRs in structure and distribution. KIR2DL4 binds HLA-G and has a single ITIM in the cytoplasmic tail and a lysine in the transmembrane region, enabling association with adaptor proteins. This inhibitory receptor was found on all decidual NK cells in the placenta at term but not on circulating maternal NK cells, suggesting that expression of KIR2DL4 is induced during pregnancy (Rajagopalan and Long, 1999).

Other KIRs or members of the C-type lectin receptor superfamily are activating receptors (Moretta et al., 2001). These receptors lack the long cytoplasmic tail of the inhibitory receptors and therefore do not contain ITIMs. Instead, they have a charged amino acid in the transmembrane region that enables receptor association with the adaptor molecule DAP12 (Lanier et al., 1998). This adaptor contains an immunoreceptor tyrosine-based activation motif (ITAM) that allows these receptors to activate NK cells. The physiologic role of these HLA class I-specific activating receptors remains unknown. NK cell-activating receptors also include *natural cytotoxicity receptors* (NKp46, NKp30, NKp44), proteins that are Ig superfamily members with little similarity to one another or to other NK-cell receptors (Moretta et al., 2001). These receptors are highly specific for NK cells and apparently interact with non-HLA molecules.

CD244 (2B4) is a member of the signaling lymphocyte activation molecule (SLAM) family of receptors expressed on all human NK cells (Ma et al., 2007). On interaction with the ligand CD48 on target cells, NK-cell signaling proceeds via interactions between the immunoreceptor tyrosine-based switch motif (ITSM) (switch motif) in the cytoplasmic tail of CD244 and one of two SH2 domain-containing adaptor proteins, SLAM-associated protein (SAP) and Ewing sarcoma-associated transcript 2 (EAT-2). SAP interactions trigger activation, as

evidenced in humans with X-linked lymphoproliferative disease, caused by loss-of-function mutations in the SAP linker. In the absence of SAP, interactions with EAT-2 may be inhibitory (Lanier, 2008).

Recent studies have explored the potential contribution of ILCs beyond NK cells. A role for lung group 2 ILCs in mediating respiratory syncytial virus (RSV)-induced IL-33-driven T_H2 -biased immunopathology has been demonstrated in neonatal mice (Saravia et al., 2015). IL-23-responsive group 3 ILCs played a role in the pathogenesis of neonatal intestinal inflammation in a murine model (Chen et al., 2015). Much remains to be learned regarding the ontogeny of ILC function with respect to both the quantity and the quality of these cells in the very young.

Polymorphonuclear Neutrophils

Neonatal polymorphonuclear neutrophils (PMNs) are present at early stages of gestation, but their functional capacities are different from those of adult PMNs. Progenitor cells that are committed to maturation along granulocyte or macrophage cell lineages (granulocyte-macrophage colony-forming units) are detectable in the human fetal liver between 6 and 12 weeks' gestation in similar proportions as in adult bone marrow (Christensen, 1989). Human fetal blood has detectable granulocyte-macrophage colony-forming units from 12 weeks' gestation to term (Christensen, 1989). Although these progenitor cells are detectable in the fetus and newborn, developmental differences between adult and mature neonatal PMNs have been demonstrated—in signal transduction, cell surface protein expression, cytoskeletal rigidity, rolling adhesion, microfilament contraction, transmigration oxygen metabolism, intracellular antioxidant mechanisms, and neutrophil extracellular trap formation (Hill, 1987; Carr, 2000; Henneke and Berner, 2006; Levy, 2007; Yost et al., 2009). The magnitude of PMN functional differences correlates with the maturity of the infant and begins to decrease within the first few weeks after birth (Carr, 2000).

In addition to intrinsic age-dependent differences in PMN function, age-dependent cell extrinsic soluble factors may developmentally regulate induction of specific functions and maturation of these cells (Christensen, 1989; Pettengill et al., 2014). For example, low concentrations of the chemoattractant complement component C5a in neonatal sera might impair establishment of chemoattractant gradients at sites of inflammation. In addition, elevated concentrations in human neonatal blood plasma of adenosine (Pettengill et al., 2013), an endogenous purine metabolite that acts via seven-transmembrane adenosine receptors to inhibit inflammatory leukocyte responses, could contribute to inhibition of newborn neutrophil function.

Systemic bacterial infection in newborns is frequently accompanied by profound neutropenia, prompting investigation of neutrophil kinetics in infected infants (Santos et al., 1980; Christensen et al., 1982). These studies have suggested diverse, developmentally specific regulatory mechanisms required for mobilization of the neutrophil response to infection. The absence of detectable neutrophil precursors in bone marrow aspirates of infected infants and systemic neutropenia motivated studies of neutrophil replacement therapy in neutropenic, infected infants (Christensen et al., 1980). Although this approach has been successful in some cases, the results have not been uniformly beneficial, and a Cochrane review suggests a need for adequately powered multicenter trials of granulocyte transfusions in neutropenic septic neonates (Pammi and Brocklehurst, 2011). Metaanalysis suggesting that granulocyte colony-stimulating factor or granulocyte-macrophage colony-stimulating factor may reduce mortality in

newborns when systemic infection is accompanied by severe neutropenia requires confirmation in adequately powered trials (Carr et al., 2003). Heterogeneity in the impact of neutrophil modulation may reflect the importance of individualizing immunologic interventions for the genetic background, developmental stage, and pathogenic microorganism being treated.

Monocytes, Macrophages, and Dendritic Cells

Cells committed to phagocyte maturation, including granulocyte or monocyte-macrophages, are detectable in the human fetal liver by 6 weeks' gestation and in peripheral fetal blood by 15 weeks' gestation. Unlike granulocytes, whose tissue half-life is hours to days, macrophages migrate into tissues and reside for weeks to months. In a tissue-specific fashion, these cells regulate availability of multiple factors, including proteases, antiproteases, prostaglandins, growth factors, reactive oxygen intermediates, and a range of cytokines and chemokines. Importantly, monocytes can migrate from the bloodstream to tissue sites, becoming tissue-based DCs. Monocytes, macrophages, and DCs share the ability to present antigens to T lymphocytes, thereby triggering the classic adaptive immune responses.

Compared with their adult counterparts, newborn monocytes, macrophages, and DCs demonstrate reduced chemotaxis and phagocytosis as well as distinct TLR signaling that is polarized toward T_H2 and antiinflammatory cytokine production (Kollmann et al., 2012). The distinct function of newborn APCs reflects both intrinsic characteristics, including reduced nucleosome remodeling for IL-12 p70 production (Goriely et al., 2004) as well as the modulatory effects of age-specific extrinsic factors such as the antiinflammatory purine metabolite adenosine, the level of which is relatively elevated in human newborn UCB plasma (Pettengill et al., 2013, 2014).

A growing literature documents that stimulation of monocytes results in a change in innate "setpoint" such that responses to subsequent stimuli are altered (Saeed et al., 2014). This phenomenon, reflecting adaptive features of the innate immune system that are mediated by epigenetic changes, has been termed *trained immunity* (Netea and van der Meer, 2017) and may contribute to the heterologous beneficial ("nonspecific") effects of live attenuated vaccines (Goodridge et al., 2016). Much remains to be learned regarding the scope, ontogeny, and mechanisms underlying innate training/innate memory.

Adaptive Immunity

Antigen-specific T and B lymphocytes bearing TCRs and BCRs, respectively, play multiple critical roles in adaptive immunity. T cells responding to a specific antigen secrete cytokines and kill infected target cells and tumor cells by cell-mediated cytotoxicity. These functions of CD4 and CD8 T cells depend on their TCRs specifically recognizing antigenic peptides bound to MHC molecules. Several T-cell populations have a more restricted expression of TCRs that recognize ligands other than peptide/MHC ligands and have an innate-like role early in the immune response; these include NK T (NKT) cells, mucosal-associated invariant T (MAIT) cells, and T cells expressing $\gamma\delta$ TCRs ($\gamma\delta$ T cells). As already discussed in the section entitled Role of Regulatory T Cells in Pregnancy, CD4⁺ Treg cells serve as negative regulators of effector responses. Analogously, antigen-specific B cells can be divided into populations that are involved in the conventional immune response and that depend on CD4 T-cell help for their differentiation into antibody-secreting cells; those that have an innate immunity-like

function and act early in immune responses, such as marginal zone B cells; and those with regulatory-like suppressive function ("Breg cells").

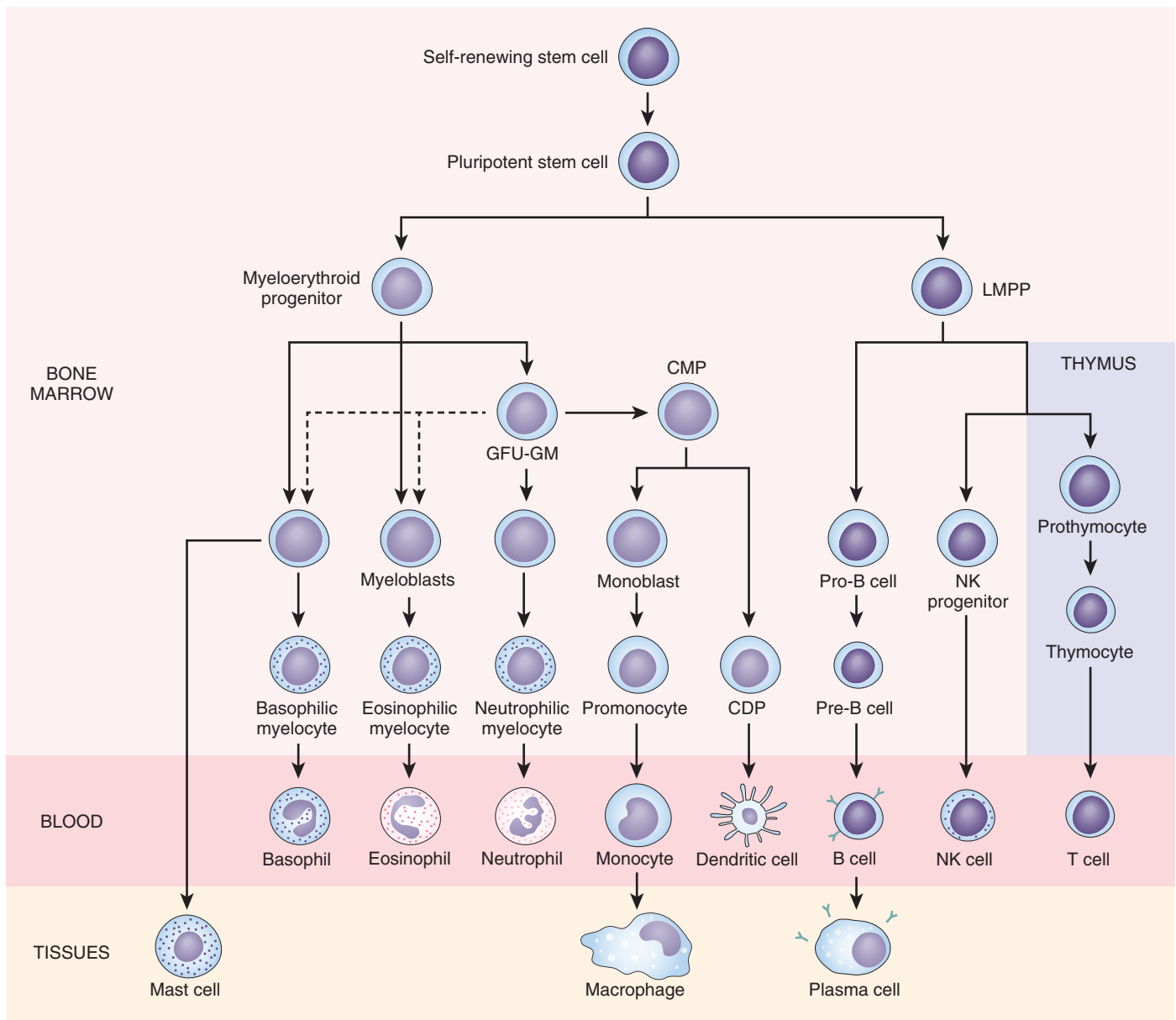
All major lymphocyte lineages, which include T cells, B cells, and ILCs, which lack antigen-specific receptors and include NK cells (Hazenberg and Spits, 2014), develop from CD34⁺CD38^{dim} pluripotent hematopoietic stem cells (HSCs) found in the fetal liver and bone marrow in a perivascular niche (Chen et al., 2016; Fig. 36.5). The process of lymphocyte differentiation and hematopoiesis in general has been traditionally viewed as a linear progressive narrowing of differentiation potential based on the sequential expression of specific transcriptional regulators. However, recent work indicates that the pathways of development of the human myeloid, erythroid, and megakaryocyte lineages may undergo major shifts during ontogeny. For example, in fetal liver, the HSCs and their CD34⁺CD38⁺ progenitor cell derivatives have a similar ratio of cells with multipotent versus unilineage potential, whereas in bone marrow, which is the definitive site of hematopoiesis starting in the second trimester of gestation, CD34⁺CD38⁺ progenitor cells predominantly have unilineage potential (Notta et al., 2016). Another example is that the HSCs of UCB have a greater potential to differentiate into the T lineage than HSCs of adult bone marrow (De Smedt et al., 2011).

T Lymphocytes

Thymocyte Development

Most T cells develop in the thymus, which includes cell types of nonhematopoietic origin, such as epithelial cells, as well as multiple cell types of hematopoietic origin, including the developing immature T cells or thymocytes, DCs, mononuclear phagocytes, and small numbers of B cells. The thymic epithelial cells are derived from the third branchial cleft and the third or fourth branchial pouch, a process that is perturbed in DiGeorge syndrome, resulting in thymic epithelial hypoplasia. Thymic lobes can be divided into four regions, which, going from outward to inward, are the subcapsular region, cortex, corticomedullary junction, and medulla. Prothymocytes, which are bone marrow-derived CD34⁺CD38⁺CD62L⁺ lymphoid cells, have the capacity to commit to the T-cell or other lymphocyte lineages depending on their receipt of instructive signals (Spits, 2002). Circulating prothymocytes enter the thymus via vessels at the cortical-medullary junction. The prothymocyte becomes committed to the T-cell lineage by the engagement of its surface notch 1 receptor by ligands displayed on the thymic epithelium, such as delta-like ligand 4. This engagement leads to the surface expression of CD1 and its differentiation into a pro-T cell that migrates to the subcapsular region just below the outer capsule.

The subcapsular pro-T cell expresses all of the internal proteins required for V(D)J recombination, including the recombinase activating gene (RAG) 1 and RAG2 endonucleases that make double-stranded breaks in DNA; the proteins involved in nonhomologous end joining repair (e.g., Artemis, XLF [Cernunnos], and DNA ligase IV); and those that are essential for generating junctional diversity at complementarity determining region (CDR) 3 (e.g., terminal deoxynucleotidyl transferase [TdT]). CDR3, which is the most variable in amino acid sequence, is located at the center of the antigen-specific binding site of both TCRs and BCRs. The pro-T cell lacks most cell surface proteins characteristic of mature peripheral T cells, including CD3, CD4, and CD8, and is therefore also referred to as a *triple-negative thymocyte* (Fig. 36.6). The pro-T cell is the first stage in which there is VDJ rearrangement of TCR gene loci, with the TCR γ gene rearrangement occurring most frequently

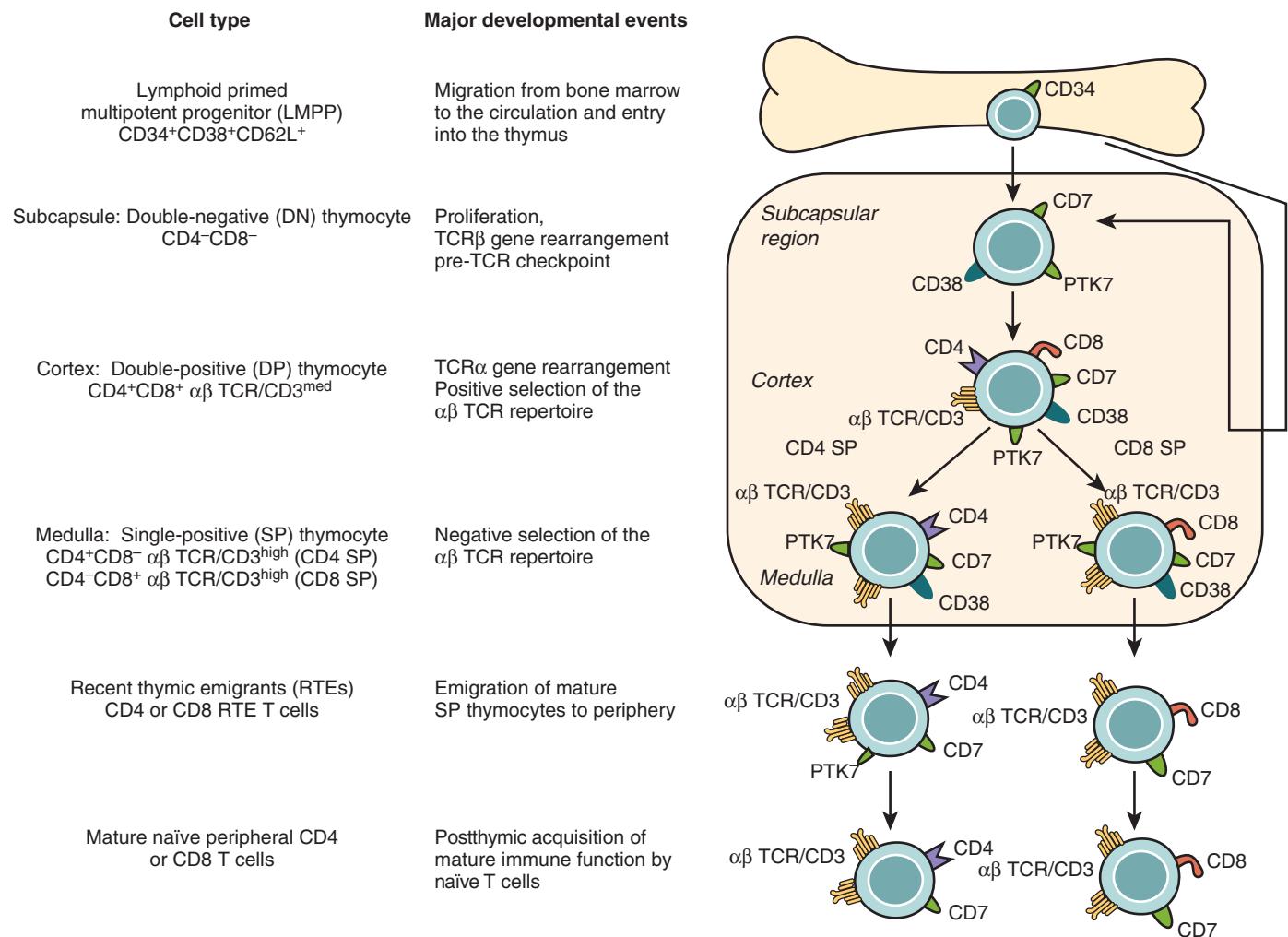


• **Fig. 36.5** Myeloid and Lymphoid Differentiation in the Bone Marrow, Blood, and Tissues. CDP, Committed dendritic cell progenitor; CFU-GM, colony-forming unit granulocyte-macrophage; CMP, common myeloid progenitor; LMPP, lymphoid primed multipotent progenitor; NK, natural killer.

(Sherwood et al., 2011). If this rearrangement is productive (i.e., capable of expressing a full-length TCR γ chain protein) and the thymocyte subsequently undergoes a productive TCR δ gene rearrangement, a $\gamma\delta$ TCR heterodimer is expressed on the cell surface, allowing the thymocyte to differentiate into a mature $\gamma\delta$ T cell that emigrates from the thymus into the periphery. More frequently (>95% of the time) these TCR γ and/or TCR δ gene rearrangements are nonproductive, and the pro-T cell attempts TCR β chain gene rearrangement. If this arrangement is productive, the TCR β chain is expressed on the cell surface in association with an invariant pre-T alpha chain forming the pre-TCR complex, which defines the pre-T-cell stage of development. Like the mature TCR, the pre-TCR is associated with the CD3 complex of proteins, which includes CD3 γ , CD3 δ , CD3 ϵ , and CD3 ζ chains, all of which have cytoplasmic tails containing specific amino acid sequences called ITAMs. These ITAMs serve

as molecular targets for tyrosine phosphorylation and binding by tyrosine kinases, such as Lck and zeta chain-associated protein of 70 kDa (ZAP-70), which generate intracellular signals leading to the induction of target genes (Lopez-Rodriguez et al., 2015). These signals direct the pre-T cell to (1) proliferate, (2) upregulate expression of CD4 and CD8 and become a double-positive (CD4⁺CD8⁺) thymocyte, (3) migrate from the subcapsular area to the thymic cortex, and (4) start rearrangement of the TCR α chain gene locus (see Fig. 36.6).

Rearrangement of the TCR α chain gene by CD4⁺CD8⁺ thymocytes is a two-step process in which there first is an internal deletion of a $\psi\delta$ rec segment that brings the unrearranged V α segments in close proximity with J α segments and the C α constant region (Hazenbergh et al., 2001). The intervening DNA, which is excised as a circular product with fused signal joint sequences,

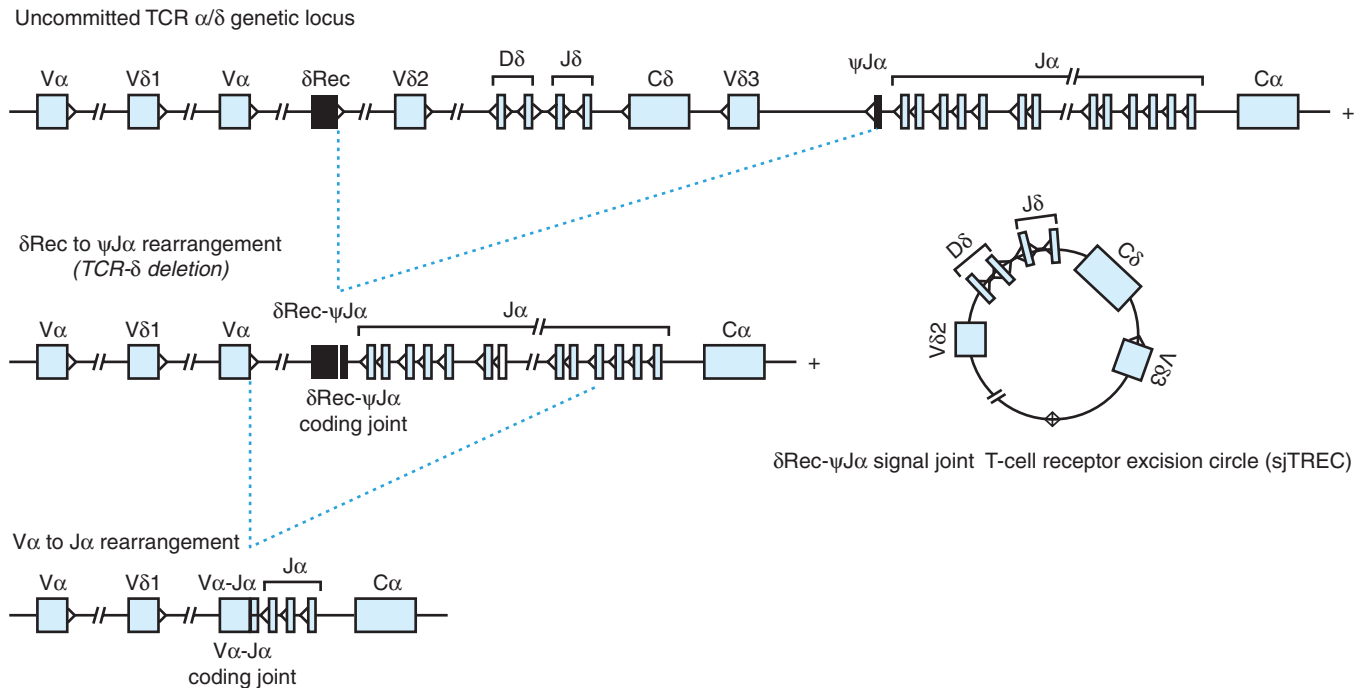


• **Fig. 36.6** Stages of Human $\alpha\beta$ T-Cell Receptor-Positive Thymocyte Development. Prothymocytes from the bone marrow or, before the third trimester, fetal liver, which express CD34, CD38, and CD62 ligand (CD62L), but lack CD4, CD8, or CD3/T-cell receptor (TCR), enter the thymus via vessels at the junction between the thymic cortex and medulla. They differentiate to progressively more mature $\alpha\beta$ TCR⁺ thymocytes, defined by their pattern of expression of the $\alpha\beta$ TCR-CD3, CD4, CD8, and CD38. Protein tyrosine kinase 7 (PTK7) is expressed throughout thymocyte development. The TCR β chain gene and then the TCR α chain gene are rearranged in the outer cortex. Positive selection occurs mainly in the central thymic cortex by interaction with thymic epithelial cells that express major histocompatibility complex (MHC) class I and class II proteins, and negative selection occurs mainly in the medulla by the interaction with thymic dendritic cells that display MHC associated with self-peptides that are derived from medullary thymic epithelial cells. Following these selection processes, medullary thymocytes emigrate into the circulation and colonize the peripheral lymphoid organs as CD4 and CD8 T cells with high levels of $\alpha\beta$ TCR/CD3. These recent thymic emigrants (RTEs) also have a high content of signal joint TCR excision circles (sjTRECs), which are a circular product of TCR gene rearrangement (see Fig. 36.7). In adults, most RTEs have modest levels of surface expression of PTK7 but probably lack CD38. In contrast, in neonates, most peripheral T cells have high levels of surface expression of PTK7 and CD38 and have relatively high amounts of sjTRECs compared with adult peripheral T cells.

referred to as a *signal joint TCR excision circle* (sjTREC), is highly stable within the cell (Fig. 36.7). The sjTREC content of the T-lineage cell subsequently decreases mainly as a result of cell proliferation, which under normal conditions is minimal until the mature T cell undergoes antigen activation-induced clonal proliferation. Thus the measurement of the sjTREC content by peripheral blood T cells is an indirect but useful assessment of the adequacy of production of new T cells by the thymus. The measurement of sjTRECs in neonatal blood spots is routinely used for newborn screening in the United States for identifying infants with impaired

production of T cells by the thymus as occurs in most forms of severe combined immunodeficiency (SCID) (see [Specific Immunologic Deficiencies of the Newborn and Their Diagnosis](#)).

Double-positive thymocytes that have productive TCR α chain gene rearrangements replace their pre-TCR with a TCR consisting of an $\alpha\beta$ TCR heterodimer in association with the CD3 proteins (see Fig. 36.6). The next major checkpoint of thymocyte development is positive selection, in which the $\alpha\beta$ TCR of the CD4⁺CD8⁺ thymocyte is tested for whether it has significant affinity for either the MHC class I alleles (HLA-A, HLA-B, and HLA-C in humans)



• **Fig. 36.7** Sequential rearrangements in the T-cell receptor (TCR) α/δ genetic loci generate signal joint TCR excision circles (sjTRECs) and $V\alpha$ - $J\alpha$ rearrangements. Rearrangement of the δ Rec segment to the $J\alpha$ segment commits the thymocyte to the $\alpha\beta$ TCR lineage as this deletes the C and J segments that are necessary to encode a productive TCR δ chain. The δ Rec- $\psi J\alpha$ rearrangement also generates an sjTREC, which is commonly used for monitoring peripheral T-cell populations for their recent thymic origin. The δ Rec- $\psi J\alpha$ rearrangement and excision of an sjTREC are followed by TCR α ($V\alpha$ - $J\alpha$) rearrangements, which if productive result in expression of an $\alpha\beta$ TCR/CD3 complex on the thymocyte cell surface. Most thymocytes that express $\alpha\beta$ TCRs have molecular evidence of nonproductive rearrangements of portions of the TCR δ gene locus (not shown).

or the MHC class II alleles (HLA-DR, HLA-DP, and HLA-DQ in humans) expressed by thymic cortical epithelial (TCE) cells. All MHC class I and class II molecules during their biosynthesis have peptides loaded into their peptide-binding grooves. In the absence of infection or vaccination with foreign proteins, these peptides are derived from self-proteins, as is the case for the MHC of TCE cells. Murine studies indicate that specialized MHC class I-binding peptides may be generated by a special type of proteasome expressed by TCE cells (the thymoproteasome) and that these play an important role in increasing the antigen responsiveness of mature peripheral T cells (Takada et al., 2015). In positive selection, thymocytes with TCRs that are unable to bind to MHC/peptide complexes on TCE cells with sufficient affinity to generate intracellular signals die by apoptosis, the default pathway. In cases where the TCR binding to MHC generates a relatively weak to moderate signal, the thymocyte is positively selected for survival. A large range of from relatively weak to medium strength “analog” signals are converted by the intracellular signaling protein Themis into a single “digital” outcome of thymocyte survival and maturation (Gascoigne and Acuto, 2015).

CD4⁺CD8⁺ thymocytes with TCRs that receive MHC class II/peptide survival signals lose CD8 expression and upregulate CD3 expression, thereby becoming CD4⁺CD8⁺ thymocytes that are CD3^{high}. CD4⁺CD8⁺ thymocytes also begin to acquire a gene expression pattern characteristic of mature peripheral CD4 T cells that is required for their capacity to carry antigen-induced effector functions, such as IL-2 secretion and CD40-ligand (CD40L) expression. CD4⁺CD8⁺ thymocytes with TCRs that receive MHC

class I/peptide survival signals lose CD4 expression and upregulate CD3 expression, thereby becoming CD4⁺CD8⁺ thymocytes; they also begin to express genes that are characteristic of peripheral CD8 T cells and that are required for their antigen-induced capacity to become cytotoxic cells. These CD4⁺CD8⁺ thymocyte-specific versus CD4⁺CD8⁺ thymocyte-specific outcomes of positive selection are directed by the master transcription factors ThPOK and Runx3, respectively (Taniuchi, 2016). In cases where the TCRs of CD4⁺CD8⁺ thymocytes receive very high levels of signaling, double-positive cortical thymocytes undergo apoptosis (Hogquist and Jameson, 2014).

A small subset of thymocytes bearing TCR α chains containing the $V\alpha 24J\alpha 18$ segments in association with TCR β chains containing $V\beta 11$ segments interact with relatively high affinity with CD1d, an MHC class I-like protein that is expressed on double-positive thymocytes (Van Kaer et al., 2016). This TCR interaction leads to the thymocyte-positive selection for NKT-lineage cells, which are distinct in function from conventional T cells in having the ability to rapidly secrete large amounts of cytokines, such as IFN- γ and IL-4, during the early phase of innate immune responses to pathogens (Brigl et al., 2011).

Another subset of thymocytes bearing TCR α chains with $V\alpha 7.2J\alpha 33$ segments paired with TCR β chains using either $V\beta 2$ or $V\beta 13$ interact with high affinity with an MHC class I-like protein, MR1, on hematopoietic cells, resulting in the positive selection of MAIT cells (Martin et al., 2009). MAIT cells, which are predominantly CD8 single positive, are found in mucosal tissues, such as the intestine, lung, and liver (Liuzzi et al., 2015).

Positively selected single-positive CD4⁺CD8⁻ or CD4⁻CD8⁺ thymocytes move into the medulla, where they undergo a final selection process before emigrating from the thymus as CD4 and CD8 T cells called *negative selection*. This selection process, which is an important mechanism for maintaining tolerance of T cells to peptides derived from self-proteins, involves the exposure of the mature thymocytes to medullary APCs expressing a highly diverse repertoire of peptides derived from self-proteins. These self-proteins include those that are normally expressed in a tissue-restricted manner (e.g., peptides derived from insulin, which is produced by pancreatic beta islet cells or parathyroid hormone) or that are characteristic of only certain stages of early development (Derbinski and Kyewski, 2010). This unusual pattern of protein expression by medullary DCs and B cells is the result of nuclear proteins, such as those encoded by the autoimmune regulator (De Martino et al., 2016) and Fezf1 (Takaba et al., 2015) genes, stochastically relieving the transcriptional repression of these tissue-specific and developmentally regulated genes. Thymocytes that have high levels of signaling for peptides derived from self-proteins are induced to undergo apoptosis, whereas intermediate levels of signaling by CD4⁺CD8⁻ thymocytes result in their differentiation into Treg cells (Perry and Hsieh, 2016). Thus thymically derived Treg cells have TCRs that have substantial affinity for self-proteins expressed in a tissue-specific or developmental-specific manner.

Recent Thymic Emigrants and the Naïve T-Cell Compartment

Following negative selection, single-positive mature thymocytes undergo additional maturation before exiting the thymus as recent thymic emigrant (RTE) naïve T cells, including upregulation of CC-chemokine receptor 7 (CCR7), IL-7 receptor alpha chain (CD127), L-selectin (CD62L), and Smad interacting protein 1 (Hogquist et al., 2015). In humans this maturation also includes downregulation of the CD45R0 isoform and upregulation of the CD45RA isoform of the CD45 protein tyrosine phosphatase. The fully mature thymocyte then enters the circulation as an RTE naïve T cell that is CD45RA⁺CD45R0⁻CCR7⁺CD62L⁺ and that retains surface expression of protein tyrosine kinase 7 (PTK7), a protein that is highly expressed during intrathymic development (Haines et al., 2009). The RTE naïve T cell recirculates between the peripheral lymphoid tissue and the blood. The entry of the circulating naïve T cells involves the interaction of T-cell surface adhesion molecules, such as L-selectin (CD62L) with sialomucins expressed on high endothelial venules and the T-cell CCR7 chemokine receptor with its chemokine ligands, which are expressed within peripheral lymphoid tissues. RTE naïve T cells also undergo postthymic antigen-independent maturation over a period of several months with the loss of PTK7, a decrease in the capacity for activation-induced chemokine (C-X-C motif) ligand 1 (CXCL8) (IL-8) production (van den Broek et al., 2016), and an increase in the capacity for IFN- γ production (Haines et al., 2009). PTK7⁺ RTE naïve T cells also undergo one to two homeostatic cell divisions as part of this maturation (Haines et al., 2009).

In the human embryo the first naïve, mature T cells appear in the circulation and lymphoid organs at approximately 11 to 12 weeks' embryonic development and have been found in the fetal intestine at 11 weeks' gestation (Spencer et al., 1986; Blom et al., 1998). Thymopoiesis continues at least through age 40 years as indicated by the presence of circulating PTK7⁺ RTEs (Haines et al., 2009) and by the results of in vivo metabolic labeling studies with deuterium (Vrisekoop et al., 2008). Thymectomy early in life (e.g., as part of open heart surgery for congenital heart disease

or for the treatment of certain autoimmune diseases, such as myasthenia gravis) results in a substantial loss of naïve T cells, including PTK7⁺ RTEs (Haines et al., 2009) and CXCL8-expressing RTEs (van den Broek et al., 2016). Thymectomy also results in an oligoclonal memory T-cell compartment (Prelog et al., 2009; Sauce et al., 2009). Some children who have undergone neonatal thymectomy appear to regenerate thymic tissue, which is associated with the reacquisition of naïve T cells with RTE features (van den Broek et al., 2016). In individuals who do not recover thymic function, the decay process is accelerated by chronic cytomegalovirus (CMV) infection, resulting in an immunosenescent T-cell phenotype similar to that seen in elderly individuals and associated with increased morbidity and mortality (Wikby et al., 2006). Naïve T cells of the peripheral lymphoid compartment can undergo homeostatic expansion in response to cytokines, such as IL-7, and this proliferation may be particularly important in disease states that impair the production of RTEs and result in profound peripheral lymphopenia, such as treatment with chemotherapy or human immunodeficiency virus (HIV) infection.

Naïve CD4 T-Cell Activation Into Effector T_h1, T_h2, T_h17, and Follicular Helper T Cells

Naïve T-cell activation requires a complex molecular signaling cascade that involves the reorganization of signaling molecules of the T-cell membrane into an "immunologic synapse" with the APCs bearing MHC/peptide (Smith-Garvin et al., 2009). For naïve T-cell activation, CD11c⁺ DCs are particularly effective as APCs. TCR engagement by a high-affinity MHC/peptide ligand results in phosphorylation of components of the CD3 complex associated with the $\alpha\beta$ TCR. The CD3 proteins contain cytoplasmic tails with specific amino acid sequences called ITAMs, which serve as molecular targets for the tyrosine kinase Lck. The CD3 ζ chain is thought to be the most critical component and is found as a homodimer. The tyrosine phosphorylated CD3 ζ chain binds the tyrosine kinase ZAP-70, which, in turn, phosphorylates linker for activation of T cells, a large protein that serves as a docking site for multiple signaling molecules. Full naïve T-cell activation also requires costimulation by engagement of CD28 on the T-cell surface by CD80 or CD86 on the APC. Together, signaling generated by the TCR and CD28 results in the activation of several parallel signaling pathways, including those of the calcineurin–NFAT, Ras–AP-1 (fos/jun), and protein kinase C delta–nuclear factor κ B (NF- κ B) pathways resulting in entry of the transcription factors nuclear factor of activated T-cells (NFAT), activator protein-1 (AP-1), and NF- κ B into the nucleus, where they bind to the *cis*-regulatory elements of hundreds of genes and alter their transcription (Smith-Garvin et al., 2009).

After their activation, naïve CD4 T cells differentiate into effector T_h1, T_h2, T_h17, or follicular helper T (T_h) cells (Zhu et al., 2010; Crotty, 2014). Differentiation into peripheral Treg cells can also occur, and these cells are discussed in the section entitled *Regulatory T Cells*. Each of these CD4 T cell types is defined by prototypical master transcription factors and by secretion of a characteristic profile of cytokines in response to antigenic stimulation and preferential expression of particular chemokine receptors. The cytokine milieu produced by non-T cells in the local environment during antigen presentation is a primary factor influencing the developmental fate of a naïve T cell following activation.

Activated T_h1 cells secrete IFN- γ , which is the signature T_h1 cytokine, IL-2, lymphotoxin α , and TNF- α , and also express surface CD40L (CD154). IL-12 produced by DCs and IFN- γ produced by NK cells promote T_h1 cell development by a signal transducer

and activator of transcription (STAT) 4-dependent mechanism, and this results in expression of the master transcription factor T-bet. T_H1 responses are generally proinflammatory, and IFN- γ secretion is particularly important in activating mononuclear phagocytes for the control of intracellular bacterial pathogens, such as *Mycobacteria*, *Salmonella*, and *Listeria*. IFN- γ also increases MHC expression, which may be particularly important in counteracting the attempt by herpesviruses to avoid antigen detection by their production of a number of proteins that decrease MHC class I and class II antigen presentation. Human neonatal CD4 T cells are biased against T_H1 polarization relative to adult T cells (Randolph and Lewis, 2006), and this bias may continue into infancy and contribute to the increased vulnerability of infants to severe and disseminated tuberculosis (Vanden Driessche et al., 2013). Both CD4 T cell–intrinsic mechanisms (Chen et al., 2006) and APC-intrinsic mechanisms, such as decreased expression of IL-12 p70 by neonatal and infant DCs (Goriely et al., 2001, 2004), appear to contribute to blunted T_H1 immunity.

Activated T_H2 cells secrete IL-4, IL-5, and IL-13 and are important in the response to infections with multicellular parasites, such as helminths, and classic allergic diseases in which the level of IgE is elevated. Their development is facilitated by a number of non-T-cell–derived cytokines, particularly from epithelial sources, including thymic stromal lymphopoietin (TSLP), IL-25, and IL-33, as well as IL-4 produced by basophils. This non-T-cell–derived IL-4 activates STAT6, which, in turn, induces GATA3, the master transcription factor for T_H2 differentiation from naïve CD4 T cells. The T_H2 cytokines IL-4 and IL-13 induce Ig heavy chain class switching to the IgE isotype (the T_H2 phenotype). IL-5 is an eosinophil growth factor and also promotes eosinophil survival in inflamed tissues. IL-13 promotes goblet cell hyperplasia and mucous secretion. Thus T_H2 cells coordinate many of the characteristic responses of the skin and mucosal tissues to parasitic infection and allergens. Murine neonatal CD4 T cells appear to be intrinsically biased toward T_H2 polarization (Adkins et al., 2004), but in vitro studies using human UCB CD4 T cells and adult allogeneic DCs as an APC source have not observed this bias (Chen et al., 2006).

Activated T_H17 cells secrete the closely related cytokines, IL-17A and IL-17F, which induce increased expression of defensins by the epithelium, leading to increased tissue resistance of the mucosa and skin to fungal and bacterial infection. In humans the most important role of IL-17 is to control mucocutaneous fungal infection, particularly with *Candida* (Okada et al., 2016). The differentiation of activated naïve CD4 T cells into T_H17 cells is promoted by IL-1 β and TGF- β and cytokines that activate STAT3, including IL-6 and IL-23. Activated STAT3 induces the expression of ROR γ , which is the master transcription factor for T_H17 cell differentiation (Bedoya et al., 2013). In addition to their role in host defense, T_H17 cells are also prominently involved in the pathogenesis of inflammatory bowel disease, psoriasis, multiple sclerosis, rheumatoid arthritis, and other autoimmune diseases. The ability of naïve CD4 T cells of the term or preterm neonate to differentiate into T_H17 cells appears to be robust (Black et al., 2012). This suggests that factors other than impaired CD4 T-cell T_H17 immunity contribute to increased susceptibility of the neonate and young infant to mucocutaneous candidiasis.

Activated T_H cells secrete IL-21 and also express surface CD40L, both of which provide essential signals for the activation and differentiation of B cells to produce antibody against protein antigens (Crotty, 2014). Human naïve CD4 T-cell differentiation into T_H cells appears to be promoted by the combination of IL-12, IL-21, IL-23, and TGF- β and engagement of inducible T-cell costimulator

(ICOS) on the T cell by ICOS ligand. Whether the differentiation of human neonatal naïve CD4 T cells into T_H cells is as robust as that of older children and adults is unclear. However, the observation that young infants, compared with older children, have reduced primary antibody responses to protein antigens, such as the hepatitis B vaccine (West, 1989), could be a reflection of less robust T_H cell generation following immunization.

Effector CD4 T-Cell Response to Herpes Simplex Virus Infection

Postnatal primary infection of the human neonate with HSV often results in high levels of the virus in blood with severe systemic disease and/or dissemination to the central nervous system. In contrast, these are rare events in infants older than 4 weeks, suggesting a unique age-related susceptibility that may reflect rapid postnatal maturation of key immunologic antiviral mechanisms. At least part of the vulnerability of the neonate may be due to limitations in HSV-specific CD4 T-cell immune responses, which are decreased and delayed for T_H1 cytokine production, compared with those adults with primary HSV infection (Burchett et al., 1992). HSV-specific antibody responses are also reduced and delayed in neonatal compared with adult primary HSV infection (Sullender et al., 1987), suggesting that neonatal HSV-specific T_H -cell generation may also be relatively impaired.

Naïve CD8 T-Cell Activation Into Cytolytic Effector Cells

Naïve CD8 T cells have a similar CD45RA⁺CD45R0[−]CD62L⁺CCR7⁺ surface phenotype as those of the CD4 T-cell subset and recirculate between the blood and secondary lymphoid tissue by the same mechanisms. As for naïve CD4 T cells, antigen presentation by CD11c⁺ DCs is particularly efficient for CD8 T-cell activation, which results in the acquisition of cytolytic effector function mediated by perforin and granzymes and the expression of FasL. Many activated CD8 T cells also secrete T_H1 cytokines, such as IFN- γ and TNF- α . Data on the ability of neonatal naïve CD8 T cells to differentiate into effector cells capable of cell-mediated cytotoxicity and eliminating infected target cells are limited. In cases of congenital CMV infection, robust CMV-specific fetal CD8 T-cell responses occur (Marchant et al., 2003), suggesting that this pathway for differentiation is intact with a strong source of antigenic stimulation. There is limited information on the neonatal and young infant CD8 T-cell responses to acute viral infection. Studies of older infants and young children indicate a relatively robust CMV-specific CD8 T-cell response to primary infection (Chen et al., 2004).

Antigen-Specific Memory T-Cell Responses

Memory T cells, which are generated as part of the primary antigen-specific T-cell response, are defined by function (i.e., more rapid effector response) and by expression of a CD45RA[−]CD45R0⁺ surface phenotype. A recently identified T memory stem cell (Tmsc) subset, which differentiates from naïve T cells early in the immune response, appears to play an important role in the long-term generation of antigen-specific CD4 and CD8 memory T cells (Gattinoni et al., 2017). This generation does not appear to require continued exposure of the Tmsc to antigen, accounting for the persistence of T-cell memory for decades following a single acute infection with pathogens that are completely cleared from the body. Tmsc give rise to central memory T cells, which have a CD45R0⁺CD62L⁺CCR7⁺ surface phenotype and survey the peripheral lymphoid tissues for specific antigen. A second population of cells, called *effector memory T cells*, have a CD45R0⁺CD62L[−] surface phenotype and express a variety of chemokine receptors

other than CCR7 and survey the nonlymphoid tissues for specific antigen. The extent to which effector memory cells are derived from central memory cells or directly differentiate from T_h1 cells remains unclear. Both central and effector memory T cells recirculate, with substantial numbers of both cell subsets found in the blood. A fourth memory cell population, T resident memory (Trm) cells, is found in nonlymphoid tissues, such as the skin and lung, and does not recirculate in the blood. On the basis of studies of the murine skin, Trm and central memory T cells appear to have a common clonal origin. Studies comparing the memory CD4 T cell responses of infants and young children with those of adults following primary CMV infection suggest that the infant/child responses are skewed toward central memory rather than effector memory responses, with an overall reduced production of T_h1 cytokines (Tu et al., 2004). Similarly, the pertussis antigen-specific CD4 T-cell response of infants following the receipt of a Diphtheria, Tetanus, acellular Pertussis [DTaP] and tetanus toxoid, reduced diphtheria toxoid, acellular pertussis [Tdap] (DTaP) vaccination series appears to be skewed toward central memory cells and limited IFN- γ production compared with that of Tdap-vaccinated adults (Sharma and Pichichero, 2012). Relative blunting of T_h1 memory cell responses in older infants and children likely also applies to the young infant and may contribute to the vulnerability of human infants to severe and disseminated primary tuberculosis (Vanden Driessche et al., 2013).

Regulatory T Cells

Treg cells have a CD25^{high}CD127^{low} surface phenotype and are a distinct subset of CD4 T helper cells critical for maintenance of immune self-tolerance and homeostasis by suppressing aberrant or excessive immune responses harmful to the host (Ohkura et al., 2013). Treg cells are defined by having an immune-suppressive phenotype with high, sustained expression of the transcription factor FOXP3 and a number of other Treg cell signature genes (Sakaguchi et al., 2013). Mutations in the *FOXP3* gene can result in an inherited multisystem autoimmune disease characterized clinically by diarrhea, insulin-dependent diabetes mellitus, thyroid disorders, and eczema, called *X-linked neonatal diabetes mellitus, enteropathy, and endocrinopathy syndrome* (Wildin et al., 2001).

Natural (thymic) Treg cells develop as a distinct lineage in the thymus, while induced (peripheral) Treg cells arise in the periphery from conventional CD4 T cells in the presence of TGF- β , retinoic acid, and microbial antigens and metabolites, such as short chain fatty acids (Tanoue et al., 2016). Naïve CD4 T cells of the second-trimester fetus are skewed toward differentiation into peripheral Treg cells rather than effector cells compared with adult naïve CD4 T cells. Murine SCID/human chimera experiments suggest that this skewing is a feature of naïve CD4 T cells that arise from fetal HSCs rather than adult HSCs (Mold et al., 2010). It is not known when in ontogeny hematopoiesis switches from the fetal-type HSC to the adult type, raising the possibility that at least some neonatal and infant T cells could be fetal HSC derivatives and, as a result, have distinct immune function. Defects in Treg cell function and number have been described in a series of different autoimmune diseases, including type 1 diabetes, systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, and inflammatory bowel disease. Clinical trials using the transfer of ex vivo expanded Treg cells for treatment of type 1 diabetes, graft-versus-host disease, and other immune-mediated diseases are under way (Bluestone et al., 2015).

Relevant to the interpretation of animal models for neonatal immunology, ontogeny of FoxP3-expressing Treg cells is much

more advanced in humans than in mice (Fontenot et al., 2005). FoxP3⁺ Treg cells are almost completely lacking in neonatal mice, while they are abundant in the thymus and mesenteric lymph nodes in the human fetus as early as 20 weeks' gestation (Michaels et al., 2006). It appears that maternal cells cross the placenta to reside in fetal lymph nodes, inducing the development of fetal Treg cells suppressing immunity against maternal antigens and probably also playing a role in immune homeostasis after birth (Mold et al., 2008). Therefore it does not seem surprising that Treg cells can be detected in neonatal tissues as early as 23 weeks' gestation (Weitkamp et al., 2009). Consistent with the increased concentration of Treg cells in fetal tissues between 12 and 20 weeks' gestation, UCB from preterm infants born between 24 and 31 weeks' gestation contains a higher percentage of Treg cells compared with that of term infants (Dirix et al., 2013). This imbalance persists until later childhood and may play a role in the immune challenges former preterm infants face. In general, the critical maturation events in the developing immune system that underlie the high vulnerability to both infection and inflammation in the (preterm) neonate remain largely understudied (Sharma et al., 2012).

$\gamma\delta$ T Cells

About 2%–5% of T-lineage cells of the thymus and peripheral blood express $\gamma\delta$ TCR. Despite the potential for a highly diverse $\gamma\delta$ TCR repertoire, $\gamma\delta$ TCR use is highly restricted in terms of variable (V) segment use, with V γ 9V δ 2 T cells being the predominant $\gamma\delta$ T-cell subset in adult peripheral blood. Gamma-delta T cells, which can be more innate-like or more adaptive-like in their immune function depending on the context (Vermijlen and Prinz, 2014), can produce T_h1- and T_h17-type cytokines, kill virally infected or tumor target cells by cell-mediated cytotoxicity, and functionally interact with other cell types of the immune system, such as B cells and NK cells. V γ 9V δ 2⁺ T cells are innate-like in their immune function in that they are not activated by their TCR-recognizing MHC/peptides but rather utilize other receptors, such as the butyrophilin subfamily 3 member A1 (BTN3A1), to recognize microbe- and host-derived phosphorylated prenol metabolites (collectively referred to as *phosphoantigens*) (Vermijlen and Prinz, 2014). Older work suggested that this V γ 9V δ 2 T-cell predominance was due to postnatal expansion in response to exposure to bacterial-derived phosphoantigens. However, studies of the human fetus suggest otherwise in that V γ 9V δ 2 T cells are also the predominant $\gamma\delta$ T-cell subset in fetal blood and, like their adult counterparts, are programmed to rapidly respond to phosphoantigens with IFN- γ and cytotoxin release (Dimova et al., 2015). These findings suggest that endogenous phosphorylated prenol metabolites are sufficient for such immune programming of $\gamma\delta$ T cells in the fetus. An example of a more adaptive-like $\gamma\delta$ T-cell response is illustrated by V γ 8V δ 1⁺ T cells, which were first identified in fetuses with congenital CMV infection (Vermijlen et al., 2010). These V γ 8V δ 1⁺ T cells have the ability to lyse CMV-infected target cells in a TCR-dependent manner and have the potential to serve as non-MHC-restricted antiviral adoptive immunotherapy (e.g., in the post-HSC transplant setting) (Deniger et al., 2014).

Natural Killer T Cells

NKT cells are so named because they express $\alpha\beta$ TCRs in conjunction with CD161 (NKR-P1A), the human orthologue of the mouse NK1.1 protein, and other NK-cell markers, including CD56, CD57, and NKG2D. Unlike conventional $\alpha\beta$ T cells, NKT cells that emigrate from the thymus into the periphery have a uniformly

CD45R0⁺ surface phenotype and the ability to rapidly secrete high levels of T_H1 and T_H2 cytokines and to carry out cell-mediated cytotoxicity. Human NKT cells have a highly restricted repertoire of $\alpha\beta$ TCR (TCR α chains containing the V α 24J α 18 segments in association with TCR β chains containing V β 11 segments) and are positively selected during thymocyte development by the nonclassical MHC molecule CD1d rather than by MHC class I or class II molecules. The invariant TCR of NKT cells can recognize endogenously produced β -linked glycolipids, such as β -galactosylceramide, bound to CD1d, which may serve as a microbial-induced danger signal (Brennan et al., 2011). The role of the invariant $\alpha\beta$ TCR in NKT-cell immunity remains controversial, and it has been proposed that activation of these cells by cytokines, such as IL-12, may be more important for their mediating an innate-like immune response (Brigl et al., 2011). NKG2D-dependent but TCR-independent activation of NKT cells (e.g., for cytotoxic activity) can also occur. NKT cells with invariant TCR can be divided into a CD4⁺(CD8⁻) subset that expresses CD62L and may be involved in recirculation in secondary lymphoid tissues and a CD4⁻CD8⁻ cell subset that may mainly serve as effector cells at sites of extralymphoid tissue inflammation. In addition to their potential role in antimicrobial host defense, NKT cells have also been implicated as negative regulators of certain T-cell-mediated immunopathologic responses, as relative deficiency of their numbers has been associated with certain autoimmune diseases, graft-versus-host disease following hematopoietic cell transplant, and asthma.

NKT-lineage cells are present in the human fetal thymus by the beginning of the second trimester of gestation, and their relative frequencies compared with those of the other thymocyte subsets decline with increasing gestational age because of the rapid expansion of thymocytes differentiating by the conventional positive selection. Fetal and postnatal thymic NKT cells, which are largely CD4⁺CD45R0⁺, express high levels of IL-7 receptors, and their development appears to be largely IL-7 dependent. Extrathymic fetal NKT-cell populations are found in the small intestine, where they may constitute up to 5% of all T cells in this tissue, and in the lung, spleen, and mesenteric lymph nodes (Loh et al., 2014). Those NKT cells found in the fetal small intestine are distinct from those of fetal peripheral lymphoid tissue in having greater capacity to produce IFN- γ and lower levels of expression of CD62L. Murine studies suggest that commensal bacteria may be an important source for NKT-cell expansion and maturation, but the finding of a human NKT-cell population with mature features in the fetal intestine before bacterial colonization of the gut suggests a different mechanism, such as the presentation of endogenous glycolipids by CD1d, which is abundantly expressed in the fetal intestine. Circulating NKT cells in the neonate, infant, and young child constitute approximately 0.06% of circulating lymphocytes, a frequency that is only modestly lower than the mean of 0.2% for adult peripheral blood (Berzins et al., 2005). At all ages there is striking individual variation in values, with frequencies ranging from less than 0.001% to more than 0.5% of circulating lymphocytes.

Mucosal-Associated Invariant T Cells

MAIT cells are a population of CD8⁺ or CD8⁻CD4⁻ $\alpha\beta$ T cells that predominantly express an invariant V α 7.2 and J α 33 TCR α chain. They are positively selected during thymic development by MR1, an MHC class I-like protein that is widely expressed on hematopoietic and epithelial cells. Antigen for the MAIT cell $\alpha\beta$ TCR consists of bacterial- or fungal-derived metabolites of the

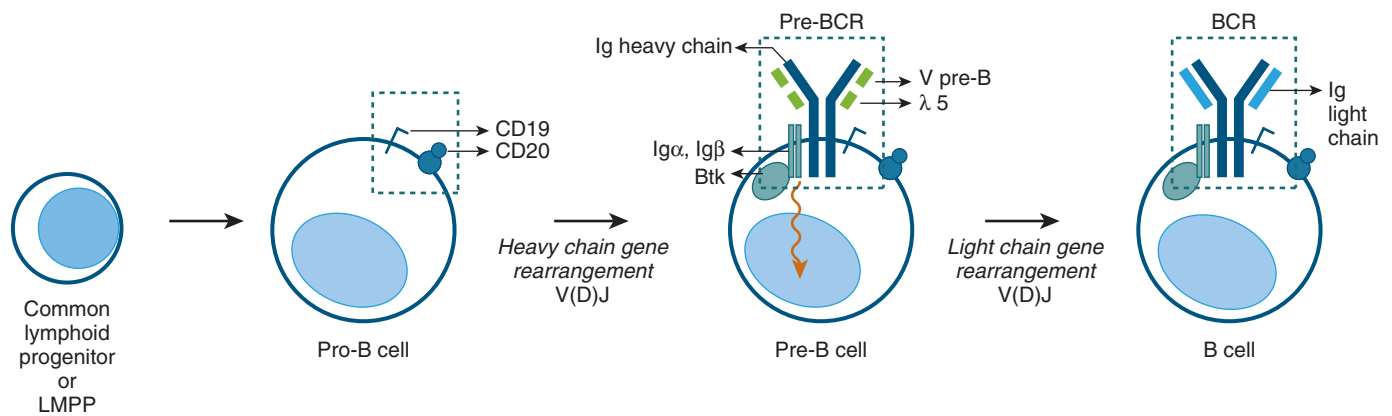
riboflavin biosynthetic pathway that bind to MR1 at a site analogous to the peptide-binding groove used by conventional MHC class I heavy chains (Birkinshaw et al., 2014). MAIT cells have the ability to produce T_H1 and T_H17 cytokines in response to polyclonal stimuli, including treatment with IL-12 or IL-18, and can also kill bacterially infected epithelial cells by cell-mediated cytotoxicity. MAIT cells constitute up to 10% of circulating $\alpha\beta$ T cells of healthy adults and uniformly have a memory (CD45RA⁻CD45R0⁺) cell surface phenotype. MAIT cells are the predominant $\alpha\beta$ T-cell type in the liver and the lamina propria of the healthy intestine (Dusseaux et al., 2011) and are also found in lung tissue. Murine studies have shown that MAIT cells can mediate protection against a variety of bacterial pathogens, including mycobacteria. Intrathymic MAIT cell precursors, which are CD161^{high}V α 7.2⁺ but IL-18R^{low}, are detectable at 18 weeks' gestation. MAIT cells with an activated and proliferative phenotype (i.e., IL-18R^{high}CD45R0⁺Ki67⁺) are easily detected in the small intestine, liver, and lung between 18 and 23 weeks' gestation (Leeansyah et al., 2014). These fetal intestinal MAIT cells secrete IFN- γ and IL-22 in response to *E. coli* and anti-CD28 monoclonal antibody. Collectively, these findings suggest that although fetal MAIT cells develop in the absence of exposure to commensal microbes, they potentially could mediate host defense against intrauterine bacterial and fungal infection in the extralymphoid tissues. MAIT cells in the UCB of neonates, which constitute approximately 0.7% of circulating $\alpha\beta$ T cells, have a surface phenotype (e.g., CD45RA⁺) that suggests that they may be RTEs that are in transit to the extralymphoid tissues, where they may mature and acquire a memory phenotype, as apparently occurs in the fetus (Leeansyah et al., 2014).

B Lymphocytes

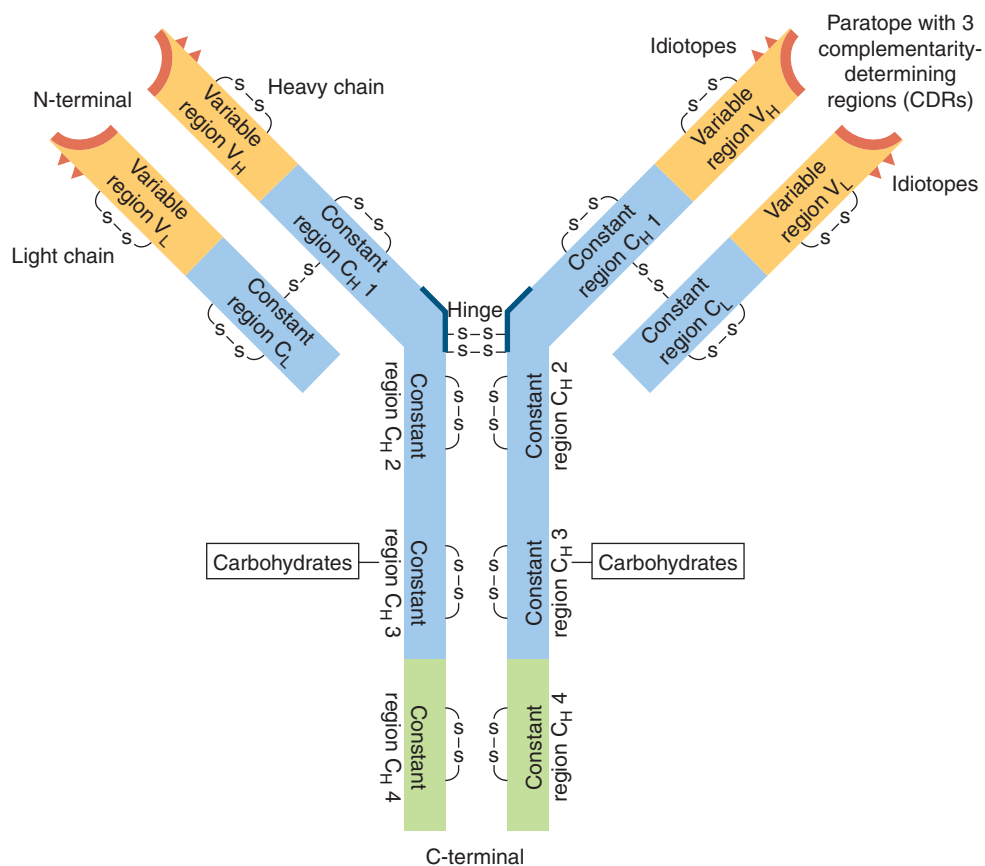
B cells are lymphocytes that express BCRs, which consist of surface Ig in association with Ig α and Ig β signaling molecules and the cytoplasmic Bruton tyrosine kinase (Btk) (Fig. 36.8). The engagement of the BCR by high-affinity antigen results in B-cell activation and clonal expansion and, in some instances, terminal B-cell differentiation into antibody-secreting plasma cells. Secreted antibodies form the humoral arm of the immune system and provide the main form of immune protection against many pathogens, particularly during the extracellular phases of their life cycle. Although the B-cell compartment is well formed before birth, full diversification of the antibody repertoire and the efficiency of somatic hypermutation, which generates higher affinity antibodies, is not achieved until late in infancy.

B-Cell Development

The early phase of B-cell development, like T-cell development, is antigen independent. The first B lineage-committed progenitors that have not undergone V(D)J recombination of the Ig genes (pro-B cells) express CD19, CD34, TdT, RAG1, and RAG2. These cells undergo heavy chain D to J gene segment rearrangements followed by V to DJ rearrangement. As in TCR gene rearrangement, TdT is used during this process to insert nucleotides between the segments to create additional junctional diversity at CDR3, which is encoded by the 3' end of the V segment and the D and J segments. CDR3 is an important determinant of antibody specificity as it forms the central portion of the antigen-binding site. If the Ig heavy chain gene rearrangement is productive, it is expressed on the cell surface in association with a surrogate light chain consisting of the V-preB and λ 5 polypeptides. Cells with a nonfunctional Ig heavy chain gene rearrangement or that express Ig heavy chain protein that assembles poorly with the surrogate light chain die by apoptosis.



• **Fig. 36.8 Human B-Cell Development.** A common lymphoid progenitor or lymphoid-primed multipotent progenitor (LMPP) cell gives rise to a pro-B cell that expresses surface CD19 and CD20 and that is committed to B-lineage-cell differentiation. The immunoglobulin (Ig) heavy chain gene locus undergoes V(D)J rearrangement, and, if productive, the B-lineage cell proceeds to the pre-B-cell stage, expressing a pre-B-cell receptor (pre-BCR). The pre-BCR consists of the Ig heavy chain in association with a surrogate light chain that consists of two polypeptides—V pre-B and λ 5—and the proteins Igα and Igβ that are required for receptor complex surface expression and signaling. The Ig light chains undergo rearrangement in the pre-B cell, and, if productive, the pre-B cell differentiates into a B cell that expresses a B-cell receptor (BCR) that contains a mature surface Ig molecule. *Btk*, Bruton tyrosine kinase.



• **Fig. 36.9 Structure of Monomeric Immunoglobulin.** (Modified from Mix E, Goertsches R, Zett UKL. Immunoglobulins: basic considerations. *J Neurol.* 2006;253:V9–V17.)

The pre-BCR signals use Btk (Fig. 36.9), which accounts for why in cases of genetic deficiency of Btk (X-linked agammaglobulinemia) there is a maturational arrest at the pre-B-cell stage. The next stage involves V_L to J_L gene rearrangement, and, if productive, the newly expressed Ig light chain replaces the surrogate light chain. The light chain can be derived from either the kappa chain (60% of B cells) or the lambda chain (40% of B cells) gene clusters. The completed BCR is antigen specific and contains a surface IgM molecule. B-lineage cells also express surface IgD with the same antigen specificity as IgM, which is generated by alternative exon usage in the constant region of the Ig heavy chain gene. B-cell development initially occurs in the fetal liver, with a switch to the bone marrow beginning in the second trimester of gestation.

B-Cell Preimmune Selection and Maturation

Immature B cells of the bone marrow that have successfully produced productive heavy and light chains express these as IgM on the cell surface. A large proportion of these BCRs are autoreactive (i.e., they bind with relatively high affinity to molecules on other cell types within the bone marrow microenvironment or peripheral lymphoid organs). An important immune tolerance mechanism is the testing of immature B-lineage cells of the bone marrow for self-reactivity. At this stage of B-cell development, BCR signaling as part of self-reactivity maintains RAG activity, allowing the B cell to undergo a secondary V-to-J light chain rearrangement, a process known as *receptor editing*. If this eliminates BCR autoreactivity and cell signaling, RAG activity ceases, and the B cell enters the circulation as a peripheral transitional B cell to complete its maturation. Otherwise, additional light chain rearrangement can occur. Most initially autoreactive immature B cells can be converted to nonautoreactivity by receptor editing, allowing most B cells with productive Ig heavy chain rearrangements to contribute to the final repertoire.

Persistently autoreactive B cells are probably eliminated either by a process of clonal deletion, which involves apoptosis induced by strong BCR signaling (Wardemann and Nussenzweig, 2007), or by anergy, in which the B cells are functionally inactivated. Anergic B cells have been identified as having an $IgD^+IgM^-CD27^-$ surface phenotype and constitute about 3% of peripheral blood B cells in adults. These anergic B cells have BCRs with autoreactive antigen specificity that mediate a decreased calcium flux and tyrosine phosphorylation after their engagement (Duty et al., 2009). Anergic B cells probably die over a period of days to weeks by apoptosis.

$IgM^{high}IgD^{high}$ transitional B cells can be identified in the bone marrow and circulation by their high levels of expression of CD5, CD10, CD24, and CD38 and low levels of adenosine triphosphate-binding cassette subfamily B member 1 (ABCB1) (Wirths and Lanzavecchia, 2005). Analogous to RTEs, these circulating transitional B cells undergo a post-bone marrow phase of peripheral maturation into fully mature naïve B cells that involves an increase in CD21 and ABCB1 expression and a decrease in CD5, CD10, CD24, and CD38 expression. The maturation of transitional B cells into fully mature antigenically naïve B cells includes negative selection of B cells with autoreactive BCRs that have entered the periphery and that have somehow escaped central tolerance mechanisms in the bone marrow (Samuels et al., 2005; Meffre, 2011). Although the mechanisms involved in this secondary checkpoint for B-cell tolerance remain poorly understood, they may involve, at least in part, the death of autoreactive B cells because of their greater dependence for survival on B-cell activating factor (BAFF), a member of the TNF ligand superfamily, than

nonautoreactive B cells. The transitional B-cell subset is also enriched in regulatory B cells, which have the capacity to express IL-10 after strong stimulation and, like Treg cells, may negatively regulate adaptive immune responses.

Most transitional B cells that escape negative selection become fully mature naïve $IgM^{high}IgD^{high}CD27^-ABCB1^+$ follicular B cells and enter follicle areas of the secondary lymphoid tissue by the interaction of their CXCR5 chemokine receptors with chemokines in the follicle (CXCL13). These cells can recirculate between the follicles of the peripheral lymphoid organs, including the spleen, lymph nodes, and Peyer patches, and the blood and lymph (Allen et al., 2007). Follicular B cells include most of those that are involved in adaptive immune responses to T-dependent antigens, such as proteins and protein-carbohydrate conjugates.

Fetal and Neonatal B-Cell Development and Surface Phenotype

B cells expressing surface IgM are present by 10 weeks' gestation. The frequency of B cells in the tissues rapidly increases, such that by 22 weeks' gestation the proportion of B cells in the spleen, blood, and bone marrow is similar to that of adults. The concentration of B cells in the circulation is higher during the second and third trimesters than at birth (Schultz et al., 2000). After birth the concentration of B cells increases to peak levels between 6 and 12 months of age, which is followed by a gradual decrease until stable adult values are reached.

Approximately 70%–75% of UCB B cells are of the transitional subset, whereas transitional B cells usually constitute 10% or less of adult peripheral blood B cells, and 25%–30% are fully naïve B cells (Wirths and Lanzavecchia, 2005; Avery et al., 2008). $CD27^+$ B cells, which include most memory B cells in adults, are at low levels or undetectable in UCB, consistent with the antigenic naïveté of the healthy newborn. Neonatal naïve B cells are functionally distinct from their adult counterparts, having significantly lower expression of the IL-4 receptor alpha chain and responsiveness to IL-4 as assessed by tyrosine phosphorylation of STAT6 (Tian et al., 2006). Circulating newborn B cells are deficient in the purine ectoenzyme CD73, impairing their capacity for extracellular purine salvage (Pettengill and Levy, 2016). Newborn naïve B cells demonstrate adult-level expression of TLRs and CD40, but responses to stimulation of these receptors are distinct, including (1) impaired neonatal TLR2- and TLR7-mediated as well as (CD40+ TLR)-mediated but enhanced TLR9-mediated cytokine production and (2) impaired CD40-mediated Ig secretion (Pettengill et al., 2016). With respect to the quantity of B cells, absolute B-cell numbers double in the first 6 months of life from those at birth, mainly due to expansion of fully mature naïve B cells and, to a lesser extent, transitional B cells.

B-Cell Activation, Somatic Hypermutation, and Isotype Switching

When mature naïve B cells contact antigen through the BCR, a signal is transduced that promotes further growth and differentiation into memory B cells and plasma cells. BCR signaling involves activation of the tyrosine kinases spleen tyrosine kinase (Syk) and Btk, which are also involved in pre-BCR signal transduction, which results in transcriptional regulation by Elk-1, c-Myc, NF- κ B, and NFAT (Corneth et al., 2016). The T-dependent B-cell response to protein antigens involves the internalization of protein bound to the BCR and its processing into peptides that are loaded onto MHC class II molecules. These peptide/MHC class II complexes that are expressed on the B-cell surface activate CD4 T cells via

their TCR, resulting a series of CD4 T cell/B-cell interactions, including between CD40L and CD40 receptor, between IL-21 and IL-21 receptor, between ICOS and ICOS ligand.

Both the B cells activated by protein antigen and the peptide antigen-specific CD4 T cells they activate and interact with enter the follicles of secondary lymphoid tissue, where these interactions continue. The activated B cells undergo massive clonal expansion and differentiation giving rise to histologically evident germinal centers. Most germinal center B cells undergo somatic hypermutation in which antigen combining site regions encoded by productively rearranged V, D, and J segments of the Ig heavy chain gene and the V and J segments of the light chain gene accumulate random point mutations that result in amino acid substitutions. Those B cells with genetic variants that increase the affinity of the BCR for antigen outcompete those having BCRs with unchanged or reduced affinity. This affinity maturation process dramatically increases the ability of antibody to perform effector function, such as neutralization or opsonization. Somatic hypermutation requires activation-induced cytidine deaminase expression by germinal center B cells as well as the action of error-prone DNA polymerases. The peak of somatic mutation is approximately 10 to 12 days after immunization with a protein antigen.

Human B cells produce five isotypes of antibody (i.e., IgM, IgD, IgG, IgA, and IgE). The IgG and IgA isotypes can be, respectively, divided into the IgA1 and IgA2 subclasses and the IgG1, IgG2, IgG3, and IgG4 subclasses. During their process of differentiation from naïve B cells into memory B cells or plasma cells, B cells are able to change from expressing IgM to expressing other antibody isotypes without changing antigen specificity. With the exception of IgD expression, which involves alternative messenger ribonucleic acid (RNA) splicing, this switching involves isotype recombination, the genetic replacement of the IgM-specific portion of the constant region (C_μ) of the heavy chain with a new isotype-specific gene segment (Fig. 36.10). As in V(D)J recombination, the intervening DNA is excised as a circle. Isotype recombination is mediated by switch regions that are positioned immediately upstream of each of the isotype-specific C regions (with the exception of IgD). Successive multiple isotype switching by a single B cell can also occur—for example, IgM to IgG and then, on reactivation by antigen, IgG to IgE. Cytokines secreted by T cells or other cell types play an important role in promoting or inhibiting switching to a specific isotype. For example, IL-4 or IL-13 is absolutely required for isotype switching to IgE, a process that can be inhibited by the presence of IFN- γ .

IgA, which is the predominant Ig isotype secreted into mucosal secretions, has a distinct CD4 T-cell-independent and CD40L-independent pathway for its generation by isotype switching that utilizes the TNF ligand superfamily—a proliferation-inducing ligand (APRIL) and BAFF. These cell surface proteins, which are expressed by a variety of cell types, including intestinal epithelium, may play an important role in the development of IgA-secreting plasma cells in the gut in response to commensal bacteria.

Neonatal Antibody Responses to T-Dependent and T-Independent Antigens

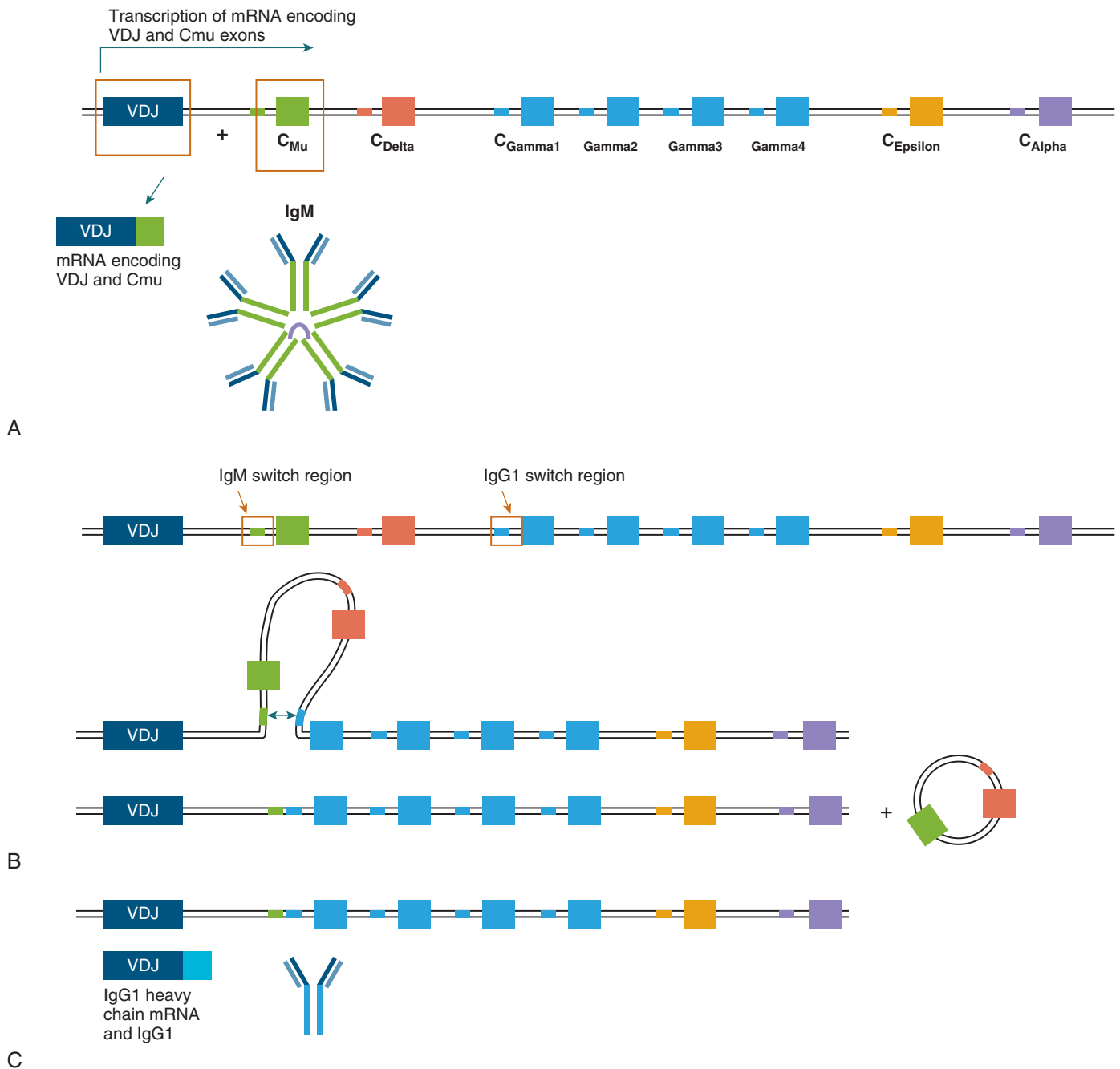
A study comparing the RSV F protein-specific heavy chain antibody responses in RSV-infected neonates and adults revealed a distinct usage of variable heavy chain segments in the neonates, suggesting that there are major age-related differences in the selection of antibody specificities. Importantly, there was also significantly less somatic hypermutation of the Ig heavy chain gene and decreased N-nucleotide addition in the antibody response of neonates

compared with adults (Williams et al., 2009). These differences would be expected to result in a relatively poor RSV-specific antibody response in the neonate and young infant. Similarly, restricted heavy chain variable gene usage and lack of somatic mutations were found in rotavirus-specific B cells of human infants (Weitkamp et al., 2003, 2005).

Whether isotype switching is limited in T-dependent responses in the neonate in response to infection is less clear. Although this is plausible, given that CD40L expression by activated neonatal CD4 T cells may be decreased (Durandy et al., 1995), studies are needed to determine if reductions in CD40L expression by antigen-specific T cells also occur in response to neonatal vaccination and, if so, whether such reduced expression correlates with an impairment of the other important roles of CD40L in promoting B-cell antibody responses (i.e., memory B-cell development and somatic hypermutation). An additional factor that may contribute to the modest reduction in T-dependent antibody production is the reduced expression on B cells (nearly all of which are naïve $\text{IgM}^+\text{IgD}^+\text{CD27}^-$ follicular B cells) from preterm neonates and, to a lesser degree, term neonates of the receptors for BAFF and APRIL (Kaur et al., 2007). Receptors for BAFF and APRIL help promote T-dependent B-cell differentiation into plasma cells, plasma cell survival, and T-independent isotype switching. Importantly, some antibody responses, such as the T-independent antibody response to bacterial capsular polysaccharides, such as those of *Streptococcus pneumoniae*, are not fully established until after 2 to 3 years of age (Mond and Kokai-Kun, 2008); the mechanisms for this delayed competency remain unclear.

Immunoglobulins

Igs, or antibodies, are a heterogeneous group of proteins that are detectable in plasma and body fluids and on the surfaces of mucosal barriers and B cells that bind specifically to antigens. The functions of Igs relevant to fetal and neonatal immunity are summarized in Table 36.2. There are five known classes of Igs: IgG, IgM, IgA, IgE, and IgD. Human IgM circulates as a pentamer or hexamer, and IgA circulates as a dimer. Multimers are formed in association with an additional J chain. The functions of individual Ig classes are different but overlapping (Table 36.2). The prototype Ig molecule consists of a pair of identical heavy chains that determine the Ig class in combination with a pair of identical light chains, which are linked by disulfide bonds (see Fig. 36.9). Each Ig molecule contains two N-terminal, identical domains with antigen-binding activity that are formed by the variable regions of a heavy chain and a light chain. The antigen-binding site is formed by three complementarity-determining regions: CDR1, CDR2, and CDR3, which are hypervariable in their amino acid sequences as a result of V(D)J recombination of the heavy and light chain genes and, in some instances, somatic hypermutation. The C-terminal region of the two heavy chains forms the Fc (fraction crystallizable) region, which is not involved in antigen binding but mediates Ig effector functions. The principal functions of the Fc region include receptor-mediated phagocytosis (IgG), antibody-dependent cellular cytotoxicity (IgG), release of inflammatory mediators from mast cells and basophils (IgE), receptor-mediated transport through mucosa (IgA and IgM) and placenta (IgG), and complement activation (IgG1, IgG2, IgG3, and IgM). The five different isotype classes of human Igs (IgG, IgM, IgA, IgD, and IgE) are defined structurally by differences in the heavy chain constant regions (CH)1 and CH2 domains of the Fc fragments. Within isotypes, there are four IgG subclasses (IgG1, IgG2, IgG3, and IgG4) and two IgA subclasses (IgA1 and IgA2).



• **Fig. 36.10** Variable (V), Diversity (D), and Joining (J) Gene Segment Recombination. (A) Transcription of messenger RNA (mRNA) encoding VDJ and constant C_μ exons. (B) Recombination highlighting the immunoglobulin M (IgM) and the immunoglobulin G1 (IgG1) switch regions. (C) IgG1 heavy chain mRNA and protein.

Immunoglobulin G

IgG is the predominant Ig isotype at all ages (Stiehm and Fudenberg, 1966). In adults, IgG1 is the predominant subclass, accounting for approximately 70% of total IgG, and IgG2, IgG3, and IgG4 account for approximately 20%, 7%, and 3% of the total, respectively. Passively derived maternal IgG is the source of virtually all of the IgG subclasses detected in the fetus and neonate. Because the IgG plasma half-life is about 21 days, these maternally derived IgG levels fall rapidly after birth. The levels of IgG synthesized by the neonate and that are derived from the mother are approximately equal at 2 months of age, and by 10 to 12 months of age nearly all

of the IgG is infant derived. IgG values typically reach a nadir of approximately 400 mg/dL in term infants at 3 to 4 months of age and rise thereafter. Premature infants have lower IgG concentrations at birth that typically reach a nadir at 3 months of age; mean IgG values of 82 and 104 mg/dL are observed in infants born at 25 to 28 weeks' gestation and 29 to 32 weeks' gestation, respectively.

Immunoglobulin M

IgM is the only isotype besides IgG that binds and activates complement. IgM has a half-life in the blood of 5 days. The concentration of IgM in the blood increases from a mean of 6 mg/

TABLE 36.2 Characteristics and Functions of Immunoglobulins

| | Molecular Mass (kDa) | Serum Concentration (g/L) | Serum Half-Life (Days) | Neutralization | Opsonization | Complement Activation | Epithelial Transport | Placental Transport | Sensitization for Killing by Natural Killer Cells | Sensitization of Mast Cells and Basophils |
|------|----------------------|---------------------------|------------------------|----------------|--------------|-------------------------------|----------------------|---------------------|---|---|
| IgG1 | 150 | 10 | 21 | ++ | +++ | Strong classic, alternative | – | +++ | ++ | + |
| IgG2 | 150 | 5 | 21 | ++ | (+) | Classical, alternative | – | + | – | – |
| IgG3 | 170 | 1 | 7 | ++ | ++ | Strong classical, alternative | – | ++ | ++ | + |
| IgG4 | 150 | 0.5 | 21 | ++ | + | Alternative | – | (+) | – | – |
| IgA1 | 160 | 3 | 7 | ++ | + | Alternative | +++ | – | – | – |
| IgA2 | 160 | 0.5 | 7 | ++ | + | Alternative | +++ | – | – | – |
| IgM | 900 | 2 | 5 | + | + | Strong classical | + | – | – | – |
| IgD | 180 | 0.03 | 3 | – | – | Alternative | – | – | – | – |
| IgE | 190 | 0.00003 | 3 | – | – | – | – | – | – | +++ |

Ig, Immunoglobulin.
Modified from Mix E, Goertsches R, Zett UKL. Immunoglobulins: basic considerations. *J Neurol*. 2006;253:V9–V17.

dL in infants born at less than 28 weeks' gestation to 11 mg/dL for those born at term (Avrech et al., 1994). This IgM is likely to be preimmune and not the result of a B-cell response to foreign antigens. Rather, it is likely enriched for polyreactive natural antibodies that may play an important role in innate defense against infection. Postnatal IgM concentrations rise rapidly for the first month and then more gradually, presumably in response to antigenic stimulation in both premature infants and term infants. By 1 year of age, the values are approximately 60% of those in adults. Because maternal–fetal transport of IgM does not occur, elevated (greater than 20 mg/dL) IgM concentrations in UCB suggest possible intrauterine infections, although many infants with congenital infections have normal values.

Immunoglobulin A

IgA does not cross the placenta, and its concentration in UCB is usually 0.1 to 5.0 mg/dL, approximately 0.5% of the levels in maternal sera (Avrech et al., 1994). The concentrations are similar in term and premature neonates (Cederqvist et al., 1978), and both IgA1 and IgA2 are present. IgA has a half-life of 6 days in blood. Secretory IgA can be detected in the saliva of neonates as early as 3 days after birth.

The concentrations of IgA in serum increase to 20% of adult levels by 1 year of age and rise progressively through adolescence. Increased UCB IgA concentrations are observed in some infants with congenital infection, such as toxoplasmosis, and are common in those infected with HIV by vertical transmission. The amount of IgA produced daily is estimated to exceed that of all Ig isotypes combined. The overall importance of IgA in host defenses is only partially understood, as individuals with complete IgA deficiency are typically asymptomatic. Recent studies suggest that mucosally secreted IgA may play an important role in regulating the composition of the human gut microbiome (Kurashima and Kiyono, 2017),

and that in cases of IgA deficiency, this role may be compensated for by mucosally secreted IgM.

Immunoglobulin E

Although IgE synthesis by the fetus is detectable as early as 11 weeks, the concentrations of IgE in UCB are typically low, with a mean of approximately 0.5% of maternal levels (Avrech et al., 1994). IgE concentrations are higher in infants born at 40 to 42 weeks' gestation than in those born at 37 to 39 weeks. The rate of postnatal increase differs and is greater in infants predisposed to allergic disease or greater environmental exposure to allergens (Edenharter et al., 1998). The concentration of IgE at birth appears to have limited predictive value for later development of atopic disease for most individuals. Maternofetal transfer of IgE appears to be a common cause of elevated IgE concentration at birth (Bonnelykke et al., 2010). On the basis of murine experiments, one potential mechanism for such transfer may be IgG anti-IgE complexes being transported via FcRn, which has substantial affinity only for IgG.

Immunoglobulin D

IgD is detectable in serum from the UCB of term and premature infants, with mean levels of approximately 0.05 mg/dL (Avrech et al., 1994). These levels increase during the first year of life. The role of IgD in normal humoral immunity remains unclear. Elevated levels of IgD are associated with certain monogenic autoinflammatory disorders, such as mevalonate kinase deficiency and familial Mediterranean fever.

Polyclonal Immunoglobulin Therapy for Prevention or Treatment of Bacterial Infections

Intravenous Ig (IVIG) has been extensively evaluated for the prevention of nosocomial or late-onset pyogenic infections in premature

neonates. A 2013 Cochrane analysis identified 19 randomized controlled trials in which IVIG was used for the prevention of sepsis in the setting of neonatal intensive care units. These trials included a total of approximately 5000 infants born at less than 37 weeks' gestation or with a birth weight of less than 2500 g (Ohlsson and Lacy, 2013). When combined, these studies showed significant reductions in both sepsis and in one or more episodes of any serious infection. However, there were no significant decreases in the IVIG-treated infants compared with those who received placebo for overall mortality, the incidence of NEC, bronchopulmonary dysplasia, intraventricular hemorrhage, or the length of hospital stay. This and another analysis both concluded that IVIG is not indicated for prophylaxis in preterm neonates and neonates with low birth weight because there is no effect on long-term outcome and little or no effect on short-term outcomes.

Similarly, IVIG has been studied as a potential therapeutic in neonates with suspected or proven sepsis. A comprehensive randomized and controlled International Immunotherapy Study (INIS) trial investigating the use of IVIG as adjunctive therapy for suspected or proven serious neonatal infections involved 3493 infants enrolled in 113 hospitals in nine countries (INIS Collaborative Group, 2011). No significant differences in the primary outcome variable—death or major disability at the age of 2 years—was observed between IVIG-treated infants and those who received placebo. There were also no significant differences between the two groups in the incidence of subsequent sepsis episodes or the rates of major or minor disability at 2 years of age. A Cochrane analysis that included the results of the INIS report as well as seven additional randomized or quasi-randomized studies also failed to find a significant impact of IVIG therapy, including that enriched in IgM, in reducing death or major disability at 2 years of corrected age or in length of hospital stay (Ohlsson and Lacy, 2015). A single-center study published after the Cochrane analysis that used historical controls found that IgM-enriched IVIG significantly reduced early mortality in very low-birth weight infants; however, it did not reduce overall mortality or the incidence of intraventricular hemorrhage, periventricular leukomalacia, NEC, or bronchopulmonary dysplasia at discharge (Capasso et al., 2013). On the basis of these results and analyses, adjunctive therapy with IVIG, including the IgM-enriched form, is not recommended in the management of neonatal sepsis.

Monoclonal Antibody Prophylaxis to Prevent Infection

Monoclonal antibodies that are fully human derived or that were originally generated in another species, usually the mouse, and then humanized (i.e., by replacement of murine constant regions of the Ig chains with those of humans) are commonly used to treat human autoimmune diseases and cancer. Palivizumab, a monoclonal antibody against RSV, is licensed for the prevention of infection in the United States and recommended for infants at high risk of severe RSV disease. This includes infants born at less than 32 weeks' gestation or with chronic lung disease of prematurity or hemodynamically significant congenital heart disease. Such infants should receive up to five monthly doses during seasons of the year when RSV is prevalent.

Specific Immunologic Deficiencies of the Newborn and Their Diagnosis

The most common reason for increased immunologic susceptibility to infection in newborns, besides prematurity, is iatrogenic

immunosuppression caused by administration of corticosteroids for treatment or prevention of bronchopulmonary dysplasia. Such therapy is particularly associated with an increased risk of fungal infection, such as candidemia, particularly in the setting of indwelling intravascular catheters used for total parenteral nutrition.

Although less common than therapeutically induced immunodeficiency, genetically inherited primary immunodeficiencies (PIDs) can present in the neonatal period and may require prompt intervention for optimal outcome. There are approximately 300 recognized PIDs (Chinen et al., 2016), most of which are monogenic disorders (Boisson et al., 2015). The physician should attempt to differentiate infants with specific genetically regulated immunologic deficiencies from those with developmentally regulated, environmentally induced, or infection-related susceptibility to microbial invasion (Stiehm et al., 2015). Documenting a full family history during a prenatal visit, including whether there is consanguinity or the family is of an ethnicity that is known to have a high incidence of a particular PID (e.g., those of Athabascan Indian heritage and SCID caused by Artemis deficiency), can be helpful in identifying families who may have a PID. However, the lack of such history does not exclude PID, as a patient can have de novo mutations causing PID. These de novo mutations are not present in either the biological mother or father or siblings and are the result of a mutation in the egg of the mother or sperm of the father, or in rare instances in the fertilized egg itself. Such de novo mutations are increasingly recognized as result of whole-exome sequencing of the biological mother, biological father, and affected child trios (Ghaoui et al., 2015). What follows is a discussion of specific PIDs that may present in the newborn period and are important to recognize promptly.

Severe Combined Immunodeficiency

SCID is a rare category of diseases that severely impacts the development and/or function of both T cells and B cells. Most forms of SCID lack peripheral T cells (i.e., are T cell negative) and are due to thymic hypoplasia and markedly impaired production of new naïve CD4 and CD8 T cells. T cell–negative SCID can be divided into four major groups on the basis of whether peripheral numbers of B cells and/or NK cells are also reduced or absent (i.e., T[−]B[−]NK[−], T[−]B[−]NK⁺, T[−]B⁺NK[−], and T[−]B[−]NK⁺) (Chinn and Shearer, 2015).

T[−]B[−]NK[−] SCID accounts for more than half of all SCID patients, most of whom are males with the X-linked form that is due to deleterious mutations of the *IL2RG* gene, which encodes the common gamma chain (γ c or CD132). This chain is a shared component of the cytokine receptors IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21. Defects in the γ c protein abrogate the development of T cells in the thymus because of the lack of IL-7 receptor function and of NK cells in the bone marrow because of the lack of IL-15 receptor function. Although B cells in X-linked SCID develop normally, they are not able to mediate T-dependent antibody responses because they do not receive CD4 T-cell help and also because they lack functional IL-21 receptors. The less common autosomal recessive form of T[−]B[−]NK[−] SCID is primarily due to biallelic mutations in Janus-associated tyrosine kinase 3 (JAK3). This kinase is expressed in cells of hematopoietic lineage and associates with γ c as part of the cytokine receptor signaling cascade and is also the target of small-molecule drugs used for immunosuppression (Pesu et al., 2005). JAK3-deficient patients are clinically similar to those with γ c deficiency except that females and males are equally involved.

Patients with autosomal recessive T⁺B⁺NK⁺ SCID most commonly have biallelic mutations of the gene encoding the IL-7 receptor alpha chain (CD127) or, less frequently, of genes encoding the proteins CD3 δ (CD3D), CD3 ϵ (CD3E), CD3 ζ (CD247), CD45 (PTPRC), or coronin 1A (CORO1A). SCID caused by coronin 1A deficiency is distinct from the other forms in having a block in the release of fully mature thymocytes into the periphery but otherwise normal intrathymic T-cell development and a normal thymic shadow with radiologic imaging.

Patients with T⁺B⁺NK⁺ SCID have biallelic mutations of genes that encode proteins that are required for TCR and Ig gene rearrangement by V(D)J recombination, including RAG1, RAG2, Artemis, protein kinase catalytic subunit (PKCS), and XLF (Cernunnos) (de Villartay, 2015). These mutations prevent the formation of pre-TCRs on thymocytes and pre-BCRs on immature B cells, resulting in early T-cell and B-cell maturational arrest and an absence of peripheral T cells and B cells.

The T⁺B⁺NK⁺ SCID phenotype is due to biallelic mutations in genes encoding the enzymes adenosine deaminase (ADA) and adenylylase kinase 2 (AK2). Patients who lack ADA, an enzyme in the purine salvage pathway, accumulate deoxyadenosine triphosphate in red blood cells and lymphocytes, and the concentration correlates with disease severity. The ADA substrates, adenosine and deoxyadenosine, are found at increased levels in the serum. Developing thymocytes are particularly sensitive to these metabolic effects. ADA deficiency may also result in neonatal hepatitis, renal dysfunction, bone abnormalities (including flaring of the costochondral junction as seen on the lateral chest radiograph [termed *rachitic rosary*]) and pelvic dysplasia), sensorineural hearing impairment, and cognitive impairment. Patients with AK2 deficiency, which is also known as *reticular dysgenesis*, have severe neutropenia and also sensorineural hearing impairment.

A T⁺ SCID-like phenotype with severe immunodeficiency and opportunistic infections can also result in cases in which the production of T cells by the thymus is normal or modestly impaired but the peripheral T cells have severe functional defects. These defects can either be T-cell intrinsic, such as biallelic mutations of the genes encoding caspase recruitment domain family member 11, CD3 γ , ZAP-70, or the calcium channel proteins Stim1 and Orai1, or that are T-cell extrinsic and are due to a lack of MHC class II-restricted antigen presentation. MHC class II deficiency is due to biallelic mutations of the regulatory factor X family member genes *RFXANK*, *RFXAP*, and *RFX5*, or of *CIITA*, which all encode proteins that positively regulate the transcription of the MHC class II genetic loci (Hanna and Etzioni, 2014). An SCID-like clinical presentation with severe T-cell lymphopenia may occur for some causes of T-cell immunodeficiencies that can also present with a less severe phenotype and retention of greater numbers of T cells, such as cartilage hair hypoplasia syndrome, *Coloboma*, *Heart defects*, *choanal Atresia*, *growth Retardation*, *Genital abnormalities*, and *Ear abnormalities* (CHARGE) syndrome, or tetratricopeptide repeat domain 7 deficiency. Finally, mutations of genes that cause SCID that are hypomorphic (i.e., preserve some degree of normal protein function) can result in atypical forms in which the T-cell numbers may range from being only moderately depressed to normal values (e.g., certain missense mutations of the *IL2RG* gene in X-linked SCID).

T⁺ SCID can also occur in Omenn syndrome, which often presents in the neonatal period with diarrhea, intense erythroderma, alopecia, hepatosplenomegaly, and lymphadenopathy. Omenn syndrome was first described in cases of biallelic hypomorphic missense mutations of the *RAG1* gene or the *RAG2* gene but has

also been reported in cases of SCID caused by *IL2RG*, *LIG4*, *IL7R*, *ADA*, *DCLRE1C*, *AK2*, *RMRP*, *ZAP70*, *CHD7*, or *CARD11* mutations. Regardless of the genetic cause, the hematologic and immune phenotype is characteristic, with markedly increased eosinophilia, highly elevated serum levels of IgE, increased numbers of CD4 T cells, and markedly reduced numbers of CD8 T cells. Unlike CD4 T cells in healthy newborns, the CD4 T cells of Omenn syndrome are uniformly CD45R0⁺ and lack CD45RA expression.

T⁺ SCID can also be the result of the engraftment of maternal T cells into the fetus. The clinical consequences of maternal engraftment can range from asymptomatic to a mild erythematous rash to full-blown symptoms of graft-versus-host disease (e.g., severe dermatitis, gastroenteritis, hepatitis, and lung disease). Maternal T-cell engraftment can also result in hemophagocytic syndrome, which can lead to secondary changes in the numbers of other cell types, such as B cells, that can obscure the SCID diagnosis. Clues to maternal engraftment include the uniform expression of CD45R0 by the circulating T cells, and unusual CD4 T cell to CD8 T cell ratios. Maternal engraftment can be readily diagnosed by means of T-cell chimerism assays, such as the polymerase chain reaction (PCR)-based short tandem repeats/variable number tandem repeats (STR/VNTR) system (Khan et al., 2004).

Most infants with SCID appear healthy at birth. In the neonatal period a morbilliform rash, probably the result of attenuated graft-versus-host disease from transplacental passage of maternal lymphocytes, may be the only symptom of SCID. During the first several months of life, as acquired maternal antibody levels drop, failure to thrive and undue susceptibility to infection become universal features. Intractable diarrhea, pneumonia, and persistent thrush, especially oral thrush, constitute the triad of findings most frequently seen in infants with this disease. A diffuse pneumonia with severe hypoxemia due to *Pneumocystis jirovecii* is a classic presentation of SCID and usually occurs between 3 and 6 months of age although it rarely may occur in the neonatal period. Patients with SCID who receive live rotavirus vaccine, which is routinely first administered at 2 months of age, often develop persistent shedding and disease (Patel et al., 2010), and such symptoms should raise the suspicion of SCID or other severe T-cell immunodeficiency.

The diagnosis of SCID is often suggested by an opportunistic or unusually severe infection in the setting of profound lymphopenia (<1000 lymphocytes per microliter). Only 10% of patients with SCID have lymphocyte counts in the normal range, and the possible reasons for T⁺ SCID have been discussed previously. The thymus gland is not seen on chest radiographs except in some cases of T⁺ SCID or SCID because of defects in thymocyte egress, such as coronin 1A deficiency. Flow-cytometric analysis of T-cell, B-cell, and NK-cell populations, including the enumeration of CD4 and CD8 T cells and their naïve (CD45RA) and memory (CD45R0) subsets, is the most important confirmatory test. Other useful tests include measurements of ADA and purine nucleoside phosphorylase activity in red blood cells. Quantitative Ig levels are not particularly helpful in the diagnosis of neonatal SCID, because most IgG is maternal in origin, and IgA and IgM levels are often low in the neonatal period. Once the immunophenotype has been established, a precise molecular diagnosis should be obtained, and genetic counseling should be provided.

An effective strategy for detecting newborns with SCID with statewide newborn screening is now in place in more than 46 states of the United States. The test is based on the real-time PCR detection of sjTRECs, which are generated during V(D)J recombination of the TCR α/δ gene locus (see Fig. 36.7). Neonatal screening

involves extraction of total DNA from standard dried blood spots on filter paper that are obtained by heel stick in the nursery and used for other routine newborn screening tests. The turnaround time of the test is usually about 3 weeks. Abnormally low values of sjTRECs should lead to prompt flow-cytometric evaluation of lymphocyte populations as described previously.

SCID is a pediatric emergency, and is invariably fatal if untreated. Most untreated patients die in the first year of life. Treatment of SCID begins with aggressive antibiotic and antiviral therapy for infections, intravenous Ig replacement, and antibiotic prophylaxis for *Pneumocystis jirovecii* and environmental mycobacterial infections. Besides greater susceptibility to opportunistic infections, these infants are also susceptible to development of graft-versus-host disease, either before birth as a result of engraftment of maternal T lymphocytes or after birth as a result of the engraftment of T lymphocytes present in transfused blood products. Therefore infants in whom SCID is suspected should receive only irradiated CMV-negative blood products and should not be given live viral vaccines.

SCID treatment usually requires allogeneic HSC transplant, ideally from an HLA-matched sibling (Slatter and Gennerly, 2013). In one approach, conditioning regimens, such as busulfan, are not used and recipients usually become chimeric, with only T and NK cells of donor origin. B-cell function is frequently deficient, and many patients continue to require Ig replacement therapy. As there is not replacement of totipotent HSCs of the recipient with those of the donor, there is eventually a drop-off in T-cell numbers, requiring that the patients receive a “boost” transplant from the original donor (Heimall et al., 2017). An alternative approach is to use standard conditioning regimens that result in the replacement of all hematopoietic cell types of the recipient with those of the donor, including HSCs. This approach has an increased risk of complications in the early posttransplant period but avoids the need for later transplants and often leads to normal B-cell function, obviating the need for potentially lifetime Ig replacement therapy. ADA deficiency can be treated with enzyme replacement. This treatment involves weekly injections of ADA coupled to polyethylene glycol. Response, consisting of decreasing deoxyadenosine triphosphate levels and increasing T-cell numbers, is seen in most patients within weeks. Finally, retroviral lentiviral gene therapy based on vector-mediated transfer of a therapeutic gene into autologous HSCs has been used to treat patients with ADA and X-linked SCID and is under development for other disorders (Thrasher and Williams, 2017). Early problems in the treatment of X-linked SCID from insertional mutagenesis leading to endogenous gene dysregulation and leukemia have been avoided with currently used lentiviral vectors. The treatment of ADA SCID by gene therapy has not encountered any major safety issues.

DiGeorge Syndrome

The embryologic anlage of the thymus, parathyroid, and a portion of the great vessels is the endodermal epithelium of the third and fourth pharyngeal pouches (McDonald-McGinn et al., 2015). When normal development of these structures is disturbed, thymic and parathyroid hypoplasia and congenital heart disease involving the great vessels can occur. Infants with this disorder, DiGeorge syndrome, can exhibit abnormalities of calcium homeostasis during the neonatal period (hypocalcemia and tetany) and variable T-cell deficits, which is usually subclinical and only severe in about 1% of cases. Often, the syndrome is suspected because of congenital conotruncal cardiac defects (about 60% of cases), low-set ears, midline facial clefts, hypomandibular abnormalities,

and hypertelorism. The cardiac anomalies associated with 22q11.2 deletion syndrome are variable but usually involve the outflow tract and the derivatives of the branchial arch arteries. These defects include interrupted aortic arch type B, truncus arteriosus, and tetralogy of Fallot. Children with 22q11.2 deletion syndrome also exhibit a higher incidence of receptive-expressive language difficulties, cognitive impairment, and behavioral problems, including psychotic illness (Jolin et al., 2009; Papangelis and Scambler, 2013).

About 95% of patients with DiGeorge syndrome have a hemizygous interstitial three-megabase deletion of the 22q11.2 chromosomal region, which is flanked by two low copy repeat regions (McDonald-McGinn et al., 2015) and which is readily diagnosed by fluorescence in situ hybridization. This deletion results in hemizygosity for 46 protein-encoding genes, including *TBX1*, 7 microRNAs, and 10 long noncoding RNAs. Cases of DiGeorge syndrome lacking the 22q11.2 deletion should be evaluated for *TBX1* loss-of-function mutations, which can result in most of the features of DiGeorge syndrome, including T-cell immunodeficiency, hypoparathyroidism, and/or conotruncal cardiac lesions (Ogata et al., 2014). Clinical features suggestive of DiGeorge syndrome can also be observed in individuals with *CHD7* mutations, the cause of CHARGE syndrome, and in some cases of gestational diabetes, fetal exposure to retinoic acid, and other chromosomal deletion disorders.

In the nursery, identification of infants with congenital conotruncal abnormalities or unexplained persistent hypocalcemia should prompt consideration of this syndrome. In most cases the T-cell deficiency is not severe, with decreases in circulating CD4 T-cell and CD8 T-cell numbers to levels that are 25%–50% of those normal for age. However, in about 1 in 400,000 deliveries, infants with features of DiGeorge syndrome may have severe T-cell lymphopenia—that is, circulating naïve ($CD45^{RA+}CD62L^{+}$) T-cell numbers less than 50 per microliter. Severe T-cell lymphopenia in association with hypoparathyroidism and conotruncal congenital heart disease is called *complete DiGeorge syndrome* and is due to the 22q11.2 deletion in about 50% of cases, CHARGE syndrome in about 25% of cases, and gestational diabetes in about 15% of cases (Markert et al., 2009). Like SCID, complete DiGeorge syndrome is a medical emergency that prompts antibiotic prophylaxis for *Pneumocystis jirovecii* and nontuberculous mycobacteria, Ig replacement therapy, and protective isolation. All patients should also be evaluated for their CMV infection status, and, if positive, antiviral therapy should be started. Patients who are not infected with CMV should not be breastfed if their mother is CMV IgG antibody positive. About 30% of complete DiGeorge syndrome patients develop an extensive erythematous rash sometimes accompanied by gastrointestinal symptoms and hepatitis, which is referred to as *atypical complete DiGeorge syndrome*. These patients often have increased levels of circulating T cells in their blood that express memory markers, such as CD45RO, with similar T cells found in skin biopsy. These T cells, which are produced by the patient and are not the result of maternal engraftment, are oligoclonal in their TCR usage and do not provide useful immune function. Patients harboring these autoreactive T cells have been treated with immunosuppressive drugs, such as calcineurin inhibitors and glucocorticoids.

The only definitive therapy for complete DiGeorge syndrome is transplant of thymic epithelial tissue, which is derived from thymus tissue removed from otherwise healthy children as part of surgery for congenital heart disease (Markert et al., 2010). Small pieces of thymic epithelia are placed within the quadriceps muscle, and the de novo production of CD4 and CD8 T cells begins approximately 4 to 6 months after transplant. The transplant is

successful in about 80% of cases, so that antibiotic prophylaxis, Ig replacement therapy, and protective isolation can be discontinued, whereas live vaccination should continue to be avoided.

Combined Immune Disorders Involving T Cells and B Cells

Many monogenic immune disorders have complex phenotypes that involve both T and B cells in which the residual T-cell function is sufficient to result in a delayed presentation after the neonatal period. For example, Wiskott–Aldrich syndrome (WAS) is a combined immune disorder involving T cells, B cells, and APCs (Chandra et al., 2016). WAS affects males and is due to mutations of a gene located on the X chromosome that encodes WAS protein (WASp), an intracellular protein. WASp is expressed in all hematopoietic cells that interact with actin cytoskeleton and can also participate in transcriptional regulation. WAS is characterized by eczema (usually severe), thrombocytopenia, increased risk of malignancy, and susceptibility to recurrent sinopulmonary infections, severe herpesvirus infection, and, less commonly, classic opportunistic infections, such as infection with *Pneumocystis jirovecii*. Other manifestations that may be present in the newborn period include petechiae and bruises, bloody diarrhea, and hemorrhage after procedures. In infants with any of these clinical findings, thrombocytopenia in a complete blood count report is an important clue to possible WAS. In contrast to immune thrombocytopenia purpura, WAS platelets have a low mean platelet volume, and this finding is pathognomonic of the disorder. Characteristic immunologic findings include moderately decreased numbers of T cells, particularly those of the CD8 subset, with relatively subtle defects in T-cell proliferation, decreased IgM levels, and increased IgA and IgE levels. Many of these immunologic findings may not be evident in the neonatal period. Flow cytometry can be used to evaluate leukocyte WASp expression to make a provisional diagnosis of WAS. As for children with SCID, all blood products given to children with WAS should be CMV negative and irradiated before administration to avoid T-cell engraftment and graft-versus-host disease. WAS can be cured by HSC transplant, with the best results obtained with transplant before 3 years of age. Lentiviral gene therapy is a promising alternative for treatment (Thrasher and Williams, 2017).

B-Cell Immunodeficiencies

Most genetic defects that target B-cell immunity but leave T-cell immunity intact, such as X-linked agammaglobulinemia (XLA), are not detected in the neonatal period without special screening tests, such as flow cytometry to evaluate circulating B-cell numbers or intracellular expression of Btk by monocytes (Locke et al., 2014). Because of maternal transfer of IgG during the last trimester of pregnancy, infants with selective immunodeficiencies of B cells typically do not develop clinically significant hypogammaglobulinemia and recurrent or severe sinopulmonary infections with encapsulated organisms until after 3 to 6 months of age. Moreover, the absence of B cells from the circulation does not usually result in clinically detectable lymphopenia on the complete blood count. Analogous to screening for severe T-cell deficiency, neonatal screening for quantitative B-cell immunodeficiency is possible by assaying by PCR for kappa receptor excision circles (KRECs), which are stable circular DNA byproducts of V(D)J rearrangement of the kappa light chain Ig gene in B-lineage cells (Hammarstrom, 2014). Currently, KREC evaluation is not part of routine newborn screening in the United States.

Innate Immune Deficiency Disorders

Immune deficiencies that include neutropenia as part of a syndrome, such as SCID due to AK2 deficiency (reticular dysgenesis), or that result in isolated quantitative or qualitative defects of neutrophils often present in the neonatal period. This reflects the essential role of neutrophils in phagocytosing pathogenic bacteria and fungi and eliminating them by oxidative and nonoxidative mechanisms within the phagosome (Dinauer, 2016). Bacterial infections, such as bacteremia with gram-negative organisms such as *E. coli* or *Pseudomonas aeruginosa* or gram-positive organisms such as *Staphylococcus aureus*, are frequent with neutrophil disorders. Delayed umbilical cord separation and omphalitis are important clinical signs that should lead to evaluation for neutrophil deficiency, such as leukocyte adhesion defect, which is most commonly due to biallelic mutations of the *CD18* gene. An important clue to leukocyte adhesion defect is that the neutrophil count is always elevated above normal values, even in the absence of infection or other inflammatory stimuli. Chronic granulomatous disease (CGD) is due to mutations of genes encoding the phagocyte oxidase system, which is required for oxidative killing within the phagosome. The clinical infections characteristic of CGD, such as deep tissue infections with bacteria or fungi, can present during the neonatal period, although in most cases this occurs later in infancy.

Although relatively rare, monogenic immune deficiencies of innate immune cell signaling may present in the neonatal period with severe gram-positive or gram-negative bacterial infections or fungal infections. Among the best characterized are biallelic mutations of the genes encoding MyD88 or IL-1 receptor–associated kinase 4 (IRAK-4), which are cytoplasmic proteins that are required for both TLR and IL-1 receptor family (IL-1R) signaling (von Bernuth et al., 2012). The lack of IL-1R signaling results in an impaired febrile response, and the absence of any fever in a neonatal patient with a severe invasive bacterial or fungal infection should lead to an evaluation for these disorders. A useful screening test for MyD88 or IRAK-4 deficiency is to evaluate peripheral blood mononuclear cells for their production of TNF- α in response to incubation with TLR ligands, such as endotoxin, flagellin, or oligodeoxynucleotides containing unmethylated CpG residues. Such production is markedly impaired or absent in these patients.

Immunization

Other than access to proper sewage systems and clean drinking water, immunization is the most effective biomedical intervention to reduce early-life infection. Approaches to protecting the newborn and the young infant from infection include maternal immunization, with subsequent passive transfer of maternal antibodies to the fetus/newborn, as well as early-life immunization of the newborn and the young infant. Each of these approaches has important advantages and potential challenges.

Maternal Immunization

Maternal immunization can provide substantial benefits for both the mother and the infant by induction before or during pregnancy of maternal IgG antibody that can be transferred to the fetus during the last trimester of pregnancy and protect both the fetus and the mother against postpartum morbidity and death. Although immunization before or during pregnancy has been effective in preventing several specific neonatal infections, including diphtheria, pertussis, tetanus, hepatitis B, and rabies (Omer, 2017), there have

• BOX 36.1 Summary of Recommendations for Immunization During Pregnancy

Live Virus Vaccines

- Influenza (live attenuated influenza vaccine) – contraindicated
- Measles – contraindicated
- Mumps – contraindicated
- Rubella – contraindicated
- Yellow fever – safety not established (travel to high-risk areas only)
- Varicella – contraindicated

Inactivated Virus Vaccines

- Hepatitis A vaccine – consider if high risk of exposure
- Influenza vaccine – recommended
- Rabies vaccine – consider if indicated

Inactivated and Recombinant Bacterial Vaccines

- Cholera vaccine – to meet international travel requirements
- Meningococcal polysaccharide vaccine – consider if indicated
- Plague vaccine – selective vaccination of exposed persons
- Typhoid vaccine – safety not established
- Pneumococcal polysaccharide vaccine – consider if indicated
- Tetanus–diphtheria vaccine – consider if indicated
- Hepatitis B vaccine – consider if indicated
- Meningococcal conjugate vaccine – consider if indicated

Pooled Immune Serum Globulins

- Hepatitis A – postexposure prophylaxis
- Measles – postexposure prophylaxis

been theoretical concerns with this approach. For example, immunization during pregnancy might result in vaccine antigens interacting with vital fetal or placental tissues that could potentially lead to unanticipated maternal or fetal morbidity. Maternal immunization can also induce an antibody response in the fetus, as has been demonstrated with tetanus toxoid (Gill et al., 1983), and potentially could induce undesirable immunologic side effects (e.g., immunologic unresponsiveness or tolerance) in the infant. In practice, none of these concerns has been documented as major issues with maternal immunization with inactivated vaccines. Limitations of and challenges for maternal immunization include (1) incomplete adherence of pregnant mothers to outpatient visits/immunization and (2) preterm birth, affecting approximately 11% of all global deliveries such that preterm newborns may be born before transfer of protective maternal antibodies.

The priorities for active vaccination during pregnancy rest on assessment of maternal risk of exposure, the maternal–fetal–neonatal risk of disease, and the potential risks from the specific vaccine. A summary of recommendations for immunizations during pregnancy is provided in Box 36.1. In general, immunization with live viral vaccines during pregnancy is not recommended, and it is preferred that live viral vaccinations be performed before pregnancy occurs. However, rare instances may occur in which live viral vaccine administration is indicated. For example, if a pregnant woman travels to an area with high risk of yellow fever, administration of that vaccine might be indicated because of the susceptibility of the mother and the fetus, the probability of exposure, and the risk of the mother and fetus contracting the disease.

Immunization during pregnancy with tetanus toxoid to prevent neonatal tetanus illustrates the importance of the maternal immunization strategy in less developed countries where neonatal tetanus

remains prevalent. Such immunization is a cost-effective method for preventing neonatal tetanus, with two or more doses appearing to be effective in reducing both the incidence of disease and death resulting from it (Demicheli et al., 2015). Older studies suggest that maternal tetanus toxoid immunization may provide up to 10 years of protection for infants (Schofield, 1986; Gill et al., 1991; Vandelaer et al., 2003).

Maternal immunization during pregnancy with acellular pertussis vaccine has become an important strategy to address the worldwide problem of pertussis of the neonate and the young infant (Bento and Rohani, 2016; Sobanjo-Ter Meulen et al., 2016). Prevention of pertussis in the neonate and the young infant is paramount as infection can rapidly progress from a nondescript mild afebrile illness, with coryza, mild cough, and sneezing, and no pulmonary findings on physical examination to a life-threatening pneumonia with pulmonary hypertension (Cherry, 2016). The complete replacement in more developed countries of the more reactogenic whole-cell pertussis vaccine with the better tolerated acellular vaccine, which induces less durable immune responses and modifies the disease but not the acquisition of infection, is likely an important contributor to the resurgence of neonatal/young infant pertussis (Sobanjo-Ter Meulen et al., 2016). In the United States, all pregnant women are recommended to receive the acellular pertussis vaccine (usually given as part of a Tdap immunization) between 27 and 36 weeks' gestation, a strategy that aims to maximize the levels of pertussis-specific IgG that are transferred to the fetus by birth (Omer, 2017). Acellular pertussis vaccine is recommended with each pregnancy to ensure that adequate pertussis-specific IgG titers are present late in gestation. The use of a similar maternal vaccination strategy in the United Kingdom has been estimated to be 90% effective in preventing the acquisition of pertussis by infants aged 2 months or younger (Amirthalingam et al., 2014; Dabrera et al., 2015). However, there is evidence that maternal immunization late in pregnancy may also blunt the infant's pertussis-specific antibody response to the primary immunization doses of acellular vaccine (Ladhani et al., 2015). Whether such blunting is clinically significant and puts older infants at greater risk of acquisition of pertussis is unclear, particularly since this effect has not been observed following the toddler booster dose of acellular vaccine (Munoz et al., 2014). Clearly, there remain many major gaps in our knowledge of immune protection of humans from infection and disease with *Bordetella pertussis* that need to be filled so as to develop more effective pertussis vaccines (Vaughan et al., 2014; Plotkin, 2016).

Immunization of the Prematurely Born Infant

In general, preterm neonates of 24 weeks' gestation or greater produce antibody to T-dependent protein antigens such as tetanus and diphtheria toxoid and inactivated poliovirus contained in combination vaccines as do term neonates when the vaccines are administered at 2, 4, and 6 months of age (Gagneur et al., 2015). However, the antibody response to acellular pertussis vaccine, particularly for pertussis toxin, is reduced in preterm infants when they are immunized at the standard chronologic age, and this reduction in the antibody response remains evident at 5 to 6 years of age. Although the clinical significance of these decreased responses to acellular pertussis vaccine remain unclear, it has been suggested that prematurely born children might benefit from an earlier administration of the fifth dose of acellular pertussis vaccine, which is usually given between 5 and 6 years of age (Esposito et al., 2002).

The antibody response in premature infants to multiple doses of hepatitis B vaccine, initially administered at birth, is reduced

compared with that of term infants (Lau et al., 1992). The ultimate antibody titers are substantially increased if immunization of the premature infant is delayed until 5 weeks of age, indicating the importance of postnatal age rather than of a particular body weight (Kim et al., 1997). The benefits of such a delay for long-term hepatitis B-specific antibody titers are evident for at least the first 3 years of life (Linder et al., 2002). The approach for hepatitis B immunization of the prematurely born infant whose mother is hepatitis B surface antigen (HBsAg) positive is described in the section entitled **Neonatal Immunization**.

In most studies the *Haemophilus influenzae* type b (Hib) polysaccharide antibody levels after three doses of Hib polysaccharide–tetanus conjugate vaccine have been significantly less in premature infants than in term infants when vaccination is begun at 2 months of age (Greenberg et al., 1994). This reduced antibody response especially applies to premature infants with chronic lung disease (Washburn et al., 1993), whose antibody titers are likely to be depressed by systemic treatment with glucocorticoids for the treatment of bronchopulmonary dysplasia. Nevertheless, protective antibody responses are ultimately achieved following the third vaccine dose in most cases.

The antibody response of premature infants to the 13-valent pneumococcal conjugate vaccine (Prevnar 13) with a schedule of 2, 3, 4, and 12 months of chronologic age resulted in lower geometric mean titers than in term infants following the initial doses; however, most infants still achieved protective levels of antibody following the booster dose. Thus a four-dose regimen is recommended for premature infants consisting of three primary doses and one booster dose of the conjugate vaccine (Gagneur et al., 2015).

Collectively, these studies support the general approach for initiating the routine childhood vaccine series at 2 months of chronologic age regardless of the gestational age at birth. Extra or earlier administration of booster doses may be considered for some vaccines. More detailed recommendations are available from the Committee on Infectious Disease of the American Academy of Pediatrics at <http://reader.aappublications.org/red-book-30th-edition-2015/>.

Neonatal Immunization

Hepatitis B vaccine, polio vaccines, and bacille Calmette–Guérin (BCG) vaccine are the only childhood vaccines routinely given at birth. Immunization shortly after birth offers important logistic advantages for providing protection against infectious diseases as birth is the most reliable point of healthcare contact worldwide, especially in less developed countries (Demirjian and Levy, 2009). However, the response of the neonate to vaccination with T-independent polysaccharide vaccines, such as unconjugated pneumococcal vaccine, is negligible and the humoral response to T-dependent antigens, such as proteins and protein–polysaccharide conjugate vaccines, is less robust and durable than that of the adult (Siegrist, 2007). Thus extending neonatal immunization to involve other routine childhood vaccines may require the development of formulations that are more immunogenic when administered in the first few days after birth.

The Advisory Committee on Immunization Practices (ACIP) and American Academy of Pediatrics (AAP) both recommend universal immunization of infants to protect against perinatal transmission of hepatitis B virus (HBV) and chronic HBV infection. Chronic HBV infection occurs in approximately 90% of infected infants and is associated with hepatocellular carcinoma and cirrhosis leading to end-stage liver disease. In all medically stable infants

born to women with a negative test result for HBsAg and weighing more than 2000 g at birth, a hepatitis immunization schedule should be initiated in the newborn period before hospital discharge. Only single-antigen hepatitis B vaccines should be used for the birth dose (Mast et al., 2005). All infants regardless of gestational age at the time of birth and whose mothers have a positive test result for HBsAg should receive passive immunization with hepatitis B Ig and active immunization with a single-antigen hepatitis B vaccine within 12 hours of birth. Repeated vaccinations are given at 1 to 2 months of age and again at 6 months. For premature infants weighing less than 2000 g and born to HBsAg-positive mothers, the birth dose is not counted as part of the vaccine series, and these infants should receive a total of four doses of the vaccine as part of the primary series (Waitz et al., 2015). Special efforts should be made to complete the hepatitis B vaccination schedule within 6 to 9 months in populations of infants with high rates of childhood hepatitis B (Peter, 1994).

BCG, the live attenuated *Mycobacterium bovis*, is the most commonly administered vaccine in world history, with billions of doses given to date. A metaanalysis of randomized controlled trials of BCG immunization of the neonate and the young infant indicated that the vaccine reduced the risk of tuberculosis by more than 50% and that this protection appeared to persist for 10 years after vaccination (Colditz et al., 1995). The mechanisms underlying this protective effect remain unclear, and defining such mechanisms has been complicated by the use in human vaccination programs of more than 10 different BCG substrains, produced at different locations, that may differ in their biologic properties and immunogenicity (Frankel et al., 2016). An important source of BCG and other vaccines for low-income countries, many of which have high rates of endemic tuberculosis, has been the Expanded Program on Immunization of the World Health Organization. BCG appears to protect not only against its target pathogen (*Mycobacterium tuberculosis*) but also against a broad array of microorganisms (Aaby et al., 2014). Such heterologous (“nonspecific”) effects may be particularly important for newborns and infants as the heterologous benefit of BCG appears to be greatest when given early in life (Pettengill et al., 2014; Goodridge et al., 2016). Although BCG administration to neonates and infants has generally been very safe, severe disseminated BCG infection (BCGosis) can occur in infant patients who have SCID, chronic granulomatous disease, or mendelian susceptibility to mycobacterial disease; for example, genetic deficiencies of IL-12 receptor, IL-12 p40, IFN- γ receptor 1 or receptor 2, or STAT1 (loss-of-function mutations) (Norouzi et al., 2012). Thus BCG vaccination is contraindicated in patients known to have or suspected of having these PIDs or who have a family history of such disorders.

A growing appreciation of distinct immunity with age has prompted efforts to develop vaccines that may induce robust protection in the newborn and the young infant (Sanchez-Schmitz and Levy, 2011). One approach for improving the response to neonatal vaccination has been to combine vaccine antigens with adjuvants to enhance immune responses, focusing on adjuvants for which human neonatal APCs have robust adult-like responsiveness in vitro (Sanchez-Schmitz, 2011; Dowling and Levy, 2014). A promising example of this approach is to combine TLR agonists that are particularly active in early life such as imidazoquinoline TLR7/8 agonists with the 13-valent pneumococcal polysaccharide protein conjugate vaccine (Prevnar 13). Immunization of 1-day-old nonhuman primates with the combination of TLR7/8 agonist and Prevnar 13 resulted in a 10-fold to 100-fold increase in specific antibody titer that was associated with robust increases in the CD4 T-cell responses to the vaccine protein conjugate

(Dowling et al., 2017). A similar approach could be potentially used for other inactivated vaccines that currently are routinely administered at 2 months of age.

Overall, there is compelling rationale to characterize early-life immune ontogeny to provide fresh perspectives on neonatal health and disease and inform novel approaches to protect the fetus and young infant (Kollmann et al., 2017).

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Viral Infections of the Fetus and Newborn

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KEY POINTS

- Viral infections of the fetus and newborn are common problems in neonatology practice and must be considered in the differential diagnosis of newborns with intrauterine growth retardation, physical examination and laboratory abnormalities, and illness in the newborn period.
- The diversity of fetal and neonatal viral infections is vast. The depth and breadth of viral disease are extensive and the differential diagnosis broad.
- Clinicians should consider specific causes and not rely on broad-based categorical consideration of Toxoplasmosis, Other, Rubella, Cytomegalovirus, Herpes Simplex Virus (TORCH) syndromes, since specific signs, symptoms, and findings are quite diverse, depending on the pathogen being considered.
- Antiviral therapies are now available for many of these pathogens, underscoring the importance of making a specific diagnosis.
- Laboratory studies focused on virologic detection, driven in most cases by molecular assays, are much more precise and reliable than serologic diagnosis.

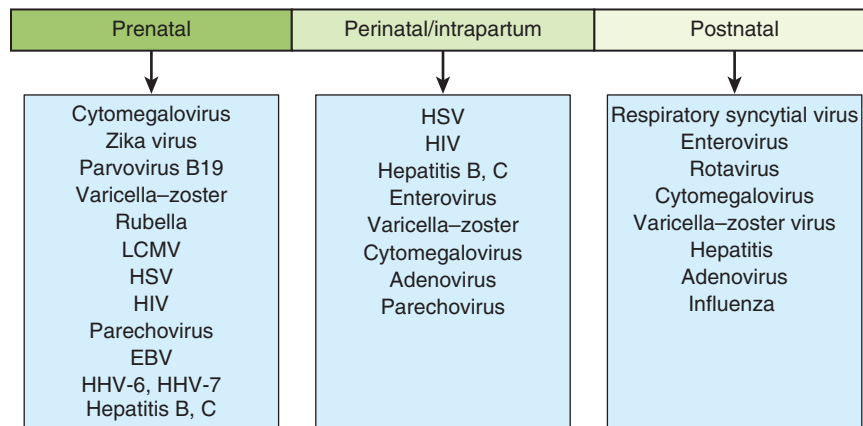
Viral infections of the fetus and newborn are common and underrecognized. Given the life-threatening nature of invasive bacterial disease in the neonate, the identification of viral infections is often relegated to a matter of secondary importance. However, identifying viral infections can also be a matter of great urgency, because antiviral agents are available for many of the most common infections. Accordingly, an appropriately high index of clinical suspicion in identifying neonatal viral infections can be lifesaving. Moreover, identification of viral disease in the neonate may be of great prognostic significance, particularly for identification of infants at high risk of long-term neurodevelopmental issues. Making a diagnosis of a neonatal viral infection can help to direct and focus anticipatory management by the child's pediatrician during the course of well-child care. This chapter reviews the epidemiology, pathogenesis, diagnosis, and short-term and long-term clinical management of many of the more common congenital and perinatal viral infections encountered in neonates.

One of the great challenges in the evaluation of viral disease in the newborn is the ascertainment of the timing of acquisition of infection. Some infections can be acquired either in utero or

in the early postnatal period. In this chapter, *congenital infection* is defined as any infection acquired in utero. *Perinatal infections* are defined as those acquired intrapartum, typically during the labor and delivery process. *Postnatal infections* are acquired in the postpartum period and are defined as infections acquired after delivery through the first month of life. In some situations, correctly identifying the timing of acquisition of infection can have substantial consequences, not only for the care of the infant but for predicting the long-term prognosis. Fig. 37.1 outlines the most common timing of acquisition of neonatal viral infections with an emphasis on the relative importance of the many viruses that can be acquired congenitally, perinatally, or postnatally. There is considerable overlap across categories for some viral infections that can be transmitted at any of these time points, and this will be considered on a pathogen-by-pathogen basis.

General Diagnostic Approach

Clinicians caring for newborns have long recognized that there are some common clinical manifestations that suggest the presence of a congenital or perinatal viral infection. These manifestations include evidence of intrauterine growth restriction (IUGR), microcephaly, hydrops fetalis, hepatomegaly, splenomegaly, pneumonitis, bone lesions, rashes, and hematologic abnormalities (Box 37.1). Because congenital viral infections are commonly encountered in neonatology practice (Alpert and Plotkin, 1986), it is appropriate for clinicians to have a high index of suspicion in any newborn with suggestive signs or symptoms. However, caution should be taken to thoughtfully consider diagnostic possibilities suggested by the history and physical examination, recognizing the specific etiologic diagnoses compatible with an infant's presentation. The unfortunate continued use of the term *TORCH titers* in the clinician's diagnostic approach to a symptomatic neonate oversimplifies and seriously underestimates the diversity of neonatal viral infections encountered in practice. The acronym *TORCH*, first coined by Nahmias et al. (1971), stands for *toxoplasmosis*, *other* infections, *rubella*, *cytomegalovirus* (CMV), and *herpes simplex virus* (HSV). Numerous variants of this acronym have been suggested in the past 4 decades (Kinney and Kumar, 1988; Ford-Jones and Kellner, 1995; Ronel et al., 1995; Tolan, 2008; Shet, 2011; Neu et al., 2015). Unfortunately, numerous clinical laboratories continue to offer the "TORCH panel," typically consisting of serologic tests



• **Fig. 37.1** Relative importance of neonatal viral infections related to the timing of acquisition of infection. Viruses are listed in declining relative order of importance relative to prenatal, perinatal (intrapartum), and postnatal timing of typical infection. Some neonatal virus infections (e.g., cytomegalovirus) can be substantial causes of disease whether acquired during gestation or acquired postpartum, whereas others (e.g., respiratory syncytial virus) are typically acquired in the postnatal period. *EBV*, Epstein–Barr virus; *HHV*, human herpesvirus; *HIV*, human immunodeficiency virus; *HSV*, herpes simplex virus; *LCMV*, lymphocytic choriomeningitis virus.

• **BOX 37.1** Clinical Features Commonly Associated With Congenital Viral Infections in Neonates

- Intrauterine growth restriction
- Nonimmune hydrops fetalis
- Echogenic bowel (prenatal ultrasonography)
- Placental villitis, chorioamnionitis
- Hepatosplenomegaly
- Jaundice (>20% direct-reacting bilirubin)
- Hemolytic anemia
- Purpura, ecchymoses, and petechiae
- Skeletal defects (“celery stalking”)
- Microcephaly and hydrocephaly
- Intracranial calcification
- Neuronal migration defects
- Pneumonitis
- Myocarditis
- Cardiac abnormalities
- Chorioretinitis
- Keratoconjunctivitis
- Cataracts
- Glaucoma

for toxoplasmosis, syphilis, HSV, CMV, and rubella. This acronym has outlived its usefulness (Lim and Wong, 1994) and should be discarded from clinical parlance, on the basis of the following considerations:

- Measurements of immunoglobulin G (IgG) antibody titers virtually always simply reflect transplacental maternal antibody and provide little information of relevance to the infant’s infection status. With the exception of the identification of antibodies to *Treponema pallidum*, which is always of interest and significance, antibodies against the other members of the TORCH panel are often of little diagnostic importance.
- Congenital and perinatal infection can occur with HSV and CMV, even in the face of preconception maternal immunity. Thus the finding of IgG antibody against these viruses in a

TORCH titer is neither diagnostic of infection nor reassuring with regard to protection against that infection.

- Highly sensitive virologic and molecular tools are available to identify virtually all pathogenic viruses. These tools include standard culture and nucleic acid identification techniques, typically based on polymerase chain reaction (PCR) amplification of viral nucleic acids. Such studies can facilitate rapid pathogen-specific diagnosis; therefore the diagnosis of neonatal viral disease should depend on diagnostic virology, not serology.
- Most importantly, the use of the acronym *TORCH* vastly underemphasizes the great diversity of viral pathogens that are associated with infections in the newborn. A list of the myriad of viral pathogens that have been reported to cause congenital infection and disease is included in Box 37.2. A discussion of many of the more unusual viral causes of congenital infection is beyond the scope of this chapter, but the list in Box 37.2 underscores the diversity of agents associated with fetal infection. A recent travel history or recent emigration might suggest consideration of some of these more unusual agents.

Rather than rely on a large battery of serologic tests, the astute clinician can typically formulate a focused differential diagnosis of a suspect neonatal or congenital viral infection with the history and physical examination, followed by the use of specific diagnostic studies emphasizing virologic and not serologic methods. Important questions include: What was the overall health of the mother during her pregnancy? What is her age and marital status (e.g., young, unmarried women have a higher risk of acquiring primary genital HSV infection)? What is her immunization history? Has she had chickenpox? Did she have other common childhood viral infections? What part of the world is she from? Is there a recent travel history? Are there potential animal exposures (e.g., exposure to cat litter or consumption of undercooked meat might suggest toxoplasmosis; exposure to rodents might suggest lymphocytic choriomeningitis virus [LCMV])? Does she have other children, and, if so, what are their ages, overall health status, and histories of group day care attendance? Have there been recent illnesses in the household? What time of year is it? (For example, respiratory syncytial virus [RSV] and enterovirus infections have characteristic seasonality in temperate zones.) What are her occupational

• BOX 37.2 Viral Pathogens Reported to Cause Congenital Infections

Adenoviridae

- Adenovirus serogroup 3

Arenaviridae

- Lymphocytic choriomeningitis virus
- Lassa fever virus

Bunyaviridae

- Bunyamwera serogroup (Cache Valley virus)
- La Crosse encephalitis virus

Flaviviridae

- Zika virus
- Hepatitis C virus
- Japanese encephalitis virus
- West Nile virus
- St. Louis encephalitis virus
- Yellow fever virus
- Dengue virus

Hepadnaviridae

- Hepatitis B virus

Herpesviridae

- Herpes simplex viruses 1 and 2
- Varicella zoster virus
- Cytomegalovirus
- Epstein–Barr virus
- Human herpesviruses 6 and 7

Orthomyxoviridae and Paramyxoviridae

- Influenza viruses
- Measles virus

Parvoviridae

- Human parvovirus B19

Picornaviridae

- Poliovirus
- Coxsackievirus
- Enteric cytopathic human orphan virus
- Parechovirus
- Hepatitis A virus

Retroviridae

- Human T-lymphotropic viruses 1 and 2
- Human immunodeficiency virus

Togaviridae

- Rubella virus
- Western equine encephalitis virus
- Venezuelan equine encephalitis virus

exposures? Did she have any symptomatic infectious illnesses during pregnancy? The answers to these types of questions, considered in the context of the infant's physical examination, can direct the next steps in establishing a definitive etiologic diagnosis.

Some of the classic presentations of the more common perinatal viral infections are reviewed in [Table 37.1](#). There can be considerable overlap of these clinical features across the different infectious categories listed; for example, the “blueberry muffin” rash of

TABLE 37.1 Clinical Findings in Selected Congenital and Perinatal Infections That Suggest a Specific Diagnosis

| Congenital Infection | Findings |
|--|---|
| Rubella | Cataracts, cloudy cornea, pigmented retina; petechiae with “blueberry muffin” rash; bone defects with longitudinal bands of demineralization (“celery stalking”); cardiovascular malformations (patent ductus arteriosus, pulmonary artery stenosis); sensorineural hearing loss; hydrops |
| Cytomegalovirus infection | Microcephaly with periventricular calcifications; chorioretinitis; petechiae with thrombocytopenia; jaundice; sensorineural hearing loss; bone abnormalities; abnormal dentition, hypocalcified enamel |
| Herpes simplex virus infection | Skin vesicles, keratoconjunctivitis, acute central nervous system findings (seizures), hepatitis, pneumonitis |
| Zika virus infection | Microcephaly, intracranial calcifications, retinitis, hearing loss |
| Parvovirus B19 infection | Hydrops, ascites, hepatomegaly, ventriculomegaly, hypertrophic cardiomyopathy, anemia |
| Varicella–zoster virus infection | Limb hypoplasia, dermatomal scarring in a cicatricial pattern, gastrointestinal tract atresia |
| Lymphocytic choriomeningitis virus infection | Hydrocephalus, chorioretinitis, intracranial calcifications |

congenital rubella syndrome (CRS) may be indistinguishable from that of congenital CMV infection, and both syndromes can include sensorineural deafness. The presence of brain calcifications and/or microcephaly, although nonspecific, should always suggest a differential diagnosis that includes CMV, toxoplasmosis, LCMV, parechoviruses, and Zika virus. Because neuroradiologic studies cannot reliably distinguish these entities, definitive diagnostic virology is necessary. Specific viral pathogens, their basic virology, the clinical manifestations of diseases they cause in the newborn, management strategies, and prospects for prevention are considered on a pathogen-specific basis in the remainder of this chapter.

Herpesviridae

Currently there are eight recognized human herpesviruses (HHVs), which are subdivided into three categories on the basis of aspects of viral biology, pathogenesis, and clinical presentations. These categories are the α -herpesviruses, consisting of HSV-1, HSV-2, and varicella–zoster virus (VZV); the β -herpesviruses, which include CMV and the roseola viruses HHV-6 and HHV-7; and the γ -herpesviruses, which include Epstein–Barr virus (EBV) and Kaposi sarcoma–associated herpesvirus (KSHV). Perinatal and congenital infections with this family of viruses have been the subject of several reviews ([Enright and Prober, 2004](#); [Schleiss, 2009](#); [Bialas et al., 2015](#)). Remarkably, all of these agents have been implicated in various degrees as causes of clinically important congenital and

perinatal infections, although these associations are less well studied for the γ -herpesviruses.

Herpes Simplex Virus Infections

HSV-1 and HSV-2 are highly related viruses. Although classically HSV-1 has been identified as a cause of oral infections (gingivostomatitis and pharyngitis), and HSV-2 has been implicated as the most common virus associated with genital herpes, in recent years these distinctions have become blurred. The greatest risk for the newborn is in the context of a first-time episode of maternal genital HSV infection occurring during pregnancy. The entity of neonatal herpes is reviewed in the following section, along with current management approaches for this infection.

Virology, Epidemiology, and Clinical Manifestations of Herpes Simplex Virus Disease

HSV-1 and HSV-2 demonstrate a high degree of similarity, both at the molecular level and in their clinical manifestations. The degree of genetic relatedness of these two viruses is approximately 45%, and the genome structures and morphology of the virion (virus particle) are virtually identical (Kieff et al., 1972). HSV-1 and HSV-2 are both acquired predominantly at mucosal surfaces and require intimate contact for transmission. After primary infection in epithelial cells, intra-axonal trafficking of viral DNA to the dorsal root ganglia results in the establishment of latency. The latent state is characterized by the cessation of virtually all gene transcription, except for the latency-associated transcript, which is expressed in the dorsal root ganglia even as the virus maintains a quiescent state (Taylor et al., 2002; Steiner et al., 2007; Nicoll et al., 2016). The function of the latency-associated transcript is unknown, but it might involve a novel ribonucleic acid (RNA)-mediated mechanism, because there does not appear to be a protein product associated with the transcript (Umbach et al., 2008). After a number of triggers, including ultraviolet radiation, stress, and immunosuppression, the virus reactivates at the level of the dorsal root ganglia and initiates a cascade of viral transcription that leads to the production of infectious virus, which can traffic via the axon to the cutaneous surface or ocular surface, producing lesions (Toma et al., 2008). The recrudescence of HSV lesions, usually manifested as vesicular or ulcerative lesions at the site of primary infection, can in turn lead to person-to-person transmission, including maternal-fetal and maternal-infant transmission. Asymptomatic or subclinical shedding of virus is well documented, particularly in the setting of genital herpes.

A wide variety of disease syndromes are associated with primary and recurrent HSV infection. Classically HSV-1 has been described as causing disease “above the belt,” whereas HSV-2 is associated with disease “below the belt.” The finding of HSV-2 antibodies in seroprevalence studies is generally viewed as indicative of genital herpes, and in the pregnant patient it can be considered to be diagnostic of genital herpes. The most common disease associated with HSV infection is herpetic gingivostomatitis, characterized typically by perioral and intraoral lesions involving the pharyngeal mucosa. HSV infection of the oropharynx may present in adolescence as herpetic pharyngitis (McMillan et al., 1993); it can be indistinguishable from other causes of pharyngitis. Other common manifestations of HSV infection include primary cutaneous infection, which can manifest itself as herpes gladiatorum or as a herpetic whitlow (Johnson, 2004; Wu and Schwartz, 2009; Sanders and Garcia, 2014), and eczema herpeticum, a serious illness that produces systemic symptoms (Bussmann et al., 2008). Exposure to an

individual (including a healthcare provider) with any of these cutaneous manifestations of HSV infection could potentially put a newborn at risk of acquisition of infection if care is not taken to protect the newborn. In contrast, HSV encephalitis is unlikely to be transmitted person-to-person, although HSV it is the most common sporadic cause of viral encephalitis in North America (Kaewpoowat et al., 2016). HSV encephalitis can be associated with primary infection or reactivation of latent infection. It is most commonly associated with HSV-1 (Baringer, 2008). Genital HSV infection is also an important risk factor for acquisition of human immunodeficiency virus (HIV) infection in the developing world (Corey et al., 2004).

From the pediatrician's perspective the most important manifestation of HSV disease is maternal genital herpes. Genital herpes can be associated with either HSV-1 or HSV-2. HSV is the most common cause of genital ulcerative disease in the developed world, and the prevalence has increased steadily in recent years (Fleming et al., 1997). Genital herpes is characterized by blisters, ulcers, or crusts on the genital area, buttocks, or both. Typically, symptomatic disease manifests itself with a mixture of vesicles, ruptured vesicles with resulting ulcers, and crusted lesions. Systemic flulike symptoms such as headache, fever, and swollen glands can accompany an outbreak of genital herpes, particularly during primary infection. Other symptoms include dysuria, urinary retention, vaginal or penile discharge, genital itching, burning or tingling, and groin sensitivity. Genital lesions differ in number, are painful in nature, and if untreated persist for up to 21 days (Whitley et al., 1998). Primary genital herpes during pregnancy can be associated with disseminated maternal disease, including severe hepatitis, disseminated intravascular coagulation (DIC), and central nervous system (CNS) infection (Young et al., 1976; Gelven et al., 1996; Frederick et al., 2002; Allen and Tuomala, 2005; Dodd et al., 2015). It has been recognized in recent years that many individuals with genital herpes are asymptomatic and unaware of their status (Wald et al., 2000; Groves, 2016). Therefore a negative maternal history of HSV *should not* dissuade the clinician from considering the possibility of neonatal herpes in an infant with compatible signs and symptoms. Patients with recurrent symptomatic episodes continue to shed virus in between episodes, even after lesions have healed and crusted over (Leone, 2005). This information has important implications for the continued evolution of the HSV epidemic in the United States. Because individuals with asymptomatic genital herpes shed virus frequently in the absence of lesions, all HSV-2-seropositive individuals are probably at risk of transmitting infection in a setting of sexual activity, labor and delivery, and intimate contact.

Neonatal Herpes

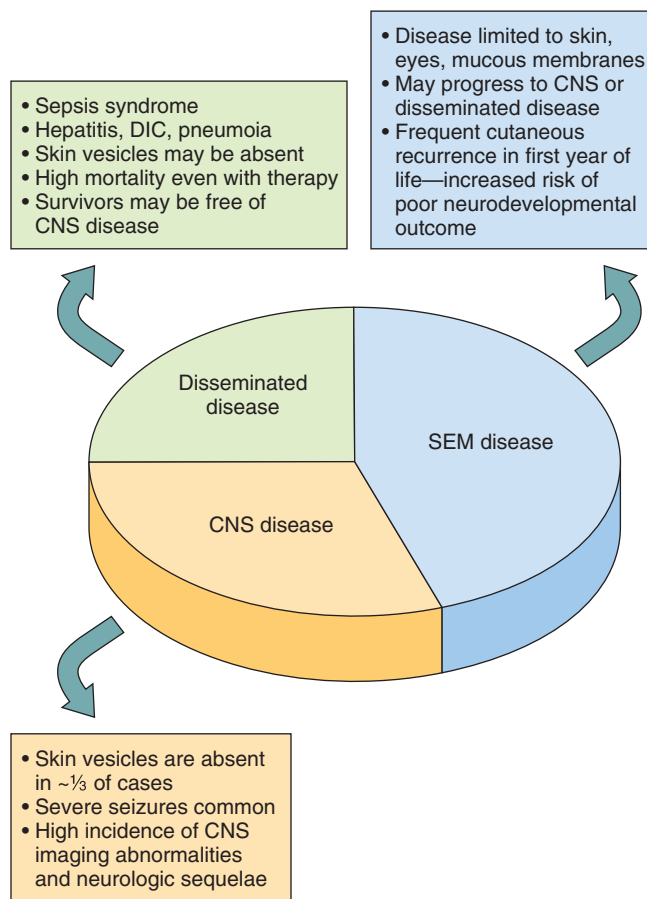
In the United States the reported incidence of neonatal herpes ranges from 1 in 2500 births to 1 in 8000 births (Whitley, 2004; Kimberlin, 2005; Corey and Wald, 2009; Chernes et al., 2012; Pinninti and Kimberlin, 2014). Approximately 70%–85% of neonatal herpes simplex infections are caused by HSV-2 (Kimberlin et al., 2001). It is estimated that 22% of pregnant women are infected genitally with HSV but that most of these women are unaware of their infection (Brown et al., 2005; Pinninti and Kimberlin, 2014). Approximately 2% of women acquire primary HSV infections during pregnancy (Brown et al., 2005). Most cases occur in neonates born to women who were recently infected, rather than to women with histories of recurrent genital herpes. Primary infection late in pregnancy poses a higher risk of transmission to the infant than do primary infections occurring before or

early in pregnancy, suggesting that the evolution of a maternal antibody response confers some measure of protection for the infant (Brown *et al.*, 2003; Caviness *et al.*, 2008a). In particular, the greatest risk of transmission to the fetus and the newborn occurs when an initial maternal infection is contracted in the second half of pregnancy (Straface *et al.*, 2012). Primary genital herpes infection in a pregnant mother results in an estimated attack rate of 33%–60% for her infant, whereas recurrent maternal infection results in a 1%–3% attack rate (Prober *et al.*, 1987; Arvin, 1991; Brown *et al.*, 1997; Pinninti and Kimberlin, 2014). Approximately 90% of these women are undiagnosed, because they are asymptomatic or have symptoms that are incorrectly attributed to other vulvovaginal disorders.

Rarely, cases of intrauterine infection have been described; these are often associated with overwhelming primary maternal infection and usually result in fetal demise. On occasion, placentitis is also observed (Florman *et al.*, 1973; Hutto *et al.*, 1987; Baldwin and Whitley, 1989; Chatterjee *et al.*, 2001; Barefoot *et al.*, 2002; Vasileiadis *et al.*, 2003; Low *et al.*, 2012; Pfister *et al.*, 2013). Most HSV infections in newborns are acquired intrapartum and related to the presence of virus in the maternal genital tract. Neonatal acquisition of HSV from an individual other than the mother, such as from a recurrent oropharyngeal lesion, is unusual (Linnemann *et al.*, 1978; Light, 1979; Yeager *et al.*, 1983). Neonatal HSV infection has been described as a complication of ritual circumcision (Rubin and Lankowsky, 2000; Gesundheit *et al.*, 2004; Leas and Umscheid, 2015). Approximately 85% of infants with neonatal HSV infection acquire the infection from the maternal genital tract at the time of delivery, but only 15%–30% of mothers who give birth to neonates with neonatal HSV infection have a known history of genital HSV infection (Whitley, 1988). Intrapartum interventions that have the risk of penetrating fetal skin, such as scalp electrode monitoring, increase the risk of transmission to the infant (Golden *et al.*, 1977; Parvey and Ch'ien, 1980).

Neonatal herpes can have devastating long-term consequences, making early recognition of paramount importance. Most newborns with perinatal or postnatal HSV infection are normal at birth. Illness typically develops after 3 days of age; therefore the presence of skin lesions, oral ulcers, and other signs and symptoms in the first 72 hours of life can suggest diagnoses other than HSV. Premature infants appear to be at greater risk, possibly because of reduced transplacental transfer of protective antibody. Approximately 40%–50% of affected infants are of less than 36 weeks' gestational age (Whitley, 1988). Although a history of maternal cervical, vaginal, or labial lesions should be sought when neonatal HSV infection is being considered in the differential diagnosis, overt herpetic disease in the maternal genital tract is evident in only approximately one-third of patients (Overall, 1994). In the remaining two-thirds of cases, infection occurs in the context of asymptomatic maternal genital tract shedding of HSV.

Infection can manifest itself in newborns in one of three forms: disease limited to the skin, eye, or mucous membrane (SEM disease); disease involving the CNS; or disseminated HSV infection, frequently manifesting itself as a sepsis-like syndrome, with pneumonia, hepatitis, and viremia (Fig. 37.2; Whitley, 2004; Kimberlin, 2005). There can be overlap in these syndromes; for example, an infant with disseminated disease may initially have only skin lesions. The relative proportion of infants with disseminated disease has been declining in recent years, probably because earlier recognition and treatment of SEM disease have resulted in more timely intervention with antiviral therapy. Disseminated disease usually begins toward the end of the first week of life. Skin vesicles may be an early sign,



• **Fig. 37.2** Characteristic presentations of neonatal herpes simplex virus (HSV) infection. Approximately 45% of neonatal HSV infections manifest themselves as skin, eye, or mucous membrane disease (SEM disease), 25% as disseminated disease, and 30% as central nervous system (CNS) disease. Characteristic features of each subtype of neonatal HSV are listed (arrows). Disease may span categories; for example, infants with SEM disease may progress to disseminated or CNS disease, and infants with CNS disease may develop skin vesicles later in the hospital course, although up to one-third of infants with CNS disease never have cutaneous manifestations. Overall, approximately half of all infants with neonatal HSV disease will have CNS involvement (CNS disease or disseminated disease with CNS involvement). DIC, Disseminated intravascular coagulation.

but they are entirely absent in almost half of patients. The scalp should be inspected carefully, particularly near the site of insertion of fetal scalp electrodes, because such lesions are easy to overlook. Systemic symptoms, although initially insidious in onset, progress rapidly. Poor feeding, lethargy, and fever may be accompanied by irritability or seizures if the CNS is involved. These symptoms are followed rapidly by jaundice, hypotension, DIC, apnea, and shock. This form of disease is indistinguishable at its onset from both neonatal enterovirus infection and bacterial sepsis. HSV infection should be considered in the differential diagnosis of infants who have fever during the first 2 weeks of life, because fever can herald the onset of systemic disease. Localized disease may begin somewhat later, with most cases appearing in the second to third weeks of life. When the CNS is the primary site of infection, the skin or eyes may or may not be involved: importantly, up to one-third of infants with documented neonatal HSV CNS disease will never have skin lesions during their clinical course (Thompson and

Whitley, 2011). These infants are lethargic, irritable, and tremulous, and seizures are common and often difficult to control.

Other less common but potentially localized findings are keratoconjunctivitis, chorioretinitis, and pneumonitis, which can manifest itself as a focal infiltrate or as diffuse bilateral disease. Secondary dissemination from a localized infection is common in the neonate. Acute retinal necrosis has been described in a series of infants with neonatal HSV infection (Venincasa et al., 2015). Intracranial hemorrhage, aseptic meningitis, and fulminant liver failure have been described (Kohl, 1994; Schlesinger and Storch, 1994; Greenes et al., 1995; Kohl, 1999; Abzug and Johnson, 2000; Erdem et al., 2002). Some of the less common presentations of neonatal HSV infection can include hydrops fetalis (Anderson and Abzug, 1999; Pfister et al., 2013), laryngitis (Vitale et al., 1993), and supraglottitis (Machin et al., 2013).

Diagnosis

The cornerstone of the diagnosis of neonatal HSV infection is virologic detection; serology is of limited use in the management of suspected infection in the infant, although type-specific serology is useful in evaluation of maternal HSV status in the setting of infants born to women with active genital HSV lesions (Kimberlin et al., 2013). HSV-1 and HSV-2 are both easily recovered by culture of clinical samples. In disseminated disease, virus is present in blood, conjunctivae, respiratory secretions, and urine; it is also present in the CNS in approximately half of patients. In SEM disease the virus can usually be found at the site of disease (i.e., within a vesicle). Definitive microbiologic diagnosis requires growth of the virus in tissue culture or detection of viral nucleic acid by PCR. HSV infection can also be confirmed by immunofluorescence of infected cells with use of HSV-specific antibodies (Sauerbrei, 2016), but this is largely being replaced by PCR. When neonatal herpes is suggested, specimens from the throat, conjunctiva, blood, stool or rectum, and urine should be obtained, as should scrapings of vesicular, pustular, and ulcerative skin lesions. Of these sites, skin and conjunctival samples have the highest yield (Kimberlin et al., 2001).

All infants with presumed neonatal HSV disease should undergo lumbar puncture, even if SEM disease is the only observed clinical manifestation. In some infants, CNS infection may be present but subclinical, and the finding of HSV DNA in the cerebrospinal fluid (CSF) has important therapeutic and prognostic implications. Blood should be sent for viral blood culture or PCR, because viremia is a common finding in neonatal HSV infection (Stanberry et al., 1994; Diamond et al., 1999). If the CNS is involved, evaluation of the CSF usually reveals a lymphocytosis, red blood cells, normal or high protein level, and low or normal glucose level. In addition to viral culture, CSF, blood, skin lesions, and other specimens should be analyzed by PCR for the presence of HSV genome (Ryan and Kinghorn, 2006). Caution should be taken with interpretation of a negative CSF PCR result: many infants with neonatal HSV disease will not have CNS involvement, so a negative CSF PCR result considered in isolation does not exclude the diagnosis of neonatal HSV. PCR of DNA extracted from the dried newborn blood spot has been reported as a way to retrospectively identify HSV infection in young infants (Lewensohn-Fuchs et al., 2003). Some experts recommend a second CSF specimen be obtained for evaluation at the end of antiviral therapy, and it has been reported that persistence of HSV DNA may be a poor prognostic factor and an indication for continuing antiviral therapy (Kimberlin et al., 1996b; Malm and Forsgren, 1999; Mejías et al., 2009). In disseminated disease, transaminase level elevations

consistent with hepatocellular injury are typically present; in severe disease, fulminant hepatitis with hepatic necrosis may be observed.

Infants with CNS disease should undergo neuroradiographic imaging with computed tomography (CT) or magnetic resonance imaging (MRI). Early in the course of illness, imaging may demonstrate nonspecific lack of gray matter–white matter junction differentiation and general signs of encephalitis. Later CT findings include dilated ventricles, parenchymal echogenicity, cystic degeneration, and intracranial calcifications (O'Reilly et al., 1995), whereas MRI images demonstrate a variable appearance (Vossough et al., 2008). Neonatal HSV-2 encephalitis can be multifocal or limited to only the temporal lobes, brainstem, or cerebellum. Deep gray matter structures are involved, and hemorrhage is observed in more than half of patients. In approximately 20% of patients, lesions are seen only by diffusion-weighted imaging. In 40% of patients, watershed distribution ischemic changes are also observed in addition to areas of presumed direct herpetic necrosis. Neurodevelopmental sequelae in infants have been correlated with MRI abnormalities, particularly with diffusion-weighted imaging, which appears to be a valuable prognostic adjunct in neonatal HSV disease (Bajaj et al., 2014). Electroencephalography (EEG) should also be considered in all infants with CNS involvement or with disseminated disease to evaluate them for seizures. EEG findings will be abnormal in approximately 80% of such patients (Kimberlin et al., 2001).

Treatment and Outcomes

The cornerstone of treatment of neonatal HSV disease is the nucleoside analogue *acyclovir*. The development of acyclovir in the 1980s was a watershed event in the management of HSV infection. The use of acyclovir and other antivirals is summarized in Table 37.2. The current recommendation of the American Academy of Pediatrics (AAP) Committee on Infectious Diseases is that neonatal HSV infections should be treated with intravenous (IV) acyclovir at a dosage of 60 mg/kg per day, divided into three doses given every 8 hours, for either 14 days for infants with SEM disease or 21 days for infants with disseminated or CNS disease. Infants with a persistent positive PCR result for HSV from blood or repeated CSF sampling at the end of 21 days of therapy should receive acyclovir for an additional 7 days (Kimberlin et al., 2013). Support for the dosage recommendation was derived from a comparison of the results from an earlier study using standard-dosage acyclovir (30 mg/kg per day) for 10 days (Kimberlin et al., 2001). There is no role for orally administered acyclovir in the initial management of neonatal HSV and no role for topically administered acyclovir in neonatal SEM disease. Herpetic keratoconjunctivitis should receive topical ophthalmic antiviral therapy along with parenteral treatment. In addition to antiviral therapy, appropriate supportive care is essential, with anticipatory management targeting complications of neonatal HSV disease such as seizures, pneumonitis, and hepatic insufficiency. A beneficial role of IV immunoglobulin (IVIG) has been suggested by animal models of neonatal HSV disease (Bravo et al., 1996), but the absence of controlled studies precludes any recommendations in infants.

Even with timely institution of antiviral therapy, the prognosis following neonatal HSV infection is guarded. The mortality rates in infants with localized CNS disease range from 4%–14% with antiviral therapy, and most survivors have long-term neurologic sequelae. Risk factors for increased morbidity and mortality associated with CNS infection include prematurity and seizures on initiation of therapy (Kohl, 1999; Kimberlin et al., 2001). Infants with disseminated disease have a high mortality rate without antiviral

TABLE 37.2 Antiviral Agents Commonly Used in Neonatology Practice

| Antiviral Agent | Indication | Dose, Route of Administration, Duration of Therapy | Comments |
|------------------|---|---|--|
| Acyclovir | Neonatal HSV infection | 60 mg/kg per day, dosing every 8 h intravenously; 21 days for disseminated infection or CNS disease; 14 days for SEM disease | Monitor CBC twice weekly; adjust dosage for renal insufficiency. |
| | Oral suppression following neonatal HSV infection | Efficacy for improving neurodevelopmental outcomes; recommended dosage regimen, 300 mg/m ² per dose, three times a day; duration of therapy, 6 months | Neutropenia observed in half to two-thirds of infants; lesions while receiving suppressive therapy should suggest an acyclovir-resistant strain |
| | VZV infection | 15 mg/kg every 8 h for a minimum of 5 to 7 days; longer courses may be needed for severe end-organ disease (pneumonia, hepatitis) | |
| | VZV postexposure prophylaxis | 10 mg/kg orally four times per day; treat for at least 7 days (from 7 days after the earliest exposure until 14 days after the last exposure) | Use in conjunction with VariZIG. |
| Trifluridine, 1% | HSV ophthalmic disease | Apply as eye drops; one drop every 2 h to the affected cornea while awake; maximum nine drops per day | Treat in consultation with an ophthalmologist. |
| Ganciclovir | Congenital CMV infection; acquired CMV infection | 12 mg/kg per day, dosing every 12 h intravenously; duration of therapy is 6 weeks for prevention of hearing loss; shorter courses of therapy (14 to 21 days) are reasonable for serious end-organ disease | Efficacy against CMV-associated hearing loss in controlled trial; benefits of shorter courses of therapy unknown; neutropenia observed in 63% of patients in controlled trial; adjust dose for renal insufficiency; consider use of G-CSF if continued therapy desired in the setting of neutropenia |
| Valganciclovir | Congenital CMV infection | 32 mg/kg per day divided twice daily; a controlled clinical trial of 6 months' oral suppression in infants with symptomatic congenital CMV infection demonstrated improved hearing and neurodevelopmental outcomes | Valine ester (prodrug) of ganciclovir; toxicity profile similar to that of ganciclovir; long-term suppressive therapy not well studied in infants; theoretical concerns of carcinogenesis, gonadal toxicity |
| Lamivudine | Hepatitis B, HIV infection | For children aged 3 months to 16 years, the recommended dosage is 4 mg/kg, up to 150 mg per dose, twice daily. | Chronic hepatitis B; also used for HIV therapy |
| Tenofovir | Hepatitis B, HIV infection | For children aged ≥12 years and weighing >35 kg, the recommended dosage is 300 mg daily. | Chronic hepatitis B |
| Entecavir | Hepatitis B | For therapy-naïve children older than 2 years and weighing >10 kg, the recommended dosages are 0.15 mg orally once a day for weight 10 to 11 kg, 0.2 mg orally once a day for weight >11 to 14 kg, 0.25 mg orally once a day for weight >14 to 17 kg, 0.3 mg orally once a day for weight >17 to 20 kg, 0.35 mg orally once a day for weight >20 to 23 kg, 0.4 mg orally once a day for weight >23 to 26 kg, 0.45 mg orally once a day for weight >26 to 30 kg, and 0.5 mg orally once a day for weight >30 kg. For children older than 2 years and weighing >10 kg who have previously received lamivudine the dosages are 0.3 mg orally once a day for weight 10 to 11 kg, 0.4 mg orally once a day for weight >11 to 14 kg, 0.5 mg orally once a day for weight >14 to 17 kg, 0.6 mg orally once a day for weight >17 to 20 kg, 0.7 mg orally once a day for weight >20 to 23 kg, 0.8 mg orally once a day for weight >23 to 26 kg, 0.9 mg orally once a day for weight >26 to 30 kg, and 1 mg orally once a day for weight >30 kg. | Chronic hepatitis B. Adjust dose for renal insufficiency |

**TABLE
37.2****Antiviral Agents Commonly Used in Neonatology Practice—cont'd**

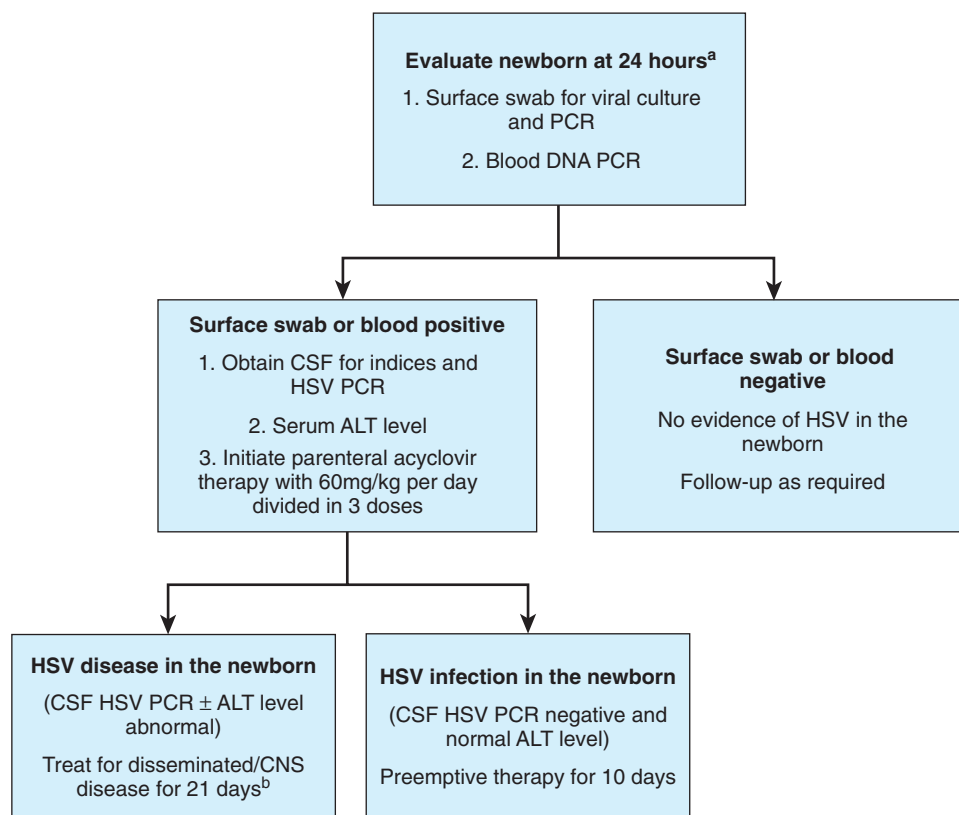
| Antiviral Agent | Indication | Dose, Route of Administration, Duration of Therapy | Comments |
|------------------------------|--|--|--|
| Interferon alfa-2b | Hepatitis B, hepatitis C | 3 million to 6 million international units per square meter three times per week; up to 24 months' duration; combined with oral ribavirin therapy for hepatitis C | Chronic hepatitis B; no data in neonates; chronic hepatitis C when administered with ribavirin; systemic side effects (fever, flulike symptoms, anorexia); leukopenia; thyroid autoantibodies |
| Pegylated interferon alfa-2b | Hepatitis B, hepatitis C | 1.5 µg/kg once per week; no information on dosing in children aged <2 years | Chronic hepatitis B; administer in conjunction with ribavirin for hepatitis C; systemic side effects (fever, flulike symptoms, anorexia); leukopenia; thyroid autoantibodies; side effects less common with pegylated formulations |
| Adefovir | Hepatitis B | Not recommended in children aged <10 years; 0.3 mg/kg orally once daily in children aged 2 to 6 years has favorable pharmacokinetics | Chronic hepatitis B; no safety or efficacy data in infants or young children |
| Ribavirin (oral) | Hepatitis C | 15 mg/kg per day orally; no information on dosing in children aged <2 years | Hemolytic anemia; teratogenic in animal models |
| Ribavirin (aerosol) | Respiratory syncytial virus infection | Standard ribavirin aerosol therapy is 6 g per 300 mL water for 18 h daily; short-duration therapy, 6 g per 100 mL water given for 2 h three times per day | Not indicated for use with mechanical ventilator; conjunctivitis; bronchospasm |
| Oseltamivir | Influenza A virus infection, influenza B virus infection | For children weighing <15 kg, the recommended dosage is 30 mg orally twice daily for 5 days (treatment) or 10 days (prophylaxis) | Not typically recommended in children aged <1 year; FDA-approved guidelines for emergency use of oseltamivir in pediatric patients younger than 1 year in 2009 in response to H1N1 influenza pandemic |
| Zanamivir | Influenza A virus infection, influenza B virus infection | Recommended dosage of zanamivir for treatment of influenza is two inhalations (one 5-mg blister per inhalation for a total dose of 10 mg) twice daily (approximately 12 h apart) | Not recommended in children aged <5 years |

CBC, Complete blood cell count; *CMV*, cytomegalovirus; *CNS*, central nervous system; *FDA*, Food and Drug Administration; *G-CSF*, granulocyte colony-stimulating factor; *HIV*, human immunodeficiency virus; *HSV*, herpes simplex virus; *SEM*, skin, eye, or mucous membranes; *VarizIG*, varicella-zoster immunoglobulin; *VZV*, varicella-zoster virus.

therapy (80% die), and most survivors have serious neurologic sequelae (Whitley, 1988; Thompson and Whitley, 2011). IV antiviral therapy has decreased disseminated disease mortality to approximately 30%, and approximately 80% of surviving infants have a normal neurologic outcome (Corey and Wald, 2009). Antiviral therapy has decreased neonatal CNS infection mortality from approximately 50% to 6%, but more than 70% of survivors have sequelae (Freij and Sever, 1988; Engman et al., 2008; Corey and Wald, 2009). Lethargy at initiation of therapy has been associated with a higher mortality rate in neonates with disseminated HSV infection (Kimberlin et al., 2001). Infants with skin involvement often have recurrent crops of skin vesicles for several years. In an infant younger than 6 months, readmission to the hospital for evaluation and IV acyclovir is appropriate when cutaneous recurrences are observed.

Treatment of asymptomatic neonates potentially exposed to HSV in the birth canal is controversial. This situation sometimes arises when a maternal perineal lesion is discovered after vaginal delivery. Some experts have in the past recommended neonatal surface cultures and administration of prophylactic antivirals, but there has little evidence to support this approach. A recent set of

guidelines endorsed by the AAP provided evidence-based guidance on the treatment of neonates born to women with active genital herpetic lesions (Kimberlin et al., 2013; Pinninti and Kimberlin, 2014). These guidelines are predicated on the availability of type-specific PCR and serology for HSV and emphasize that decision making should be predicated on the nature of the maternal infection (primary versus nonprimary) and the results of diagnostic virology evaluation of the newborn using samples obtained at 24 hours of age. For treatment of newborns born to women with HSV genital lesions at delivery and a history of genital herpes, careful coordination between the pediatric and obstetric teams is required, and maternal PCR and culture of suspect lesions coupled with serologic assessment are required, along with virologic testing of the newborn at 24 hours of age. These recommendations are summarized in Fig. 37.3 and are applicable to infants born by caesarean delivery or by the vaginal route. For infants born to women with lesions at delivery and no history of genital herpes before pregnancy, it is recommended that maternal PCR and culture of vaginal lesions be performed (both to identify and to type HSV) and that the newborn be evaluated at 24 hours of age by obtaining surface swabs for culture and PCR, blood for HSV PCR, CSF for HSV



^aWaiting for 24 hours after delivery is recommended to differentiate contamination of neonatal skin by maternal secretions versus true HSV infection of the baby.

^bAfter completion of parenteral therapy, treat with oral acyclovir suppressive therapy for 6 months (300mg/m² per dose three times per day).

• **Fig. 37.3** Treatment of a newborn born to a woman with presumed active genital herpes simplex virus (HSV) lesions at delivery in the setting of recurrent genital HSV infection. A waiting period of 24 hours is currently recommended in this setting before surface cultures are obtained and polymerase chain reaction (PCR) for HSV genome detection (and blood for HSV PCR) from the neonate. This approach aids in differentiation of contamination of neonatal skin from maternal secretions from true infection of the newborn. ALT, Alanine aminotransferase; CSF, cerebrospinal fluid.

PCR, and serum alanine aminotransferase (ALT) level and that parenteral acyclovir therapy (60 mg/kg per day in three divided doses) be commenced. The duration of therapy is then dictated by the results of the diagnostic evaluation. For infants born in settings where PCR and type-specific serology may not be readily available, the most important variable informing clinical management is the maternal history. Infants born to women with primary, first-episode genital HSV infection have an up to approximately 60% risk of neonatal HSV infection (Brown et al., 2003). On the other hand, term infants exposed to HSV in the birth canal and born to women with long-standing histories of recurrent genital herpes are at low risk (<1%) of infection, and if the infant is asymptomatic, observation without antiviral therapy is sufficient. If the maternal HSV history is unclear, the infant is premature, or other obstetric complications or risk factors are present (e.g., prolonged rupture of membranes, maternal fever, signs or symptoms of chorioamnionitis, fetal scalp electrode monitoring), then empiric antiviral therapy is warranted. In settings where PCR and type-specific serology may not be readily available, after the appropriate specimens have been collected and sent for virologic analysis, the infant should receive parenteral acyclovir therapy (60 mg/kg per day) pending the results of diagnostic virology studies.

Similarly controversial is the question of whether empiric acyclovir therapy should be administered to neonates who are admitted for evaluation of febrile illnesses in the first 30 days of life, the so-called rule-out sepsis evaluation. Standard practice in most children's hospitals is to rule out sepsis in this setting by administration of broad-spectrum antibiotics pending the result of diagnostic cultures of blood, urine, and CSF. Some experts recommend the use of empiric acyclovir therapy in this setting (Long, 2008), citing data that indicate that the prevalence of neonatal HSV infection is similar to that of invasive bacterial infection in this setting and that infants may exhibit fever and sepsis-like syndrome as the only manifestations of HSV infection (Caviness et al., 2008b). Other experts recommend a more selective approach, based on analysis of history, risk factors, and laboratory and radiographic analyses, such as liver function tests and chest radiograph (Kimberlin, 2008; Gaensbauer et al., 2014). A retrospective analysis of an empiric acyclovir strategy restricted to newborns with onset of febrile illness at 21 days of age or earlier, who would typically receive empiric parenteral antibiotic therapy, captured 90% of HSV cases and anticipated a rate of HSV CNS infection similar to that of bacterial meningitis (Long et al., 2011). An estimated 1.3% of empirically treated patients in this study had

HSV infection. Another study, of 49 infants evaluated for fever at less than 42 days of age, observed that most of the infants with documented HSV infection (84%) had seizure, vesicular rash, or a critical level of illness. A subset of patients (16%) lacked classic signs at hospitalization; most manifested signs suggestive of HSV within 24 hours (Curfman et al., 2016).

In addition to its critical role in the management of the acute clinical syndromes of neonatal HSV infection, acyclovir may be of benefit when administered as long-term suppressive therapy in the first 6 months of life. The Collaborative Antiviral Study Group (CASG) has reported results of controlled studies evaluating long-term oral suppressive acyclovir therapy after neonatal HSV infection aimed at prevention of both recurrent skin lesions and neurologic sequelae. The observation driving this study is that recurrent skin lesions (more than three episodes) within the first 6 months of life predict an adverse neurologic prognosis, possibly because recurrent skin lesions are a surrogate marker for subclinical reactivation events in the CNS (Whitley, 1991). Phase I and phase II trials demonstrated fewer cutaneous recurrences in the treatment group but did not have enough participants to analyze the effect on neurologic outcomes (Kimberlin et al., 1996a). These observations supported the hypothesis that reduction of cutaneous recurrences in the first year of life could result in improved neurodevelopmental outcome (Gutierrez and Arvin, 2003). That long-term oral suppressive therapy may be beneficial for improving long-term neurodevelopmental outcomes with regard to neonatal HSV infection was suggested by a 2-year pilot study of oral suppressive therapy in a cohort of 16 infants (Tiffany et al., 2005). In this uncontrolled study, all children were independently mobile, free of seizures, and had normal vision and speech development at the time of final neurodevelopmental assessment. A phase III, placebo-controlled trial performed by the CASG confirmed that acyclovir suppressive therapy for 6 months after completion of IV therapy for neonatal HSV disease resulted in improved neurodevelopmental outcomes (Kimberlin et al., 2011). Infants with CNS disease randomized to receive oral acyclovir therapy had improved neurodevelopmental outcome and fewer frequent recurrences of skin lesions while receiving therapy. The results of this study have led to a recommendation that infants with neonatal HSV infection of any disease classification should receive oral acyclovir therapy (recommended dosage regimen 300 mg/m² per dose, three times a day) for 6 months. Absolute neutrophil counts should be monitored at 2 and 4 weeks, and monthly thereafter, after commencement of suppressive therapy (Pinninti and Kimberlin, 2014). The emergence of acyclovir-resistant HSV strains is a concern, both during treatment for the acute episode of neonatal infection and in infants receiving long-term suppressive therapy (Kimberlin et al., 1996b; Oram et al., 2000; Levin et al., 2001; Kakiuchi et al., 2013; Bache et al., 2014). More data are needed on the long-term risks of antiviral resistance and on novel treatment alternatives (Birkmann and Zimmermann, 2016). In contrast, no long-term benefit of suppressive nucleoside therapy was noted in adult survivors of HSV encephalitis (Gnann et al., 2015).

Prospects for Prevention

Recent developments focused on preventing HSV infections among women of childbearing age include consideration of maternal screening programs and the use of antiviral agents in women at risk of transmission of infection. The availability of reliable kits that allow specific serodiagnosis of HSV-1 and HSV-2 infections has enabled the identification of asymptomatic women with genital herpes who can be counseled appropriately during pregnancy

(Sauerbrei and Wutzler, 2007). Although routine seroscreening of pregnant women is not currently considered a standard of care, screening in some instances, including situations where high maternal anxiety exists, seems justified. There is no evidence that routine administration of suppressive antivirals during pregnancy can improve outcomes or reduce disease in newborns (Sheffield et al., 2006), although this approach has become common in many obstetric practices (Hollier and Wendel, 2008). One benefit of suppressive antiviral therapy during pregnancy for a woman with a history of genital herpes may be a reduced likelihood of caesarean delivery (Brown et al., 2003; Hollier and Wendel, 2008). It is important to recognize that transmission of virus and subsequent symptomatic neonatal HSV infections have been described even in the face of maternal suppressive therapy with antivirals (Pinninti et al., 2012). Strategies for preventing neonatal herpes can also target prevention of intrapartum transmission by caesarean delivery. If a mother has active genital HSV infection at the time of delivery, and if the membranes are intact or have been ruptured for less than 4 hours, both the AAP and the American College of Obstetricians and Gynecologists recommend caesarean delivery. The role of intrapartum antiviral therapy or IVIG has not been studied in this setting.

Even with careful histories and meticulous physical examination during labor and delivery, many at-risk deliveries cannot be predicted or identified, because so many HSV-2-seropositive women are asymptomatic, do not know that they have genital herpes, and may unknowingly shed virus at the time of delivery. Infants in whom HSV infection is known or highly suspected should be isolated with contact precautions, and skin lesions should be covered. Finally, any healthcare provider with active herpetic whitlow or other skin lesions should not have direct patient care responsibilities for neonates.

Several phase I, II, and III clinical trials of HSV vaccines are ongoing (Stanberry and Rosenthal, 2005). A vaccine that prevented genital HSV infection, or reduced HSV shedding, would in principle reduce the incidence of neonatal HSV infection. A double-blind, randomized trial of an HSV-2 glycoprotein D subunit vaccine given with alum adjuvant and 3-O-deacylated monophosphoryl lipid A was reported in a study of individuals whose regular sexual partners had a history of genital herpes. The vaccine was efficacious in women who were seronegative for both HSV-1 and HSV-2, but it was not efficacious in women who were seropositive for HSV-1 and seronegative for HSV-2 at the baseline. The vaccine had no efficacy in men, regardless of serostatus (Stanberry et al., 2002). Other live-attenuated and subunit HSV vaccines are currently in development (Iwasaki, 2016; Johnston et al., 2016).

Varicella–Zoster Virus

VZV is a member of the α -herpesvirus subfamily of the *Herpesviridae*. Like the related HSV-1 and HSV-2, VZV can infect neurons, where it establishes latent infection. Primary varicella infection, commonly known as *chickenpox*, usually results in a fever and a characteristic vesicular exanthem. The illness often includes other systemic symptoms, such as headache and malaise. Reactivation from latency, which can occur years or decades after the primary VZV infection, is usually referred to as *zoster* or *shingles*. Zoster is characterized by a painful vesicular rash in a dermatomal distribution and in older patients can lead to postherpetic neuralgia.

The neonatologist may encounter consequences of maternal VZV infection in two different clinical presentations. In *congenital* varicella, VZV is transmitted to the fetus in the first or second

trimester of pregnancy, where it can produce a number of teratogenic consequences (Laforet and Lynch, 1947; Sraubstein et al., 1974; Auriti et al., 2009; Smith and Arvin, 2009). In contrast, *neonatal* varicella occurs in the setting of primary maternal varicella acquired late in the third trimester, and the affected infant can exhibit symptoms and signs in the neonatal period. This section reviews both of these presentations of VZV-related disease in infants.

Epidemiology of Maternal and Perinatal Varicella–Zoster Virus Infection

Before the advent of routine childhood vaccination against chickenpox, it was estimated that the VZV seroprevalence in women of childbearing age was greater than 95%, because of the formerly ubiquitous nature of this infection. In this era, primary VZV infections during pregnancy occurred with a frequency of 5 to 7 per 10,000 pregnancies in the United States (Balducci et al., 1992; Brunell, 1992). The major concern in the setting of primary maternal VZV infection is the risk of congenital varicella syndrome (CVS). For pregnant women with primary varicella infection, the rate of transmission to the fetus is estimated to be approximately 25%. Only a subset of infected fetuses exhibit symptomatic disease. Approximately 130 cases of CVS were reported in the literature between 1947 and 2013 (Sauerbrei and Wutzler, 2000; Ahn et al., 2016). The risk of symptomatic intrauterine VZV infection after maternal varicella occurring during the first 20 weeks of pregnancy is approximately 1%–2% (Siegel, 1973; Paryani and Arvin, 1986; Enders et al., 1994; Pastuszak et al., 1994). In a systematic review the estimated incidence of CVS was 0.59% for women infected with VZV during the entire pregnancy and 0.84% for those infected during the first 20 weeks of pregnancy (Ahn et al., 2016). Other smaller case series have found fetal infection rates in the range of 0.8% in women with primary infections (varicella-like rash and positive immunoglobulin M [IgM] serologic findings; Sanchez et al., 2011). Symptomatic disease appears to be more common in female infants (Sauerbrei and Wutzler, 2000). CVS has been reported in two pregnancies following maternal zoster (Ahn et al., 2016), although this is very rare insofar as a prospective study of infants born to 366 mothers with a clinical history of zoster during pregnancy found no infants with CVS (Enders et al., 1994).

Maternal infection in the third trimester is not associated with CVS, presumably because this falls outside the time frame when VZV is teratogenic to the developing fetus. Maternal infection just before or after delivery poses a high risk of neonatal varicella. Before the advent of VZV vaccination, neonatal varicella was encountered much more commonly than CVS. For infants in whom maternal illness begins 5 days or less before delivery or up to 2 days after delivery, the infant attack rate is 17%–31% (Meyers, 1974; Feldman, 1986; Brunell, 1992). Because the incubation period for varicella is between 10 and 21 days, cases beginning in the first 10 days of life are considered to have been acquired in utero.

Pathogenesis and Clinical Manifestations

CVS is acquired from a maternal primary varicella infection that occurs during the first or second trimester. The virus is thought to be transmitted transplacentally during the viremia that precedes or accompanies the rash of chickenpox. Ascending infection from cervical infection has been proposed as a potential mechanism of transmission but is probably much less common (Sauerbrei and Wutzler, 2000). It has been proposed that some of the congenital malformations associated with CVS may be a consequence of zoster-like virus reactivation events in the infected fetus rather

than the direct effects of the primary viral infection. This explanation is supported by the common finding of unusual cicatricial rashes in dermatomal distributions (more compatible with zosteriform reactivation events than primary skin infection) in newborns with CVS. Other clinical manifestations include asymmetric muscular atrophy with limb hypoplasia, low birth weight, neurologic abnormalities (cortical or spinal cord atrophy, seizures, microcephaly, encephalitis, Horner syndrome), and ophthalmologic abnormalities (chorioretinitis, microphthalmia, atrophy, and cataracts; Brunell, 1992; Feldman, 1986; Sauerbrei and Wutzler, 2000). Gastrointestinal (GI) abnormalities are reported in 15%–23% of cases; findings include duodenal stenosis, dilated jejunum, small descending colon, intestinal atresia or bands, and hepatic calcifications (Alkalay et al., 1987; Jones et al., 1994). Immature fetal cell-mediated immune response may explain the short latency period and the inadequate protection from the consequences of episodes of reactivation in utero (Higa et al., 1987; Kustermann et al., 1996). Pathology reports have noted destruction of neural tissue with residual dystrophic calcifications, chronic active inflammation in nonneural tissues surrounding viral inclusions, and evidence of chronic placental villitis (Qureshi and Jacques, 1996; Bruder et al., 2000; Petignat et al., 2001).

Maternal varicella near term or immediately postpartum can lead to neonatal varicella. Neonatal varicella is usually caused by maternal chickenpox acquired during the last 3 weeks of pregnancy. Infection may be transmitted perinatally by transplacental viremia (most cases) or by ascending infection from the birth canal; it can also be contracted postnatally by the aerosol route or by direct contact with infectious lesions. Serious postnatal infection acquired from maternal varicella via breastfeeding has not been reported. Neonatal varicella may develop despite the administration of varicella–zoster immunoglobulin (VariZIG) to the infant at birth. Transplacentally transmitted infections occur in the first 10 to 12 days of life, whereas chickenpox after that time is most likely acquired by postnatal infection. The clinical presentation differs markedly for cases in which maternal rash began 5 days or more before delivery from those in which maternal illness occurred from 5 days before to 2 days after delivery. When maternal varicella is noted more than 5 days before delivery, neonatal disease usually begins within the first 4 days of life and is typically mild. In contrast, neonatal varicella in a newborn whose mother develops varicella from 5 days before until 2 days after delivery has a high risk of morbidity and mortality (Isaacs, 2000; Tan and Koren, 2006). In this second group, neonatal disease typically begins between 5 and 10 days after delivery, and a fatal outcome has been reported in 23%–30% of cases (Brunell, 1966; Sauerbrei and Wutzler, 2001). When the disease appears in this setting, it closely resembles varicella in the immunodeficient or immunosuppressed host. Recurrent crops of skin vesicles develop over a prolonged period. Typical presenting signs are fever, hemorrhagic rash, and visceral dissemination with involvement of the liver, lung, and brain. Secondary bacterial infection may occur.

Prospective studies of the long-term outcomes of CVS have not been performed, and there is probably a wide continuum of disease. Among the 96 infants reviewed by Sauerbrei and Wutzler (2000), 14 (15%) had clinical signs of zoster during early infancy; these infants had been exposed to varicella between 8 and 24 weeks' gestation. Some experts consider early zoster infections as one criterion for diagnosis of CVS. Between 1% and 2% of infants without clinical evidence of CVS but whose mothers had chickenpox in the second and third trimesters develop zoster in the first few weeks of life; if this occurs, consideration should be given to

ophthalmologic evaluations to rule out the possibility of CVS (Sauerbrei and Wutzler, 2000; Enders et al., 2004). Overall mortality rates for CVS are estimated at 30%. Deaths occur in the first few months of life, usually secondary to severe pulmonary disease (Pastuszak et al., 1994; Sauerbrei and Wutzler, 2000).

Diagnostic Studies

Prenatal diagnosis—by means of quantification of varicella-specific IgM on fetal blood obtained by cordocentesis or through PCR analysis of chorionic villi, fetal blood, and amniotic fluid—has been attempted (Cuthbertson et al., 1987; Kustermann et al., 1996; Mouly et al., 1997). Although the yield of PCR on amniotic fluid or fetal blood is higher than that of serologic analysis of fetal IgM or viral cultures, large prospective studies of the correlation of such findings with clinical outcomes have not been performed. Reported prenatal ultrasonographic findings include polyhydramnios, hydrops, progressive IUGR, microcephaly, limb hypoplasia, and liver hyperechogenicities (Pretorius et al., 1992; Petignat et al., 2001). Abnormal ultrasonographic findings might not develop in a fetus for at least 5 weeks after maternal infection (Kerkering, 2001).

Serologic studies can be performed postnatally on infants (Paryani and Arvin, 1986). Suspected congenital infection with varicella can be confirmed by the finding of persistent VZV IgG beyond the presumed duration of passive transfer of maternal antibodies (at least 6 to 7 months). Detection of fetal IgM can also confirm infection, but it is less useful because only one-fourth of infants reported with classic CVS have positive VZV IgM titer test results (Enders et al., 1994; Sauerbrei and Wutzler, 2000). Highly sensitive direct fluorescent antibody (DFA) tests are available and demonstrate high levels of sensitivity and specificity (Coffin and Hodinka, 1995; Chan et al., 2001). VZV can also be detected from skin lesions by PCR (Leung et al., 2010). DFA and PCR are often extremely low yield in infants with suspected congenital infection compared with infants and children who have acquired varicella infections. Neuroradiographic demonstration of intracranial calcifications has been reported with CVS, but this is not a common finding (Kerkering, 2001).

Treatment

No controlled studies examining the effect of antiviral therapy to prevent or treat CVS have been conducted. Some experts have recommended oral acyclovir therapy for pregnant women with varicella, especially during the second and third trimesters (American Academy of Pediatrics Committee on Infectious Diseases, 2015). IV treatment with acyclovir for the infected pregnant woman is recommended for patients with serious complications of varicella, particularly pneumonia. Anecdotal observations suggest a potential effect of acyclovir on the progression of eye disease in CVS (Sauerbrei and Wutzler, 2000). However there are no recommendations for the use of acyclovir or IVIG for treatment or prevention of CVS. For neonatal varicella, treatment with IV acyclovir, 60 mg/kg per day divided into doses every 8 hours, is recommended, particularly for infants at the highest risk of adverse outcomes (i.e., the infant born to a woman who develops varicella from 5 days before until 2 days after delivery; Table 37.2).

Prevention

Women who develop VZV infection in pregnancy should be informed of the potential adverse maternal and fetal sequelae, the risk of transmission to the fetus, the options available for prenatal diagnosis, and potential therapies (Shrim et al., 2012). Treatment of a pregnant patient following exposure to VZV or on the

• BOX 37.3 Candidates for Varicella–Zoster Immunoglobulin After Significant Exposure to Varicella–Zoster Virus Infection in Perinatology and Neonatology Practice

- Pregnant women without evidence of immunity
- Newborn whose mother develops chickenpox within 5 days before delivery or within 48 hours after delivery
- Hospitalized premature infant (≥28 weeks' gestational age) whose mother has no history of chickenpox or serologic evidence of prior infection
- Hospitalized preterm infants (<28 weeks' gestational age or birthweight ≤1000 g) regardless of the maternal history of varicella or varicella–zoster virus serostatus

development of an illness compatible with a primary VZV infection is challenging (Benoit et al., 2015). In late 2012, VariZIG was approved by the Food and Drug Administration (FDA) for postexposure prophylaxis of varicella for individuals at high risk of severe disease (Centers for Disease Control and Prevention, 2013). If a susceptible pregnant woman has a significant exposure to varicella, administration of VariZIG to her and her newborn should be considered. These recommendations are summarized in Box 37.3. Pregnant patients who lack a history of VZV infection should receive VariZIG as soon as possible following VZV exposure, up to 10 days but ideally within 96 hours of exposure, for the greatest effectiveness (Bapat and Koren, 2013). Newborns of mothers in whom varicella develops from 5 days before to 2 days after delivery should receive VariZIG as soon as possible. VariZIG is given intramuscularly at a recommended dose of 125 units per 10-kg bodyweight, up to a maximum of 625 units. If VariZIG is unavailable, IVIG (400 mg/kg) can be a substitute. Passive immunoprophylaxis has been shown to prevent chickenpox in exposed older children (Brunell et al., 1969) but does not always prevent neonatal disease (Reynolds et al., 1999). Approximately 50% of exposed infants treated with immunoglobulin can still develop varicella, but the disease is often attenuated, and approximately 10% have severe disease (Hanngren et al., 1985). For healthy term newborns exposed postnatally to varicella, including newborns whose mother's rash began more than 48 hours after delivery, VariZIG is not generally indicated. VariZIG is not indicated for an infant whose mother has zoster. Breastfeeding is not contraindicated in newborns born to women with VZV infection.

Follow-up of infants exposed to VZV and treated with VariZIG can include consideration of serologic testing (enzyme immunoassay, latex agglutination, or indirect fluorescent antibody staining for IgG) to determine whether asymptomatic infection has elicited immune protection. Some experts recommend repeated administration of VariZIG after a repeated exposure of an infant in whom varicella did not develop more than 3 weeks after administration of the initial dose of VariZIG, although the risk to these infants is less well defined. Infants receiving VariZIG should also be placed in respiratory isolation for 28 days or until discharge, because administration of VariZIG can prolong the incubation period.

In the event of a significant varicella exposure in a newborn nursery, infants whose mothers have no history of chickenpox and who have undetectable antivariella antibody titers should be considered candidates for VariZIG. All exposed infants of less than 28 weeks' gestational age or with birthweights less than 1000 g,

regardless of maternal history, should receive VariZIG (Box 37.3). The decision to use VariZIG in the premature infant older than 28 weeks' gestation should be predicated on maternal history of chickenpox or serologic evidence of protection.

Acyclovir has also been recommended by some experts for postexposure prophylaxis in the setting of a nursery outbreak of VZV infection (Hayakawa et al., 2003; Shinjoh and Takahashi, 2009). A suggested dosage is 10 mg/kg by mouth four times per day for 7 days. All exposed healthcare professionals without evidence of immunity should be excused from patient contact from day 8 to day 21 after exposure to an infectious patient or to day 28 if the individual has received VariZIG (American Academy of Pediatrics Committee on Infectious Diseases, 2015).

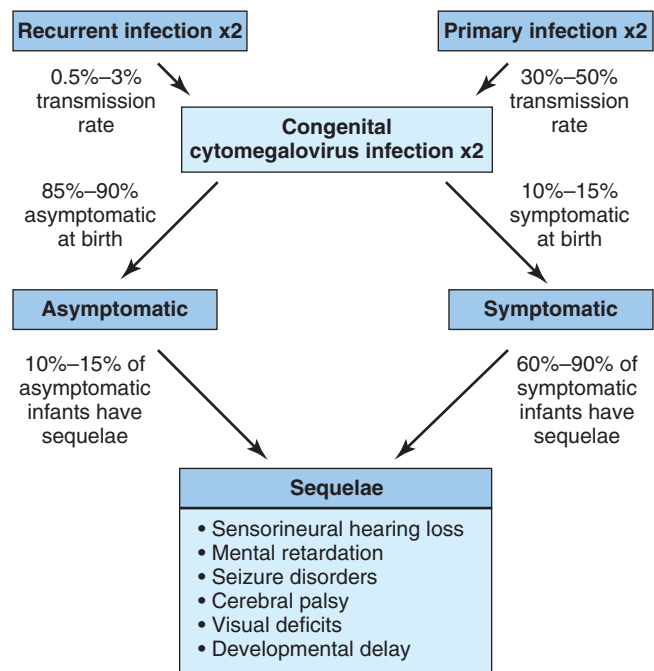
The best means of prevention is to follow current recommendations for universal varicella immunization of all children at 12 to 15 months of age, as well as vaccination of all susceptible adolescents and adults. VZV vaccines have been licensed in three formulations: a monovalent formulation for children and young adults, a combination vaccine given with the measles–mumps–rubella vaccine in children and young adults (not currently manufactured), and a monovalent formulation for adults older than 60 years for prevention of herpes zoster (shingles). There is no evidence that CVS occurs after exposure to varicella vaccine during pregnancy. From March 17, 1995, through March 16, 2005, 981 women were enrolled in a pregnancy registry for women exposed to varicella vaccine (Shields et al., 2001; Wilson et al., 2008; Marin et al., 2014). Pregnancy outcomes were available for 629 prospectively enrolled women. Among the 131 live births to VZV-seronegative women, there was no evidence of CVS. Nonetheless it is recommended that adolescents and women of childbearing age should avoid pregnancy for at least 1 month after immunization.

Cytomegalovirus

CMV infection is ubiquitous in the general population and generally produces few if any symptoms in the immunocompetent infant, child, or adult. The mild nature of primary infection in most individuals belies the severe nature of CMV-induced illness in those with impaired, suppressed, or immature immune systems, including infected newborns. Among the perinatally acquired viral infections in the developed world, CMV infection imposes the largest economic burden and produces the greatest long-term neurodevelopmental morbidity.

Epidemiology

In retrospect, the first description of congenital CMV disease was in 1904, when Ribbert (1904) observed the large inclusion-bearing cells that represent the typical histopathologic finding of CMV end-organ disease in a stillborn infant. In 1920 a viral cause was proposed for the “cytomegaly” seen in tissue sections of these inclusion-bearing cells (Goodpasture and Talbot, 1921), and it would be several more decades before the ubiquitous nature of this virus and the depth and breadth of the disease it produces would be elucidated. In the developed world, CMV transmission occurs in 0.5%–2% of all live births, making CMV infection the most common congenital viral infection (Demmler, 1991; Kenneson and Cannon, 2007; Sharon and Schleiss, 2007; Manicklal et al., 2013; Swanson and Schleiss, 2013; Lanzieri et al., 2014). Approximately 40,000 neonates are born with CMV infection every year in the United States. Consequently, congenital CMV infection has a much greater impact than other more commonly recognized causes of birth defects in newborns. More than 8000 children per



• **Fig. 37.4** Profiles of congenital cytomegalovirus (CMV) epidemiology, infection, and outcome. The rates of transmission to the fetus are highest in the setting of primary maternal infection (up to 50%), although 0.5%–3% of women with preconception immunity may nonetheless transmit CMV because of reinfection or reactivation of latent infection. Among all infants with congenital CMV infection, regardless of maternal immune status during pregnancy, approximately 10%–15% have symptoms or signs at birth (e.g., microcephaly, chorioretinitis, hepatosplenomegaly, petechiae, purpura, thrombocytopenia, hepatitis, seizures, pneumonitis). Symptomatic infants have the highest risk of neurodevelopmental sequelae, although any infant with congenital CMV infection is potentially at risk of sequelae. Among asymptomatic congenitally infected infants with sequelae, the most common manifestation is sensorineural hearing loss, which may not be present at birth.

year will be permanently disabled by congenital CMV infection (Fig. 37.4). This number is higher than the number affected by other, better known childhood conditions such as Down syndrome, fetal alcohol syndrome, and spina bifida (Ross et al., 2006; Cannon et al., 2012). Rates of congenital CMV infection tend to parallel those of maternal seropositivity (Britt, 2015) and differ substantially across populations (Mustakangas et al., 2000; Manicklal et al., 2013). Although the lifetime risk of acquiring CMV infection is high, approaching 90% by the eighth decade of life (Staras et al., 2006), seroprevalence is substantially lower among women of childbearing age. Seronegative women are therefore at risk of acquiring primary infections during pregnancy. Primary infections pose an increased risk of transmission to the fetus and possibly a higher risk of sequelae. Every year in the United States, approximately 27,000 seronegative women acquire a primary CMV infection (Colugnati et al., 2007). Among pregnant women, the overall estimated annual seroconversion rate in the United States is 2.3% (Hyde et al., 2010).

Seroprevalence rates for CMV differ significantly globally and are generally inversely correlated with socioeconomic status. CMV seroprevalence is greater in childhood in developing countries. In developed countries, seroprevalence tends to be higher in blacks and Hispanics, low-income groups, and individuals without higher

education. Young maternal age, single marital status, and nonwhite race are associated with higher rates of congenital CMV infection. Women with increased occupational exposure to young children (including day care providers) are at elevated risk of acquisition of primary CMV infections (Pass et al., 1986; Adler, 1989; Pass et al., 1990; Stagno and Cloud, 1994; Hyde et al., 2010). Healthcare providers in contrast are not at increased risk of acquisition of a primary CMV infection (Dworsky et al., 1983).

Pathogenesis

Morphologically and at the genome level, CMV has the largest genome of any human pathogen (Boeckh and Geballe, 2011). There are approximately 160 known CMV genes, although on the basis of its coding potential, CMV has the potential to encode more than 250 open reading frames. The mechanisms by which CMV injures the fetus involve a complex interplay of viral gene products, maternal immune response, and placental biology. CMV encodes genes modifying the cell cycle, cellular apoptosis mechanisms, inflammatory responses, and evasion of host immune responses. The pathogenesis of disease associated with acute CMV infection has been attributed to lytic virus replication, with end-organ damage occurring either secondary to virus-mediated cell death or from pathologic host immune responses targeting virus-infected cells (Britt, 2008; Schleiss, 2011). The factors that contribute to fetal injury include the timing of infection relative to the gestational age of the fetus (Pass et al., 2006), the maternal immune status (Fowler et al., 1992), the extent of associated placental injury (Fisher et al., 2000), the magnitude of the viral load in the amniotic fluid (Lazzarotto et al., 2000), the induction of host genes occurring in response to infection (Challacombe et al., 2004), and possibly the genotype of the particular strain of CMV infecting the fetus (Arav-Boger et al., 2006; Arav-Boger, 2015). The relative contribution of maternal, placental, and fetal compartments in the pathogenesis of disease remains incompletely defined. Much of the injury that CMV produces in the newborn may be caused by placental insufficiency and not by viral infection of the fetus (Schleiss, 2006a). Delivery of oxygen, substrate, and nutritional factors to the fetus is impaired for a CMV-infected placenta. Moreover, CMV infection of the placenta might contribute to IUGR and fetal injury via induction of proinflammatory cytokines and modulation of normal trophoblast gene expression (Yamamoto-Tabata et al., 2004; Chan and Guilbert, 2005; Chou et al., 2006; La Torre et al., 2006; Maidji et al., 2007). Identified as a site for major pathogenic virus-induced injury, the placenta is increasingly considered to be a target for novel therapeutic interventions, such as CMV hyperimmune globulin for women with primary CMV infection during pregnancy (La Torre et al., 2006; Adler and Nigro, 2013; Adler, 2015).

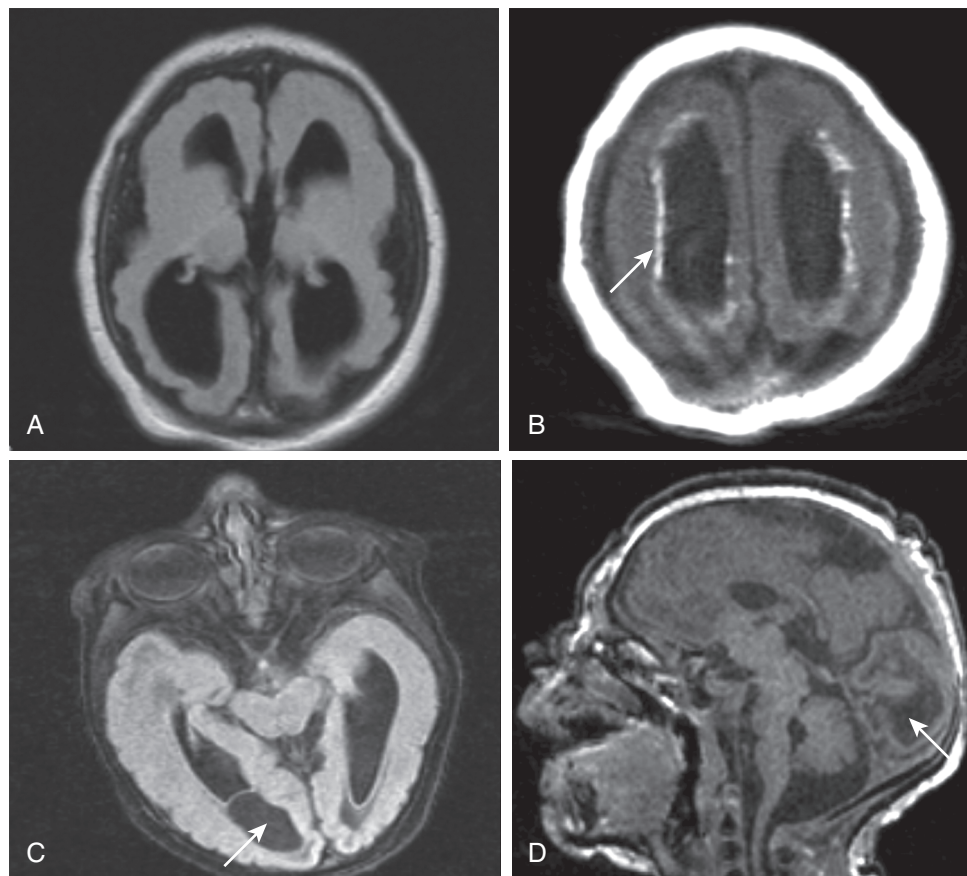
Congenital CMV infection leads to more severe disease and an increased risk of sequelae in the setting of primary maternal infection during pregnancy. Primary maternal infection confers a 40%–50% risk of intrauterine transmission during gestation, versus the 0.5%–2% transmission risk in women with preconceptional immunity (Fowler et al., 2003). Although preconception immunity confers some level of protection for the fetus, congenital infection in immune women can lead to symptomatic disease and sequelae. Congenital CMV infection in previously immune mothers appears to be related to reinfection with new strains of virus, with variations in epitopes of virally encoded proteins that may correlate with decreased maternal immunity (Boppana et al., 1999, 2001; Ross et al., 2010; Yamamoto et al., 2010). Many of the pathologic manifestations of congenital CMV infection that reflect visceral

organ involvement (hepatitis and pneumonitis) are also observed in immunocompromised adults with disseminated CMV disease. The developing fetal brain is also highly susceptible to CMV-induced injury (Gabrielli et al., 2012; Lanari et al., 2012; Teissier et al., 2014). The pathogenesis of CMV infection in the CNS seems to be strongly related to perturbations in neural migration, neural death, cellular compositions, and the immune system of the brain (Cheeran et al., 2009). In infants with severe symptomatic congenital CMV infection, histopathologic evidence of viral dissemination is commonly found in the brain, ear structures, retina, liver, lung, kidney, and endocrine glands (Bissinger et al., 2002). Distinctive features include large cells with large nuclei containing oval inclusions (“owl’s eye” appearance). Inclusion-bearing epithelial cells have been described in the semicircular canals, vestibular membrane, cochlea, and other structures of the ear (Teissier et al., 2011; Gabrielli et al., 2013). Temporal bone anomalies with cochlear, vestibular, and auditory canal defects have been noted in association with hearing loss in affected infants (Davis, 1969; Myers and Stool, 1968; Bernard et al., 2015).

Clinical Presentation, Sequelae, and Prognosis

Prenatal ultrasonography provides clues to the possible diagnosis of fetal CMV infection. Findings include IUGR, microcephaly, ventriculomegaly, periventricular calcifications, echogenic bowel, polyhydramnios, pleural effusion, pericardial effusion, hepatosplenomegaly, intrahepatic calcifications, pseudo-meconium ileus, and placental enlargement (Nelson and Demmler, 1997; Guerra et al., 2008; Swanson and Schleiss, 2013). Fetal hydrops is also a common finding (Sampath et al., 2005). Abnormal prenatal findings on ultrasound examination were associated with increased risk of sequelae (Lipitz et al., 2013). However, the sensitivity of maternal ultrasonography to detect fetal CMV infection is unpredictable, given that most congenitally infected infants are asymptomatic. In a study of 600 pregnant women with primary CMV infection, abnormal ultrasound findings were detected in only 51 of 600 pregnancies (8.5%) and in only 23 of 154 fetuses (14.9%) in which congenital infection was documented. Thus the positive predictive value of an abnormal ultrasound finding that predicted symptomatic congenital infection in women with primary CMV infection was only 35.3% when fetal infection status was unknown, compared with 78.3% when congenital CMV infection was confirmed (Guerra et al., 2000, 2008). Another similar study noted that fetal ultrasound anomalies were detected in 37.7% of pregnant women with primary CMV infection acquired in early pregnancy in the setting of proven fetal infection (Leyder et al., 2016). When maternal ultrasonographic imaging was normal in the setting of proven fetal infection, CNS symptoms or signs were nonetheless noted in 53% of infants (Amir et al., 2016).

Signs and symptoms are apparent at birth in 10%–15% of all children with congenital CMV infection. Table 37.1 outlines the clinical manifestations of symptomatic CMV infection. Infection in the symptomatic infant can involve any organ and manifests itself along a spectrum from mild illness to severe disseminated multiorgan system disease. The mortality rate associated with symptomatic congenital CMV disease in the first year of life is estimated to be greater than 10% (Boppana et al., 1992). Clinical features include jaundice, hepatosplenomegaly, lethargy, respiratory distress, seizures, and petechial rash. Infants with symptomatic disease are often premature and small for their gestational age. A wide spectrum of disease can be observed, including hemolysis, bone marrow suppression, hepatitis, pneumonitis, enteritis, and nephritis. Long bone abnormalities have been described, reminiscent



• **Fig. 37.5** Magnetic resonance image abnormalities in infants with congenital cytomegalovirus (CMV) infection with neurologic manifestations. (A) T1 axial flair image of a neonate with congenital CMV infection with a diagnosis of central nervous system malformation in utero by prenatal ultrasonography, demonstrating severe hydrocephalus and cortical dysplasia and polymicrogyria. (B) Axial T1 image of an infant with symptomatic congenital CMV infection demonstrating ventriculomegaly and periventricular enhancement (arrow). (C, D) T1 fast low-angle shot axial (C) and sagittal (D) images of a symptomatic, congenitally infected infant demonstrating ventriculomegaly, polymicrogyria, and porencephalic cyst (arrow).

of those observed in congenital syphilis (Alessandri et al., 1995). Common laboratory abnormalities include thrombocytopenia, anemia, abnormal levels of liver enzymes (particularly elevated levels of transaminases), and elevated conjugated bilirubin levels.

Of particular concern are the CNS diseases observed with symptomatic congenital CMV infection, including meningoencephalitis, calcifications, microcephaly, neuronal migration disturbances, germinal matrix cysts, ventriculomegaly, and cerebellar hypoplasia (Cheeran et al., 2009; Gabrielli et al., 2012; Lanari et al., 2012). CNS disease is usually characterized by at least one of the following signs and symptoms: lethargy, microcephaly, intracranial calcifications, hypotonia, seizures, hearing deficit, or an abnormal eye examination finding, such as chorioretinitis or optic atrophy. The finding of microcephaly at birth typically indicates CNS involvement (Fig. 37.5). Long-term neurodevelopmental disabilities are observed in 50%–90% of children who are symptomatic at birth. In contrast, long-term neurodevelopment impairment appears to be less likely in congenitally infected infants who are asymptomatic at birth: when it does occur, injury is typically limited to sensorineural hearing loss (SNHL). Several studies have suggested that the intellectual development of asymptomatic congenitally infected infants appears to be normal (Conboy et al., 1986, 1987). Among symptomatic congenitally infected infants, long-term sequelae can include microcephaly, hearing loss, motor deficits (paresis or paralysis),

cerebral palsy, intellectual disability, seizures, ocular abnormalities (chorioretinitis, optic atrophy), and learning disabilities (Sharon and Schleiss, 2007; Cheeran et al., 2009).

The incidence of SNHL among children with congenital CMV infection ranges from 10% to 15% of those who are asymptomatic at birth to up to 60% of those who are symptomatic as newborns (Pass, 2005). SNHL can be progressive and fluctuating in both asymptomatic and symptomatically congenitally infected infants (Rosenthal et al., 2009). Among asymptomatic congenitally infected infants, SNHL tends to be high frequency in nature; it ranges in severity from a unilateral, mild hearing deficit to severe, bilateral, profound deafness. CMV-induced SNHL is a dynamic, evolving lesion; it may be present at birth or can appear later in childhood. Delayed-onset hearing loss usually occurs before 4 years of age (Williamson et al., 1992; Fowler et al., 1997; Dahle et al., 2000; Rivera et al., 2002; Madden et al., 2005) but has been reported to evolve and progress through 6 years of age and beyond. The pathogenesis of SNHL is related to an inflammatory labyrinthitis (Schleiss and Choo, 2006; Teissier et al., 2011; Gabrielli et al., 2013; Teissier et al., 2016). Some temporal bone and cochlear abnormalities have been described in a small case series of hearing-impaired infants with congenital CMV infection (Bauman et al., 1994), but CMV-associated SNHL is not associated with enlarged vestibular aqueduct syndrome (Pryor et al., 2005). Vestibular

disorders may occur independently of SNHL (Karlton et al., 2014; Bernard et al., 2015). The presence of petechiae and IUGR at birth are associated with the development of SNHL (Rivera et al., 2002). All congenitally infected infants, regardless of the results of functional hearing assessment at birth, should be monitored prospectively for SNHL. For severe SNHL, cochlear implantation has been used with success (Lee et al., 2005; Yoshida et al., 2009; Shin et al., 2011).

Diagnosis and Infant Assessment

Congenital CMV infection is best diagnosed by detection of virus, either through culture or via PCR, in samples collected in the first 2 to 3 weeks of life. The timing of collection of these samples is important because subsequent viral isolation beyond 3 weeks of age may represent neonatal infections acquired in the birth canal or after exposure to breast milk (Schleiss, 2006b) and not congenital infection. Urine and saliva are the clinical samples of choice for virus detection. Rapid confirmation of infection can be made with centrifugation culture ("shell vial") assays with a high sensitivity and specificity (Gleaves et al., 1984; Rabella and Drew, 1990; Revello and Gerna, 2002), although culture-based assays are largely being replaced in clinical virology laboratories by PCR. The magnitude of the systemic viral load measured by PCR in the congenitally infected infant may be a predictor of neurodevelopmental prognosis (Lanari et al., 2006).

Serodiagnosis of congenital CMV infection is problematic. In congenital CMV infection, antibody production by the infected fetus begins in utero; however, serodiagnosis of congenital infection is complicated by the presence of maternal IgG antibodies that cross the placenta. Although a negative antibody titer test result in infant and maternal sera provides sufficient evidence to exclude the diagnosis of congenital CMV infection, positive IgG titer test results in the newborn by no means confirm congenital infection. The presence of IgM antibodies to CMV in cord or neonatal blood is highly specific and in principle represents fetal antibody response, but IgM serologic tests have limited sensitivity in diagnosing congenital CMV infection (Revello et al., 1999b).

A recent development in molecular diagnostics for congenital CMV infection has been the use of dried blood spots (DBSs) as a source of CMV DNA for PCR-based detection (Barbi et al., 2006; Scanga et al., 2006; Yamagishi et al., 2006). DBS screening is amenable to long-term storage, so diagnosis can be made retrospectively, even after several years. There has been considerable interest in development of this test for implementation of widespread population-based newborn screening for congenital CMV infection (Walter et al., 2008; Dollard et al., 2010; Kharrazi et al., 2010). However, recent evidence from a large multicenter study suggests that DBS screening is not sufficiently sensitive for diagnosis of congenital CMV compared with detection in other bodily fluids such as saliva and urine (Boppana et al., 2010; Atkinson et al., 2013). An alternative approach would be to analyze the DBSs obtained from infants who failed newborn hearing screens, because congenital CMV infection can be identified in approximately 3% of such cases (Choi et al., 2009; Ross et al., 2017). This approach would allow early detection and more timely intervention; however, it would fail to identify those congenitally infected infants who pass the newborn hearing screening but later develop SNHL.

Cranial ultrasonography, head CT, and brain MRI are used to detect brain lesions associated with congenital CMV infection. CNS anomalies can be detected in some infants in utero. Fetal MRI can also detect abnormalities, including microcephaly and cortical anomalies, even when the ultrasonographic findings are

normal; this appears to be the preferred modality for diagnosis of fetal CNS involvement (see Fig. 37.5; Benoist et al., 2008; Doneda et al., 2010). Because the finding of CNS disease is a potential harbinger of permanent sequelae, diagnostic CNS imaging is warranted in all suspected cases of congenital infection (Boesch et al., 1989; Boppana et al., 1997; Kylat et al., 2006; Ancora et al., 2007; Gabrielli et al., 2012; Lanari et al., 2012; Capretti et al., 2014). Any of the standard imaging modalities is valuable in assessing CNS involvement. Ultrasonography, because of its convenience, is an appropriate initial study and is particularly valuable and sensitive in detecting periventricular calcifications and lenticulostriate vasculopathy associated with mild to moderate ventricular dilatation. MRI provides important additional information, particularly the presence of associated polymicrogyria, hippocampal dysplasia, and cerebellar hypoplasia (de Vries et al., 2004; Capretti et al., 2014); therefore the staged sequential use of an initial cranial ultrasound followed by MRI is probably the preferred approach to CNS imaging in this setting.

Careful initial hearing evaluation and longitudinal monitoring for SNHL are required in all infants with documented congenital CMV infection, given that this complication is the most common late sequela of congenital CMV infection. Early recognition of SNHL and institution of appropriate interventions (speech-language therapy, centers for deafness education, and cochlear implants) can markedly improve the developmental, social, and language skills of a child with hearing impairment. Any child born with congenital CMV infection, whether symptomatic or not, deserves careful and recurring evaluation for cognitive delays, visual impairment, or motor disabilities. Follow-up evaluation should consist of a multidisciplinary team approach including an infectious diseases specialist, pediatric otolaryngologist, and child behavioral-developmental specialist, in addition to a physical therapist, ophthalmologist, and neurologist as needed. Mothers of infants with congenital CMV infection should be counseled regarding future pregnancies. For infants with symptomatic congenital CMV infection born to women with low CMV IgG avidity antibodies (Society for Maternal-Fetal Medicine et al., 2016), some authorities recommend they be monitored for emergence of high-avidity antibody before future pregnancies are contemplated.

Treatment: Antiviral Intervention in the Newborn and the Pregnant Patient

Successful results with the use of antiviral therapies against CMV in immunosuppressed patients (Razonable, 2005; Boeckh and Ljungman, 2009; Vora and Englund, 2015) have helped drive the advent of antiviral treatment for congenital CMV infection (Hilgendorff et al., 2009; Lombardi et al., 2009; Amir et al., 2010; Yilmaz Çiftdoğan and Vardar, 2011). The benefits of ganciclovir in congenitally infected infants have been demonstrated in controlled trials. In a phase III, randomized, nonblinded controlled trial of ganciclovir for newborns with congenital CMV disease (Kimberlin et al., 2003), a group of infants with virologically confirmed congenital CMV infection received a 6-week course of IV ganciclovir therapy (6 mg/kg every 12 hours). The primary end point was improved hearing or retention of normal hearing. A statistically higher likelihood of normal or improved hearing at 6 months of age was noted in treated infants compared with controls. In a follow-up assessment, infants with symptomatic congenital CMV infection involving the CNS who received IV ganciclovir therapy had fewer developmental delays at 6 and 12 months, as assessed by the Denver Developmental Screening Test, compared with untreated infants (Oliver et al., 2009). On the basis of these

encouraging results, a randomized, placebo-controlled trial of oral valganciclovir therapy in neonates with symptomatic congenital CMV disease was recently conducted. In this study, all participants received valganciclovir (at a dosage of 16 mg per kilogram of bodyweight orally twice daily) for 6 weeks. Participants then underwent randomization in a 1:1 ratio to receive either continued valganciclovir therapy or placebo for 4.5 months. Hearing was more likely to be improved or to remain normal at 12 months in the 6-month treatment group than in the 6-week treatment group, and this group also had better neurodevelopmental scores on the Bayley Scales of Infant and Toddler Development (Nassetta et al., 2009; Kimberlin et al., 2015). Because antiviral therapy improved hearing and neurodevelopmental outcomes, 6 months of oral valganciclovir therapy should be considered for all infants with congenital CMV infection with any evidence of CNS involvement (microcephaly, abnormal CNS imaging, CSF positive for CMV DNA, chorioretinitis, or evidence of SNHL). Careful monitoring for neutropenia, the major side effect of valganciclovir therapy, is essential.

Ganciclovir therapy should also be used in any infant with severe or life-threatening end-organ CMV disease, whether acquired via congenital infection or by a postnatal route, such as via breastfeeding in a low birth weight premature infant (Table 37.2; Schleiss and McVoy, 2004). CMV retinitis in the congenitally infected infant can be a particularly problematic management issue; it has been reported that antiviral therapy for up to 6 months may be required to control chorioretinitis in the symptomatic congenitally infected infant (Shoji et al., 2010). All infants with documented congenital infection should have an ophthalmologic evaluation. If chorioretinitis is present, it should be managed in consultation with an ophthalmologist and infectious diseases expert.

Although ganciclovir improves neurodevelopmental outcomes for symptomatic infants, it is not yet clear whether ganciclovir holds the promise of improving outcomes in infants with asymptomatic congenital CMV infection. Because some of these infants are at risk of progressing to SNHL, clinical trials are warranted to explore whether antiviral therapy could prevent development of hearing loss.

The prospect of treating the pregnant patient to prevent transmission of CMV to the fetus is an area of active investigation. Ganciclovir has demonstrated teratogenic risk in some studies (Schleiss and McVoy, 2004); although this has never been demonstrated in humans, there is limited research in this area. A case report of the use of orally administered ganciclovir in a pregnant liver transplant patient did not show any evidence of teratogenicity (Pescovitz, 1999). Ganciclovir has been demonstrated to cross the placenta, and therefore could theoretically be used to treat CMV infection in utero (Brady et al., 2002). An observational study of 20 women with 21 fetuses, with confirmed congenital CMV infection treated with orally administered valacyclovir, demonstrated placental transfer of valacyclovir with measurable concentrations in the amniotic fluid and a subsequent reduced viral load in the fetal blood (Jacquemard et al., 2007). There have been several case reports of treatment of congenital CMV infection in utero with oral, parenteral, or intraamniotic administration of ganciclovir with various degrees of success (Revello et al., 1993; Miguelez et al., 1998; Revello and Gerna, 1999; Puliyanda et al., 2005). Maternal therapy with high-dosage oral valacyclovir therapy (8 g/d) has also been studied in women known to be carrying a CMV-infected fetus (Leruez-Ville et al., 2016; Leruez-Ville and Ville, 2017).

Passive immunization with CMV human immunoglobulin (HIG) has been studied for the in utero treatment and prevention

of congenital CMV infection. CMV HIG is a pooled, high-titer immunoglobulin preparation derived from donors with high levels of CMV antibody. Nigro et al. (2005) completed an uncontrolled and unblinded prospective study of CMV HIG for the treatment of pregnant women with primary CMV infection, including some women with confirmed fetal CMV infection (Nigro et al., 2005). Improvement was reported in the CMV HIG-treated patients with respect to ultrasonographic abnormalities in fetal brain and in placenta (La Torre et al., 2006; Schleiss, 2006a; Nigro et al., 2008), and reduced infection rates were observed compared with untreated controls. However, a randomized, blinded placebo-controlled trial of CMV HIG for the treatment and prevention of congenital CMV infection failed to demonstrate a benefit (Revello et al., 2014). It thus remains unclear if CMV HIG is useful in prevention of viral transmission or sequelae. A National Institutes of Health-funded multicenter randomized, placebo-controlled trial of CMV HIG during pregnancy in the setting of primary maternal infection is currently ongoing, and it is hoped that this study will shed light on this question (<https://clinicaltrials.gov/ct2/show/NCT01376778?term=brenna+anderson&rank=3>).

Natal Acquisition of Cytomegalovirus Infection: Implications for the Premature Infant

In addition to congenital infection, CMV can produce disease in the newborn after natal acquisition; this can occur via one of three mechanisms: (1) transmission in the birth canal during vaginal delivery after exposure to infectious cervicovaginal secretions, (2) through ingestion of breast milk, and (3) via blood transfusion. Of these potential mechanisms, the most common is via breast milk (Schleiss, 2006b), with transmission in the birth canal occurring less commonly. It has long been recognized that CMV is shed by the cervix (Montgomery et al., 1972) and excreted in the breast milk of seropositive women (Hayes et al., 1972; Stagno et al., 1982). CD14⁺ cells appear to play a major role in transmission of CMV by lactation (Maschmann et al., 2015). The risk of CMV transmission in infants who are breastfed by seropositive women shedding virus in their breast milk has been reported to be between 58% and 69% (Stagno et al., 1980; Dworsky et al., 1983). CMV infection acquired in the postnatal period in healthy term infants by this route is typically asymptomatic, only rarely producing any morbidity. There is no convincing evidence that acquisition of CMV via breast milk leads to any adverse neurodevelopmental sequelae (Kurath et al., 2010). In a study of CMV transmission through breastfeeding, all the infants who acquired CMV infection had normal neurodevelopment at a mean follow-up of 51 months of age (Vollmer et al., 2004). There is evidence that acquisition of CMV infection in the premature infant may contribute to the development of chronic lung disease (Kelly et al., 2015; Mukhopadhyay et al., 2016). Moreover, a recent study that examined long-term neuropsychologic sequelae in adolescents born before term identified early postnatal CMV infection as a risk factor for scoring significantly lower than those without CMV with respect to their overall cognitive abilities (Brecht et al., 2015; Wright and Permar, 2015).

Breast milk-acquired CMV can cause high infection rates and significant symptomatic disease in premature infants. In one study of very low birth weight (VLBW) premature infants (<32 weeks' gestation, <1500 g) exposed to CMV via breast milk, virus transmission occurred in 33 of the 87 exposed infants (Maschmann et al., 2001). Approximately half of these infants were ill and exhibited symptoms such as hepatopathy, neutropenia, thrombocytopenia, and sepsis-like deterioration. Other reports of early postnatal CMV

infection acquired by preterm infants from breast milk in highly immune populations have suggested a lower rate of symptomatic CMV infection (Mussi-Pinhata et al., 2004; Kurath et al., 2010; Josephson et al., 2014). The Centers for Disease Control and Prevention (CDC) has estimated that up to 4.5% of VLBW and premature infants in the United States may develop CMV sepsis syndrome because of breast milk–acquired CMV infections, resulting in approximately 2000 infants annually (Lanzieri et al., 2013). Proposed efforts to reduce the infectivity of breast milk from seropositive mothers have included freezing breast milk at -20°C , Holder pasteurization, and short-term pasteurization (Hamprrecht et al., 2004). Of these methods, freezing is the most studied and most likely to maintain the salutary immunologic properties of breast milk. Some experts recommend freeze-thawing of all breast milk before the VLBW premature infant is fed if the mother is known to be CMV seropositive or if her CMV serostatus is unknown. Although freezing of breast milk may lower the incidence of postnatally acquired CMV infection, it does not entirely eliminate the risk (Maschmann et al., 2006; Omarsdottir et al., 2015). Other interventions, such as microwave irradiation, can eliminate CMV from human milk (Ben-Shoshan et al., 2016), but it is unknown what impact this intervention has on the nutritional and immunologic components of milk. It is presumed that VLBW premature infants are at increased risk because they possess fewer transplacentally acquired antibodies against CMV than do term babies and thus are more likely to develop disease on infection. Treatment of the VLBW infant with IVIG appeared to reduce the likelihood of transmission of CMV by breast milk (Capretti et al., 2009), although this is not currently considered to be an indication for the use of IVIG in the premature infant. Further evidence is necessary to make recommendations regarding what, if any, interventions are appropriate in low birth weight, preterm infants receiving breast milk from CMV-seropositive mothers.

Transfusion-associated CMV infections were at one time a major problem in the neonatal intensive care setting (Yeager et al., 1981; Adler et al., 1983, 1984; Adler, 1986). Two approaches are currently used to decrease the risks of transfusion-associated CMV infection: leukocyte reduction and directed transfusion of CMV-negative blood products (Lamberson et al., 1988). Although leukocyte reduction has had a dramatic effect on the risk of transfusion-associated CMV infection, reports are conflicting in the literature regarding the question of whether this intervention is completely effective at eliminating the risk of transfusion-transmitted CMV infection (Fergusson et al., 2002; Vamvakas, 2005; Allain et al., 2009). A survey of the American Association of Blood Banks physician membership revealed that 65% of those responding believed that leukocyte-reduced and CMV-negative blood components were equivalent in their ability to prevent transfusion-associated transmission of CMV (Smith et al., 2010). A prospective, multicenter birth-cohort study conducted at three neonatal intensive care units (NICUs) in the United States found no evidence of transfusion-associated CMV infection when leukocyte-reduced blood was used for transfusion of VLBW infants (Josephson et al., 2014). These data support the practice of transfusing leukocyte-reduced blood products to premature infants, including blood from CMV-seropositive donors, since this practice should be adequate for preventing virtually all cases of transfusion-associated CMV infection in the NICU.

Prevention

One important strategy for addressing the problem of congenital CMV infection is the education of women of childbearing age

about the risks of transmission and strategies for prevention. Child care providers (including day care workers, special education teachers, and therapists) appear to have a higher risk of occupational exposure to CMV because of extensive contact with infants and young children (Pass et al., 1986; Adler, 1989; Pass et al., 1990). Education on the potential occupational risk in this group is essential. In contrast to these child care providers, healthcare workers who appropriately use routine infection control practices are not at increased risk of CMV acquisition. CMV infections in pregnant women are typically clinically “silent.” Like most healthy individuals, more than 90% of pregnant women with primary CMV infections have no symptoms. When symptoms occur, they are nonspecific and vague, often described as a flulike syndrome. Potential manifestations include fever, fatigue, headache, myalgia, lymphadenitis, and pharyngitis, but these are the exception and not the rule. Because most maternal CMV infections are asymptomatic, a major goal is education of all women of childbearing age on hygienic practices (American College of Obstetrics and Gynecologists, 2002; Jeon et al., 2006; Ross et al., 2006). Hygienic strategies are important because the saliva and urine of infected children are significant sources of CMV infection among pregnant women. Strategies include washing hands whenever there is contact with a child’s saliva or urine, not sharing food, utensils, or cups, and not kissing a child on the mouth or cheek (Cannon and Davis, 2005; Anderson et al., 2008a). It is also essential that women become better educated on the importance of CMV infection. A survey of women in 2005 showed that only 14% of women knew what CMV was, but most believed that preventative measures for an infection that could harm an unborn baby would generally be acceptable (Ross et al., 2008). The effectiveness of educating pregnant women on methods to prevent CMV transmission has been demonstrated (Adler et al., 1996). In a study in which seronegative mothers with a child in group day care were instructed on measures to prevent CMV transmission, pregnant mothers had a significantly lower rate of CMV infection than nonpregnant mothers attempting conception (Adler et al., 2004). In addition, a study in France demonstrated a lower CMV seroconversion rate after pregnant women had been counseled on hygienic measures (Vauloup-Fellous et al., 2009).

Prenatal maternal screening for CMV antibodies is controversial. Because women who are CMV immune can be reinfected with new strains that can then be transmitted to the fetus, with subsequent sequelae (Boppa et al., 1999, 2001; Ross et al., 2010; Yamamoto et al., 2010), a positive preconception titer test result for CMV IgG antibody may provide a false sense of reassurance and decrease a pregnant patient’s motivation to engage in careful hygienic practices. A study that evaluated three screening strategies suggested that universal maternal screening for CMV could be a cost-effective strategy if a treatment were available that could achieve a 47% reduction in disease incidence (Cahill et al., 2009).

Ultimately the control of congenital CMV infection could be realized by the development of an effective vaccine. No CMV vaccines are currently licensed; however, because of the enormous economic impact of congenital CMV infection, the Institute of Medicine has identified a CMV vaccine as the highest-level priority for new vaccine development (excluding HIV vaccines) for the United States (Stratton et al., 2001). A vaccine based on the CMV major envelope glycoprotein gB has demonstrated modest efficacy against acquisition of primary CMV infection in young, CMV-seronegative women in clinical trials (Pass et al., 2009; Bernstein et al., 2016). A consensus workshop sponsored by the US FDA concluded that CMV vaccine studies should be targeted at evaluating protection against congenital CMV infection, an essential precursor

to the development of congenital CMV disease with attendant sequelae (Krause et al., 2013). Other clinical trials are ongoing, including evaluation of live, attenuated CMV vaccines; subunit vaccines targeting other key proteins involved in the humoral and cellular immune responses to CMV infection; and disabled, single-cycle, replication-deficient vaccines that express key CMV proteins but are incapable of establishing infection (Schleiss, 2008; Fu et al., 2014).

Human Herpesvirus 6 and 7

HHV-6 was isolated in tissue culture in 1986 from peripheral blood leukocytes of patients with both lymphoproliferative disorders and HIV infection (Bernstein and Schleiss, 1996; Schleiss, 2009). For several years after its discovery, its role in disease was unclear, but it is now known to be the major etiologic agent of roseola infantum (exanthem subitum) and has been implicated in other clinical syndromes. HHV-6 is a prototypical β -herpesvirus, with a double-stranded DNA genome contained within an icosahedral capsid, surrounded by an outer envelope. HHV-6 is subclassified as either variant A or variant B, on the basis of differences in nucleotide sequence, restriction enzyme profile, and reactivity with monoclonal antibodies. HHV-6B is the subtype typically associated with roseola infantum (Yamanishi et al., 1988). HHV-6 and HHV-7 are ubiquitous in nature and typically cause infection in the first 2 years of life. A recent study in Ugandan infants demonstrated that more than 75% of infants acquire HHV-6 infection in the first year of life (Gantt et al., 2016).

HHV-7 is highly related to HHV-6 and, like HHV-6, is responsible for roseola infantum (Tanaka et al., 1994). HHV-7 is a β -herpesvirus, structurally and molecularly similar to CMV and HHV-6. As with HHV-6, infection with HHV-7 appears to be ubiquitous, although infection appears to be acquired somewhat later in life than HHV-6 infection (Suga et al., 1997; Caserta et al., 1998). Approximately 40%–45% of children have antibodies to HHV-7 by 2 years of age, and 70% of children are seropositive by 6 years of age. Both HHV-6 and HHV-7 can be found by PCR in cervical secretions, suggesting a possible (but unconfirmed) route for intrapartum transmission (Okuno et al., 1995; Dahl et al., 1999; Caserta et al., 2007).

Vertical transmission of HHV-6 DNA is described. Examination of 305 umbilical cord blood samples in one study identified HHV-6 DNA by PCR in 1.6% of infants (Adams et al., 1998). Congenital HHV-6 transmission was first definitively reported in a study of 5638 umbilical cord bloods; 57 samples (1%) had HHV-6 DNA by PCR, but none had HHV-7 (Hall et al., 2004). Of note, these infections were all asymptomatic, and it is not clear if this vertically transmitted DNA reactivates to produce bona fide virus particles. Vertical transmission of HHV-6 most often occurs (90% of cases) not from actively replicating virus but because of the germline passage of HHV-6 integrated into the chromosome (Hall et al., 2008). This germline mode of inheritance of the HHV-6 genome in this form of vertical transmission appears to be exclusively maternal (Hall et al., 2010). The clinical consequences of such transmission and the differences in germline transmission of the viral genome and the more common postnatal acquisition of HHV-6 in early childhood remain unknown, although recent evidence suggests that there may be long-term neurodevelopmental concerns in infants who have germline transmission of viral DNA (Caserta et al., 2014). The HHV-6 genome appears to integrate into the telomere—a chromosomal component important in cellular aging and in cancer—suggesting that there may be long-term consequences

associated with vertical germline transmission (Nacheva et al., 2008; Arbuckle et al., 2010). HHV-6 DNA can also be found in breast milk. Perinatal transmission via this mechanism has been postulated (Joshi et al., 2000) but has not been demonstrated.

Kaposi Sarcoma–Associated Herpesvirus and Epstein–Barr Virus

In 1994 a novel herpesvirus, KSHV (HHV-8), was identified in patients with AIDS-associated Kaposi sarcoma (KS) (Chang et al., 1994). This virus was assigned to the γ -herpesvirus subfamily of the *Herpesviridae*, on the basis of its molecular and sequence similarity to the other prototypical γ -herpesvirus, EBV. Subsequent studies have linked KSHV to both AIDS-associated KS and the endemic forms of KS that are prevalent in elderly Mediterranean men. A cross-sectional study of the seroprevalence of KSHV in children and adolescents in the United States indicated a prevalence of approximately 1% (Anderson et al., 2008b). There appears to be considerable regional variation in prevalence in the United States. In a population of children in south Texas, the seroprevalence was 26%, strongly suggesting that nonsexual modes of transmission predominate (Baillargeon et al., 2002). In sub-Saharan Africa, prevalence in children is even higher, approaching 60% in some studies (Sarmati, 2004). Endemic forms of pediatric KS are relatively common in sub-Saharan Africa and are associated, in some cases, with single-gene inborn errors of immunity (Jackson et al., 2016). There are reports of infections in infants that suggest possible vertical transmission, but congenital infection has not been demonstrable by PCR techniques (Sarmati et al., 2004). KSHV can also be transmitted by blood transfusion (Hladik et al., 2006); this observation suggests that transplacental transmission is at least theoretically feasible. In a study of 89 KSHV-seropositive women, KSHV DNA was detected in the peripheral blood mononuclear cells of 13 mothers (14.6%); KSHV DNA was detectable in the peripheral blood mononuclear cells of 2 of 89 samples drawn at birth from neonates born to these mothers. KSHV has also been demonstrated to infect placental cells, suggesting that a transplacental route of infection is feasible (Di Stefano et al., 2008). These findings suggest that KSHV can be transmitted perinatally but infrequently (Mantina et al., 2001). As serologic and nucleic acid–based diagnostic tests become more widely available, a better assessment of the worldwide seroepidemiology of KSHV infection and an increased understanding of its modes of transmission will be achievable. The clinical presentation of KSHV infection in immunocompetent children has been described (Andreoni et al., 2002). Fever and rash appear to be important findings with primary infection. Additional information on the epidemiology and modes of transmission of this pathogen, particularly in the prenatal and intrapartum period, is needed.

As for KSHV, there is a minimal amount of information available about prenatal and perinatal modes of transmission of EBV. EBV is the causative agent of infectious mononucleosis and is associated with nasopharyngeal carcinoma, Burkitt lymphoma, and lymphoproliferative disease in immunocompromised patients (Schleiss, 2009). Primary EBV infection during pregnancy appears to be rare (Le et al., 1983; Avgil and Ornoy, 2006). It is not clear whether transplacental passage of EBV in seropositive pregnant women occurs. It has been postulated that high-titer antibodies cross the placenta and protect the fetus from hematogenous transmission of virus in women who reactivate EBV during pregnancy (Purtilo and Sakamoto, 1982). A solitary case report describes the occurrence of severe EBV disease in a premature

infant, born at 28 weeks' gestation, who was examined on the 42nd day of life with hepatosplenomegaly, hemolytic anemia, thrombocytopenia, and atypical lymphocytosis (Andronikou et al., 1999). In another study, the potential for EBV vertical transmission from a seropositive mother to her child was evaluated in 67 pregnant women by nested PCR (Meyohas et al., 1996). Two of 67 neonates were positive for EBV DNA. In six pregnant women with evidence of primary EBV infection, four pathologic births were observed: one spontaneous abortion, two premature babies, one of whom died, and one stillborn with multiple malformations (Icart et al., 1981), although intrauterine EBV infection was not demonstrated. Placentas and some fetuses were studied in five cases of pregnancy interruption caused by maternal infectious mononucleosis in early gestation (Ornoy et al., 1982). Studies of pregnancy outcomes in the setting of primary maternal EBV infection have demonstrated necrotizing deciduitis, endovascularitis, perivascularitis, and occasional vascular obliteration in placental villi, as well as mononuclear and plasma cell infiltrates, although once again direct virologic evidence for EBV infection of these tissues was lacking (Ornoy et al., 1982). EBV DNA was identified in amniotic fluid and placenta in another case report (Tomai, 2011). A 2% congenital EBV transmission rate has been described in HIV-infected newborns (Gumbo et al., 2014). EBV DNA has also been detected in newborn blood spot screening cards from children with cerebral palsy (McMichael et al., 2012).

Human Parvovirus B19

Parvovirus B19, a small, single-stranded DNA virus, is the only member of the parvovirus family that causes human disease. The virus was identified in 1975 (Cossart et al., 1975) and was first linked to a disease in 1981—aplastic crisis in children with sickle cell anemia (Pattison et al., 1981). Primary infection with parvovirus B19 is commonly known as *fifth disease* or *erythema infectiosum*; it is classically described as a childhood exanthem with a “slapped cheek” appearance (Anderson et al., 1984). Considerable interest in the role of this virus in the pathogenesis of hydrops fetalis (nonimmune) and fetal aplastic crisis has evolved since the first cases of fetal death associated with maternal parvovirus B19 infection were reported in the 1980s (Brown et al., 1984; Kinney et al., 1988). The spectrum of parvovirus B19–associated diseases continues to expand and includes neurologic disease, arthritis, and autoimmune disease (Watanabe and Kawashima, 2015; Kerr, 2016; Mauermann et al., 2016; Palermo et al., 2016).

Epidemiology

Parvovirus B19 infection is common in childhood and continues at a low rate throughout adult life. One study identified an annual seroconversion of 1.5% in women of childbearing age unrelated to their occupation (Koch and Adler, 1989). The peak incidence of erythema infectiosum is in the late winter and early spring. Periodic epidemics at intervals of a few years are typical. The virus is spread by respiratory droplets (Anderson and Cohen, 1987), by blood products (especially pooled clotting factor concentrates; Jordan et al., 1998), and transplacentally (Ergaz and Ornoy, 2006). Approximately 50%–80% of adults in the United States are seropositive for human parvovirus B19 (Anderson, 1987; Vyse et al., 2007). A significant proportion of childbearing women are thus susceptible to infection (Markenson and Yancey, 1998; Yaegashi et al., 1998). Preconception seroprevalence to parvovirus B19 ranges from 24% to 84% (Ergaz and Ornoy, 2006). During pregnancy

the risk of acquiring parvovirus B19 infection is low, ranging from 0% to 16.5% in different studies (Ergaz and Ornoy, 2006). The risk of primary maternal infection is higher during epidemics of erythema infectiosum (Woernle et al., 1987; Kerr et al., 1994).

It is estimated that one-fourth to half of maternal parvovirus B19 infections result in transmission of infection to the fetus (Gratacos et al., 1995; Alger, 1997; Koch et al., 1998). The vast majority of pregnancies are unaffected (Berry et al., 1992; Sheikh et al., 1992). The risk of adverse fetal outcome is increased if maternal infection occurs during the first two trimesters of pregnancy (Skjoldbrand-Sparre et al., 2000), particularly before 20 weeks' gestation. There are conflicting reports regarding the prognosis once fetal infection has been established. A longitudinal study of fetal morbidity and mortality in more than 1000 women with primary parvovirus B19 infection in pregnancy demonstrated a risk of fetal hydrops of 3.9% and a risk of fetal death of 6.3%; fetal death was observed only if maternal infection occurred before the 20th week of gestation (Enders et al., 2004). A retrospective analysis of intrauterine parvovirus B19 infection at a single site suggested that the rate of adverse fetal outcome is much higher than previously appreciated, with fetal hydrops and demise occurring in more than 10% of pregnancies (Beigi et al., 2008), although the total number of cases reported in this series was low; this primarily represented a referral population to a tertiary care center. In an analysis of nearly 1000 cases of fetal and newborn demise caused by various infectious agents, parvovirus B19 was found to be responsible for 63% of all of the virus-related deaths occurring before live birth (Williams et al., 2013), even in the absence of a history of frank hydrops fetalis. Thus the spectrum of diseases associated with parvovirus B19 infection remains incompletely defined.

Pathogenesis

The most common mode of transmission of parvovirus B19 is via a respiratory route. Typically, once the virus establishes infection, viremia occurs, followed by mild systemic symptoms such as fever and malaise. Viremia is short lived, lasting only 1 to 3 days, and the characteristic immune-mediated rash develops 1 to 2 weeks later. Once the rash appears, an individual is no longer infectious. Arthropathy caused by parvovirus B19 is common; it is observed more frequently in adults with primary infection than in children. Symptoms usually subside within 1 to 3 weeks, although approximately 20% of affected women have persistent or recurring arthropathy for months to years (Woolf et al., 1989).

Potential pathogenic mechanisms involve the recognized affinity of parvovirus B19 for progenitor erythroid cells of bone marrow. The blood group P antigen is a main cellular receptor for parvovirus B19, and it is found on red blood cells and on placental trophoblast cells (Jordan et al., 2001). The P antigen is also expressed on fetal cardiac myocytes, enabling parvovirus B19 to infect myocardial cells (Rouger et al., 1987), leading to myocarditis (von Kaisenberg et al., 2001). Myocarditis induced by parvovirus B19 can contribute to high-output cardiac failure, and the myocardial inflammation and subendocardial fibroelastosis may also contribute to fetal hydrops (Morey et al., 1992). Fetal infection most likely occurs hematogenously via the placenta during maternal viremia. Parvovirus B19 infection in utero causes a pronormoblast arrest, which leads to fetal anemia, nonimmune hydrops, and sometimes progressive congestive heart failure (Kinney et al., 1988; Ergaz and Ornoy, 2006). The fetus is especially susceptible to adverse consequences

of red blood cell infection, secondary to the intrinsic short fetal erythrocyte life span and rapidly expanding blood volume, especially during the second trimester. It has also been postulated that parvovirus B19 infection leads to cytotoxicity and subsequent anemia by inducing apoptosis of infected red blood cells (Yaegashi et al., 1999, 2000). The nonstructural 1 (NS1) protein of parvovirus B19 induces cell death by apoptosis in erythroid-lineage cells by a pathway that involves caspase 3, whose activation may be a key event during parvovirus B19-induced cell death (Moffatt et al., 1998). The NS1 protein also has a key role in the arrest of infected cells at the G₁ phase of the cell cycle before apoptosis induction (Chisaka et al., 2003).

In addition to erythroid precursors and myocytes, other organs appear to be involved in fetal parvovirus B19 infection. Fetal brain infection has been reported. Neuropathologic findings in the infected hydropic fetus include perivascular calcifications, primarily in the cerebral white matter, as well as multinucleated giant cells. Viral DNA has been demonstrated in the brain and liver (Isumi et al., 1999). Data also suggest that the maternal cell-mediated immune response at the placental level contributes to the pathogenesis of congenital infection. In one study, placentas from women whose pregnancies were complicated by parvovirus B19 infection had increased infiltration of CD3 T cells and elevated levels of interleukin-2 (Jordan et al., 2001).

Clinical Spectrum

Parvovirus B19 infection causes erythema infectiosum, or fifth disease, in normal hosts, aplastic crisis in patients with hemolytic disorders, and chronic anemia in immunocompromised hosts. A substantial proportion of infected adult women may also have arthropathy in association with parvovirus B19 infection (Woolf et al., 1989). Maternal symptoms have been present in up to two-thirds of documented cases of nonimmune hydrops fetalis associated with parvovirus B19 infection (Yaegashi et al., 1998).

The major clinical presentation of parvovirus B19 infection in the fetus is hydrops fetalis. Various estimates suggest that human parvovirus B19 infection contributes from 10%–27% of cases of nonimmune hydrops fetalis (Yaegashi et al., 1994; Essary et al., 1998; Markenson and Yancey, 1998). Nonimmune hydrops fetalis was the main complication in 0.9%–23% of pregnancies among proven maternal infections with parvovirus B19 (Ergaz and Ornoy, 2006). A risk of 7.1% for hydrops fetalis has been described for pregnant women who acquire parvovirus B19 infection between 13 and 20 weeks' gestation (Enders et al., 2004). The role that parvovirus B19 plays in intrauterine fetal demise in the absence of hydrops fetalis is incompletely defined (Riipinen et al., 2008; Williams et al., 2013). In addition to hydrops fetalis, in recent years there have been increasing numbers of case reports of neurologic and ophthalmologic anomalies associated with fetal parvovirus B19 infections. Although some studies undertaken to examine the association between fetal infection and congenital anomalies failed to reveal any associations (Mortimer et al., 1985; Kinney et al., 1988), in other studies parvovirus B19 has been a cause of neuronal migration defects (Pistorius et al., 2008). The role of parvovirus B19 in neurodevelopmental injury has not been fully explored. There have been at least three case reports of fetal encephalopathy associated with in utero infection with parvovirus B19 (Alger, 1997).

Parvovirus B19 has been implicated in some cases of congenital anemia (Heegaard and Brown, 2002). In one series of 11 children with a diagnosis of Diamond–Blackfan syndrome, 3 of 11 bone

marrow aspirates revealed evidence of parvovirus B19 DNA. All three of these children, but none of the parvovirus B19 PCR-negative cases, underwent spontaneous remission (Heegaard et al., 1996). In light of these data, all infants undergoing evaluation for congenital anemias should be evaluated for the possibility of parvovirus B19 infection. Long-term persistence of parvovirus B19 DNAemia (>1 year) with concomitant red blood cell aplasia has been described in the context of congenital infection (Lejeune et al., 2014; Hudson et al., 2015; Nadimpalli et al., 2015). Congenital parvovirus B19 infection has also been associated with bone lesions in long and axial bones (Cantey et al., 2013).

Laboratory Evaluation

In primary care the diagnosis of human parvovirus B19 infection is most commonly made clinically through recognition of the characteristic rash. Serologic confirmation is necessary in high-risk situations, such as after a significant exposure of a pregnant woman to a child with erythema infectiosum. Both radioimmunoassays and enzyme-linked immunosorbent assays are available for detection of human parvovirus B19-specific IgG and IgM antibodies (Kinney and Kumar, 1988). The presence of anti-parvovirus B19 IgM in fetal blood or amniotic fluid may confirm fetal infection but is not a sensitive test as it may be detected in only one-fifth of infected fetuses (Torok et al., 1992). False-positive results of parvovirus B19 IgM testing have been reported, including cross-reactions with anti-rubella IgM (Dieck et al., 1999).

Monitoring of the pregnant patient with evidence of a primary parvovirus B19 infection is important. The woman should be counseled regarding risks of fetal transmission, fetal loss, and hydrops, and serial ultrasound examinations should be performed every 1 to 2 weeks, up to 12 weeks after acute infection, to detect development of anemia and hydrops (Crane et al., 2014). The findings of echogenic bowel, ascites, pleural or pericardial effusion, or scalp edema are considered to be important markers of fetal infection and disease. Middle cerebral artery Doppler imaging for evaluation of fetal anemia may be another useful prospective surveillance tool, because fetal anemia can be detected with this technique before fetal hydrops is evident (Feldman et al., 2010). In the context of a human parvovirus B19 infection in a symptomatic, pregnant woman, elevated or rising weekly measurements of maternal alpha fetoprotein suggest fetal infection, and rising concentrations may be a marker for an increased risk of hydrops fetalis (Carrington et al., 1987). However, some studies have failed to demonstrate any association between the magnitude of the elevation of the alpha fetoprotein level and the severity of fetal anemia (Simms et al., 2009). A number of commercial PCR assays are available, and these can be performed on serum, amniotic fluid, or fetal tissue. Presumptive diagnosis can also be made on the basis of finding IgM antibody in the maternal and fetal blood. PCR for parvovirus B19 DNA or in situ hybridization studies can be performed with maternal blood, amniotic fluid, cord blood, or fetal tissues. The detection of parvovirus B19 DNA in maternal blood has excellent diagnostic sensitivity, and new-generation enzyme immunoassays and IgG avidity assays appear to hold promise for improved serodiagnosis during pregnancy (Enders et al., 2006, 2008).

Treatment

Spontaneous resolution of fetal hydrops with normal neonatal outcome has been reported in approximately one-third of cases

(Humphrey et al., 1991; Sheikh et al., 1992; Rodis et al., 1998a). Because two-thirds of fetuses do not recover without intervention, fetal transfusion is usually recommended (Boley and Popek, 1993; Brown et al., 1994). The earlier fetal transfusion is attempted, the more likely it is to be successful. Cordocentesis allows precise assessment of the magnitude of fetal anemia, which can then be corrected by blood transfusion, typically using packed red blood cells. With this approach, outcomes have been favorable in most reported series, even among severely anemic fetuses. In one report, packed red blood cell transfusion was performed in 30 patients with fetal anemia (hemoglobin values ranging from 2.1 to 9.6 g/dL). The overall survival rate was 83.8% (Schild et al., 1999). In a report of 13 patients with severe hydrops fetalis who received intrauterine transfusion, 11 survived (84.6%), whereas all the nontransfused fetuses with severe hydrops fetalis died (Enders et al., 2004). A retrospective review of 20 fetuses with parvovirus B19 infection complicated by severe anemia and/or fetal hydrops reported a survival rate of 76% for fetuses undergoing one or more transfusions (Macé et al., 2014).

Prognosis

Mortality rates for fetal hydrops resulting from all nonimmune causes continue to exceed 50%, even with current aggressive therapies and intensive care (Wy et al., 1999; Huang et al., 2007; Macé et al., 2014). Hydrops secondary to parvovirus B19 infection seems to have a better outcome than that from other causes (Ismail et al., 2001; Enders et al., 2004). Although few long-term prospective studies of infants born to mothers with documented primary parvovirus B19 infection have been conducted, most report normal developmental outcome. Parvovirus B19 has recently been recognized as a cause of CNS injury in older children, including encephalitis, meningitis, stroke, and peripheral neuropathy (Douvoyiannis et al., 2009), but two prospective studies in the United Kingdom of approximately 300 congenitally exposed infants found the risk of major congenital or developmental abnormality to be less than 1% (Miller et al., 1998), and there is minimal risk of long-term morbidity or death in childhood following primary maternal infections in pregnancy (Lassen et al., 2013). A case-control study involving approximately 200 mother-infant pairs found no differences in frequency of developmental delay between infants born to women with confirmed primary parvovirus B19 infection during pregnancy and infants born to women with evidence of preconceptional immunity (Rodis et al., 1998b). Other studies have also suggested a favorable long prognosis in children born to women with primary parvovirus B19 infections during pregnancy (Miller et al., 1998).

Prevention

If a pregnant woman has a significant exposure to an infectious case of parvovirus B19, counseling should be provided regarding the potential risk of infection. Anti-parvovirus IgM and IgG serologic analyses and serum PCR should be performed; if they show evidence of primary infection, then serial fetal ultrasonographic evaluations should be performed. Postexposure passive immunization with immunoglobulin is not currently recommended because the period of maternal viremia has passed by the time the diagnosis of acute parvovirus B19 infection is made (Boley and Popek, 1993). Although high-dose IVIG has been used to attempt to prevent hydrops fetalis during pregnancy in the setting of acute infection (Selbing et al., 1995), treatment with this modality in the pregnant

woman or the neonate has not been shown to improve fetal outcomes. Human IgG monoclonal antibodies with potent neutralizing activity have been generated, and these are suggested as candidates for the development of immunotherapeutic approaches for individuals chronically infected with parvovirus B19 or for acutely infected pregnant women (Gigler et al., 1999), but these interventions are not commercially available. There has been limited progress in the development of a candidate parvovirus B19 vaccine. Phase I studies of a recombinant vaccine based on baculovirus-produced capsids have been conducted, and this vaccine was found to have a favorable safety and immunogenicity profile (Bansal et al., 1993). Efforts are under way to research and develop vaccines to prevent parvovirus B19 infections with use of other expression systems (Lowin et al., 2005).

Pregnant healthcare providers should be counseled about the potential risks to their fetus from parvovirus B19 infections and should, at a minimum, wear masks and use standard droplet precautions when caring for immunocompromised patients with chronic parvovirus B19 infection or patients with parvovirus B19-induced aplastic crises. Some hospitals exclude pregnant healthcare providers from caring for these high-risk patients, but this issue remains controversial.

Rubella

Rubella virus is an enveloped, single-stranded, positive-sense RNA virus belonging to the family *Togaviridae*. Humans are the only known natural host for rubella virus. Rubella, commonly known as *German measles*, usually results in a mild illness with an accompanying exanthem in adults and children; however, rubella produces serious consequences in pregnant patients, in whom fetal infection can lead to serious anomalies. An ophthalmologist, Norman Gregg, offered the first description of CRS in 1941 while investigating an epidemic of neonatal cataracts (Gregg, 1941). Not until the global pandemic of 1964 to 1965, however, were the multiple teratogenic manifestations of CRS fully appreciated and the permanent neurodevelopmental consequences for newborns fully recognized. The capacity to grow the virus in tissue culture led rapidly to the development of a vaccine and subsequently an immediate reduction in the incidence of CRS in the United States and other developed countries, followed by efforts to eradicate indigenous CRS in the Western hemisphere (Papania et al., 2014). However, CRS is still encountered in the developing world, and unfounded concerns about the safety of measles-mumps-rubella vaccine have set the stage for potential reemergence of these diseases, because of decreased vaccine adherence in the context of routine well-child care (Omer et al., 2009; Grabenstein, 2013; Bahta and Ashkir, 2015). Therefore a working knowledge of the identification of the clinical manifestations of CRS remains highly relevant to the neonatologist.

Epidemiology

Since the development of rubella vaccine in 1969, the incidence of rubella in the United States has decreased dramatically. The annual incidence of rubella cases has dropped 99%, from 58 per 100,000 population in 1969 to less than 0.5 per 100,000 population in 1997 to 1999 (Danovaro-Holliday et al., 2001). CRS cases in the United States have demonstrated a similar dramatic decline, and in 2004 the CDC concluded, against the background of few or no reports of rubella activity from the 50 states and the virtual absence of reported CRS, that endemic rubella had been eliminated

from the United States (Centers for Disease Control and Prevention, 2005; Plotkin, 2006; Reef et al., 2006), although occasional importation cases had been described through the early 2000s (Castillo-Solórzano et al., 2003; Andrade et al., 2006). However, by mid-2015, endemic rubella had been declared to be eliminated from the Americas (Lambert et al., 2015; Kirby, 2015; Shrivastava et al., 2015). CRS may nonetheless be encountered in the United States in infants of mothers emigrating from countries with no or suboptimal national vaccination programs (Fang et al., 2013). Rubella is still an important and potentially preventable cause of birth defects globally, with more than 100,000 cases of CRS occurring annually in developing countries (Vynnycky et al., 2016).

Maternal rubella that occurs between the month before conception and the second trimester of pregnancy may be associated with transmission of infection and, depending on the timing of infection, disease in the infant. The frequency of congenital infection after maternal rubella with a rash is 70%–85% if infection occurs during the first 12 weeks of gestation, 30%–54% during the first 13 to 16 weeks of gestation, and 10%–25% at the end of the second trimester (Miller et al., 1982; South and Sever, 1985). The classic findings of congenital rubella are most typically associated with the onset of maternal infection during the first 8 weeks of gestation (Miller et al., 1982). Both the risk of fetal infection and the severity of disease decline after the first trimester, and the risk of any teratogenic effect is extremely low after 17 weeks' gestation (Lee and Bowden, 2000).

Pathogenesis

Rubella virus is transmitted via respiratory droplets. Once the oral or nasopharyngeal mucosae have been infected, viral replication occurs in the upper respiratory tract and nasopharyngeal lymphoid tissue. The virus then spreads contiguously to regional lymph nodes and hematogenously to distant sites. Fetal infection is believed to occur as a consequence of maternal viremia. The mechanism by which rubella infection of the fetus leads to teratogenesis has not been fully determined, but the cytopathology in infected fetal tissues suggests necrosis, apoptosis, or both, as well as inhibition of cell division of precursor cells involved in organogenesis (Lee and Bowden, 2000; Atreya et al., 2004). The rubella replicase protein p90 interacts with a cellular cytokinesis-regulatory component (i.e., the citron kinase) in the process leading to tetraploidy and cell cycle arrest. In tissue culture a number of unusual manifestations of rubella virus replication have been observed, including mitochondrial abnormalities and disruption of the cytoskeleton. Characteristic markers of apoptosis such as DNA fragmentation, nuclear chromatin condensation, and annexin V staining can be observed following rubella virus infection in cell culture. Microarray analysis following rubella virus infection in cell culture demonstrated upregulation of cytokines and interferon (IFN), suggesting that the induction of inflammatory responses may serve as another possible mechanism of injury induced by rubella (Adamo et al., 2008). Cells infected with rubella virus form a unique cytoplasmic fiber network resembling microtubules, which appears to facilitate cell-to-cell spread of the rubella virus genome even in the absence of infectious virus particles (Matthews et al., 2010). Astrocytes have been shown to be permissive for rubella virus replication in cell culture (Chantler et al., 1995). Macrovascular fetal endothelial cells have also been found to be highly permissive for rubella virus replication, suggesting that the vascular diseases in CRS are triggered by persistent rubella virus infection of endothelial cells (Perelygina et al., 2013, 2015). The importance of endothelial cells as targets

of infection in the developing fetus was also demonstrated by histopathologic examination of three cases of fatal CRS that demonstrated rubella virus antigen in interstitial fibroblasts in the heart, adventitial fibroblasts of large blood vessels, alveolar macrophages, progenitor cells of the outer granular layer of the brain, and capillary endothelium and basal plate in the placenta (Lazar et al., 2016). Fetuses infected with rubella virus demonstrate cellular damage in multiple sites, and a noninflammatory necrosis is observed in multiple target organs, including eyes, heart, brain, and ears (Lee and Bowden, 2000).

Clinical Spectrum

The peak incidence of endemic rubella is in the late winter and early spring months. Up to 50% of primary infections are asymptomatic. The period of maximal communicability extends from a few days before until 7 days after onset of the rash. Often a prodrome of mild systemic symptoms precedes the rash by 1 to 5 days. Viremia can be detected as early as 9 days before the onset of rash. Lymphadenopathy, which can precede a rash, is often present in the posterior auricular or suboccipital region. The rash classically begins on the face and spreads caudally to the trunk and extremities. Symptoms generally last up to 3 days, and the incubation period ranges from 14 to 21 days. Transmission by breastfeeding in a case of postpartum acquisition of infection has been described (Klein et al., 1980). The typical illness in adults and children with acquired rubella consists of an acute generalized maculopapular rash, fever, and arthralgias, arthritis, or lymphadenopathy. Conjunctivitis is also common. Encephalitis (1 in 5000 cases) and thrombocytopenia (1 in 3000 cases) are complications (Chaari et al., 2014).

Infants with congenital rubella are usually born at term but are often small for their gestational age. The most common isolated sequela is hearing loss (Ueda et al., 1979; Miller et al., 1982). The next most common findings are heart defects, cataracts, low birth weight, hepatosplenomegaly, and microcephaly. The triad of deafness, cataracts, and congenital heart disease constitutes the classic syndrome (Bouthry et al., 2014). In addition, systemic illness can occur and can be characterized by purpura, hepatosplenomegaly, jaundice, pneumonia, and meningoencephalitis; 45%–70% of infants have cardiac lesions, including patent ductus arteriosus, peripheral pulmonic stenosis, and valve abnormalities (Schluter et al., 1998; Reef et al., 2000). An extensive review of published reports of cardiovascular disease in the setting of CRS demonstrated that branch pulmonary artery stenosis is the most commonly identified isolated lesion (Oster et al., 2010). Death caused by pulmonary hypertension has been described in the setting of untreated patent ductus arteriosus caused by CRS (Toizumi et al., 2014). Additional ocular findings are pigmentary retinopathy, microphthalmia, and strabismus. Skin lesions have been described as resembling a blueberry muffin and represent extramedullary dermal hematopoiesis; an identical rash can be observed in congenital CMV infection (Brough et al., 1967; Bowden et al., 1989; Avram et al., 2007; Mehta et al., 2008).

The clinical manifestations of CRS differ to some extent depending on the timing of fetal infection. In a prospective study following up pregnant women with confirmed rubella by trimester, a full range of rubella-associated defects (including congenital heart disease and deafness) were observed in nine infants infected before the 11th week. Thirty-five percent of infants (9 of 26) infected between 13 and 16 weeks' gestation had deafness alone (Miller et al., 1982). Cataracts typically occur secondary to maternal rubella occurring before day 60 of pregnancy; heart disease is found almost exclusively

when maternal infection is before the 80th day (i.e., first trimester). Disease manifestations that may have their onset after birth (late-onset disease) include a generalized rash with seborrheic features that may persist for weeks, acute or chronic interstitial pneumonia, abnormal hearing resulting from presumed labyrinthitis, central auditory imperception, and progressive rubella panencephalitis (Phelan and Campbell, 1969; Sever et al., 1985; Reef et al., 2000; Franklin and Kelley, 2001). Infants with congenital rubella appear to shed virus in the oropharynx for the first several months of life (Nagasawa et al., 2016).

A higher than expected incidence of autoimmune diseases, such as thyroid disorders and diabetes mellitus, has also been reported years after the diagnosis of congenital rubella (McEvoy et al., 1988; Reef et al., 2000; Forrest et al., 2002; Gale, 2008). Abnormalities in dental development, including evidence of hypoplastic enamel of the developing teeth, have been described in children with CRS (Bhatia et al., 2012). Infants with late-onset disease have demonstrated immunologic abnormalities, including dysgammaglobulinemia or hypogammaglobulinemia (Soothill et al., 1966; Hayes et al., 1967; Hancock et al., 1968). Hyper-IgM syndrome has also been described in association with autoimmune disease in a child with CRS (Palacin et al., 2007). Other studies have demonstrated dysfunction of cellular immune responses in children with CRS (Fuccillo et al., 1974; South et al., 1975; Verder et al., 1986). Psychiatric disturbances, including some with features of autism spectrum disorders, have been observed decades after CRS (Hwang and Chen, 2010).

Laboratory Evaluation

It is common obstetric practice to screen all pregnant women for rubella virus antibodies, and because of the devastating consequences of CRS, this is a reasonable policy to continue, even in the setting of eradication of endemic rubella in the United States. Women who are exposed to rubella should be screened for evidence of previous immunity; if none is found, they should be tested for rubella virus IgG and IgM antibodies. A positive IgM titer or a rise in paired IgG titers is indicative of recent infection. Women with such findings should also be evaluated to try to determine the likely gestational age at the time of infection to assess the potential risk to the fetus.

The laboratory diagnosis of congenital rubella can be made definitively only during the first year of life, unless the virus can be recovered later from an affected site, such as the lens. Diagnosis can be confirmed by a positive rubella IgM titer; a significant acute-to-convalescence change in IgG titer; isolation of rubella virus from nasal, blood, throat, urine, or CSF samples; or detection of viral nucleic acid by reverse transcriptase (RT) PCR from the throat, CSF, or lens (obtained during cataract surgery). An infected infant can excrete the virus for many months after birth despite the presence of neutralizing antibody and thus may pose a hazard to susceptible individuals (Nagasawa et al., 2016).

Other laboratory findings include thrombocytopenia, hyperbilirubinemia, and leukopenia. There is relatively little information about maternal ultrasonographic findings in CRS (Yazigi et al., 2017), largely because the syndrome had become rare by the time routine ultrasonographic screening during pregnancy became standard. Radiographic findings include a large anterior fontanel, linear areas of radiolucency in the long bones (i.e., celery stalking), increased densities in the metaphyses, and irregular provisional zones of calcification (Reed, 1969; Chapman, 1991). The radiographic changes seen in rubella are not pathognomonic of the

disease but resemble those seen in other congenital viral infections, including congenital CMV infection (Alessandri et al., 1995).

Treatment and Prognosis

There is no specific antiviral therapy for congenital rubella. Initially the infant may need general supportive care, such as administration of blood transfusion for anemia or active bleeding, seizure control, and phototherapy for hyperbilirubinemia. Long-term care requires a multidisciplinary approach consisting of occupational and physical therapy, close neurologic and audiologic monitoring, and surgical interventions as needed for cardiac malformations and cataracts (Shah et al., 2014).

The consequences of fetal rubella may not be evident at birth. In one study of 123 infants with documented congenital rubella, 85% of cases were not diagnosed until after discharge from the nursery (Hardy, 1973). Communication disorders, hearing defects, some mental or motor retardation, and microcephaly by 1 to 3 years of age were among the major problems that were discovered after the newborn period. A predisposition for inguinal hernias was also noted. Longitudinal studies of somatic growth show that most infants with congenital rubella remain smaller than average throughout infancy but grow at a normal rate. Stunting of growth was more common after rubella virus infection in the first 8 weeks of pregnancy than after later infection. Even in the absence of intellectual disability, neuromuscular development is commonly abnormal. A study of neurodevelopmental outcomes in 29 affected children without intellectual disability found that 25 had other abnormalities: hearing loss, difficulties with balance and gait, learning deficits, and behavioral disturbances (Desmond et al., 1978).

Prevention

The critical intervention in prevention of CRS is to ensure that women who are considering pregnancy have been appropriately vaccinated. The Advisory Committee on Immunization Practices recommends screening of all pregnant women for rubella immunity and postpartum vaccination of those who are susceptible (McLean et al., 2013). Immunity to rubella appears to confer almost complete protection against CRS. Rare cases of documented subclinical maternal reinfection with rubella virus have been reported (Saule et al., 1988; Morgan-Capner et al., 1991). In rare cases, maternal reinfection can lead to CRS (Banerji et al., 2005). Approximately 20 cases of CRS after maternal reinfection have been reported in the literature, and none have caused symptomatic CRS when the known reinfection occurred after 12 weeks' gestation (Bullens et al., 2000).

Live attenuated rubella virus vaccine is safe and effective, although the duration of immunity is uncertain. It is currently administered in the United States in a trivalent formulation in combination with measles–mumps–rubella vaccine. The vaccine is recommended for children at 12 to 15 months of age and at 4 to 5 years of age. It is also recommended for women of childbearing age in whom the results of both a hemagglutination inhibition antibody test and a pregnancy test are negative. Although no cases of symptomatic congenital rubella have been reported as a consequence of vaccination during pregnancy in the more than 500 cases monitored, vaccination is not recommended during pregnancy because of the theoretical hazard to the fetus (Josefson, 2001; Tookey, 2001; Nasiri et al., 2009). A mild rubella-like illness is sometimes seen after immunization, with arthralgia occurring 10 days to 3 weeks after

injection. If a woman is found to be nonimmune, vaccine should be administered during the immediate postpartum period before discharge. Breastfeeding is not a contraindication to postpartum immunization. Immunization in the postpartum period has rarely produced polyarticular arthritis, neurologic symptoms, or chronic rubella viremia (Tingle et al., 1985).

The problem of treatment of the pregnant woman who is exposed to rubella or who contracts the disease should be resolved after the known risks have been weighed. If serum antibody is detectable at the time of exposure, the fetus is probably protected. If no antibody is detectable, additional serum samples should be obtained at 2 to 3 weeks after exposure and again at 4 to 6 weeks after exposure. These samples can be run concurrently with the first serum sample to ascertain whether infection has occurred (i.e., seroconversion). There is some evidence that administration of IVIG to a pregnant woman may prevent rubella virus infection or viremia (Young et al., 2015). IVIG can reduce the likelihood of fetal infection but will not eliminate the risk; therefore the CDC does not routinely recommend the use of IVIG in a pregnant woman for postexposure prophylaxis unless she does not wish to terminate the pregnancy. Decisions about the termination of pregnancy should be made only after maternal infection has been proved and should also account for the risk of rubella-associated damage to the fetus, which is highest when maternal infection occurs during the first 8 weeks of pregnancy.

Lymphocytic Choriomeningitis Virus

LCMV is a member of the family *Arenaviridae*. Rodents are the primary reservoir, particularly mice and hamsters. Like other arenaviruses, LCMV has a bisegmented negative-strand RNA genome. The S segment encodes the virus nucleoprotein and glycoprotein, whereas the L segment encodes the virus polymerase (L) and Z protein. Sequelae of human exposure to LCMV range from asymptomatic infection to nonspecific, flulike symptoms; a proportion of infections have neurologic manifestations. LCMV was first described as a cause of congenital infection in England in 1955 in a 12-day-old infant (Komrower et al., 1955) and later in the United States (Barton et al., 1993; Larsen et al., 1993; Barton et al., 2001; Barton and Mets, 2001). Because LCMV has only recently been recognized as a source of congenital infection, it is likely underdiagnosed (Jamieson et al., 2006).

Epidemiology

Human seroprevalence ranges between less than 1% and 10% worldwide and differs extensively with geographic region (Childs et al., 1991; Stephensen et al., 1992; Ambrosio et al., 1994; Marrie and Saron, 1998). One study noted a higher prevalence in women (Marrie and Saron, 1998). Studies conducted in the 1940s through the 1970s found that approximately 8%–11% of cases of aseptic meningitis and encephalitis were associated with LCMV infection (Meyer et al., 1960; Park et al., 1997a). In temperate climates, human exposure is more common during the fall and winter, when rodents move indoors. Outbreaks have been reported in laboratory personnel working with hamsters and mice (Hinman et al., 1975; Vanzee et al., 1975; Dykewicz et al., 1992). Multiple outbreaks associated with pet hamsters have also been reported in the United States (Biggar et al., 1975; Maetz et al., 1976) and Europe (Deibel et al., 1975; Brouqui et al., 1995); however, congenital infection is relatively rare. As of the early 2000s, 54 cases had been published worldwide since the initial reported case in 1955, and 27 of these

cases had occurred in the United States (Barton and Mets, 2001; Greenhow and Weinrub, 2003). There has been increased recognition of congenital LCMV in recent years; 34 of the cases described in a review were reported since 1993 (Jamieson et al., 2006). The true frequency of congenital LCMV infection is unknown, because there is no active surveillance. As with other congenital infections, there may be a wide spectrum of disease, including asymptomatic and subclinical or nonspecific infections.

Pathogenesis

Humans acquire LCMV infection from aerosolized particles, bites, or fomite contact with virus excreted from rodents (Jahrling and Peters, 1992). Human-to-human horizontal transmission by organ transplant has been documented (Fischer et al., 2006). The pathogenesis of LCMV infection is poorly understood, although it is likely an immunopathologic process mediated by the host CD8⁺ T-cell response (Craighead, 2000). It is also postulated that the high rate of spontaneous mutations that arise during LCMV replication allows both variability in pathogenicity and a mechanism of escape from humoral response during the initial phase of infection (Ciurea et al., 2001).

Like other arenaviruses, LCMV replicates either at the site of infection or in corresponding lymph nodes; this localized replication is followed by viremia. During the viremic stage the virus travels to parenchymal organs and the CNS. Pathologic findings include lymphocytic infiltration and extramedullary hematopoiesis. Chemokine and cytokine responses appear to be responsible for neurologic disease (Christensen et al., 2009), although LCMV inactivates the host innate type 1 IFN response, presumably as an immune evasion strategy (Borrow et al., 2010). These immune evasion mechanisms allow viral persistence in the infected host.

Clinical Spectrum

It is estimated that asymptomatic or mild LCMV infections occur in approximately one-third of patients infected; however, the classic presentation of LCMV infection is a nonspecific, flulike, or mononucleosis-like illness that is often biphasic. The symptoms include fever, malaise, nausea, vomiting, myalgias, headache, photophobia, pharyngitis, cough, and adenopathy. After defervescence and resolution of these constitutional symptoms, a second phase of CNS disease may develop. Neurologic manifestations occur in approximately one-fourth of infectious episodes and range from aseptic meningitis to meningoencephalitis. Transverse myelitis, Guillain-Barré syndrome, and deafness have also been reported. Other manifestations include pneumonitis, arthritis, myocarditis, parotitis, and dermatitis. Recovery may take months but usually occurs without sequelae (Craighead, 2000).

When LCMV infection occurs during pregnancy, maternal symptoms typically appear during the first and second trimesters, but only 50%–60% of mothers of infants with a diagnosis of congenital LCMV infection recall having symptoms (Wright et al., 1997). Known maternal exposure to rodents is reported in approximately one-fourth to one-half of cases (Barton and Mets, 2001). Usually, exposed women are from rural settings, come from lower socioeconomic settings with substandard housing conditions, or have pet rodents in the home (i.e., hamsters).

The complete spectrum of disease secondary to congenital LCMV infection is still uncertain, although the virus clearly targets the fetal brain and retina, potentially causing ventriculomegaly, hydrocephalus, chorioretinitis, and neurodevelopmental abnormalities

(Bonthius, 2012; Anderson et al., 2014). Chorioretinitis and hydrocephalus are the predominant characteristics reported among children with a diagnosis of congenital LCMV infection. Chorioretinitis is present in more than 90% of cases. Other ocular findings include chorioretinal scars, optic atrophy (usually bilateral), nystagmus, esotropia, exotropia, leukocoria, cataracts, and microphthalmia (Enders et al., 1999; Brezin et al., 2000; Barton and Mets, 2001). Some ophthalmologic findings resemble those described in the lacunar retinopathy of Aicardi syndrome (Wright et al., 1997). A wide range of neurologic defects are described, including microencephaly, encephalomalacia, chorioretinitis, porencephalic cysts, neuronal migration disturbances, periventricular infection, and cerebellar hypoplasia (Bonthius et al., 2007, 2012). Congenital LCMV infection should be considered in the differential diagnosis of neonatal hydrocephalus (Schulte et al., 2006).

Most infants are born at term, and birthweights are generally appropriate or large for gestational age. Thirty-five percent to 40% of infants reported with congenital LCMV infection have had microcephaly or macrocephaly at birth (Barton and Mets, 2001). Systemic symptoms are rare, although hepatosplenomegaly and jaundice have been noted. Other individual case report findings include pes valgus, dermatologic findings resembling staphylococcal scalded-skin syndrome (Wright et al., 1997), spontaneous abortion (Biggar et al., 1975), and intrauterine demise secondary to hydrops fetalis (Enders et al., 1999; Meritet et al., 2009; El Feghaly and Hunstad, 2013). It is important to note that because systemic symptoms are typically minimal at birth, the diagnosis of congenital LCMV infection may not be considered until an affected infant is a few months old, when microcephaly, macrocephaly, visual loss, or developmental delay may be noted.

Laboratory Evaluation

Serology is the most reliable and feasible method to diagnose LCMV infection. In most reports the diagnosis was established by testing of the infant's serum, CSF, or both; in some, maternal serum testing was the key. Testing of all three fluids provides the most information. Because of the low baseline population seroprevalence, positive LCMV titer test results are much more useful for diagnosis than detection of antibodies to microbes such as CMV and *Toxoplasma gondii*. There is a commercially available immunofluorescent antibody test that detects both IgM and IgG for LCMV. It has better sensitivity than the complement fixation and neutralizing antibody tests (Lewis et al., 1975; Lehmann-Grube et al., 1979). Complement fixation titers generally do not rise until more than 10 days after onset of infection, but immunofluorescent antibody results may be positive within the first few days of illness (Deibel et al., 1975). The CDC also has an enzyme-linked immunosorbent assay test for IgM and IgG; it may be more useful for diagnosis in an older child because it can detect increased IgG levels later than and persistent IgG for longer than the immunofluorescent antibody test. Some studies have found antibody as late as 30 years after suspected exposure. RT-PCR has been used in serum and CSF to diagnosis LCMV infection and as a surveillance tool; it may become more available in the future (Park et al., 1997b; Enders et al., 1999; McCausland and Crotty, 2008).

Information about routine laboratory data in patients with a diagnosis of congenital LCMV infection is minimal, but thrombocytopenia and hyperbilirubinemia have been reported. CSF findings are variable. Up to half of cases demonstrate a mild increase in white blood cell count (up to 64 cells per microliter in one case

series of 18 infants), the serum protein concentration may be normal or mildly elevated, and the serum glucose concentration may be normal or mildly decreased (Wright et al., 1997). Among infants reported in whom neuroradiographic imaging was performed, 89% (17 of 19) had hydrocephalus or periventricular calcifications. Flattened gyri, lissencephaly, and schizencephaly have been reported (Barton and Mets, 2001), compatible with a role for LCMV in fetal neuronal migration defects (Bonthius et al., 2007).

Treatment

Ribavirin has been used for management of other arenavirus infections and inhibits LCMV growth in vitro (Géssner and Lother, 1989). Although novel approaches are being used to develop antivirals against LCMV and other pathogenic arenaviruses (de la Torre, 2008), there are currently no recommendations for the use of antiviral agents against these viruses. In the outbreak associated with solid organ transplant, one affected recipient received ribavirin and reduced levels of immunosuppressive therapy and survived (Fischer et al., 2006).

Prognosis

Because congenital LCMV infections have been recognized relatively recently and the existing data come from case reports, there may be a wider spectrum of disease than is currently appreciated. The proportion of asymptomatic infected infants is unknown. In a review of 26 serologically confirmed infant cases, 9 infants (35%) died, and 10 (63%) of the 16 reported survivors had severe neurologic sequelae (Wright et al., 1997). Neurologic sequelae reported in the setting of congenital LCMV infection include microcephaly, spastic quadriplegia, mental retardation, developmental delay, seizures, and visual loss (Barton and Mets, 2001; Bonthius, 2012). SNHL is also described (Wright et al., 1997; Barton and Mets, 2001; Anderson et al., 2014; Cohen et al., 2014). Retinal disease is an underrecognized complication of congenital LCMV infection. A study in Chicago of patients prospectively diagnosed with chorioretinitis and patients in a home for severely mentally retarded children with chorioretinal scars found six children with elevated LCMV titers and negative evaluation for toxoplasmosis, CMV, rubella, and HSV (Mets et al., 2000). Similarly, two children with chorioretinal scars and elevated LCMV titers, suggesting congenital infection, have been reported in France (Brezin et al., 2000).

Prevention

Public health officials and clinicians should be aware that (1) wild, laboratory, and pet rodent exposure can lead to intrauterine infection with LCMV and (2) congenital infection has been associated with potentially devastating ophthalmologic and neurologic sequelae. Pregnant women need to be educated about the risks of exposure to infected rodent excreta and instructed to avoid rodents and rodent droppings. Obstetricians and neonatologists should seek a history of pet or wild rodent exposure for counseling purposes and to aid in the evaluation of infants with unexplained CNS diseases. The diagnosis of LCMV infection should be considered in all cases of infant hydrocephalus. There are currently no vaccines approved by the FDA for the prevention of arenavirus disease, although candidate multivalent arenavirus vaccines capable of providing T cell–mediated protection against a variety of pathogenic arenaviruses are currently in development (Botten et al., 2010).

Attenuated LCMV is also itself being used as a vector for vaccine development for heterologous pathogens (Ring and Flatz, 2016).

Enteroviruses

Enteroviruses are single-stranded, positive-sense RNA viruses. These viruses belong to the family *Picornaviridae* (*pico* means *very small* in Spanish). The enteroviruses of humans include polioviruses 1, 2, and 3, coxsackieviruses A and B (named after Cocksackie, New York, the city where these viruses were first identified and characterized), and the echoviruses (*echo* is an acronym for *enteric cytopathic human orphan*). Of these, poliovirus infection has historically been responsible for the greatest morbidity in infants. Severe, often fatal poliovirus disease used to occur with great frequency in infants infected in the perinatal period, with a high incidence of residual paralysis in survivors. Fortunately, poliovirus infections have become rare in the developed world, because of the widespread implementation of effective immunizations. However, neonatal diseases associated with coxsackieviruses and echoviruses, for which there are no vaccines, remain common and can be associated with serious morbidity and occasional death. Typically acquired from a maternal source, these agents are associated with a wide range of clinical syndromes in the NICU, including CNS infection, myocarditis, and a sepsis-like syndrome. Enteroviruses are also responsible for a large number of hospital readmissions for evaluation of febrile syndromes in infants younger than 2 months.

Epidemiology

Enterovirus infections are seasonal, occurring most commonly during summer and autumn in temperate climates. The incidence varies from year to year, with outbreaks sometimes caused by a single coxsackievirus or echovirus serotype and sometimes by several serotypes (Sawyer et al., 1994). In older children, enteroviruses are transmitted by the fecal–oral route and are typically associated with a variety of febrile syndromes, including febrile exanthematous syndromes, aseptic meningitis, pneumonia with or without pleural effusion, and myocarditis. Disease in newborns is relatively uncommon but reflects the frequency of infection in the general population (Kraiden and Middleton, 1983). Enteroviruses may also be associated with nosocomial outbreaks. Nursery and obstetric clinic outbreaks of both coxsackievirus B (Brightman et al., 1966; Rantakallio et al., 1970; Bhambhani et al., 2007) and echovirus (Nagington et al., 1978; Jankovic et al., 1999; Chen et al., 2005) infections have been reported and associated with severe, and sometimes fatal, illnesses.

Enterovirus infections account for at least one-third of neonatal febrile admissions for suspected sepsis and for between half and two-thirds of all admissions during the peak enterovirus season (Dagan, 1996; Byington et al., 1999). Neonatal aseptic meningitis is also frequently caused by enterovirus infections. In a review of neonatal meningitis seen over a 15-year period in Galveston, Texas, enterovirus was the most common cause of meningitis in newborns older than 7 days (Shattuck and Chonmaitree, 1992). Enteroviruses, along with other viruses, have been implicated as a potential cause of sudden infant death syndrome (SIDS), possibly from myocarditis or pulmonary infection (Shimizu et al., 1995; Grangeot-Keros et al., 1996), but this association has been controversial. In one study of SIDS victims, a comprehensive assessment was undertaken to attempt to identify potential viral infection of the myocardium. Overall, 62 SIDS victims and 11 controls were studied. Enteroviruses

were detected in 14 cases (22.5%), adenoviruses in 2 cases (3.2%), EBVs in 3 cases (4.8%), and parvovirus B19 in 7 cases (11.2%), whereas control group samples were completely negative for viral nucleic acid (Dettmeyer et al., 2004). However, another evaluation of histopathologic features and PCR analysis from 24 SIDS cases failed to demonstrate any association with viral infection (Krous et al., 2009), leaving this putative association unclear.

Etiology and Pathogenesis

Neonates can acquire enterovirus infections secondary to in utero transmission, intrapartum transmission during labor and delivery, or postnatally. Intrauterine infections appear to occur via transplacental spread, secondary to maternal viremia, and this mode of transmission appears to be responsible for up to 22% of cases of neonatal enterovirus infection (Kaplan et al., 1983; Modlin, 1986). Enteroviruses have been implicated as a cause of fetal demise (Nielsen et al., 1988; Johansson et al., 1992; Konstantinidou et al., 2007). Intrapartum or postnatal transmission is more common than transplacental transmission. The dominant mode of transmission of serious neonatal infection is through contact with maternal blood, fecal material, or vaginal or cervical secretions, most likely during or shortly after delivery (Jenista et al., 1984; Hawkes and Vaudry, 2005). After acquisition of infection, viremia ensues in the infant, leading to a variety of end-organ diseases. The presence or absence of transplacental maternally derived antibody also dictates the severity of the disease in the infected infant. Breastfeeding appears to provide a relative degree of protection against acquisition of infection in neonates (Sadeharju et al., 2007).

One of the most comprehensive analyses of the incidence of neonatal enterovirus disease came from a prospective study in Rochester, New York. This study demonstrated that approximately 13% of all newborns tested positive for enterovirus from throat or stool cultures during a typical season (June to October; Jenista et al., 1984). Although this result represented a remarkably high incidence, 79% of these infections were asymptomatic. The most common clinical findings in the 21% of symptomatic infections were lethargy and fever. These infants were typically admitted to a hospital for an evaluation to rule out sepsis, making enterovirus infection a more common reason for hospitalization because of an infectious disease than group B streptococcus, HSV, and CMV infections combined.

It appears that any of the nonpolio enteroviruses can cause disease in the newborn. A variety of clinical syndromes are associated with certain enteroviruses. A retrospective medical record review of 24 neonatal enterovirus infections in Toronto, Canada, found that 10 infants died, 12 had aseptic meningitis, and 5 had myocarditis (Kraiden and Middleton, 1983). Of the 24 isolates, 7 were echovirus, 15 were coxsackievirus B, 1 was coxsackievirus A, and 1 was nontypable. In infants with acute enterovirus disease requiring hospitalization, coxsackievirus B is associated primarily with myocarditis and aseptic meningitis (Kibrick and Benirschke, 1958). Recent reports have identified coxsackievirus B1 as an emerging cause of life-threatening myocarditis and other severe, fatal syndromes in neonates (Verma et al., 2009; Wikswo et al., 2009). Echoviruses are associated with severe nonspecific febrile illnesses with DIC (Nagington et al., 1978), aseptic meningitis (Cramblett et al., 1973), or hepatitis (Modlin, 1980). With both coxsackievirus and echovirus, nonspecific febrile illnesses, with or without an exanthem, are commonly observed. Enterovirus 71 is particularly notable for its etiologic role in epidemics of severe neurologic diseases in children (Chen et al., 2010). Nursery-based outbreaks

of infection with this neurovirulent enterovirus have also been described (Huang et al., 2010).

Except for transplacental infections, the portal of entry for enterovirus is via the oral or respiratory route. After replication in the pharynx and the GI tract, virus seeds the tonsils, cervical and mesenteric nodes, and Peyer patches. The pathogenesis of enterovirus disease stems from the ensuing viremia, which can lead to infection of the heart, CNS, liver, pancreas, adrenal glands, skin, mucous membranes, and respiratory tract. In coxsackievirus B infections, myocardial necrosis and inflammation may be seen that are patchy or diffuse, with extensive infiltration by lymphocytes, mononuclear cells, histiocytes, and polymorphonuclear leukocytes. Similar infiltrates are seen in the meninges in both coxsackievirus and echovirus aseptic meningitis. Brainstem encephalitis can be observed with enterovirus 71 infection, often in association with pulmonary edema (Wang and Liu, 2009). Fatal fulminant infections are often associated with echovirus 11 (Mostoufzadeh et al., 1983). Recently a severe enteroviral pneumonitis in children has been described caused by enterovirus 68, which was associated with wheezing, hypoxia, and pediatric intensive care unit admissions (Du et al., 2015; Esposito et al., 2015; McAllister et al., 2015; Principi and Esposito, 2015). Development of antibody is associated with recovery, although virus may continue to be shed in the stool for several weeks. Failure to clear enterovirus infection, particularly from the CNS, should suggest an underlying humoral immunodeficiency, although this would not typically be recognized in the neonatal period because of the presence of transplacentally acquired IgG (McKinney et al., 1987; Misbah et al., 1992).

Clinical Spectrum

When neonatal disease is acquired vertically from the mother, the infant is typically asymptomatic at birth, although premature delivery is more common. The mother may be febrile at this time or may have a history of recent high temperatures and GI symptoms. Fever, anorexia, and vomiting develop in the baby after an incubation period of 1 to 5 days. The onset of illness occurs in the first week of life in more than 50% of affected infants (Krajden and Middleton, 1983). At that point the clinical evolution depends on the infecting virus and the extent of end-organ involvement. In most instances the disease is mild and self-limited. Symptomatic infections may be characterized by rash, aseptic meningitis, hepatitis, and pneumonia. A review of 29 infants younger than 2 weeks with enterovirus infections reported that 5 of the infants had severe multisystem disease, and all survived (Abzug et al., 1993). In another study in Salt Lake City, 1779 febrile infants younger than 90 days and undergoing evaluation for sepsis were enrolled; 1061 were tested for enterovirus, and 214 (20%) were enterovirus positive (57% from blood and 74% from CSF). The mean age of infants with enterovirus infection was 33 days; 91% were admitted, and 2% required intensive care (Rittichier et al., 2005).

These observations underscore the generally benign and self-limited nature of neonatal enterovirus disease; however, morbidity can be substantial in severe disseminated disease. A viral sepsis syndrome—characterized by DIC, refractory hypotension, and death—may occur in the setting of severe disease. Typically these severe infections are acquired in the immediate perinatal period. The mother is commonly symptomatic and may have been empirically treated with broad-spectrum antibiotics for possible chorioamnionitis. A maternal history of suspected chorioamnionitis in the absence of positive bacterial cultures should suggest this

association, particularly during the typical “enterovirus season” observed in temperate climates. If myocarditis is present, congestive heart failure is often severe. Some infections, particularly those with echoviruses, are characterized by a rampant and overwhelming hepatitis (Modlin, 1980). Others exhibit primarily pulmonary disease or GI involvement, including diarrhea and necrotizing enterocolitis (Lake et al., 1976). Intracranial bleeding ranging from small to massive, severe hemorrhage has also been reported as a complication of neonatal enterovirus infection (Swiatek, 1997; Abzug and Johnson, 2000; Abzug, 2001). Other rarely associated findings include disseminated vesicular rash, dermal hematopoiesis, and hemophagocytic syndrome (Bowden et al., 1989; Barre et al., 1998; Sauerbrei et al., 2000). The severity of CNS infection is similarly variable. Moderate or mild meningitis is characterized by temporary irritability, lethargy, fever, and feeding difficulty.

Acquired postnatal enterovirus disease in infants is characterized primarily by high temperature, irritability, lethargy, or poor feeding. One-fourth of infected infants develop diarrhea or vomiting with or without an erythematous maculopapular rash. Conjunctivitis has also been observed. Respiratory tract symptoms are less common (Dagan, 1996). As noted, there is significant overlap between enteroviral and bacterial neonatal infections, and the two syndromes are difficult to distinguish; therefore many febrile infants will be admitted to the hospital and treated with broad-spectrum antibiotics and, if the CNS is involved, with acyclovir for possible bacterial sepsis and neonatal HSV infection. Such treatment seems unavoidable, except in circumstances in which enterovirus infection can be diagnosed rapidly and definitively. The duration of illness ranges from less than 24 hours to longer than 7 days but generally is 3 to 4 days.

Laboratory Evaluation

Viral culture from stool or rectal swab, nasopharyngeal swab, blood, buffy coat, urine, or CSF is the gold standard diagnosis. Stool or rectal swab cultures can remain positive for several weeks following the initial infection, underscoring the importance of fecal–oral transmission in the epidemiology of these infections. Recently, RT-PCR assays have become standardized and are available from many commercial and reference laboratories. Serologic tests for enteroviruses have been reported but are less useful than culture and PCR (Swanink et al., 1993).

Laboratory evaluation is predicated on the clinical syndrome and the end organs involved. Infants with aseptic meningitis typically have moderate CSF pleocytosis, which can be either lymphocytic or polymorphonuclear but may lack pleocytosis even in the setting of documented CNS infection (Seiden et al., 2010). Accordingly, during periods of active enterovirus infection in the community, CSF should be sent for PCR even if pleocytosis is absent. Thrombocytopenia, elevated transaminase levels, hyperbilirubinemia, hyperammonemia, hematologic abnormalities consistent with DIC, anemia, peripheral leukocytosis, and abnormal chest radiographs are among other potential laboratory findings. When myocarditis is a diagnostic possibility, echocardiogram and electrocardiogram are indicated and may reveal diminished left ventricular function or dysrhythmias. Liver biopsy and histopathologic examination may be warranted in cases of fulminant hepatic failure (Abzug, 2001; Muehlenbachs et al., 2015). For neonatal enterovirus disease, the dried umbilical cord has been used for retrospective, PCR-based diagnosis of in utero–acquired infection (Morioka et al., 2014).

Treatment

The cornerstone of treatment of neonatal enterovirus disease is supportive care. Myocarditis and heart failure can be treated with inotropic support, diuretics, aggressive fluid management, and other supportive measures. DIC should be treated with blood products and other supportive measures as indicated. There is no evidence that steroids are of benefit. IVIG has been reported anecdotally to treat neonatal enterovirus infections with various degrees of success (Valduss et al., 1993; Kimura et al., 1999). Only one randomized trial has systematically studied its use, in 16 neonates with severe enterovirus infection; nine of these infants were randomized to receive IVIG, at a dose of 750 mg/kg. Decreased viremia and viruria along with faster resolution of irritability, jaundice, and diarrhea were demonstrated in patients given IVIG with high titers of neutralizing enteroviral-specific antibodies. However, there were no significant differences in other major clinical outcomes, such as the duration of hospitalization, fever, and symptoms of acute illness between treatment and control groups (Abzug et al., 1995). To attempt to augment the enterovirus type-specific antibody level in the setting of symptomatic neonatal disease, maternal plasma transfusion has been attempted (Jantausch et al., 1995; Rentz et al., 2006), based on the rationale that neonates typically acquire infection from their mothers in the peripartum period. Although reports of success with use of IVIG for treatment of neonatal enterovirus infections are largely based on anecdotal reports in limited numbers of patients, its use should be considered for severely symptomatic infections with life-threatening end-organ disease.

The antiviral drug pleconaril has been developed specifically to treat picornavirus infections (enteroviruses and rhinoviruses). A small case series of infants with severe enteroviral hepatitis suggested a beneficial effect (Aradottir et al., 2001). Another small case series indicated that five of six neonates with severe enterovirus infection who were treated with pleconaril survived, with minimal or no sequelae (Rotbart and Webster, 2001). A multicenter study of pleconaril treatment of enteroviral meningitis in children younger than 12 months conducted by the CASG and sponsored by the National Institutes of Health demonstrated no significant differences in the duration of positivity by culture or PCR, hospitalization, or symptoms for the treatment and placebo groups (Abzug et al., 2003). Pleconaril did appear to have an effect on enteroviral meningitis in adults (Desmond et al., 2006), but the role of this drug in infants and children remains undefined. In a recent study of pleconaril for treatment of neonatal enteroviral sepsis, symptomatic infants with hepatitis, coagulopathy, and/or myocarditis and onset of disease at 15 days of life or earlier were randomized to receive either orally administered pleconaril or placebo for a 7-day treatment course. Shorter times to both culture and PCR negativity and a greater overall survival rate were observed among the pleconaril recipients (Abzug et al., 2016). Although other small-molecular inhibitors of picornavirus replication are in preclinical development (Smeets et al., 2016), additional trials of pleconaril for treatment of serious enterovirus disease in infants and young children are warranted.

Short-Term and Long-Term Prognosis

Prognostic factors for severe neonatal disease include peripartum maternal illness, earlier age of onset of neonatal disease, absence of serotype-specific antibody, and absence of fever and irritability (Abzug et al., 1993). All these risk factors are most consistent with

vertical intrauterine enterovirus infection rather than postnatally acquired infection. The highest mortality rates are associated with the combination of severe hepatitis, coagulopathy, and myocarditis. Severe hepatitis caused by enterovirus infection is associated with mortality rates ranging from 30% to 80% (Modlin, 1986; Abzug, 2001). By the time DIC has developed, the prognosis is grave. Prothrombin time longer than 30 seconds was a risk factor for death in one retrospective case review (Abzug, 2001).

Few long-term follow-up studies have been published, but the available information suggests that infants who survive severe enteroviral neonatal disease have a complete recovery in most instances. Outcomes of 6 of 11 survivors with follow-up ranging from 9 to 48 months included normal growth and no residual medical problems or liver dysfunction (Abzug, 2001). The long-term prognosis following CNS infection is unclear. A number of early studies of infants younger than 3 months with aseptic meningitis suggested that there may be some impairment of intellectual development in comparison with carefully selected control groups (Farmer et al., 1975; Sells et al., 1975). However, in a series of nine children with enteroviral meningitis and nine matched controls evaluated for sequelae at approximately 4 years of age, no differences in mean intelligence quotient, head circumference, detectable SNHL, or intellectual functioning were detected (Wilfert et al., 1981). A similar case-control follow-up study of 33 participants, compared with siblings used as controls, reported no neurodevelopmental sequelae (Bergman et al., 1987). Older children with enterovirus 71 CNS infection, however, are at risk of significant neurodevelopmental sequelae (Chang et al., 2007).

Prevention

Anecdotal reports of the use of IVIG in nursery outbreaks to prevent further horizontal transmission of enterovirus infection have produced conflicting results (Nagington et al., 1983; Caroleane et al., 1985; Kinney et al., 1986). Because there are multiple nonpolio enteroviral serotypes that cause clinical disease, design of antienteroviral immunization is conceptually difficult, although vaccines are in development for enterovirus 71 (Zhang et al., 2010; de Crom et al., 2016). Standard contact precautions should be used for the treatment of hospitalized infants with known or suspected enterovirus infections.

Human Parechovirus

Recently two viruses formerly classified with the echoviruses, echoviruses 22 and 23, were shown to comprise a separate genus within the family *Picornaviridae*, the genus *Parechovirus*. Bank voles and humans serve as natural hosts. There are currently four species in this genus: *Parechovirus A* (also known as *human parechovirus*), *Parechovirus B* (more commonly known as *Ljungan virus*), *Parechovirus C* (also known as *Sebokele virus*), and *Parechovirus D* (*Ferret parechovirus*). Seventeen distinct human parechovirus genotypes have now been recognized (Drexler et al., 2009; Pajkrt et al., 2009; Harvala et al., 2010; Renaud and Harrison, 2015). Genotype 1 is most commonly associated with GI illness, while genotype 3 is associated with neonatal sepsis and CNS infection (de Crom et al., 2016). These viruses have been recognized recently as significant neonatal pathogens (Harvala et al., 2010; Sharp et al., 2013). The primary sites of parechovirus replication are believed to be the respiratory tract and the GI tract. Replication in the GI tract is associated with prolonged shedding of infectious virus in the feces. As a result, fecal-oral transmission, as with enteroviruses,

appears to be the predominant route of infection. Parechovirus infections may also be acquired via a respiratory route, with subsequent virus shedding detectable in respiratory secretions. Viremia leads to secondary seeding of other organs and systemic symptoms.

Neonates with parechovirus infection, particularly parechovirus 3 infection, have a clinical presentation similar to infants with severe enterovirus disease, with a sepsis-like illness in severe cases (Verboon-Macielek et al., 2008a; Wolthers et al., 2008). Hepatitis is also a prominent feature of neonatal infection. The most frequent signs are fever, seizures, irritability, rash, and feeding problems. It has been postulated that parechovirus 3 infection is a newly emerging infection, and the relative lack of maternal antibody may predispose the neonate to more severe disease (Harvala et al., 2010). Parechovirus can also cause aseptic meningitis and encephalitis in the neonate, with the predominant site of injury being the periventricular white matter (Verboon-Macielek et al., 2008b; Levorson et al., 2009; Gupta et al., 2010). The findings noted by MRI can mimic those observed with hypoxic-ischemic encephalopathy (Amarnath et al., 2016). Parechovirus can cause aseptic meningitis in the absence of CSF pleocytosis (Sharp et al., 2013), usually associated with the type 3 parechovirus genotype (Harvala et al., 2011). Parechovirus RNA has also been found at autopsy in children younger than 2 years (Sedmak et al., 2010), including in infants with otherwise unexplained deaths. Having an older sibling in the household is an independent risk factor for acquisition of neonatal disease (Nielsen et al., 2016). In a case series of 13 patients with suspected CNS infection who were evaluated for parechovirus infection, nine infants had confirmed parechovirus encephalitis, and five demonstrated neurodevelopmental sequelae, including two with cerebral palsy and one with central visual impairment (Britton et al., 2016). In a retrospective analysis of more than 300 CSF samples collected over a 10-year period, the prevalence of parechovirus was higher than that of HSV, CMV, and VZV (Vollbach et al., 2015).

Ljungan virus, first described in 1998, has been the subject of recent interest because of its apparent role in the pathogenesis of type 1 diabetes in rodents and because of a possible emerging role in human disease (Tolf et al., 2009). The virus does not appear to have a role in the development of diabetes in humans (Tapia et al., 2010), although seroprevalence studies demonstrate that this infection does circulate by person-to-person transmission (Jääskeläinen et al., 2016). This zoonotic virus has recently emerged as a recognized cause of fetal infection associated with severe CNS abnormalities, including hydranencephaly and hydrocephalus and, possibly, SIDS (Niklasson et al., 2007, 2009a, 2009b). A study from Sweden identified a strong epidemiologic association between small rodent abundance and the incidence of intrauterine fetal death in humans. Ljungan virus antigen was detected in this study in half of the intrauterine fetal death cases tested (Niklasson et al., 2009b).

Zika Virus

Zika virus is a newly emerging pathogen that has recently been demonstrated to cause serious disease in newborns, most notably microcephaly, in the context of congenital transmission. Congenital infections with related flaviviruses, such as West Nile virus, have been described (Alpert et al., 2003) but are rare (O'Leary et al., 2006; Pridjian et al., 2016). The emergence of Zika virus infection, in contrast, has had a devastating impact on newborn health. Zika virus was first isolated in Uganda in 1947 in rhesus macaques (Dick, 1952; Dick et al., 1952) but received little attention until

early 2015, when an outbreak of Zika virus was identified in northeast Brazil. By late 2015, reports of an increase in the number of infants born with microcephaly in Zika virus-affected areas emerged, and viral nucleic acids were identified in the amniotic fluid of women whose fetuses had been found to have microcephaly (Schuler-Faccini et al., 2016). Since these reports, congenital Zika virus infection has been recognized throughout the Western Hemisphere, including congenital infections identified in the United States (Culjat et al., 2016; Driggers et al., 2016), and this infection has had a devastating impact on newborns. Zika virus infection has been declared a public health emergency of international concern by the World Health Organization (WHO) (Heymann et al., 2016). Although much remains to be learned about Zika virus, it is important for neonatologists and pediatricians to be aware of the clinical manifestations of this emerging neonatal viral infection.

Epidemiology

Zika virus infection was first recognized in Africa, where it was associated with occasional reports of febrile syndromes in humans (MacNamara, 1954) but otherwise was of little clinical significance. Through mechanisms that remain incompletely defined, Zika virus underwent a migration out of Africa into Asia, Micronesia, and the Pacific Islands over the ensuing decades (Haddow et al., 2012), eventually producing outbreaks of disease on the island of Yap (in the Federated States of Micronesia) in the early 2000s (Lanciotti et al., 2008; Duffy et al., 2009) and on Easter Island in 2014 (Tognarelli et al., 2016). No fetal abnormalities were identified initially during a French Polynesia outbreak described in 2013 to 2014, but a retrospective analysis of this outbreak identified 19 cases of congenital cerebral malformations, including septal and callosal disruption, ventriculomegaly, neuronal migration defects, cerebellar hypoplasia, occipital pseudocysts, and brain calcifications (Besnard et al., 2016). Thus it was clear even before the Brazilian outbreak that congenital Zika virus infection could cause major brain malformations.

The extent of the Zika virus epidemic in the Americas as of mid-2016 indicated that virtually all countries in South America had identified active Zika virus transmission, with approximately 50 countries on the official CDC Zika virus travel advisory list (<http://www.cdc.gov/zika/geo/active-countries.html>). Most cases have been identified in Brazil, where in 2015, there were an estimated 440,000 to 1,300,000 Zika virus cases, with approximately 5000 suspected cases of microcephaly, most of them in the northeast region of the country, and an estimated 76 deaths (Heukelbach et al., 2016). Estimates of the total number of cases of brain injury caused by congenital Zika virus infection have been complicated by the lack of a uniform definition of microcephaly (Nunes et al., 2016). In the United States, cases are tracked by the CDC (<http://www.cdc.gov/zika/geo/united-states.html>). As of early 2017, more than 5000 cases of documented Zika virus infection had been reported in US states and territories, and more than 1500 of these have been in pregnant women.

Zika virus infection is predominantly a vector-borne infection, transmitted by the mosquitoes *Aedes aegyptii* and *Aedes albopictus* (Rabaan et al., 2017). Both of these mosquitoes are endemic in the United States (Hahn et al., 2016). Nonvector modes of transmission, particularly sexual transmission, are known to occur (Atkinson et al., 2016; Grischott et al., 2016; Matheron et al., 2016) but appear rare. Zika virus infection has also been documented to be transmitted by blood transfusion (Barjas-Castro et al.,

2016). Autochthonous transmission to humans by mosquitoes has been documented in the United States (in Florida) as of mid-2016.

Pathogenesis

Zika virus is a member of the family *Flaviviridae* (Faye et al., 2014). This family includes West Nile virus, chikungunya virus, yellow fever virus, and dengue virus. Zika virus is an enveloped, positive-sense, single-stranded RNA virus with a genome of approximately 10,800 bases in length. The RNA serves as a single messenger RNA species that encodes a polyprotein that is subsequently cleaved into capsid, precursor membrane (pRM), envelope (E), and nonstructural (NS) proteins. The E protein composes the majority of the virion surface and is involved in aspects of replication, including binding to target receptors and membrane fusion. The E protein is also a candidate subunit vaccine target. The NS1 protein has emerged as a potential diagnostic marker of early infection and a possible vaccine candidate (Rastogi et al., 2016).

Zika virus appears to reach the developing fetus via a transplacental route of transmission. Viral RNA has been identified in amniotic fluid (Calvet et al., 2016), and viral antigens have been identified in fetal brain tissue and placenta/chorionic villi (Martines et al., 2016). Viral particles have also been identified in fetal brain by electron microscopy in the setting of primary maternal infection with associated fetal transmission during pregnancy (Mlakar et al., 2016). The likelihood of microcephaly and associated brain anomalies depends on the timing of infection.

The mechanism of brain injury induced by Zika virus appears to involve direct infection of human neural progenitor cells in the developing fetal brain. These cells, derived from induced pluripotent stem cells, undergo lytic infection and release infectious progeny virus. Both direct virus-mediated cell death and dysregulation of cell cycle progression contribute to brain injury (Tang et al., 2016), as does a virus-induced alteration of immune response and apoptotic gene transcription (Li et al., 2016). Zika virus also interferes with fetal neuronal stem cell differentiation (McGrath et al., 2017; van den Pol et al., 2017). The candidate viral receptor, AXL, is expressed in glial cells, astrocytes, endothelial cells, and microglia in the developing human cortex and in progenitor cells in developing retina (Nowakowski et al., 2016).

Clinical Manifestations

Only one in five infected individuals have symptoms in the setting of primary infection. Symptomatic acute Zika virus infection in the nonpregnant individual produces headache, arthralgia, myalgia, nonpurulent conjunctivitis, erythematous (and often pruritus) rash, and lower back pain (Brasil et al., 2016). Fever, when present, is of low intensity and short lived. Symptoms usually begin within 2 weeks of exposure, and viremia persists for approximately 1 week. Zika virus has also been causally associated with Guillain-Barré syndrome (GBS) (Cao-Lormeau et al., 2016). The antiglycolipid IgG antibodies commonly detected in GBS were detected in less than 50% of cases in one investigation, suggesting a role for direct viral neurotoxicity rather than indirect (immune-mediated) pathogenesis for some patients. Nerve conduction studies demonstrated the acute motor axonal neuropathy type of GBS, and clinical improvement during convalescence suggested a reversible conduction failure component (Cao-Lormeau et al., 2016).

For the congenitally infected fetus, vertical transmission of Zika virus is primarily associated with clinical manifestations related to the CNS. Microcephaly is the prominent potential consequence

of congenital Zika virus infection early in pregnancy. The risk of microcephaly is very much dependent on the trimester of infection, with estimated rates of 0.88%–13.2% following first-trimester infection (Johansson et al., 2016). Although microcephaly has not been reported in the offspring of women infected in the third trimester (Brasil et al., 2016), the brain and placenta can still become infected, and clinical manifestations in infants congenitally infected during this trimester include stillbirth, growth restriction, and EEG abnormalities with motor and tone abnormalities. Other CNS abnormalities include ventriculomegaly, global hypoglyria, and hydranencephaly (Besnard et al., 2016; Hazin et al., 2016; Sarno et al., 2016). Ocular manifestations are common, may occur in the absence of microcephaly, and include pigmentary and hemorrhagic retinopathy, chorioretinal atrophy, abnormal vascular development, optic nerve abnormalities, microphthalmia, and cataracts (de Paula Freitas et al., 2016; Ventura et al., 2016a, 2016b). Hearing loss is described in congenital Zika virus infection (de Barros Miranda-Filho et al., 2016). A case of hydrops fetalis has also been observed (Sarno et al., 2016).

Laboratory Evaluation and Diagnostic Studies

Laboratory abnormalities in the setting of acute Zika virus infection are often nonspecific and may include leukopenia, thrombocytopenia, and transaminitis (Musso and Gubler, 2016). Laboratory diagnosis of Zika virus infection relies on detection of Zika virus-specific RNA by RT-PCR of serum and/or urine. Samples should be obtained within 7 days of symptom onset for suspected cases. Serum PCR results are positive only for a brief window (3–7 days), when the infected individual has viremia; hence a negative result cannot exclude infection. Since urine PCR results may be positive for up to 14 days following onset of symptoms, this may be a useful adjunct to diagnosis. For an individual presenting 4 to 7 days after onset of symptoms with a negative Zika virus PCR result, serologic testing should be performed. Serologic testing for the highly related chikungunya, dengue, and West Nile viruses should also be considered. If Zika virus IgM test results are positive, equivocal, or inconclusive, testing for neutralization antibodies using the plaque reduction neutralization test (PRNT) should be performed. A Zika virus-specific PRNT titer greater than 10 should be interpreted as evidence of infection with Zika virus when the PRNT titer for the other flaviviruses tested is less than 10 (Rabe et al., 2016).

Treatment

No specific treatment is available for Zika virus infection, although intense efforts are under way to identify and develop antivirals for this and other flavivirus infections (Makhluף et al., 2016). A recent cell culture screen identified 2'-C-methylated nucleosides as promising lead candidates for further development (Eyer et al., 2016), but none are currently licensed for clinical use. There are currently no recommendations for passive antibody-based immunoprophylaxis for prevention of maternofetal transmission of Zika virus, although recent animal model studies have demonstrated that adoptive transfer of purified IgG from mice and rhesus macaques vaccinated with various investigational Zika pRM/E vaccines confers passive protection against viral challenge (Abbink et al., 2016; Larocca et al., 2016). These observations provide hope that passive immunoprophylaxis strategies might be developed that are capable of protecting the fetus from disease following maternal exposure during pregnancy (Heinz and Stiasny, 2017).

Clinical Evaluation and Prognosis

The long-term outcome of infants with congenital Zika virus infection remains uncertain. The CDC has provided recommendations for the evaluation and long-term follow-up of infants with possible congenital Zika virus infection (Karwowski et al., 2016). Congenital infection is defined as the presence of Zika virus RNA in any sample collected at birth, including amniotic fluid, placenta, umbilical cord, newborn serum, newborn urine, or newborn CSF (Staples et al., 2016). Investigation for congenital infection should also include testing of newborn serum for the presence of Zika virus IgM antibody, with confirmatory PRNT testing. Infants with microcephaly but negative Zika virus diagnostic evaluation findings should be evaluated for other perinatal infections, particularly CMV infection (Schleiss, 2016). Evaluation by a geneticist is also recommended. For infants with positive or inconclusive Zika virus test results, evaluation for neurologic abnormalities, dysmorphic features, hepatosplenomegaly, and rash is recommended. Cranial ultrasonography is recommended, along with ophthalmologic evaluation within 1 month after birth. Evaluation of hearing before hospital discharge or within 1 month after birth is recommended, along with a repeated audiologic evaluation at 6 months of age. Careful evaluation of developmental milestones, as well as head circumference, is recommended through the first year of life. Ongoing consultation with pediatric neurologists, developmental pediatricians, and physical and speech therapists is appropriate if any abnormalities are noted.

Although Zika virus particles have been observed in breast milk (Dupont-Rouzeyrol et al., 2016), there have to date been no reports of Zika virus transmission by breastfeeding. Currently there is no contraindication to breastfeeding in infants born to women with Zika virus infection, since the theoretical risk of Zika virus transmission through breast milk is outweighed by the known benefits of breastfeeding. Moreover, no developmental complications have been observed in otherwise healthy children with postnatal Zika virus infection or exposure.

Prevention

Avoidance of travel to high-risk areas currently is the only prevention strategy for men and women of reproductive age. If travel is unavoidable, the CDC recommends use of an FDA-approved insect repellent containing *N,N*-Diethyl-*meta*-toluamide (DEET), picaridin, oil-of-lemon eucalyptus, *para*-methane diol, or IR3535. The WHO currently recommends that individuals practice 8-week abstinence or safe sex precautions after return from Zika virus–endemic regions (McCarthy, 2016). However, late sexual transmission because of longer periods of persistence of Zika virus in semen has been described (Turmel et al., 2016). For this reason, couples in whom a man had confirmed Zika virus infection or clinical illness consistent with Zika virus disease should wait at least 6 months after symptom onset to attempt conception.

Vaccines targeting the Zika virus pRM/E protein have been shown to be effective at preventing disease and blocking transmission in mouse and primate models (Larocca et al., 2016; Barouch et al., 2017). Insect vector control is also a critical component of prevention. Proposed interventions include increased use of larvicides (Faraji and Unlu, 2016) and the intentional environmental release of genetically modified mosquitoes incapable of producing progeny after mating (Adalja et al., 2016). Safeguards to protect the blood supply against the potential risk of transfusion-associated Zika virus infections will also need to be developed and implemented (Katz and Rossmann, 2017).

Hepatitis Viruses

There are six distinct viruses known to cause viral hepatitis—hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D (delta agent) virus, hepatitis E virus, and hepatitis G virus (HGV). HGV is of importance for the pregnant patient, but only HBV and HCV are of major importance in the newborn. The main features of each virus type are listed in Table 37.3 (Krugman, 1992; Jonas, 2000; Koff, 2007). HAV is passed by fecal–oral transmission and is a rare cause of neonatal disease, although it has been nosocomially transmitted in the setting of a NICU. Hepatitis E virus is similar to HAV in its mode of transmission and clinical manifestations, except for an increased mortality in pregnant women infected with hepatitis E virus (Aggarwal et al., 2009; Teshale et al., 2010). There are no data regarding perinatal transmission of hepatitis E virus. Hepatitis D virus may cause only coinfection or superinfection with HBV. Its only clinical significance is that HBV infection may become more severe when hepatitis D virus is present. Perinatal transmission has been described (Ramia and Bahakim, 1988) but is uncommon.

HGV, also known as *GB virus type C*, has been associated with acute and chronic hepatitis, and HGV infection is usually noted as a coinfection with HBV or HCV. This virus has a tropism for lymphocytes and may influence the course and prognosis in HIV-seropositive patients (Reshetnyak et al., 2008). Perinatal transmission can occur in 60%–80% of infants born to HGV-viremic mothers, but there has been no report of clinical hepatitis attributed to HGV infection in this setting (Zanetti et al., 1998; Wejstal et al., 1999; Zuin et al., 1999; Ohto et al., 2000). The clinical significance and effects of HGV infection are still poorly understood. HBV and HCV are both transmitted vertically and, among the viral hepatitis, are of the greatest importance to the care of newborns.

Hepatitis B Virus

The issues that are most important to neonatologists are the frequency with which HBV is transmitted to neonates at the time of birth, the short-term and long-term consequences of these infections, the importance of greater surveillance for maternal carriage of HBV, the role of antiviral therapy to prevent transmission and disease progression, and the availability of effective HBV immunoprophylaxis (Krugman, 1988; Arfaoui et al., 2010; Clemente and Vajro, 2016).

Incidence

In certain parts of the world and among certain ethnic groups, as many as 7%–10% of all infants acquire HBV infections at the time of birth, and almost all of these infections become chronic. In the United States, it has been estimated that approximately 20,000 infants are born annually to mothers who are chronic HBV carriers (Mast et al., 1998). Since 1990 the incidence of acute HBV infection in the United States has declined dramatically, with the largest declines occurring in children younger than 15 years (98%) and in young adults aged 15 to 24 years (90%). The frequency of transmission depends primarily on the prevalence of the HBV carrier state among women of childbearing age. HBV is a carcinogenic virus, and infections acquired perinatally can lead to chronic liver failure and hepatic carcinoma in adult life (Beasley and Hwang, 1984; Balistreri, 1988; Chan and Sung, 2006).

The incidence of neonatal HBV infection depends on the timing of infection and the overall prevalence of the disease in the

TABLE 37.3 Viral Hepatitis Types A, B, C, D, E, and G: Comparison of Clinical, Epidemiologic, Immunologic, and Therapeutic Features

| Feature | Hepatitis A | Hepatitis B | Hepatitis C | Hepatitis D | Hepatitis E | Hepatitis G |
|-----------------------------|--------------------------|-----------------------|----------------------------|---|---|--------------------------------------|
| Virus | Hepatitis A virus | Hepatitis B virus | Hepatitis C virus | Hepatitis D virus (hepatitis delta virus) | Hepatitis E virus | Hepatitis G virus (GB virus type C) |
| Family | <i>Picornaviridae</i> | <i>Hepadnaviridae</i> | <i>Flaviviridae</i> | Unassigned | <i>Hepeviridae</i> | <i>Flaviviridae</i> |
| Genome | RNA | DNA | RNA | RNA | RNA | RNA |
| Incubation period | 15 to 40 days | 50 to 180 days | 1 to 5 months | 2 to 8 weeks | 2 to 9 weeks | Unknown |
| Mode of Transmission | | | | | | |
| Oral (fecal) | Usual | No | No | No | Usual | No |
| Parenteral | Rare | Usual | Usual | Usual | No | Usual |
| Perinatal | Rare | Yes | Yes | Only with hepatitis B virus | Unknown | Yes |
| Other | Food-borne or waterborne | Sexual contact | Sexual contact less common | Sexual contact less common | Waterborne transmission in developing countries | Sexual contact; probably less common |
| Sequelae | | | | | | |
| Carrier state | No | Yes | Yes | Yes | No | Yes |
| Chronic disease | No cases reported | Yes | Yes | Yes | No cases reported | Yes; controversial |
| Interventions | | | | | | |
| Immunoglobulin | Yes | Yes | No | No | No | No |
| Vaccine | Yes | Yes | No | No | No | No |
| Antiviral therapy | No | Yes | Yes ^a | No | No | No |

^aNot approved for pediatric use.
From Saul Krugman. Viral Hepatitis: A, B, C, D and E—Infection, *Pediatrics in Review* Jun 1992;13(6):203–212; DOI: 10.1542/pir.13-6-203.

population under study. Women with acute HBV infection during the first or second trimester rarely transmit the virus to their infants (Krugman, 1988; Stevens, 1994). Transplacental transfer has been described but appears to be rare. The carriage rate for hepatitis B surface antigen (HBsAg) ranges from 0.1% in the United States and Europe to 15% in Taiwan and parts of Africa, with intermediate rates in Japan, South America, and Southeast Asia. Transmission rates among immigrant women in Western countries appear to parallel the rates in their countries of origin (Krugman, 1988).

The most important route of transmission is transmission that occurs during labor and delivery. The likelihood of transmission is great if symptomatic acute disease is present (60%–70% transmission; Gerety and Schweitzer, 1977). Infants of hepatitis B e antigen (HBeAg)-positive mothers have an 80%–90% chance of becoming HBsAg carriers (Okada et al., 1976; Lee et al., 1978). The risk of an infant acquiring HBV infection if born to an HBsAg-positive but HBeAg-negative mother is 5%–20%. Chronic neonatal infection occurs in less than 10% of infants of HBeAg-negative mothers (Krugman, 1988). Although HBsAg has been found in breast milk, breastfeeding does not appear to have any influence on the rate of transmission (Beasley et al., 1975). The WHO currently recommends that all mothers who are hepatitis B–positive breastfeed their infants and that their infants be immunized at birth.

Etiology and Pathogenesis

HBV is a DNA virus that localizes primarily in hepatic parenchymal cells but circulates in the bloodstream, along with several subviral

antigens, for periods ranging from a few days to many years. Several distinct genotypes have been identified, and these subtypes show biologic variability in transmission and disease progression (Magnius and Norder, 1995; Schaefer et al., 2009; Kramvis, 2016). Transplacental leakage of HBeAg-positive maternal blood is a potential source of intrauterine infection (Lin et al., 1987). During either acute or persistent viremia in the mother, the virus itself or viral antigens may rarely cross the placenta and cause intrauterine infection, but more commonly infection occurs perinatally during labor or delivery (Chisari and Ferrari, 1995; Xu et al., 2001). The finding of hepatitis virus antigens in the newborn might not indicate the presence of infection but rather might indicate the passive transfer of antigen only; therefore antigen test results should be interpreted cautiously in this setting. HBV infection of placental trophoblast has been documented (Bai et al., 2007). Innate immune responses in the trophoblast appear to play a key role in defense against transplacental transmission, particularly Toll-like receptors 7 and 8 (Tian et al., 2015). Immunoglobulin against HBV also plays a role in prevention of transmission at the placental level, because of antibody deposition in Hofbauer cells that serves as an immune barrier to transmission between the mother and the fetus (Liu et al., 2015). HBeAg plays a role in immune tolerance to infection in the newborn. HBeAg is the only HBV antigen to cross the placenta, and its presence leads to specific unresponsiveness of helper T cells to the viral capsid protein in the newborn (Kramvis, 2016). HBeAg is tolerated in utero, acts as a tolerogen after birth, and its presence in maternal serum is a biomarker for an increased

risk of perinatal transmission. Most infants born to mothers infected with HBV have a negative test result for HBsAg at birth but in the absence of prophylaxis are at risk of becoming HBsAg-positive during the first 3 months of life, suggesting that transmission is primarily peripartum and not transplacental (Krugman, 1988, 1992; Shapiro, 1993; Mulligan and Stiehm, 1994). Prevention of peripartum HBV transmission is not considered to be an indication for primary caesarean delivery.

Clinical Spectrum and Laboratory Evaluation

Infants with HBV infection do not show clinical or chemical signs of disease at birth. Without immunoprophylaxis the usual pattern is the development of chronic antigenemia with mild and often persistent enzyme level elevations, beginning at 2 to 6 months of age (Mulligan and Stiehm, 1994). Less commonly the infection becomes clinically manifest, with jaundice, fever, hepatomegaly, and anorexia, followed by either recovery or chronic active hepatitis. Rarely, fulminant hepatitis is seen and can be fatal (Delaplane et al., 1983).

Laboratory tests are essential in the diagnosis of HBV infection. Evaluations of serum enzymes and bilirubin reflect the extent of liver damage. Serologic tests identify the virus involved (Krugman, 1988). HBsAg appears early, usually before liver disease is found; it persists in those who become long-term carriers or disappears in the 5% of infants in whom the infection is resolved. HBeAg and anti-HBeAg testing can be used to assess infectivity; although they are not as frequently used for serodiagnosis, they are useful markers of the likelihood of perinatal transmission. Risk factors that increase the likelihood of maternofetal transmission include the magnitude of maternal viral load, presence of HBeAg, and a maternal thalassemia minor genotype (Dunkelberg et al., 2014; Zhang et al., 2014). PCR and other nucleic acid detection techniques are important in confirming diagnosis, assessing viral load, and monitoring the response to therapy.

Prevention

The primary goal of prevention is to prevent chronic HBV infection and chronic liver disease. In the United States, in the past 3 decades a comprehensive immunization strategy (summarized in Table 37.4) has been implemented consisting of four components:

1. Universal immunization of all infants beginning at birth
2. Prevention of perinatal infection through routine screening of all pregnant women and appropriate immunoprophylaxis of infants born to HBsAg-positive women (or women whose HBsAg status is unknown)
3. Routine immunization of children and adolescents who have not been immunized previously
4. Immunization of nonimmunized adults at increased risk of infection

For infants the three-dose vaccination schedule should be initiated in the neonatal period or by 2 months of age (Table 37.4). Four doses are acceptable if a birth dose is given and a combination vaccine is used to complete the series. Vaccination can be delayed until just before hospital discharge in preterm (birthweight <2000 g) infants born to HBsAg-negative mothers.

All infants born to HBsAg-positive women should receive both active and passive immunization within 12 hours of birth. The doses and recommended options for administration of the HBV vaccines that are currently licensed in the United States are provided in Table 37.4 (American Academy of Pediatrics Committee on Infectious Diseases, 2015). If the maternal HBsAg status is unknown, it should be checked immediately, and the infant should receive the first dose of HBV vaccine immediately. Infants born to women who test positive or to women in whom the HBsAg status is unknown should be given hepatitis B immune globulin as soon as possible within 1 week after birth. The highest immunization failure rates have been observed in infants of HBeAg-positive women and those infected in utero (Farmer et al., 1987; Tang et al., 1998). Infants who received hepatitis B immunoglobulin and HBV vaccine at birth, followed by two additional immunizations, should be tested for anti-HBsAg and HBsAg at 9 to 12 months of age or 1 to 2 months after the last dose. This is important because about 5% of such infants develop chronic HBV infection even after optimal immunoprophylaxis. Infants who do not receive the vaccine should be tested as soon as they are identified.

In addition to HBV immunoglobulin and vaccination, oral antiviral therapies in highly viremic women can reduce the risk of transmission of HBV to newborns. The use of telbivudine, lamivudine, and tenofovir appears to be safe in pregnancy, with

TABLE 37.4 Licensed Monovalent and Combination Hepatitis B Vaccines

| Clinical Scenario | MONOVALENT VACCINES | | COMBINATION VACCINES | | |
|---|----------------------------------|---------------------|--------------------------------|-------------------------------|----------------------------------|
| | Recombivax Hepatitis B Dose (μg) | Engerix-B Dose (μg) | Twinrix ^a Dose (μg) | Comvax ^b Dose (μg) | Pediatric ^c Dose (μg) |
| Newborns born to HBsAg-negative mothers and children and adolescents aged <20 years | 5 (0.5 mL) | 10 (0.5 mL) | NA | 5 (0.5 mL) | 10 (0.5 mL) |
| Newborns born to HBsAg-positive mothers ^d | 5 (0.5 mL) | 10 (0.5 mL) | NA | 5 (0.5 mL) | 10 (0.5 mL) |
| Adolescents aged 11 to 15 years | 10 (1 mL) | NA | NA | NA | NA |
| Adults aged >20 years | 10 (1.0 mL) | 20 (1.0 mL) | 20 (1.0 mL) | NA | NA |

^aTwinrix is a combination of Engerix-B (20 μg) and hepatitis A vaccine, licensed for use in people aged 18 years or older in a three-dose series in a 0-, 1-, and 6-month schedule.

^bComvax is a combination of Recombivax HB (5 μg) and *Haemophilus influenzae* type B (PRP-OMP) recommended for use at 2, 4, and 12 to 15 months of age. This vaccine should not be administered at birth, before 6 weeks of age, or after 71 months of age.

^cPediatric is a combination of diphtheria and tetanus toxoids and acellular pertussis (i.e., DTaP), inactivated poliovirus, and hepatitis B (Engerix-B, 10 μg). It is recommended for use at 2, 4, and 6 months of age but should not be administered at birth, before 6 weeks of age, or after 7 years of age.

^dHepatitis B immunoglobulin (0.5 mL) should be administered simultaneously with vaccination.

HBsAg, Hepatitis B surface antigen; NA, not applicable.

no increased adverse maternal or fetal outcome (Brown et al., 2016; Piratvisuth et al., 2016). Recently, a prevention study examined whether the use of tenofovir in women with chronic HBV infection (HBeAg positive) could modify the risk of transmission to the newborn. In a cohort of HBeAg-positive mothers with an HBV DNA level of more than 200,000 international units per milliliter during the third trimester, the rate of mother-to-child transmission was lower among those who received tenofovir therapy than among untreated controls (Pan et al., 2016).

Treatment

Currently, seven antiviral agents have been approved by the FDA for treatment of adults with chronic HBV infection in the United States (Bitton Alaluf and Shlomai, 2016). These agents, categorized as either IFNs (IFN alfa-2b and pegylated IFN alfa-2a) or nucleoside or nucleotide analogues (lamivudine, adefovir, entecavir, tenofovir, telbivudine), are used as monotherapy or in combination. Lamivudine is now considered the first-line therapy in adult patients, eclipsing IFN. Experience with and indications for antiviral therapy for HBV infection in children are limited (Clemente and Vajro, 2016). IFN alfa, lamivudine, and adefovir, a RT inhibitor, were the first drugs approved for treatment of chronic HBV infection in children, but success is widely variable (Kurbegov and Sokol, 2009; Giacchino and Cappelli, 2010). In September 2012, tenofovir was approved by the FDA for use in children with chronic HBV infection aged 12 years or older weighing more than 35 kg. More recently, entecavir was approved in the United States for use in children aged 2 years or older with chronic HBV infection and evidence of active viral replication and disease activity and, with IFN alfa, is emerging as a first-line antiviral regimen for children with HBV infection who are candidates for antiviral therapy (Chang et al., 2016; Jonas et al., 2016).

These antiviral therapies are summarized in Table 37.2. IFN alfa appears to be the most effective (approximately 30% HBeAg seroconversion, 10% HBsAg seroconversion), although benefits are primarily observed in children with ALT levels more than twice the upper limit of normal. The virologic response rates for lamivudine mirror those for IFN alfa (23%–31% HBeAg seroconversion), with easier administration and a more favorable safety profile but lower HBsAg seroconversion (2%–3%) and high rates of drug resistance. A 5-year study of entecavir in children demonstrated an HBV DNA negativity rate of 89%–98% at 1 to 5 years of follow-up and an HBeAg seroconversion rate of 18.2% at 5 years; resistance was rare (<1%) (Ray, 2016). Adefovir demonstrates a favorable safety profile and is less likely to select for resistance than lamivudine, but virologic response was limited to adolescent patients and was lower than that of lamivudine (16% HBeAg seroconversion, <1% HBsAg seroconversion). Most experts recommend “watchful waiting” of children with chronic HBV infection, because current therapies are only modestly effective at best, and evidence of long-term benefit is scant. Young children are often believed to be immune tolerant of HBV infection (i.e., they have viral DNA present in serum but normal transaminase levels and no evidence of active hepatitis). In these children, transaminase levels and viral load should be monitored, but they are not typically considered to be candidates for antiviral therapy.

Prognosis

Most long-term follow-up studies have shown that children vaccinated at birth have high levels of protection until at least 5 years of age. Approximately 5%–10% of infants born to HBeAg-positive mothers become long-term HBV carriers despite combined active

and passive immunoprophylaxis with HBV immunoglobulin and HBV vaccine (Kato et al., 1999). Failure of immunoprophylaxis may be associated with the level of maternal viremia and specific HBV genetic variants (Nguie et al., 1998). Infants who become infected with HBV perinatally have a 90% risk of chronic infection, and 15%–25% of those with chronic infection die of HBV-related liver disease (primarily hepatocellular carcinoma) as adults. There is some evidence that the risk of carcinoma correlates with specific HBV genotypes (Sherman, 2010).

Hepatitis C Virus

In 1989, HCV was found to be the main cause of non-A, non-B, parenterally transmitted hepatitis; subsequently, HCV was found to account for a significant portion of the cases of sporadic acute and chronic hepatitis (Choo et al., 1989; Reyes et al., 1990; Weiss and Persing, 1995). HCV is a small, single-stranded RNA virus that is a member of the family *Flaviviridae*; this family consists primarily of vector-borne infections, such as St. Louis encephalitis virus and West Nile virus. HCV is an exception in this family because it is not transmitted by insect vectors. Seven genotypes are described, with significant biologic differences in regard to disease progression and responsiveness to therapy (Klennerman et al., 2009; Smith et al., 2014). Women with chronic HCV infection are more likely to have premature infants or infants with anomalies (Connell et al., 2011). Vertically transmitted HCV infection in infants has been described (Thomas et al., 1998) but is rare. It is associated with maternal viremia and a high rate of chronic hepatitis in the affected infant, but there is less overall liver injury compared with adult HCV infections (Tovo et al., 2000; El-Guindi, 2016).

Incidence

The seroprevalence for anti-HCV antibody in pregnant women ranges from 0.7% to 4.4% worldwide (Conte et al., 2000). In the United States, seroprevalence of HCV decreased during the 1990s and had remained low and stable until recent years; however, rates were unfortunately noted to increase between 2011 and 2014, particularly among women of childbearing age (Koneru et al., 2016). It is estimated that approximately 4.6 million people in the United States have been infected with HCV and that 3.5 million are currently infected (Edlin et al., 2015). Most HCV infections are in men. Forty percent to 50% of women with HCV have no identified known risk factors for infection (Bortolotti et al., 1998). Estimates from the 1990s suggested that the vertical transmission rate of HCV is approximately 5%–11% in HIV-negative mothers and ranges from 10% to 20% from mothers coinfecting with HIV (Palomba et al., 1996; Polywka et al., 1997; Hillemanns et al., 2000; Tajiri et al., 2001). More recent studies, however, suggest a lower rate of vertical transmission, ranging from 2.7% to 3.6% (Ferrero et al., 2003; Syriopoulou et al., 2005). One study has shown, in a multivariate analysis, that higher risk of vertical transmission is related more to maternal use of injection drugs than to HIV infection itself, although the mechanism for this finding is still unclear (Resti et al., 2002). Maternal viral load, HIV coinfection, prolonged rupture of membranes, fetal exposure to maternal infected blood, and invasive monitoring of the fetus increase the risk of viral transmission, and caesarean delivery and breastfeeding increase the transmission risk in women coinfecting with HCV and HIV (Tosone et al., 2014). The risk of HCV infection from transfused blood after the advent of HCV screening is estimated to be less than 1 in 1 million units transfused.

Etiology and Pathogenesis

HCV is transmitted less efficiently by sexual contact than HBV. Risk factors for HCV infection include transfusion, IV drug use, frequent occupational exposure to blood products, and household or sexual contact with an infected person (Weiss and Persing, 1995). In children, perinatal transmission is the most common route of infection (Mohan et al., 2010). Preparations of IVIG contaminated with HCV were reported between April 1993 and February 1994, but since that time, routine screening for HCV with PCR and application of a viral inactivation process during manufacture have been implemented to reduce the risk of transmission (Schiff, 1994).

Perinatal transmission is the leading cause of childhood HCV infection (Mohan et al., 2010). Transmission of HCV from mother to child is thought to occur either in utero or at the time of delivery. HCV has been shown to productively infect placental trophoblasts in cell culture (Nie et al., 2012), although a direct transplacental route of infection remains unproven. Placental innate immunity mediated by natural killer cell responses may prevent direct transplacental transmission of virus in most cases (Hurtado et al., 2010). Viral genotypes and infection and replication in maternal peripheral blood monocytes can also affect the ability of the virus to infect the fetus or newborn (Zuccotti et al., 1995; Azzari et al., 2000). Perinatal transmission is confined almost always to women with HCV RNA in the peripheral blood detectable by PCR, but all children born to women with anti-HCV antibodies should be tested for HCV. Data regarding the correlation of HCV RNA titer in the mother with the risk of vertical transmission are conflicting, although most studies have reported an association (Lynch-Salamon and Combs, 1992; Ohto et al., 1994; Resti et al., 1998; Conte et al., 2000; Tajiri et al., 2001). Maternal peripheral blood mononuclear cell infection by HCV, membrane rupture of longer than 6 hours before delivery, and procedures exposing the infant to maternal blood infected with HCV during vaginal delivery are associated with an increased risk of transmission (Tosone et al., 2014). Internal fetal monitoring is also a risk factor for transmission (Mast et al., 2005). Maternal coinfection with HCV and HIV, maternal history of IV drug use, and HCV infection of the sexual partner of the mother are also risk factors for transmission (Fiore and Savasi, 2009; Indolfi and Resti, 2009). Transmission rates as high as 25% have been reported in HCV-infected, HIV-positive women. The effect of vaginal delivery versus cesarean delivery on transmission rates is unclear (Paccagnini et al., 1995; Gibb et al., 2000); current recommendations do not support the practice of cesarean delivery in women with HCV infection. Primary infection is asymptomatic in most infants. At least one-quarter of infected children will demonstrate spontaneous viral clearance before 6 years of age. Polymorphisms in the *IL28B* gene, and infection with HCV genotype 3, have been associated with greater chances of spontaneous viral clearance (Tovo et al., 2016). In general, HCV progression is mild or moderate in children with chronic infection who grow regularly, although cases with marked liver fibrosis or hepatic failure have been described.

Transmission of HCV via breast milk has not been demonstrated conclusively, although HCV can be found in breast milk and colostrum (Gurakan et al., 1994; Zimmermann et al., 1995). Studies comparing breastfed and bottle-fed infants born to HCV-infected mothers have not shown a statistically significant difference in vertical transmission (Lin et al., 1995; Resti et al., 1998). However, breastfeeding increases the transmission risk in women coinfecting with HCV and HIV (Tosone et al., 2014). Overall, maternal HCV

infection is not a contraindication to breastfeeding (Mast, 2004); however, it may be prudent for mothers who are infected with HCV and who choose to breastfeed to consider abstaining from breastfeeding if their nipples are cracked and bleeding.

Clinical Spectrum and Laboratory Evaluation

Acquired HCV infection typically causes jaundice in one-third of cases and significant increases in ALT in almost all infected individuals. Most neonates perinatally infected with HCV demonstrate little in the way of clinical symptoms; they may have elevated liver ALT levels either transiently or intermittently. The increases in ALT values, when tested, are most commonly noted between 3 and 6 months of age. HCV RNA PCR results may be negative initially at birth or within the first few days of life but typically become positive by 1 to 2 weeks of age and remain so until at least 5 years of age. The highest sensitivity of PCR is reported after 1 month of age (Thomas et al., 1997; Polywka et al., 2006). Confirmatory anti-HCV IgG serology should be delayed until exposed infants are at least 15 to 18 months old, when at least 99% will have cleared maternal antibody (Dunn et al., 2001). Before 18 months of age, only a positive HCV PCR result can confirm the diagnosis of neonatal infection; positive anti-HCV IgG results reflect passively acquired maternal antibody. IgM assays are not available or reliable for perinatal diagnosis of HCV. Liver ultrasonographic findings are usually normal or may consist of a mild diffuse increase in echogenicity. Liver biopsies, when performed in symptomatic children, demonstrate mild to moderate chronic persistent hepatitis (Palomba et al., 1996; Tovo et al., 2000).

Treatment

Until recently, only IFN and ribavirin were approved by the FDA to treat adults and children with chronic HCV infection. The recent development of novel and highly effective antivirals for HCV infection has revolutionized the care of infected patients (Zopf et al., 2016). These drugs are not yet licensed for pediatric use. Novel drugs include ledipasvir, sofosbuvir, daclatasvir, elbasvir, beclabuvir, grazoprevir, paritaprevir, ombitasvir, velpatasvir, and dasabuvir. Ledipasvir, ombitasvir, daclatasvir, elbasvir, and velpatasvir inhibit the virally encoded phosphoprotein nonstructural protein 5A (NS5A), which is involved in viral replication, assembly, and secretion, while sofosbuvir is metabolized to a uridine triphosphate mimic, which functions as an RNA chain terminator when incorporated into the nascent RNA by the NS5B polymerase. Dasabuvir and beclabuvir are also NS5B inhibitors. Paritaprevir and grazoprevir inhibit the nonstructural protein 3 (NS3/4) serine protease, a viral nonstructural protein that is the 70-kDa cleavage product of the HCV polyprotein.

Efforts to treat HCV before the advent of new, “direct” therapy (Zopf et al., 2016) yielded mixed results. Whereas only 10%–25% of adults treated with IFN had a sustained remission of disease (Bonkovsky and Woolley, 1999) treatment with a combination of IFN and ribavirin achieves remission in closer to half of treated adults (Cornberg et al., 2002). Randomized controlled trials indicated that patients treated with pegylated IFNs (so called because they are formulated and stabilized with polyethylene glycol), both as dual therapy with ribavirin and as monotherapy, experienced higher sustained viral response rates than those treated with nonpegylated IFN (Shepherd et al., 2004). The advent of new, direct therapy has led to permanent remission in adult patients. Data on the use of these agents in infants and children are limited, and none are currently approved for pediatric use. The use of IFN alfa-2b in combination with ribavirin has been approved by the

FDA (Palumbo, 2009). In a case series of four pediatric patients treated with IFN for 1 year, viral load decreased to undetectable levels during treatment in all four patients, but two patients became viremic again once the treatment was stopped (Tovo et al., 2000). There are significant genotype-dependent differences in responsiveness to antiviral therapy; patients with genotype 1 had the lowest levels of sustained virologic response, and patients with genotype 2 or 3 had the highest (Shepherd et al., 2004; Palumbo, 2009). The use of IFN alfa-2b in combination with ribavirin provides a much more favorable sustained virologic response in children with HCV genotype 2 or 3 (84%) than in those with HCV genotype 1 (36%; González-Peralta et al., 2005). For genotype 1 HCV infection treated with pegylated IFNs combined with ribavirin, it has been shown that genetic polymorphisms near the human *IL28B* gene, encoding IFN lambda 3, are associated with significant differences in response to the treatment (Ge et al., 2009; Thomas et al., 2009). Older IFN therapies were available only in parenteral formulations and are associated with significant side effects; therefore experience in children has been minimal.

Infants and children with persistent elevations in liver transaminase levels should be referred to a pediatric gastroenterologist for evaluation and treatment. The necessity for as well as the frequency of screening tests of liver function has not been established.

Prognosis

In 60%–80% of HCV-infected adults, chronic hepatitis occurs, and in one-third of HCV-infected adults, chronic HCV infection leads to cirrhosis or liver failure within 20 to 30 years after infection (Iwarson et al., 1995; Leone and Rizzetto, 2010). In patients with cirrhosis, the incidence of hepatocellular carcinoma is 2%–5% per year. There are limited data regarding long-term follow-up in HCV-infected infants, but existing knowledge indicates that most children remain viremic until at least 5 to 6 years of age, and most children who do not achieve spontaneous viral clearance in early life will develop chronic, persistent hepatitis. Studies monitoring infected children beyond 6 years of age have not been completed, but they are at risk of cirrhosis and hepatocellular carcinoma. Transient HCV viremia with subsequent resolution has been reported (Zanetti et al., 1995; Padula et al., 1999; Ruiz-Extremiera et al., 2000). All of the infants who have been followed up for an average of 3 to 4 years have been reported to have normal growth and development (Tovo et al., 2000).

Prevention

Although development of a HCV vaccine is considered to be a major public health priority (Houghton and Abrignani, 2005), currently no vaccine is available. Some experts have noted that, given the safety and effectiveness of new HCV therapies, a vaccine may not be necessary (Stone et al., 2016), although as noted these drugs are not yet available for infants and children. Like all other infants, infants with HCV should receive routine HBV immunization. In addition, they should receive HAV vaccination at 2 years of age. Parents should be advised to avoid unnecessary administration of medicines known to be hepatotoxic. Standard precautions are recommended for the hospitalized infant.

Adenovirus

Adenoviruses are so named because they were originally recovered from human adenoidal tissue; they are medium-sized (90–100 nm), nonenveloped icosahedral viruses composed of a nucleocapsid and

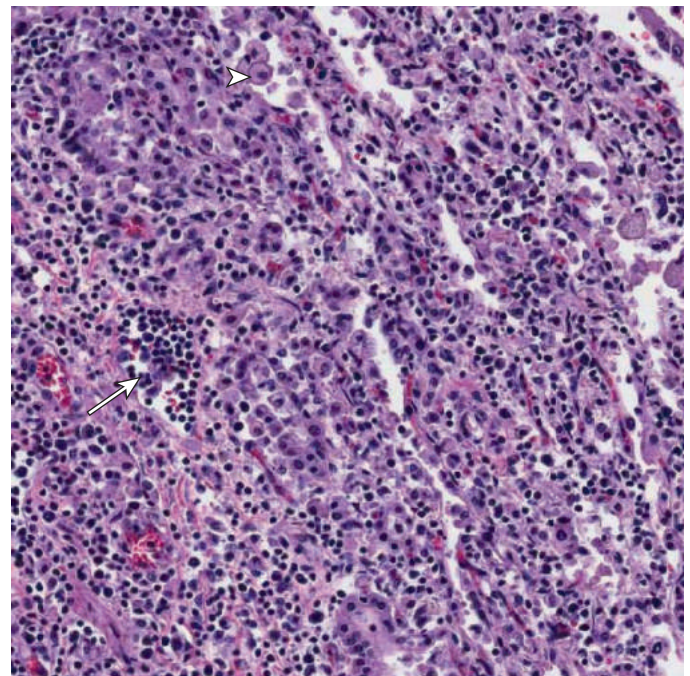
a double-stranded linear DNA genome. They are the largest of the nonenveloped viruses. There are 53 described adenovirus serotypes in humans.

Epidemiology and Clinical Manifestations

Adenoviruses are responsible for a wide variety of clinical syndromes, including conjunctivitis, respiratory tract disease, and gastroenteritis. Adenoviruses cause 5%–10% of upper respiratory tract infections in children and many infections in adults as well. They are a common problem in solid organ and hematopoietic stem cell transplant recipients. No clear seasonality has been described for adenovirus infections.

Adenovirus Infections in Newborns

There are a limited number of reports of adenovirus infection in young infants, but published case series indicate that adenovirus can cause serious, life-threatening disease in the neonate. A review of neonatal adenovirus infection (Abzug and Levin, 1991) identified several characteristic historical features, including prolonged rupture of membranes, history of maternal illness, vaginal mode of delivery, and onset of illness within the first 10 days of life. Serotypes 2, 3, 7, 11, 13, 19, 21, 30, and 35 have been implicated (Andiman et al., 1977; Sun and Duara, 1985; Matsuoka et al., 1990; Abzug and Levin, 1991; Pinto et al., 1992; Osamura et al., 1993). Clinical findings in various case reports and case series have included lethargy, fever or hypothermia, anorexia, apnea, hepatomegaly, bleeding, and progressive pneumonia (Fig. 37.6). Laboratory abnormalities include thrombocytopenia, coagulopathy, and hepatitis. Acquisition



• **Fig. 37.6** Postmortem histologic analysis from a newborn who died of disseminated adenovirus infection at 2 weeks of age. Hematoxylin and eosin stain of lung demonstrating inflammatory infiltrates (arrow) and intranuclear inclusions (arrowhead). This infant had a viral sepsis syndrome characterized by hepatic failure, disseminated intravascular coagulation, and pneumonitis from adenovirus infection presumed to have been acquired intrapartum.

of infection from the mother via vaginal delivery is the presumed mode of transmission in most cases, although transplacental spread has also been implicated.

Although most neonatal adenovirus infections are believed to be acquired in the birth canal, congenital infections have been described, presumably because of transplacental transmission. A wide range of fetal diseases, including pleural effusion, hepatitis, myocarditis, meningitis, and CNS abnormalities, have been described (Meyer et al., 1985; Rieger-Fackeldey et al., 2000; Baschat et al., 2003; Piedra, 2005). The use of steroids and the presence of bronchopulmonary dysplasia appear to increase the risk of adenoviral pneumonia (Faden et al., 2005) in the NICU setting. Disseminated adenovirus infection in neonates younger than 14 days old was noted in a retrospective review to carry a nearly 50% risk of death (Kelley, 2010; Ronchi et al., 2014a). Adenovirus has been implicated as a potential cause of chorioamnionitis and premature birth (Van den Veyver et al., 1998; Tsekoura et al., 2010). Neonatal group C adenovirus infection has also been hypothesized to play an etiologic role in the pathogenesis of childhood leukemia (Gustafsson et al., 2007; Vasconcelos et al., 2008; Ornelles et al., 2015), although this association remains speculative.

Prevention and Intervention

IV ribavirin therapy has been administered to a neonate with disseminated adenovirus infection undergoing extracorporeal membrane oxygenation, with evidence of viral clearance noted within 48 hours of initiation of therapy (Aebi et al., 1997). Adenovirus vaccines were at one time available for use in military personnel (Gaydos and Gaydos, 1995; Tucker et al., 2008) but are not currently in production. Adenoviruses are hardy and resistant to inactivation by physical and chemical methods that kill most viruses, adding to the challenge of hospital infection control during outbreaks of infection. Nosocomial outbreaks in NICUs have been described in association with ophthalmologic procedures (Calkavur et al., 2012) and by healthcare worker-mediated transmission (Henquell et al., 2009). Although cidofovir has not been approved by the FDA for pediatric use, it has been noted to be an effective antiviral agent for adenovirus infections (Ganapathi et al., 2016), although experience with the use of this agent in newborns is very limited.

Respiratory Viruses

Although relatively uncommon, any one of the respiratory viruses can cause symptomatic respiratory disease in newborns. The association has been described for rhinoviruses, adenoviruses, parainfluenza viruses, influenza virus, and RSV (Kujari et al., 2014; Ronchi et al., 2014b). Adenovirus, rhinovirus, and parainfluenza virus infections are generally characterized by mild rhinorrhea in neonates. All of these viruses, however, can cause clinical symptoms indistinguishable from those of bacterial infection, leading to increased diagnostic testing and empiric antibiotic treatment. Influenza virus infections are usually mild, but in the absence of maternally transmitted antibody, they can be life threatening, with severe pneumonia, hypoxia, and a prolonged course. During the H1N1 influenza pandemic of 2009 to 2010, pregnant women were at a uniquely high risk of severe influenza (Jamieson et al., 2009), and infections were described in neonates (Sert et al., 2010).

The most extensive nursery outbreaks of viral respiratory disease, however, have been caused by RSV (Hall et al., 1979; Wilson et al., 1989). Because of the importance that RSV plays in the newborn nursery, this virus is considered in greater detail in the following section.

Respiratory Syncytial Virus

RSV is the major cause of viral pneumonia and bronchiolitis in infants and children. In temperate climates it causes large annual epidemics during the winter and early spring months (typically ranging from November through April). During these months, RSV appears to be responsible for up to 20% of all pediatric hospital admissions (Hall et al., 2009). Nosocomial infections are frequent during these times, and illness among hospital staff members is a major factor in its spread from infant to infant. Several nursery outbreaks have been described. In one of these outbreaks, cultures were obtained prospectively so that a full picture of the virus's pathogenicity and epidemiology could be drawn (Hall et al., 1979). Twenty-three of 66 infants hospitalized for 6 days or more were infected. Virtually all infants were symptomatic. Clinical manifestations included pneumonia, upper respiratory tract infection, apneic spells, and nonspecific signs. Pneumonia and apnea were seen almost exclusively in infants older than 3 weeks, and nonspecific signs were most commonly observed in younger infants. Four infants (17%) died, two unexpectedly, during the course of infection. The spread of infection in the unit was difficult to interrupt; infants in incubators did not seem to be protected against acquisition of the infection. Eighteen of the 53 nursery personnel were infected during the outbreak; 83% of the infected nursery providers were symptomatic. Of particular importance is the observation that RSV infection, both in term infants and in preterm infants, is commonly associated with a new onset of apnea (Bruhn et al., 1977). Boys are at greater risk than girls of serious RSV disease. RSV-associated apnea has been the probable explanation for some case reports of deaths attributed to SIDS (Eisenhut, 2006). RSV lower respiratory tract disease can be slow to resolve. After discharge from initial hospitalization, risk factors for infant rehospitalization secondary to RSV infection include premature gestational age, chronic lung disease, siblings in day care or school, chronologic age of less than 3 months, and exposure to tobacco smoke (Carbonell-Estrany and Quero, 2001).

Diagnosis can be confirmed by DFA staining of nasopharyngeal or tracheal aspirate, nasopharyngeal swab, or other respiratory secretions. Culture of RSV can require 3 to 5 days and can be used if DFA staining is not available. PCR is also available for the rapid diagnosis of infection and is rapidly emerging as the preferred diagnostic study for RSV infection, commonly performed as a component of a "multiplex" assay that simultaneously tests for multiple viral pathogens (El Kholy et al., 2016).

Treatment and prevention of RSV infections in infants attracted considerable attention during the 1990s because of the clinical and economic impacts of these infections (Groothuis, 1994; Levin, 1994; Meissner, 1994; Kinney et al., 1995). Considerable debate has ensued concerning the efficacy, safety, and potential effect on healthcare workers of ribavirin therapy, so its use remains controversial (Wald and Dashefsky, 1994; Meissner, 2001; Ventre and Randolph, 2004). Treatment consists of nebulization of ribavirin by a small-particle aerosol generator supplied by the manufacturer into an oxygen hood, tent, or mask from a solution containing 20 mg of ribavirin per milliliter of water (Table 37.2). The aerosol has been administered on various schedules for 3 to 5 days (e.g., 12 to 20 hours per day). The efficacy of ribavirin in this setting remains unclear. Some long-term benefit with regard to recurrent wheezing may be realized (Ventre and Randolph, 2004). Corticosteroids are not effective in the treatment of RSV infection.

Prevention efforts have focused on passive and active immunization. Standard IVIG therapy has not been shown to be efficacious

• BOX 37.4 Guidelines for Prophylaxis in Preterm Infants at the Start of the Respiratory Syncytial Virus Season

- Infants born at ≤ 29 weeks' gestation who are younger than 12 months at the start of the respiratory syncytial virus (RSV) season (Monthly prophylaxis should be discontinued if an infant is hospitalized with documented RSV disease.)
- In the first year of life, palivizumab is recommended for premature infants with chronic lung disease of prematurity, defined as birth at < 32 weeks' gestation and a requirement for more than 21% oxygen for at least 28 days after birth.
- Clinicians may administer palivizumab prophylaxis in the first year of life to certain infants with hemodynamically significant heart disease (consultation with a pediatric cardiologist is recommended).
- Clinicians may administer up to a maximum of five monthly doses of palivizumab (15 mg/kg per dose) during the RSV season to infants who qualify for prophylaxis in the first year of life (monthly prophylaxis should be discontinued if an infant is hospitalized with documented RSV disease).
- Palivizumab is not recommended in the second year of life except for children with chronic lung disease of prematurity who continue to require medical interventions such as supplemental oxygen, diuretics, and/or corticosteroids.
- Palivizumab may be considered in infants with pulmonary abnormalities, neuromuscular diseases, or profoundly immunocompromised states (including heart transplant in children younger than 2 years) or in Alaskan Native or other remote Native American populations in which costs associated with medical transport are high.
- Palivizumab therapy is not recommended for children with Down syndrome.

for prevention of RSV infection in high-risk infants (Meissner et al., 1993). The efficacy of monthly prophylactic administration of RSV-specific immunoglobulin (750 mg/kg or 150 mg/kg) in 249 infants with cardiac disease or bronchopulmonary dysplasia was examined in a multicenter trial (Groothuis, 1994). In the high-dose (750 mg/kg) group, there were fewer lower respiratory tract infections, hospitalizations, days in hospital, and days in the intensive care unit as well as less use of ribavirin. Subsequently, a mouse monoclonal antibody was developed, which provides similar protection against RSV (i.e., palivizumab). Unlike anti-RSV immunoglobulin, which is a pooled human blood product, the monoclonal antibody does not confer the theoretical risk of acquiring blood-borne pathogens. Palivizumab is also substantially easier to administer because it is given as an intramuscular injection. Current recommendations by the AAP for the use of palivizumab are summarized in Box 37.4 (Committee on Infectious Diseases and Bronchiolitis Guidelines Committee, 2014). Additional preventive measures for high-risk infants include elimination or minimization of exposure to tobacco smoke, avoidance of crowds and situations in which exposure to infected individuals cannot be controlled, careful hand hygiene education of parents, vaccination against influenza beginning at 6 months of age, and restriction of participation in child care during the RSV season whenever feasible. The prospects for infant or maternal immunization with live attenuated and protein subunit vaccines are under active investigation (Piedra, 2000; Crowe, 2001; Fretzayas and Moustaki, 2010; Saso and Kampmann, 2016). Since RSV produces its most significant morbidity in the newborn, an RSV vaccine may one day ideally be used in pregnant women (Gerds et al., 2016).

Nosocomial spread of RSV and other respiratory viruses can be minimized by emphasis on hand washing by care providers

between contacts with patients. Without additional special precautions, an attack rate of approximately 26% has been observed (Madge et al., 1992). Use of standard contact precautions, such as cohort nursing and the use of gowns and gloves for all contacts with RSV-infected children, can reduce the risk of nosocomial RSV infection to 9.5% (Madge et al., 1992). Palivizumab has been reported to be a useful adjunct in the control of nursery-associated outbreaks of RSV infection (Hammoud et al., 2016).

Gastrointestinal Viruses

Historically, the most important of the viruses that cause diarrhea from the perspective of the neonatal nursery are the rotaviruses. This important group of viruses, with at least four serotypes, is responsible for a large proportion of significant and sometimes severe diarrhea in infants aged 6 to 24 months (Cohen, 1991; Haffee, 1991; Taylor and Echeverria, 1993; Greenberg et al., 1994). Although the overall burden of rotavirus disease has been reduced substantially by the licensure of a number of rotavirus vaccines for use in the routine childhood immunization series (Bernstein, 2009; Dennehy, 2015), rotavirus still continues to produce disease in infants, particularly in parts of the developing world where vaccine is not available. Evidence suggests that rotavirus causes an unexpectedly significant burden of disease in infants as young as 2 to 3 months (Clark et al., 2010). Nursery-acquired infections are common; surprisingly, such infections appear to be benign in most infants. Two studies performed in nurseries in Sydney, Australia, and in London found that 30%–50% of 5-day-old babies shed the virus (Murphy et al., 1977; Chrystie et al., 1978). However, more than 90% of the infected infants were asymptomatic.

Noroviruses are a genetically diverse group of single-stranded, positive-sense RNA, nonenveloped viruses. These viruses belong to the family *Caliciviridae*. The genus *Norovirus* has one species, which is designated as *Norwalk virus*, although there are many serotypes and strains. Outbreaks of norovirus infection have been described in the NICU setting (Tzialla et al., 2011). IUGR infants have been observed to be more likely to shed norovirus and other enteric viruses than infants who are appropriate for gestational age (Naing et al., 2013). Therapy is supportive.

Human Immunodeficiency Virus Infection

Introduction

The HIV epidemic has seen dramatic shifts since the first cases were identified in the early 1980s. Great strides and vast achievements have been made globally in identifying infected individuals, increasing access to treatments, and implementing prevention programs (Royce et al., 1997). The first major breakthrough for mother-to-child transmission came in February 1994 with results of the Pediatric AIDS Clinical Trials Group (PACTG) protocol 076 study (Connor et al., 1994) demonstrating that risk decreased from 25.5%–8.3% when the mother and infant were treated with zidovudine. Now with universal prenatal HIV counseling and testing, antiretroviral (ARV) prophylaxis, scheduled caesarean deliveries, and avoidance of breastfeeding, the rate of mother-to-child transmission has dropped to 2% or less in the United States and Europe.

Still, there is much work to be done. The WHO reported statistics from 2014 showing that 73% of all pregnant women with HIV globally received medicines to prevent transmission to their infants,

and only 32% of HIV-infected children received ARV therapy (ART), compared with 41% of adults. The “Global Plan” was devised in 2010 by a “Global Task Team” consisting of a group of stakeholders from 25 countries and 30 representative international organizations cochaired by Joint United Nations Programme on HIV/AIDS (UNAIDS) and the US government. This plan outlines several steps leading “towards the elimination of new HIV infections among children and keeping their mothers alive by 2015” and prioritizes 22 countries with the highest numbers of pregnant women with HIV. On the basis of the progress report released in 2015, the goals have not yet been reached. The first main goal is to reduce new HIV infections among children by 90% from the benchmark established in 2009. The countries targeted in the Global Plan have collectively reduced new infections by 48% so far. Another main goal is to reduce the mother-to-child transmission rate to 5% or less among breastfeeding groups and to 2% or less among nonbreastfeeding groups. The rates are 28% and 14%, respectively, as last reported from the target countries. This important work will continue until the original goals are met (UNAIDS, 2015a).

The WHO released a guideline in September 2015 stating that “antiretroviral therapy (ART) should be initiated in everyone living with HIV at any age at any CD4 cell count” (World Health Organization, 2015). This caused a significant shift in the global approach, with the new emphasis on treatment for all. With the increasing availability of ARVs globally, children who acquired HIV through the perinatal period or through breastfeeding are living longer and reaching adolescence in record numbers. There are an estimated 2 million adolescents aged 10 to 19 years with HIV globally. However, AIDS is still the leading cause of death among adolescents in Africa and the second leading cause globally. AIDS-related deaths in adolescents have tripled since 2000 while declining among all other age groups (Idele et al., 2014; UNAIDS, 2015b).

Among these adolescents infected via mother-to-child transmission is an emerging group of young women reaching childbearing age. They too are becoming pregnant and delivering their own children, bringing new challenges to prevention efforts. The psychosocial and emotional needs of this group are unique. Some studies suggest higher rates of preterm or small for gestational age births and found that these patients are more likely to have lower median CD4⁺ T lymphocyte counts, detectable viral loads, and genotypic drug resistance (Badell et al., 2013; Munjal et al., 2013).

Prevention of Mother-to-Child Transmission of Human Immunodeficiency Virus

Antepartum

All pregnant women with an unknown HIV status should be tested for HIV with their first routine prenatal screening. The best screening test is an FDA-approved fourth-generation HIV antibody–antigen combination immunoassay. This test detects both HIV-1 and HIV-2 antibodies as well as the HIV-1 p24 antigen. The addition of antigen testing provides a shorter window to detect infection of 2 weeks since initial infection compared with 4 weeks by older-generation Western blot testing. When HIV infection occurs early, the antibody test results may be negative or indeterminate while the antigen test is positive (Centers for Disease Control and Prevention and Association of Public Health Laboratories, 2014).

Women with an initial negative HIV test who are considered to be high risk should be tested again with an HIV antibody–antigen combination test in the third trimester, ideally before their 36th week of gestation. This includes women with a new sexually

transmitted infection, with a known HIV-positive partner, with high-risk HIV-associated behaviors, who are incarcerated, who live in areas with elevated HIV incidence, or who receive care in facilities with an HIV incidence of at least 1 case per 1000 pregnant women per year.

Acute HIV infection during pregnancy (or breastfeeding) poses an increased risk of mother-to-child transmission of HIV because of the rapid development and high levels of HIV viral load. Furthermore, the diagnosis can easily be missed because the acute retroviral syndrome can mimic symptoms of infections with other common viruses such as influenza virus or EBV. These cases represent a significant portion of the transmission still occurring in developed countries such as the United States (Nesheim et al., 2013). Providers must be diligent and quick to test or retest pregnant women who are high risk or who present with a constellation of symptoms suggestive of acute retroviral syndrome such as pharyngitis, lymphadenopathy, myalgia, arthralgia, rash, or fever.

Whenever infection is suspected within less than 2 weeks, the results of antibody testing are unclear, or antigen testing is not available, then a plasma HIV RNA PCR (for viral load) should also be performed in addition to the HIV antibody–antigen combination immunoassay.

Once a pregnant woman has been identified as infected with HIV, ART should be started as soon as possible. Current consensus is that all pregnant women with HIV-1 infection should receive combination ART regardless of the absolute CD4 count or viral load. ARV drug resistance testing should be performed for each pregnant woman who is ARV naive with a viral load of more than 500 to 1000 copies, but ART can be started while the results are awaited and adjusted accordingly later because of the importance of viral load suppression (Drake et al., 2014). Viral resistance testing is important because a woman can acquire a new HIV infection with a resistant strain, irrespective of her antiviral treatment history.

The choice of ARV drug combination is based on many factors, but in general ARV-naïve pregnant women should receive at least three active ARVs. This usually consists of a backbone of two nucleoside/nucleotide RT inhibitors (several options) along with a nonnucleoside RT inhibitor (efavirenz therapy initiated after 8 weeks of pregnancy) or an integrase inhibitor (raltegravir) or a protease inhibitor (several options). If a protease inhibitor is chosen, it should be combined with ritonavir, which is a potent inhibitor of the cytochrome P450 pathway and thus significantly increases the serum concentration of the protease inhibitor. This is known as a *ritonavir-boosted protease inhibitor regimen*. However, it is important to note that known HIV-infected pregnant women currently receiving ART should continue with their combination therapy if it is tolerated and effective at viral suppression.

Generally, the literature is reassuring regarding the rate of birth defects related to ARV teratogenicity. The Pediatric HIV/AIDS Cohort Study overall first-trimester ARV exposure was not associated with an increased risk of birth defects. Because of study findings in nonhuman primates that are largely debated, FDA labeling for efavirenz advises against efavirenz use in pregnancy in the first trimester. Guidelines suggest that treatment with efavirenz should be avoided during the first 8 weeks of pregnancy (the primary period of fetal organogenesis) whenever possible. However, they also note that if a pregnant woman is doing well with efavirenz therapy at presentation, risks and benefits should be weighed given that most pregnant women present to care after organogenesis is already complete (Ford et al., 2011).

The goal of ART is to maintain a viral load below the limit of detection throughout pregnancy. To that end, viral load should

be tested several times during the pregnancy with a plasma HIV RNA PCR. Testing should be performed at the initial visit, 2 to 4 weeks after initiation or change of ART, monthly until viral load is undetectable, and at least every 3 months after suppression is achieved. An additional test must be done in all cases at 34 to 36 weeks' gestation to ensure viral suppression at delivery and to plan for the mode of delivery.

Early ultrasonography is recommended for assessment of gestational age to allow the careful planning of the mode of delivery. Protease inhibitor regimens in general have been associated with an increased risk of hyperglycemia, and so there is a theoretical risk of this increasing in the setting of pregnancy. Most studies have not shown an increased glucose intolerance; however, some experts recommend early and aggressive glucose screening for those women on ARV regimens containing protease inhibitors (Hitti et al., 2007). There is no recommendation or practice for additional glucose screening in the exposed infant.

Guidelines on the prevention of HIV-2 (in contrast to HIV-1) transmission during pregnancy are based largely on expert opinion. Data suggest that the rates of perinatal transmission of HIV-2 are low regardless of intervention (0%–4% reported). As a result, opinions are mixed on whether or not the mothers need to receive ART during pregnancy. Most suggest ARV prophylaxis during pregnancy with use of agents known to be active against HIV-2 and allow discontinuation after delivery if the mother is otherwise well and the absolute CD4 count is greater than 500 (Burgard et al., 2010).

Intrapartum

HIV-1-infected pregnant women who have plasma viral loads with more than 1000 copies/mL or unknown viral loads near the time of delivery should have a scheduled caesarean delivery at 38 weeks. This timing is recommended to decrease the possibility of the onset of labor or rupture of membranes before delivery can occur. Studies in 1999 (both a multicenter randomized controlled trial and a metaanalysis) showed a dramatic decrease in the risk of transmission by 50%–80% when caesarean deliveries were performed at this juncture. The same year, the Women and Infants Transmission Study showed that no viral transmissions to infants occurred when women had viral loads of less than 1000 copies/mL (Garcia et al., 1999). Later studies continued to support the decrease in transmission rates with caesarean delivery, but there are some reports of transmission occurring despite low viral loads, albeit at very low rates. Therefore the decision to deliver vaginally when viral loads are less than 1000 copies/mL should be individualized for each woman and infant by weighing the risks of caesarean delivery overall with the potential benefits.

The original PACTG protocol 076 study administered IV zidovudine therapy to all women with HIV in labor. Now with many women achieving viral suppression with ART, intrapartum therapy is no longer universal. The French Perinatal Cohort group demonstrated that IV zidovudine therapy was not needed for women with HIV RNA loads of less than 1000 copies/mL (Briand et al., 2013), and several other studies were able to duplicate these results. Therefore IV zidovudine therapy should be given to HIV-infected women in labor with HIV RNA loads greater than 1000 copies/mL, but it is not required for HIV-infected women receiving ART who have documented viral loads of less than 1000 copies/mL near delivery. If women have unknown viral loads, they should also receive IV zidovudine therapy. In women who present in labor with unknown HIV status, HIV testing should be expedited, and if the results are positive, a confirmatory test should be done and

IV zidovudine therapy should be started immediately. Ideally, administration should begin 3 hours before delivery or as soon as possible. The dual purpose of the use of zidovudine intrapartum is to reduce the maternal HIV viral load and to provide preexposure and postexposure prophylaxis to the infant against intrapartum transmission. See Fig. 37.7 for a suggested approach for a pregnant woman with unknown HIV status with delivery imminent.

It is still recommended to administer IV zidovudine therapy intrapartum even when the mother's virus has known resistance to zidovudine when the HIV RNA load is greater than 1000 copies/mL near delivery. It is recommended to use 2 mg/kg IV continuous infusion during the first hour and then 1 mg/kg IV continuous infusion each hour thereafter until the cord is clamped. This should begin at least 3 hours before delivery. Any oral ARVs the mother was taking before delivery should continue to be taken. This recommendation is in part due to the understanding that perinatal transmission of resistant virus has been reported but appears to be infrequent. There are some data suggesting that drug resistance mutations that lead to decreased viral fitness may also decrease viral transmissibility (Chen et al., 2011). Furthermore, zidovudine has a long-established track record of reducing the risk of transmission, as discussed previously. Zidovudine also has unique characteristics, including crossing the placenta readily, providing a high maternal-to-cord blood ratio, and penetrating the CNS well.

There is a potential increased risk of transmission with some obstetric procedures, including artificial rupture of membranes, invasive fetal monitoring such as with fetal scalp electrodes, and use of forceps or vacuum-assisted delivery. Episiotomy should also be avoided if possible.

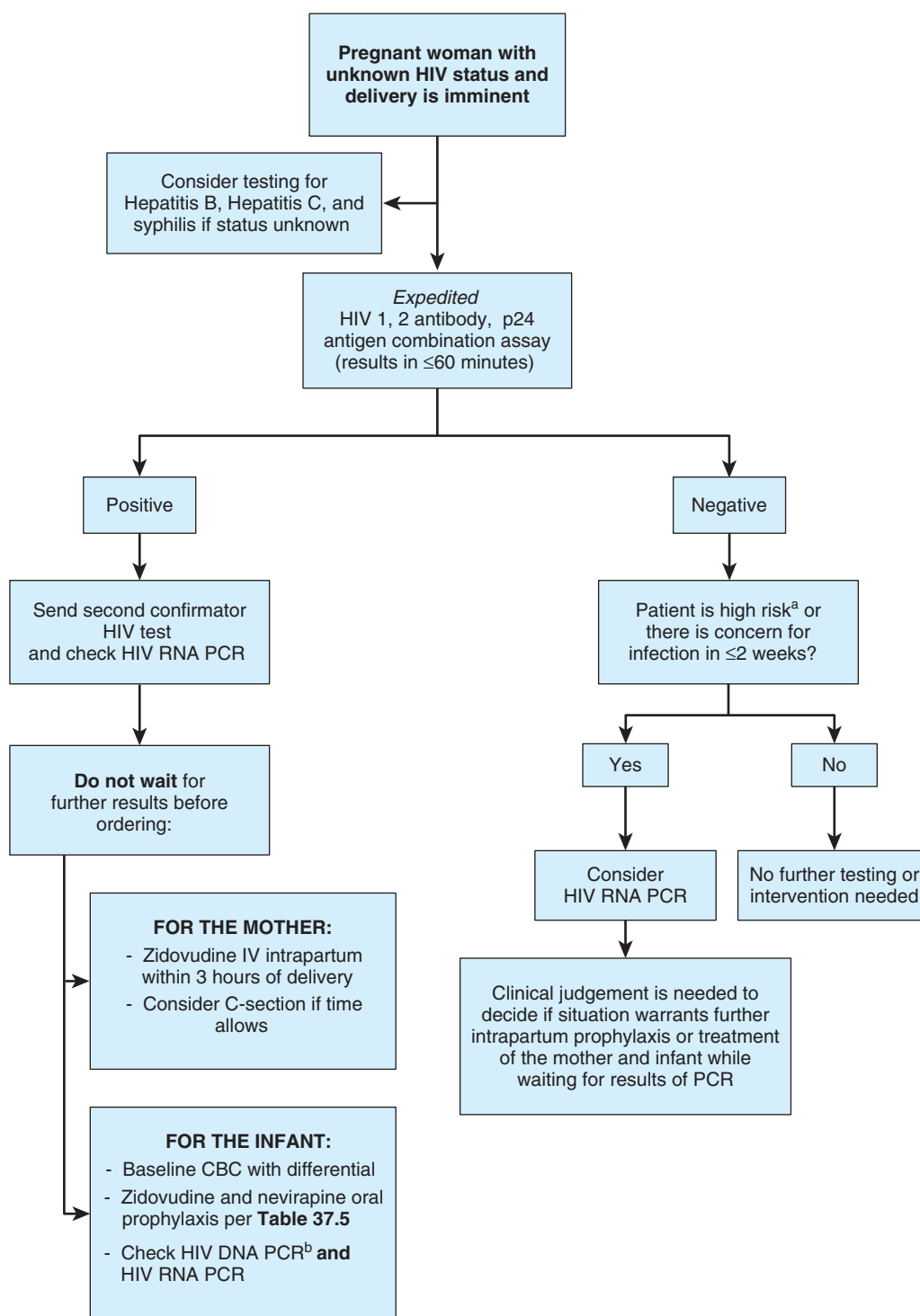
Postpartum

Mothers with HIV-1 infection should continue their ARV regimen after delivery, as ART is now recommended for all HIV-1-infected individuals. The unique challenges of labor, delivery, and motherhood may increase the barriers to adherence, and so the mother should receive any additional support that is needed to continue therapy. Contraception options should be discussed early. As the mother will no longer receive obstetric care after her postpartum visit, careful attention should be made to ensure that she is following up with an HIV care provider and that she has full linkage to care.

All HIV-exposed infants require ART for at least the first 4 to 6 weeks regardless of the mother's viral load at the time of delivery (see the next section).

Breastfeeding by HIV-infected mothers is not recommended in the United States and other developed countries where replacement feeding (formula) is affordable, the water supply is safe, and the risk of infant death due to diarrheal and respiratory infections is low. Infant prophylaxis with ART and maternal postpartum ART cannot completely eliminate the risk of infection. The AAP released a policy statement in 2013 recommending that HIV-infected mothers in the United States not breastfeed their infants, regardless of maternal viral load and ART (Committee on Pediatric AIDS, 2013).

In the developing world where these conditions are not met, many HIV-infected mothers do continue to breastfeed. Several studies of infant prophylaxis with ART and maternal combination ART postpartum were shown to decrease the risk of transmission through breast milk (Bedri et al., 2008). Another study showed that exclusive breastfeeding leads to lower concentrations of HIV in breast milk, while decreases in the frequency of breastfeeding can cause viral levels to increase (Kuhn et al., 2008). In 2010 the



• **Fig. 37.7** Proposed algorithm for treatment of a neonate born to a woman with indeterminate or unknown human immunodeficiency virus (HIV) status. A rapid turnaround time HIV antibody/p24 assay should be performed. In women who present in labor with unknown HIV status, HIV testing should be expedited, and if the results are positive, a confirmatory test should be done and intravenous (IV) zidovudine therapy should be started immediately if the results are positive or the index of suspicion is high. The recommendation is 2 mg/kg IV continuous infusion during the first hour and then 1 mg/kg IV continuous infusion each hour thereafter until the cord is clamped. This should begin at least 3 hours before delivery. Recommendations for empiric therapy of the newborn with zidovudine and nevirapine, pending the results of nucleic acid testing, are outlined in Table 37.5. ^aHigh risk can be defined as a pregnant woman with a new sexually transmitted infection, with a known HIV-positive partner, with high-risk HIV-associated behaviors, who is incarcerated, and/or who lives in an area (or receives care in facilities) with an elevated HIV incidence. ^bIf available. CBC, Complete blood count; C-section, caesarean section; HIV, human immunodeficiency virus; IV, intravenous; RNA, ribonucleic acid; PCR, polymerase chain reaction.

WHO recommended that national authorities in each country decide which infant feeding practice should be promoted in their locality: either complete avoidance of all breastfeeding or exclusive breastfeeding while taking ARVs. However, the ART exposure may be associated with development of ARV drug resistance in infants who become infected despite the prophylaxis (Fogel et al., 2011).

There may be times when an HIV-infected mother breastfeeds inadvertently. There is no guideline as to what to do in this instance, and practice differs. Some experts consider 4 to 6 weeks of post-exposure prophylaxis with ART after the breastfeeding stopped, but this remains controversial.

Another potential mode for mother-to-child transmission is via premasticated food (Ivy et al., 2012). Healthcare providers should be aware of this risk and counsel mothers and other HIV-infected caregivers to avoid this practice.

Treatment of the Human Immunodeficiency Virus–Exposed Infant

All Human Immunodeficiency Virus–Exposed Infants

ARV prophylaxis should be started as soon as possible and ideally within 6 to 12 hours of birth in all HIV-exposed newborns. Oral zidovudine therapy for 6 weeks is still the recommended course based on the PACTG protocol 076 study (Connor et al., 1994). With use of more recent data (Ferguson et al., 2011), guidelines suggest that a 4-week oral zidovudine regimen can be considered in term infants with mothers who had viral suppression throughout pregnancy and adhered to their combination ART; see Table 37.5, which indicates recommended zidovudine dosing based on gestational age, birthweight, and the status of maternal antepartum ARV regimens. A complete blood count with differential should

be performed before zidovudine therapy is started. Thereafter, hematologic monitoring is needed, but there is no specific recommendation as to when to test or how often. Anemia is the primary complication seen in infants with a 4- to 6-week prophylactic zidovudine regimen, and neutropenia also occurs. The PACTG protocol 076 trial showed that zidovudine-exposed infants had lower hemoglobin levels at birth compared with the placebo group. A difference in hemoglobin values persisted throughout the 6 weeks of treatment (Connor et al., 1994). If the infant also had in utero exposure to maternal combination ART, there may be more anemia and/or neutropenia than found in infants exposed to zidovudine alone, as found in the PACTG 316 trial (Dorenbaum et al., 2002). Most experts will check a complete blood count with differential again when they are also drawing blood for a nucleic acid amplification test or if the infant has symptoms of anemia.

Newborns considered to be at higher risk of HIV transmission should have 6 weeks of oral zidovudine therapy and also three doses of nevirapine in the first week of life (Table 37.5). This is at birth, 48 hours later, and 96 hours after the second dose. Higher-risk newborns include those born to mothers who did not receive any antepartum or intrapartum ART and/or mothers with more than 1000 viral copies/mL near delivery and/or mothers whose viral load is unknown. This recommendation is based on data from the National Institute of Child Health and Human Development HIV-1 Prevention Trials Network (NICHD-HPTN) 040/PACTG 1043 study (Nielsen-Saines et al., 2012). Some experts advocate the use of triple ARV prophylaxis in these high-risk infants. However, there are no data demonstrating improved efficacy for a three-drug regimen over a two-drug regimen in the prevention of transmission. The most common regimen is the use of zidovudine, lamivudine, and nevirapine in combination (McKeegan et al., 2011).

TABLE
37.5

Antiretroviral Prophylaxis Dosing for All Human Immunodeficiency Virus–Exposed Infants

| Dosing | | Duration |
|---|--|--|
| Zidovudine | ≥35 weeks' gestation at birth: 4 mg/kg per dose orally twice daily, started as soon after birth as possible and preferably within 6 to 12 h of delivery (or, if unable to tolerate oral agents, 3 mg/kg per dose intravenously, beginning within 6 to 12 h of delivery, then every 12 h) | Start at birth and continue for 4 to 6 weeks ^a |
| Zidovudine | ≥30–<35 weeks' gestation at birth: 2 mg/kg per dose orally (or 1.5 mg/kg per dose intravenously), started as soon after birth as possible, preferably within 6 to 12 h of delivery, then every 12 h, advanced to 3 mg/kg per dose orally (or 2.3 mg/kg per dose intravenously) every 12 h at age 15 days | Start at birth and continue for 6 weeks |
| Zidovudine | <30 weeks' gestation at birth: 2 mg/kg body weight per dose orally (or 1.5 mg/kg per dose intravenously) started as soon after birth as possible, preferably within 6 to 12 h of delivery, then every 12 h, advanced to 3 mg/kg per dose orally (or 2.3 mg/kg per dose intravenously) every 12 h after age 4 weeks | Start at birth and continue for 6 weeks |
| Additional Antiretroviral Prophylaxis Agents for HIV-Exposed Infants of Women WHO Received No Antepartum Antiretroviral Prophylaxis (Initiated as Soon After Delivery as Possible) | | |
| Nevirapine (in addition to zidovudine as shown above) | Birthweight 1.5 to 2 kg: 8 mg per dose orally Birthweight >2 kg: 12 mg per dose orally | Three doses in the first week of life: 1. Within 48 h of birth 2. 48 h after first dose 3. 96 h after second dose |

^aA 6-week course of neonatal zidovudine therapy is generally recommended. A 4-week neonatal zidovudine prophylaxis regimen may be considered when the mother has received standard antiretroviral therapy during pregnancy with consistent viral suppression and there are no concerns related to maternal adherence.

HIV, Human immunodeficiency virus.

Modified from Panel on Treatment of HIV-Infected Pregnant Women and Prevention of Perinatal Transmission. Recommendations for use of antiretroviral drugs in pregnant HIV-1 infected women for maternal health and interventions to reduce perinatal HIV transmission in the United States. <http://aidsinfo.nih.gov/contentfiles/lvguidelines/PerinatalGL.pdf>; 2016.

All HIV-exposed newborns should be tested for HIV infection at the baseline (usually 14 days of life, but some experts would also test at birth, especially for newborns with mothers with unknown HIV status or who are not virologically suppressed). Testing should be repeated at 4 to 6 weeks of age and again at 4 to 6 months of age. A nucleic acid amplification test should be performed during this period, as antibody testing will reflect the mother's serologic status until the infant is older than 18 months. HIV DNA PCR may be preferable over HIV RNA PCR because the ARV prophylaxis may lower infant plasma viral RNA to undetectable levels. However, not all laboratories offer HIV DNA PCR. Also, not all HIV DNA PCR assays are able to detect non-subtype B or group O HIV, and so if this is a concern, both assays should be performed. Many experts will confirm an HIV-negative status with an HIV antibody test after age 18 months.

Infants are considered presumptively negative if they have had two negative nucleic acid amplification test results by age 4 to 6 weeks. More definitive testing is at the 4- to 6-month age mark. If the HIV status is unknown or not delineated by the time the prophylaxis course is complete at 4 to 6 weeks, infants should start prophylaxis for *Pneumocystis jirovecii* pneumonia.

HIV-infected mothers may have an increased incidence of coinfection with other pathogens that can be transmitted from mother to child. Pathogens to consider include HBV, HCV, *Treponema pallidum*, *Mycobacterium tuberculosis*, HSV, CMV, and *Toxoplasma gondii*. Infants may need additional testing depending on any known coinfections in the mother and the clinical history of the mother, including evidence of any of these diseases during the perinatal period. HBV vaccine may be given per the usual schedule and preferably within the first 12 hours of birth as the immunoprophylaxis of perinatal infection is most effective within this period. The routine primary immunization schedule may be followed thereafter. The schedule for live virus vaccines may need to be adjusted for infants identified as infected with HIV.

Human Immunodeficiency Virus–Infected Infant

If the result of a nucleic acid amplification test is positive, the test should be repeated for confirmation. Viral resistance testing should then be performed, and the ART regimen should be adjusted as

needed. Both genotypic and phenotypic resistance testing is available. Genotype testing reflects the actual genetic mutations that may or may not confer clinically important resistance. Phenotype testing measures the ability of a specific patient's virus to replicate while exposed to each individual ARV tested. This is compared with a "wild-type virus" control. Both types of tests should be performed if available, but either can provide resistance information needed to adjust an ART regimen if indicated.

With the more rapid turnaround of nucleic acid amplification tests, diagnoses are made earlier than previously experienced. There are very limited dosing and safety data for ARVs in the early newborn period.

There is growing interest in early and aggressive treatment with combination ART, especially in light of the so-called Mississippi baby case of an HIV-infected newborn in whom combination ART was started at 30 hours of life and who demonstrated a prolonged remission after ART was stopped against medical advice at age 18 months (Persaud et al., 2013). Viremia recurred, but there was 2 years of viral suppression despite the absence of ART. This has been attributed to the knowledge that HIV can hide in reservoirs despite an undetectable plasma viral load, and this may lead to rebound viremia (Luzuriaga et al., 2015). Despite cases like this, there is a lack of evidence to support the practice of very early treatment. We recommend consultation with a pediatric HIV specialist to determine the optimal regimen and dosing at the time of a confirmed positive nucleic acid amplification test result, especially in newborns less than 2 weeks old.

Zidovudine remains the only drug recommended for treatment of preterm infants. They may transition to therapy with another drug, or another drug may be added once they reach 14 days past their original due date. In term neonates, zidovudine, lamivudine, emtricitabine, and stavudine can be used for treatment starting at birth. Once the term neonates reach 14 days of age, didanosine, ritonavir-boosted lopinavir, and nevirapine can also be used.

Current dosing for nevirapine is based on the regimen used in the NICHD-HPTN 040 study. This was designed to target prophylaxis concentrations above 0.1 µg/mL rather than the 3.0 µg/mL target for treatment concentrations. A dosing regimen designed to reach the plasma target for therapeutic levels is currently under investigation in the IMPAACT P1115 clinical trial.

Summary

ARVs used during pregnancy, labor, and breastfeeding and for infant prophylaxis have dramatically reduced mother-to-child transmission of HIV. However, not all women and infants are offered ART, and not all have access to care. We can also identify infection in neonates earlier and more reliably with nucleic acid amplification tests, but not all infants at risk are tested. UNAIDS announced the "90-90-90" targets toward ending the AIDS epidemic by 2020: 90% of people with HIV will be diagnosed, 90% of those with a diagnosis will be receiving sustained ART, with 90% viral suppression in those receiving ART. Women and children are at the center of this campaign (Davies et al., 2015). Also, the programs initiated as part of the Global Plan "towards the elimination of new HIV infections among children and keeping their mothers alive" will continue. The National Institute for Allergy and Infectious Diseases announced plans for a new vaccine trial at HIV Vaccine Awareness Day 2016. This new trial (HVTN 702)

commenced in November, 2016, and plans to enroll 5400 subjects to assess the safety, tolerability, and effectiveness of a vaccine regimen designed to prevent acquisition of HIV infection in South African adults. This vaccine is based on a prime-boost regimen of two vaccines: ALVAC-HIV vaccine (the primer dose), a modified canarypox vaccine–vectored vaccine, and AIDSVAX B/E vaccine (the booster dose), a glycoprotein 120 vaccine. In a previous phase III study in Thailand (the RV144 study), this vaccine demonstrated an efficacy of 31% in prevention of HIV infection (Rerks-Ngarm et al., 2009).

Guidelines for the treatment of women during pregnancy, ART, infant prophylaxis, and pediatric ART change rapidly. We recommend readers also access the Web-based program AIDS Info (<https://aidsinfo.nih.gov>) of the US Department of Health and Human Services for the current guidelines and information.

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Congenital Toxoplasmosis, Syphilis, Malaria, and Tuberculosis

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KEY POINTS

- Maternal transmission of *Toxoplasma gondii* in the first trimester causes the greatest damage to the fetus, while infections later in pregnancy are more readily transmissible to the fetus.
- No neonate should be discharged from a birth hospital without documentation of maternal syphilis testing. If maternal syphilis testing is positive, the adequacy and timing of maternal treatment, comparison of neonate and maternal serologic test results, and the neonate's examination findings must be taken into consideration.
- Symptoms of congenital tuberculosis (TB) are protean but should be considered in any ill neonate whose mother is from a TB-endemic area.
- Symptoms of congenital malaria are nonspecific, though fever is universal. Parasitemia with *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, or *Plasmodium malariae* has been reported with congenital malaria and should be considered in any infant whose mother is from a malaria-endemic region.

Congenital Toxoplasmosis

Epidemiology

Toxoplasmosis in the fetus and newborn is due to maternal infection with the parasite *Toxoplasma gondii*. This obligate protozoan parasite is ubiquitous in nature. While the cat is the definitive host, *T. gondii* can infect most warm-blooded animals. Human seroprevalence differs both geographically and by socioeconomic status. In the United States, seroprevalence studies obtained from the National Health and Nutrition Examination Survey 2009–2010 show a continuous decrease in seroprevalence, being around 9% for women of childbearing age, compared with prior surveys conducted from 1988–1994 and 1999–2004 (Jones et al., 2014). Accordingly, more than 90% of women of childbearing age in the United States are susceptible to primary infection with *T. gondii* during pregnancy with risk of transmission to the fetus. Seroprevalence is increased for those born outside the United States, living below the poverty level, and with a lower level of education. Global data on seroprevalence show the highest rates in Latin America, parts of eastern/central Europe, the Middle East, and parts of Southeast Asia and Africa (Pappas et al., 2009).

However, seroprevalence rates differ considerably from one country to another, from one region of a country to another, and even from one ethnic group to another in the same region. These widely disparate seroprevalence rates among different adult populations throughout the world have been explained by differences in eating and sanitation practices that contribute to acquisition of infection. Eating undercooked or raw meat or unwashed raw fruits and vegetables, drinking unpasteurized goat's milk, working with meat, having three or more kittens, and even certain climactic conditions have been associated with higher risks of infection (Jones et al., 2009). Waterborne transmission particularly from untreated well water has been noted (Jones et al., 2009; Krueger et al., 2014).

Older studies showed the prevalence of congenital infection in Massachusetts and New Hampshire to be 0.08 per 1000 births through immunoglobulin (Ig) M screening of newborn blood specimens collected on filter paper (Guerina et al., 1994). Higher rates of 310 per 1000 live births in Paris and Vienna have been noted, where maternal seroprevalence rates were approximately 70% and 40%, respectively. In Massachusetts, a case–control study involving 14 years of newborn screening for congenital toxoplasmosis found that the mother's birth outside the United States, particularly in Cambodia and Laos, and the mother's educational level and higher gravidity were strongly predictive of congenital infection (Jara et al., 2001). With approximately 4 million live births annually in the United States, there are an estimated 400–4000 babies born each year with congenital toxoplasmosis (Feldman et al., 2010).

Pathogenesis, Clinical Presentation, and Natural History

T. gondii exists in three forms:

1. An oocyst that is shed in cat feces from sporozoites formed within the cat's intestinal tract
2. A tachyzoite or endozoite that is the proliferative form and was formerly referred to as a *trophozoite*
3. A tissue cyst that has an intracystic form termed *cystozoite* or *bradyzoite*

Nonfeline mammals or birds ingest infective oocysts from contaminated soil. Tissue cysts then accumulate in the organs and skeletal muscle of these animals. The possible routes of transmission from animal to human are direct contact with infected cat feces, ingestion of undercooked meat containing infective cysts, and

ingestion of fruits or vegetables that have been in contaminated soil. Rarer methods of transmission can be from infected blood transfusions or organ transplantation (Schwartz et al., 2013). Congenital infection results from placental infection and subsequent hematogenous spread to the fetus.

Infection of the fetus occurs as a consequence of maternal primary infection during pregnancy or, rarely, just before conception (Villena et al., 1998b). Reactivation of latent *T. gondii* infection during pregnancy does not lead to fetal infection, except among immunocompromised women such as those infected with human immunodeficiency virus (HIV) or those undergoing chemotherapy (Langer, 1963; Mitchell et al., 1990; O'Donohoe et al., 1991; European Collaborative Study and Research Network on Congenital Toxoplasmosis, 1996; Dunn et al., 1997; Bachmeyer et al., 2006). Even under those circumstances the risk is low. Rarely, maternal reinfection can result in congenital toxoplasmosis (Hennequin et al., 1997). The severity of disease is related to both host and parasite factors as well as the stage of pregnancy (Jamieson et al., 2008; McLeod et al., 2012).

Infection of the fetus occurs transplacentally during maternal parasitemia. Placental infection is an important intermediary step, and up to 16 weeks may elapse between placental infection and subsequent infection of the fetus. This time delay has been termed the *prenatal incubation period* (Remington et al., 2001) and explains the success of intervention during pregnancy. Infections in twins show similar clinical manifestations in monozygotic twins, whereas discrepancies in clinical findings are common in dizygotic twins (Wiswell et al., 1984; Sibalic et al., 1986; Couvreur et al., 1991).

Overall, approximately 40% of infants born to mothers who acquired toxoplasmosis during pregnancy are congenitally infected with *T. gondii*. The rate of vertical transmission differs according to the trimester in which the mother became infected, with fetal infection rates increasing as pregnancy advances (Wong and Remington, 1994; Dunn et al., 1999; Remington et al., 2001). Only 15% of infants are infected when maternal infection occurs in the first trimester, whereas the transmission rates are 30% and 60% with maternal infection in the second and third trimesters respectively. The severity of clinical manifestations is greatest, however, when maternal infection occurs early in pregnancy. Maternal infection in the first trimester results in severe disease in as many as 40% of infected fetuses and in stillbirth or perinatal death in an additional 35% of infants (Wong and Remington, 1994). Conversely, maternal infection in the third trimester is rarely if ever associated with severe fetal disease or stillbirth, and approximately 90% of infants in such situations have subclinical infection (Wong and Remington, 1994).

Postnatally, transmission of *T. gondii* can occur from transfusion of blood or blood products or from transplantation of organ or bone marrow from a seropositive donor with latent infection. Although the organism has been detected in human milk, transmission by breastfeeding has not been documented.

Most newborns with congenital toxoplasmosis are asymptomatic, with apparent disease present in approximately 10%–25% of infected infants (Alford et al., 1969, 1974; Guerina et al., 1994), although thorough evaluation may demonstrate eye or neurologic abnormalities in approximately 20% of cases. The clinical manifestations of toxoplasmosis are often indistinguishable from those seen with other congenital infections, such as cytomegalovirus infection or congenital syphilis. Approximately one-third of infants have a generalized form of the disease that principally involves organs of the reticuloendothelial system. The abnormalities include temperature instability, hepatosplenomegaly, jaundice, pneumonitis,

TABLE 38.1 Clinical Findings Among Infants With Congenital Toxoplasmosis

| Finding | INFANTS WITH FINDINGS (%) | |
|------------------------------|--|--|
| | Neurologic Disease ^a (108 Cases) | Generalized Disease ^b (44 Cases) |
| Chorioretinitis | 94 | 66 |
| Abnormal cerebrospinal fluid | 55 | 84 |
| Anemia | 51 | 77 |
| Convulsions | 50 | 18 |
| Intracranial calcification | 50 | 4 |
| Jaundice | 29 | 80 |
| Hydrocephalus | 28 | 0 |
| Fever | 25 | 77 |
| Splenomegaly | 21 | 90 |
| Lymphadenopathy | 17 | 68 |
| Hepatomegaly | 17 | 77 |
| Vomiting | 16 | 48 |
| Microcephaly | 13 | 0 |
| Diarrhea | 6 | 25 |
| Cataracts | 5 | 0 |
| Eosinophilia | 4 | 18 |
| Abnormal bleeding | 3 | 18 |
| Hypothermia | 2 | 20 |
| Glaucoma | 2 | 0 |
| Optic atrophy | 2 | 0 |
| Microphthalmia | 2 | 0 |
| Rash | 1 | 25 |
| Pneumonitis | 0 | 41 |

^aInfants with otherwise undiagnosed central nervous system diseases in the first year of life.

^bInfants with otherwise undiagnosed nonneurologic diseases during the first 2 months of life.

Modified from Remington JS, McLeod R, Thulliez P, Desmonts G. Toxoplasmosis. In: Remington JS, Klein JO, Wilson CV, Nizet V, eds. *Infectious Diseases of the Fetus and Newborn Infant*. 7th ed. Philadelphia, PA: Saunders; 2011:918–1041.

generalized lymphadenopathy, rash, chorioretinitis, anemia, thrombocytopenia, eosinophilia, and abnormal cerebrospinal fluid (CSF) indices (Table 38.1; Eichenwald, 1960). The other two-thirds of infected infants principally manifest neurologic disease.

Central nervous system involvement is the hallmark of congenital *T. gondii* infection (Diebler et al., 1985; McAuley et al., 1994; Remington et al., 2001). Chorioretinitis, intracranial calcifications, and hydrocephalus are the most characteristic findings, occurring in approximately 86%, 37%, and 20% of symptomatic infants respectively (Eichenwald, 1960; Remington et al., 2001). This constellation of findings has been referred to as the *classic triad of congenital toxoplasmosis*; its presence should alert the clinician to the diagnosis. Intracranial calcifications may be single or multiple

but typically are generalized and located in the caudate nucleus, choroid plexus, meninges, and subependymal zone (Mussbichler, 1968); they also may occur periventricularly, as in cytomegalovirus infection. They are visualized best by computed tomography (CT) but are often detected on ultrasonography as well. Intracranial calcifications may resolve with appropriate antimicrobial therapy (McAuley et al., 1994). Hydrocephalus may be the only manifestation of disease; it results from the extensive periaqueductal and periventricular vasculitis with necrosis that causes obstruction of the ventricular system. Ventriculoperitoneal shunting is often required (Martinovic et al., 1982; McAuley et al., 1994). Abnormalities of the CSF are common; characteristically, they consist of lymphocytic pleocytosis and a markedly elevated protein content. Microcephaly, when present, indicates severe brain injury. Hypothermia and hyperthermia may occur secondary to hypothalamic involvement. *T. gondii* has been detected in the inner ear and mastoid, with the associated inflammation resulting in deafness. An ascending flaccid paralysis with myelitis has also been reported (Campbell et al., 2001).

Chorioretinitis secondary to congenital toxoplasmosis can manifest at any age. It usually manifests as strabismus in infants. Defects in visual acuity are more common in older children who had never received treatment. Typically the eye lesion consists of a focal necrotizing retinitis that is often bilateral with involvement of the macula and even the optic nerve. Complications include blindness, iridocyclitis, and cataracts (Arun et al., 2007; Phan et al., 2008).

Other less common manifestations of congenital toxoplasmosis are nonimmune hydrops fetalis, myocarditis, nephrotic syndrome, and Ig abnormalities, with both hypergammaglobulinemia and hypogammaglobulinemia described. Bony abnormalities consisting of metaphyseal lucencies similar to those seen in congenital syphilis have also been reported (Milgram, 1974). A variety of endocrine abnormalities may occur, including hypothyroidism, diabetes insipidus (Yamakawa et al., 1996; Oygur et al., 1998), precocious puberty, and growth hormone deficiency.

Diagnosis

Attempts to diagnose congenital toxoplasmosis are made either during pregnancy as part of a surveillance protocol because of maternal disease or abnormal fetal findings or after birth because of concern for infection. The test methods differ depending on the scenario and can be complicated. While commercial laboratories are able to perform some assays, reference laboratories are needed for more intensive evaluation and can be very useful with the availability of their consultative services, such as the Palo Alto Medical Foundation *Toxoplasma* Serology Laboratory (Palo Alto, CA, USA) (email toxolab@pamf.org, telephone 650-853-4828, <http://www.pamf.org/serology/>).

Serologic assays for measurement of antibodies to *T. gondii* in serum and body fluids are the most widely used methods of diagnosing toxoplasmosis in a pregnant woman and the fetus or newborn, but the findings can be difficult to interpret (Dannemann et al., 1990; Wong and Remington, 1994; Foudrinier et al., 1995; Naessens et al., 1999; Robert-Gangneux et al., 1999a; Villena et al., 1999; Boyer, 2001; Pinon et al., 2001; Madi et al., 2010). Most nonreference laboratories will be able to test for *Toxoplasma* IgG and IgM and perform polymerase chain reaction (PCR) assays. The more commonly used tests that detect *T. gondii*-specific IgG antibodies are the IgG enzyme-linked immunosorbent assay (ELISA), the indirect immunofluorescent antibody test, and direct

agglutination. The Sabin–Feldman dye test is considered the gold standard but requires live organisms and accordingly is performed by reference laboratories (Pomares and Montoya, 2016). If a pregnant woman is known to be seronegative before pregnancy and followed up serially, then these assays are useful to document seroconversion during pregnancy and risk to the fetus. However, most countries, including the United States, do not have routine prenatal screening, in which case it is important to decipher whether a positive test result represents recent infection or chronic infection without a risk to the fetus. Reference laboratories can perform a differential agglutination test that has been developed as a confirmatory test to differentiate acute from chronic maternal infection. This test compares the IgG serologic titer obtained with the use of formalin-fixed tachyzoites (HS antigen) with that obtained with acetone-fixed or methanol-fixed tachyzoites (AC antigen). The latter preparation contains stage-specific *T. gondii* antigens that are recognized by IgG antibodies only during early infection. An additional assay to assist in ruling out maternal infection acquired in the first 3 months of pregnancy is the IgG avidity test performed by the ELISA technique. This test is based on the principle that, although the antibody-binding avidity or affinity for an antigen is initially low after primary antigenic stimulation, IgG antibodies that are present from previous antigenic stimulation are usually of high avidity. Therefore a high-avidity result in the first trimester would exclude an infection acquired in the previous 12 weeks. Finally, an enzyme-linked immunofiltration assay has been developed that allows discrimination between IgG antibodies of maternal origin and IgG antibodies synthesized by the fetus as well as identification of antibody subtypes in infected neonates (Zufferey et al., 1999).

Tests that detect *T. gondii*-specific IgM include (1) the double-sandwich IgM ELISA, which has a sensitivity of 75%–80% and a specificity of 100% (Guerina et al., 1994), (2) the IgM immunosorbent agglutination assay, which is the most sensitive test but should not be performed on umbilical cord blood, because even small quantities of maternal IgM antibodies contaminating the specimen will yield a false-positive result, and (3) the IgM immunofluorescent antibody test. The last test is not recommended because it has a much lower sensitivity than either the IgM ELISA or the IgM immunosorbent agglutination assay, and it has poor specificity secondary to rheumatoid factors and antinuclear antibodies, contributing to false-positive results. Other tests used in reference laboratories include a *T. gondii*-specific IgA ELISA and IgA immunofiltration assay; a *T. gondii*-specific IgE immunofiltration assay; and IgG, IgM, and IgA immunoblotting tests, which are generally used in comparing samples from the neonate and the mother to help determine true infection. Interferon γ release assays (IGRAs) on infants have been used to diagnose congenital infection but are not available commercially (Pomares and Montoya, 2016).

PCR analysis has been used successfully to detect *T. gondii* DNA in amniotic fluid, placenta, CSF, brain, urine, and fetal and infant blood (Grover et al., 1990; Hohlfeld et al., 1994; Guy et al., 1996; Fricker-Hidalgo et al., 1998; Jenum et al., 1998; Foulon et al., 1999b; Romand et al., 2001). PCR performed on amniotic fluid obtained by amniocentesis is the preferred method of confirming in utero infection (Kasper et al., 2009). False-negative results have been reported, however, and interlaboratory variability in the performance of PCR assays has been documented (Guy et al., 1996; Romand et al., 2001). PCR performed on neonatal CSF is recommended for the evaluation of possible central nervous system (CNS) involvement.

Isolation of *T. gondii* from body fluids and tissues provides definitive evidence of infection. The organism can be isolated from placenta, amniotic fluid, fetal blood obtained by cordocentesis, umbilical cord blood, infant peripheral blood, and CSF by means of intraperitoneal and subcutaneous inoculation of laboratory mice (Wong and Remington, 1994; Foulon et al., 1999a; Remington et al., 2001). Mouse inoculation may require as long as 4–6 weeks for demonstration of the presence of the parasite. Although it is not a practical method, isolation of the organism should be attempted to confirm a diagnosis and is available (at the Palo Alto Medical Foundation *Toxoplasma* Serology Laboratory). In addition, tissue culture has been used to isolate *T. gondii* from amniotic fluid.

Histopathologic examination of the placenta and tissues obtained at postmortem examination or by biopsy from stillborns or infants should be performed because the specimens may demonstrate the presence of tachyzoites. In addition, tachyzoites have been demonstrated in CSF, ventricular fluid, and aqueous humor by specialized staining techniques.

Because most adults with acquired *T. gondii* infection are asymptomatic, evaluation of the pregnant woman and fetus is usually prompted by either seroconversion or an elevated maternal *Toxoplasma* species (spp.) IgG titer (Couvreur et al., 1988; Daffos et al., 1988; Montoya and Remington, 2008). The latter may reflect chronic past infection; therefore the acuity of the maternal infection is determined serologically with the HS–AC differential agglutination test, where agglutination titers to formalin-fixed tachyzoites (HS antigen) are compared with titers against acetone-fixed or methanol-fixed tachyzoites (AC antigen). In general, an acute pattern demonstrates high AC and HS titers, while a nonacute pattern demonstrates high AC titers and low HS titers. This method can differentiate an acute from a remote infection in pregnant women, whereas the levels of IgM and IgA antibodies detectable by ELISA or immunosorbent agglutination assay are elevated for prolonged periods. If recent maternal infection is documented by an acute pattern on the HS–AC test, seroconversion, or rising IgG antibody titers, the fetus should be evaluated by ultrasonography, and amniotic fluid should be tested for specific *Toxoplasma* spp. DNA with PCR. PCR has supplanted the need for cordocentesis, and a positive result confirms fetal infection (Hohlfeld et al., 1994). Postnatally, serologic testing of paired maternal and infant sera should be performed by a reliable laboratory that will include assays for *Toxoplasma* spp. IgG, IgM, and IgA antibodies (Pomares and Montoya, 2016). Waiting until the newborn is 10 days of age avoids contamination with maternal blood and false-positive IgM and IgA results. Subinoculation of mice with placental tissue, amniotic fluid, and umbilical cord blood should be considered. If the results of these tests suggest possible infection, the newborn should be evaluated fully with complete blood cell count and platelet determination, liver function tests, CSF evaluation (including tests for IgG and IgM antibodies and PCR) (Wallon et al., 1998), cranial ultrasound imaging or CT of the head, ophthalmologic examination, and hearing evaluation. The presence of neonatal IgM antibody in serum or CSF, or a positive PCR result for blood or CSF, indicates congenital infection. In addition, at-risk infants should undergo serologic follow-up to detect rising serum IgG titers during the first year after birth or persistence of IgG antibody beyond 12–15 months of age, when maternal IgG antibody has disappeared (Robert-Gangneux et al., 1999a, 1999b; Pomares and Montoya, 2016). Uninfected infants show a continuous decline in *T. gondii* IgG titer, which usually is gone by 7 months of age, with no detectable IgM or IgA antibodies.

With low IgG titers and an HS–AC differential agglutination test result that indicate remote maternal infection, further evaluation of the mother or infant is not necessary unless the mother is severely immunosuppressed. Because fetal infection has occurred during chronic *T. gondii* infection in very immunosuppressed women such as those with poorly controlled HIV infection, their infants should be evaluated serologically at birth for evidence of congenital infection. It has been suggested that HIV-infected pregnant women who have low CD4+ T-lymphocyte counts and who are seropositive for *T. gondii* antibody should receive prophylaxis to prevent fetal infection (Beaman et al., 1992; Wong and Remington, 1994). However, insufficient data are currently available to recommend that such therapy be given routinely for this indication. Nevertheless, if such women have had toxoplasmic encephalitis, prophylaxis with pyrimethamine, sulfadiazine, and leucovorin (folinic acid) should be considered (Masur et al., 2002).

Therapy

It is currently recommended that fetuses and infants younger than 1 year who are infected with *T. gondii* receive specific therapy effective against this congenital pathogen, even if they have no clinical signs of disease (Koppe et al., 1986; Couvreur et al., 1988; Daffos et al., 1988; Hohlfeld et al., 1989; McGee et al., 1992; McAuley et al., 1994; Wong and Remington, 1994; Roizen et al., 1995; Vergani et al., 1998; Foulon et al., 1999b; Friedman et al., 1999; Wallon et al., 1999; Gilbert et al., 2001; Peyron and Wallon, 2001; McLeod et al., 2009). On the basis of comparison with untreated historical controls, outcome is improved substantially by neonatal treatment. The effectiveness of maternal and fetal treatment is less clear. Spiramycin has been used in pregnant women with acute toxoplasmosis to reduce transplacental transmission of *T. gondii*. If fetal infection is confirmed after the 17th week of pregnancy, however, treatment with pyrimethamine, sulfadiazine, and folinic acid is recommended. Prenatal treatment of congenital toxoplasmosis is believed to reduce the clinical severity of infection in the newborn, while shifting the disease to a more subclinical form. This effect in turn may ameliorate the long-term neurologic complications that are commonly seen among infants who have clinical manifestations in the neonatal period. A metaanalysis of the effectiveness of prenatal treatment of toxoplasmosis infection found no evidence that such treatment significantly decreased clinical manifestations of disease in infected infants (SYROCOT Study Group, 2007). However, studies in Brazil and Germany suggest efficacy (Cortina-Borja et al., 2010; Hotop et al., 2012).

Neonatal treatment has resulted in reductions in sensorineural hearing loss and neurodevelopmental and visual handicaps. Table 38.2 shows the recommended guidelines for the treatment of congenital toxoplasmosis. In infants with congenital toxoplasmosis, the treatment consists of pyrimethamine, sulfadiazine, and folinic acid (McLeod et al., 1992; McAuley et al., 1994; Remington et al., 2001). The ideal duration of therapy is not known, although prolonged courses of at least 1 year are preferred. Currently most experts recommend combined treatment until the patient is 1 year old (Vergani et al., 1998; Villena et al., 1998a; Remington et al., 2001). Accessing pyrimethamine in the United States has recently become difficult because of extreme cost escalation. Assistance with access is available through <http://www.daraprimdirect.com> (telephone 1-877-258-2033).

Complete blood cell counts and platelet counts must be monitored closely while the patient is receiving therapy, because granulocytopenia, thrombocytopenia, and megaloblastic anemia

TABLE 38.2 Treatment Guidelines for Toxoplasmosis

| Condition | Therapy | Dose (Oral Unless Specified) | Duration |
|---|--|--|--|
| Pregnant woman with acute toxoplasmosis | Spiramycin for first 21 weeks of gestation or until term if fetus not infected ^a | 3 g, divided twice a day without food | Until fetal infection documented or excluded at 21 weeks; if fetal infection documented, replaced with pyrimethamine, leucovorin, and sulfadiazine |
| | Pyrimethamine (if fetal infection confirmed after 18th week of gestation or if infection acquired in last few weeks of gestation) <i>and</i> | Loading dosage: 100 mg/d in two divided doses for 2 days followed by 50 mg/d | Until delivery |
| | Sulfadiazine ^a <i>and</i> | 3 g per day (twice daily) | Until delivery |
| | Leucovorin ^b | 5–20 mg/d | Until delivery |
| Congenital <i>Toxoplasma gondii</i> infection in infant | Pyrimethamine <i>and</i> | Loading dosage: 2 mg/kg per day for 2 days; then 1 mg/kg per day for 6 months; then 1 mg/kg per day on Monday, Wednesday, and Friday each week | 1 year |
| | Sulfadiazine <i>and</i> | 100 mg/kg per day in two daily divided doses | 1 year |
| | Leucovorin ^b | 5–10 mg three times per week | 1 year |
| | Corticosteroids (prednisone) ^c | 1 mg/kg per day in two daily divided doses | Until resolution of elevated (≥ 1 g/dL) CSF protein level or active chorioretinitis that threatens vision |

^aDose may need to be adjusted for renal insufficiency.

^bMonitor blood and platelet counts weekly; adjust dosage for megaloblastic anemia, granulocytopenia, or thrombocytopenia.

^cWhen signs of inflammation or active chorioretinitis have subsided, dose can be tapered and eventually discontinued; use only in conjunction with pyrimethamine, sulfadiazine, and leucovorin. CSF, Cerebrospinal fluid.

Data from McAuley JB, Boyer KM, Remington JS, McLeod RL. Toxoplasmosis. In: Cherry JD, Harrison GJ, Kaplan SL, Steinbach WJ, Hotez PJ, eds. *Feigin and Cherry's Textbook of Pediatric Infectious Diseases*. 7th ed. Philadelphia, PA: Elsevier; 2014;2986–3005.

can occur. The counts usually increase once a higher dose of folinic acid is administered or pyrimethamine therapy and sulfadiazine therapy are discontinued temporarily. The indications for adjunctive therapy with corticosteroids such as prednisone (0.5 mg/kg twice per day) are a CSF protein concentration of 1 g/dL or higher and chorioretinitis that threatens vision; corticosteroid treatment is continued until either condition resolves. Current therapies are not effective against encysted bradyzoites and therefore might not prevent reactivation of chorioretinitis and neurologic disease.

Prognosis

Maternal toxoplasmosis acquired during the first and second trimesters has been associated with stillbirth and perinatal death secondary to severe fetal infection in approximately 35% and 7% of cases respectively. Among infants born with congenital toxoplasmosis, the mortality rate has been reported to be as high as 12%. In addition, infants with congenital toxoplasmosis are at high risk of ophthalmologic, neurodevelopmental, and audiologic impairments, including mental retardation (87%), seizures (82%), spasticity and palsies (71%), and deafness (15%) (Eichenwald, 1960; Koppe et al., 1986; Hohlfield et al., 1989; McAuley et al., 1994). Among neonates with subclinical infection, long-term follow-up reveals eye or neurologic disease in as many as 90% by the time they reach adulthood (Couvreur and Desmonts, 1962; Saxon et al., 1973; Wilson et al., 1980; Couvreur et al., 1984;

McLeod et al., 2000). While data from the US National Collaborative Treatment Trial show that severity is influenced by host and parasite factors, treatment of neonates with congenital toxoplasmosis early and for 1 year resulted in more favorable outcomes than were reported for untreated infants or infants who were treated for only 1 month (McLeod et al., 2006).

Prevention

Pregnant women whose serologic status for *T. gondii* is negative or unknown, as well as women who are attempting to conceive, should be educated on the prevention of congenital toxoplasmosis through avoidance of at-risk behaviors that may expose them to cat feces or encysted bradyzoites in raw meat (Wilson and Remington, 1980; Eskild et al., 1996; Lopez et al., 2000; Jones et al., 2001). Instructions to wear gloves when such women are changing cat litter boxes or gardening and to wash their hands after such activities should be given. Daily changing of cat litter will also decrease the chance of infection, because oocysts are not infective during the first 1–2 days after passage. In addition, keeping domestic cats inside and feeding them commercially prepared foods rather than undercooked meats or wild rodents reduce the likelihood of their becoming infected and capable of transmitting the infection to a pregnant woman. Oral ingestion of *T. gondii* can be prevented by either cooking meat to well done, smoking it, or curing it in brine and by washing kitchen surfaces and utensils that come into

contact with raw meat. Vegetables and fruits should be washed, and hands and kitchen surfaces should be cleaned after fruits, vegetables, and raw meat have been handled. Flies and cockroaches may serve as transport hosts for *T. gondii*, so their access to food must be prevented.

Routine serologic screening of women during pregnancy has been an effective means of prevention in France and Austria, where the incidence of congenital toxoplasmosis is high. No such screening is currently recommended in the United States. However, high-risk women, including those who are immunocompromised, should be screened early in pregnancy. Neonatal screening for IgM antibody has also been advocated so that asymptomatic infants can be detected and treated before neurologic symptoms develop (Petersen and Eaton, 1999). This strategy, however, has been hampered by the lack of readily available and reliable IgM test kits. Moreover, such screening will not detect the approximately 25% of infected infants who lack anti-*Toxoplasma* spp. IgM antibody. Further study involving cost analyses is needed to define the best preventive strategy for congenital toxoplasmosis in specific populations, regions, and countries.

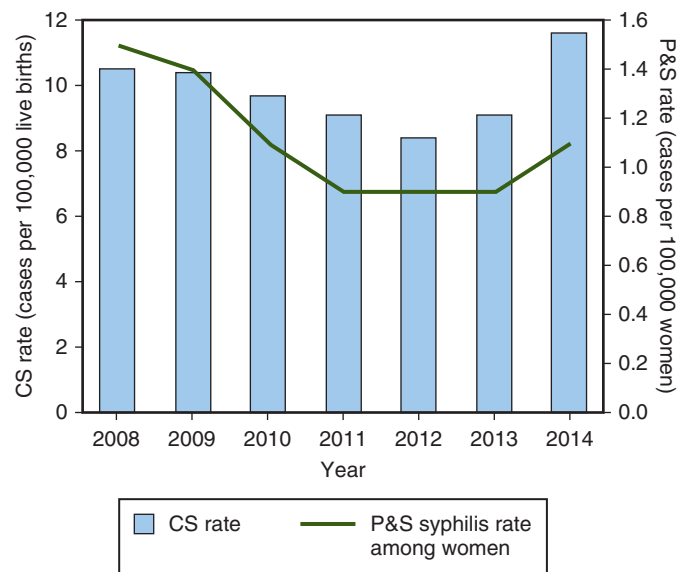
Congenital Syphilis

Congenital syphilis, a result of fetal infection with the spirochete *Treponema pallidum*, remains a major public health problem worldwide. While adults acquire the infection sexually, infants are infected mostly in utero by a transplacental route or possibly during delivery by contact with a genital lesion of an infected mother. When maternal infection is detected during pregnancy, congenital syphilis is both preventable and treatable. However, if infected infants are not identified in a timely fashion, they may experience lifelong consequences. Accordingly, the Centers for Disease Control and Prevention (CDC) recommends that no mother or newborn be discharged from the hospital without the maternal serologic status for syphilis having been documented at least once during the pregnancy and preferably again at delivery if the mother is at increased risk or lives in a community with high prevalence of syphilis infection.

Epidemiology

The incidence of congenital syphilis mirrors the rates of primary and secondary syphilis in women. Overall, congenital syphilis disproportionately affects infants of black women whose prenatal care was lacking or inadequate. Less prenatal care has been associated with increased risk of fetal death that most often occurs by 31 weeks' gestation. From 1999–2013 in the United States, neonatal mortality secondary to congenital syphilis was 12 per 1000 live births, with a case fatality rate of 6.5%. Of the 418 reported deaths, 82% were stillbirths, and 89% of the mothers had untreated or inadequately treated syphilis (Su et al., 2016).

During the prepenicillin era of the 1930s and 1940s, 60–80 infants and children attended the congenital syphilis clinic of the Harriet Lane Home (Baltimore, MD) each week for arsenic therapy. Many more were lost to follow-up before completion of their 2–3-year course of treatment. It was unusual if fewer than three or four new cases were discovered in the general outpatient department in the course of 1 week. Subsequently, the frequency of congenital syphilis declined for several decades, only to increase dramatically in the late 1980s and early 1990s. This increase was fueled by the crack cocaine epidemic with women exchanging drugs for sex with multiple and anonymous partners. At the same time, in 1988, the CDC surveillance case definition for reporting cases of congenital syphilis was broadened to include all liveborn



• **Fig. 38.1** Congenital syphilis rate among infants younger than 1 year and the rate of primary and secondary syphilis among females 10 years or older. National Electronic Telecommunication System for Surveillance, United States, 2008–2014. CS, Congenital syphilis; P&S, primary and secondary. (Data from Bowen V, Su J, Torrone E, Kidd S, Weinstock H. Increase in incidence of congenital syphilis - United States, 2012–2014. *MMWR Morbid Mortal Wkly Rep.* 2015;64:1241–1245.)

and stillborn infants, irrespective of clinical findings, who had reactive serologic test results for syphilis and had been delivered to women with untreated or inadequately treated syphilis. This change resulted in a fourfold increase in reported cases of congenital syphilis when compared with the previously used Kaufman criteria that included only symptomatic infants (reviewed in *Centers for Disease Control*, 1989). For unclear reasons, the rate of maternal syphilis subsequently declined and the rate of congenital syphilis decreased from 1991–2005 but increased slightly from 2005–2008. From 2008–2012 the overall rate of congenital syphilis again decreased from 10.5 cases per 100,000 live births to 8.4 cases per 100,000 live births, reflecting decreasing trends in primary and secondary syphilis among women. However, in 2014, the rate of congenital syphilis increased across all regions of the United States, to 11.6 cases per 100,000 live births (458 cases), the highest rate reported since 2001 (Bowen et al., 2015) (Fig. 38.1).

Worldwide, congenital syphilis remains a major cause of fetal and neonatal death, with more newborns affected by congenital syphilis than by any other neonatal infection (Schmid, 2004). The World Health Organization estimates that globally, 1.5–1.85 million pregnant women are infected with syphilis annually, and half of them have neonates with adverse outcomes, such as stillbirth or prematurity in 17%–40%, congenital infection including nonimmune hydrops fetalis in 10%–30%, and death in 10%–23% (Gomez et al., 2013). The global burden of congenital syphilis is confounded further by the high prevalence of infection with HIV, as syphilis is a known risk factor for acquisition of HIV.

Pathogenesis, Clinical Presentation, and Natural History

The causative agent for syphilis is *T. pallidum*, a thin, corkscrew-shaped, flagellated, highly motile spirochete. *T. pallidum* is able to

invade the fetal compartment at any time during gestation, although the risk of fetal infection increases as the stage of pregnancy advances. Spirochetes have been detected in fetal tissue from spontaneous abortion as early as 9 and 10 weeks' gestation and recovered from amniotic fluid at 14 weeks of pregnancy by rabbit infectivity testing. Vertical transmission is related directly to the maternal stage of syphilis, with early syphilis resulting in significantly higher transmission rates than late latent infection. [Ingraham \(1950\)](#) reported that among 251 women with syphilis of less than 4 years' duration, 41% of their neonates were born alive and had congenital syphilis, 25% were stillborn, 14% died in the neonatal period, 21% had low birth weight but no evidence of syphilis, and 18% were normal full-term neonates. In contrast, only 2% of neonates born to mothers with late latent disease had congenital syphilis. [Fiumara et al. \(1952\)](#) reported that untreated maternal primary or secondary syphilis resulted in 50% of neonates having congenital syphilis, while the other 50% were stillborn, premature, or died in the neonatal period. With early and late latent infection, 40% and 10% of neonates, respectively, had congenital syphilis ([Fiumara et al., 1952](#)). These data are supported by a study in which mothers with primary, secondary, early latent, and late latent infection had transmission rates of 29%, 59%, 50%, and 13%, respectively ([Wendel, 1988](#)).

Because *T. pallidum* enters the fetal bloodstream directly, the primary stage of infection is completely bypassed. There is no chancre and no local lymphadenopathy. Instead, there is widespread hematogenous spread to all organs and tissues, including the liver, spleen, pancreas, intestine, kidney, skin, mucous membranes of the lips, nose, and anus, bones and cartilage, and the CNS. Invasion of the lung results in a characteristic "pneumonia alba" that is seen more frequently in developing countries.

Microscopically, the tissue alterations consist of interstitial fibrosis and perivascular inflammation with plasma and round cell infiltration, with visualization of spirochetes by silver or fluorescent staining. Gumma formation is infrequent in neonates, while extramedullary hematopoiesis involving the liver, spleen, dermis, kidneys, and other organs is common.

The placenta of neonates with congenital syphilis is often large, thick, and pale. Histopathologic features include villous enlargement, acute villitis, and erythroblastosis. Intense inflammation of the umbilical cord results in a "barber's pole" appearance where the edematous portions have a spiral striped zone of red and pale blue discoloration, interspersed with streaks of chalky white. Histologically, the umbilical cord exhibits abscess-like foci of necrosis within Wharton jelly and umbilical vessels. Placental and umbilical cord histopathology should be performed on every case of suspected syphilis.

The clinical, laboratory, and radiographic abnormalities of congenital syphilis are a consequence of active infection with *T. pallidum* and the resultant inflammatory response induced in various body organs and tissues. The severity of these manifestations is highly variable and can range from overwhelming involvement of multiple organs and body systems as occurs in nonimmune fetal hydrops to only laboratory or radiographic abnormalities. Most neonates born to mothers with untreated syphilis appear normal and have no clinical or laboratory evidence of infection at birth but may develop manifestations of disease several months to years later if left untreated. Two characteristic syndromes of clinical disease have been described. *Early congenital syphilis* refers to those clinical manifestations that appear in the first 2 years after birth, while those features that occur after 2 years and usually at puberty are designated as *late congenital syphilis*.

The signs and symptoms of early congenital syphilis are summarized in [Table 38.3](#). Prematurity and low birth weight is seen

TABLE 38.3 Clinical Features of Congenital Syphilis in the Neonatal Period

| Feature | Prevalence (%) |
|--|-----------------|
| Hepatomegaly with or without splenomegaly ^a | 60–100 |
| Radiographic bone changes (periostitis; osteochondritis); ^a pseudoparalysis of Parrot | 75–100; 12 |
| Lymphadenopathy | 20–50 |
| Jaundice | 50–70 |
| Rash ^a | 40 |
| Hepatitis (elevated transaminase concentrations) | 40 |
| Anemia and/or thrombocytopenia | 20–50 |
| Respiratory distress (pneumonia) | 34 |
| Fever | 10 ^b |
| Small for gestational age | 10 |
| Nonimmune hydrops | 5 |
| Rhinitis, mucous patch, condyloma lata, nephrotic syndrome, myocarditis, diarrhea (malabsorption), pancreatitis, chorioretinitis, cataract | Rare (<5) |
| Central nervous system (leptomeningitis, cranial nerve palsies, cerebral infarction, seizures, hypopituitarism) | Rare (<5) |

^aProminent feature.

^bMore common when presentation is at more than 3 weeks of age.

Data from Kollman TR, Dobson S. Syphilis. In: Remington JS, Klein JO, Wilson CV, Nizet V, eds. *Infectious Diseases of the Fetus and Newborn Infant*. 7th ed. Philadelphia, PA: Saunders; 2011:524–563; and Saloojee H, Velaphi S, Goga Y, et al. The prevention and management of congenital syphilis: an overview and recommendations. *Bull World Health Organ*. 2004; 82:424–430.

in 10%–40% of neonates ([Saloojee et al., 2004](#)). Hepatosplenomegaly is frequent, with extramedullary hematopoiesis a prominent finding in both the liver and the spleen. Approximately one-third of neonates have direct and indirect hyperbilirubinemia and elevated transaminase levels that may worsen transiently after initiation of penicillin therapy. Liver abnormalities may require months to resolve, but they rarely lead to cirrhosis. Generalized lymphadenopathy occurs in about 20% of infants, with characteristic enlargement of epitrochlear nodes. Anemia secondary to hemolysis or infection of the bone marrow with hematopoietic suppression may be severe ([Nolt et al., 2002](#)). Thrombocytopenia with petechiae and purpura occurs frequently and may be the sole manifestation of congenital infection. Other less common manifestations include ocular findings (chorioretinitis, cataract, glaucoma, and uveitis), pneumonitis, pneumonia alba, nephrotic syndrome, myocarditis, pancreatitis, and inflammation and fibrosis of the gastrointestinal tract leading to malabsorption and diarrhea.

Mucocutaneous lesions occur in 40%–60% of affected infants. The rash of congenital syphilis is usually oval and maculopapular but becomes copper colored with desquamation ([Fig. 38.2](#)) mostly in the palms and soles. A characteristic vesicular bullous eruption known as *pemphigus syphiliticus* may develop with erythema, blisters, and eventual crusting and skin wrinkling. Mucocutaneous junctions may also be involved, with the lips becoming weepy, thickened,



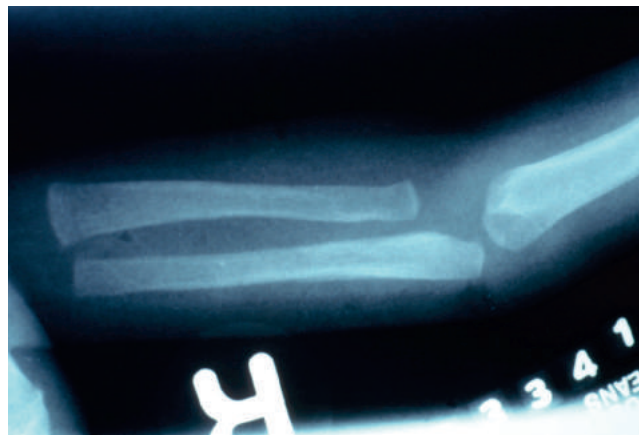
• **Fig. 38.2** Congenital Syphilis With Desquamation Over the Hand. (From American Academy of Pediatrics Committee on Infectious Diseases. Syphilis: clinical manifestations images. In: *Red Book Online Visual Library*. Elk Grove Village, IL: American Academy of Pediatrics; 2015. Available at <http://redbook.solutions.aap.org/chapter.aspx?resultClick=1&bookID=1484§ionID=88187246#91040951>.)

and rough. Radial cracks may traverse the vermillion zone surrounding the margins of the lips and are the beginning of the radiating scars called *rhagades* that are seen with late congenital syphilis. Rarely, mucous patches of the lips, tongue, and palate as well as white, flat, moist, raised plaques known as *condylomata lata* in the perioral and perianal areas may occur.

Rhinitis (“snuffles”), a watery nasal discharge that may become thick, purulent, and blood tinged, occurred in almost two-thirds of patients reported in the early literature but is now less prevalent (Ingall et al., 2006). Both the nasal discharge and vesicular fluid contain large concentrations of spirochetes and are highly infectious.

Bone radiographs demonstrate characteristic osteochondritis and periostitis in 60%–80% of infants with clinical signs of congenital syphilis and 20% of well-appearing, congenitally infected infants (Fig. 38.3). These abnormalities tend to involve the long bones (tibia, humerus, femur), ribs, and cranium and are usually symmetric, with the lower extremities involved more often than the upper extremities. Rarely, the bone lesions may be painful and result in subepiphyseal fracture and epiphyseal dislocation with pseudoparalysis of the affected limb (pseudoparalysis of Parrot). Osteochondritis involves the metaphysis and is visualized on the long bone radiographs approximately 5 weeks after fetal infection. There is metaphyseal demineralization with a radiolucent band representing a zone of osteoporosis below a radiodense band below the epiphyseal plate that is a widened and enhanced zone of provisional calcification. Radiographically, this results in the classic transverse saw-toothed appearance of the metaphysis, whose margins become serrated, jagged, and irregular. Bilateral demineralization and osseous destruction of the proximal medial tibial metaphysis are referred to as *Wimberger sign*. Periostitis requires 16 weeks for radiographic demonstration and consists of multiple layers of periosteal new bone formation in response to diaphyseal inflammation. After several months, complete healing of the affected bones occurs, even without antibiotic therapy.

CNS invasion by *T. pallidum* occurs in about 50% of infants with clinical, laboratory, or radiographic signs of congenital syphilis. Clinical signs of CNS involvement, however, are rare in the neonatal



• **Fig. 38.3** The radiograph displays the characteristic “celery stalking” and widening of the metaphyses in long bones found in untreated congenital syphilis. (From American Academy of Pediatrics Committee on Infectious Diseases. Syphilis: clinical manifestations images. In: *Red Book Online Visual Library*. Elk Grove Village, IL: American Academy of Pediatrics; 2015. Available at <http://redbook.solutions.aap.org/chapter.aspx?resultClick=1&bookID=1484§ionID=88187246#91040949>.)

period but can occur later if infected infants are not identified and treated (Nolt et al., 2002). Such manifestations include bulging fontanelle, seizures, leptomeningitis, cranial nerve palsies, hydrocephalus, cerebral infarction, and pituitary gland dysfunction with hypoglycemia and diabetes insipidus.

The clinical manifestations or stigmata of late congenital syphilis result from persistent inflammation or scarring associated with early congenital syphilis infection and are prevented by treatment during gestation or within the first 3 months of age. Infants with late congenital syphilis are not infectious. Late manifestations include dental stigmata such as Hutchinson teeth, where the permanent upper central incisors are small, widely spaced, barrel shaped, and notched, and mulberry molars, where the first lower molar has many small cusps instead of the usual four. Osteochondritis affecting the otic capsule may lead to cochlear degeneration and fibrous adhesions resulting in eighth nerve deafness, for which corticosteroid treatment may be beneficial. Late ocular manifestations include uveitis and interstitial keratitis. The constellation of interstitial keratitis, eighth cranial nerve deafness, and Hutchinson teeth is known as the Hutchinson triad.

The sequela of periostitis of the skull is frontal bossing, of the tibia is saber shins, and of the clavicle is Higouménakis sign with sternoclavicular thickening. Clutton joints, or painless synovitis and hydrarthrosis, are rare. The sequelae of syphilitic rhinitis include rhagades and a short maxilla with a high palatal arch. If the inflammation of the nasal mucosa extends to the underlying cartilage and bone, perforation of the palate and nasal septum occurs, resulting in a “saddle nose” deformity. Sequelae of CNS infection include mental retardation, hydrocephalus, seizure disorder, cranial nerve palsies, paralysis, and optic nerve atrophy (Fiumara and Lessell, 1970; Brosco et al., 2006).

Diagnosis

The diagnosis of congenital syphilis is established by the observation of spirochetes in body fluids or tissue and suggested by serologic test results. *T. pallidum* may be identified by dark-field microscopy, PCR testing, and fluorescent antibody or silver staining of

mucocutaneous lesions, nasal discharge, vesicular fluid, amniotic fluid, placenta, umbilical cord, or tissue obtained at autopsy. Its diagnosis, however, remains challenging since *T. pallidum* cannot be cultivated in artificial media, and many infected infants lack clinical, laboratory, and radiographic signs of disease. In addition, the diagnosis can often only be inferred since maternal nontreponemal and treponemal IgG antibodies are transferred transplacentally to the fetus, complicating the interpretation of reactive serologic test results for syphilis in neonates. A diagnosis of congenital syphilis is supported by an infant's serum quantitative nontreponemal antibody titer that is at least fourfold higher than the mother's titer. The absence of such a finding, however, does not exclude a diagnosis of congenital syphilis.

Serologic tests for syphilis are classified into nontreponemal and treponemal tests. Nontreponemal tests include the Venereal Disease Research Laboratory (VDRL) test and the rapid plasma reagin test. The same nontreponemal test should be performed on the mother and the infant so that accurate comparisons can be made. Serologic testing of the infant should be performed on serum and not umbilical cord blood since false-positive test results have been reported secondary to contamination of the specimen with maternal blood or Wharton jelly. False-negative test results also may occur when the maternal nontreponemal titer is of low dilution (Rawstron and Bromberg, 1991). Measurement of total umbilical cord IgM levels and use of treponemal IgM ELISAs and the fluorescent treponemal antibody absorption IgM test have not proved useful in the diagnosis of congenital syphilis and are not recommended (Herremans et al., 2010).

Treponemal tests include the *T. pallidum* particle agglutination test, the fluorescent treponemal antibody absorption test, treponemal enzyme immunoassay (EIA), and chemiluminescence immunoassay (CIA). These treponemal tests are used to confirm the diagnosis of syphilis.

The diagnosis of congenital neurosyphilis is difficult to establish, with treponemal infection of the CNS only inferred from abnormalities of the CSF such as a reactive VDRL test result, pleocytosis (more than 18–25 white blood cells per microliter), and elevated protein content (>150 mg/dL; >170 mg/dL if infant is premature). However, a reactive CSF VDRL test result in neonates may be caused by passive transfer of nontreponemal IgG antibodies from serum into the CSF. By inoculation of rabbit testes with CSF, with resultant syphilitic infection of the rabbit, Michelow et al. (2002) found that invasion of the CNS with *T. pallidum* occurs in 41% of infants who have clinical, laboratory, or radiographic abnormalities of congenital syphilis and in 60% of those who have abnormal physical examination findings consistent with a diagnosis of congenital syphilis. The sensitivity and specificity of a reactive CSF VDRL test result, pleocytosis, and elevated protein content were 53% and 90%, 38% and 88%, and 56% and 78%, respectively (Sanchez et al., 1993). Therefore if clinical, laboratory, or radiographic evaluation supports a diagnosis of congenital syphilis, then therapy effective against CNS disease is warranted.

A practical approach to the evaluation and treatment of infants born to mothers with reactive serologic test results for syphilis is presented in Fig. 38.4. All pregnant women and their sexual partner(s) who have syphilis should be tested for coinfection with HIV, although infants born to mothers coinfecting with syphilis and HIV do not require different evaluation, therapy, or follow-up. In infants born to mothers with reactive serologic test results for syphilis, a serum quantitative nontreponemal test should be performed, and they should be carefully examined for physical signs of congenital syphilis. In neonates who have an abnormal physical

examination finding that is consistent with congenital syphilis, a serum quantitative nontreponemal serologic titer that is fourfold or greater than the mother's titer, or a positive dark-field or fluorescent antibody test result, or a positive PCR of lesions or body fluid(s), a complete blood cell count and platelet count should be performed as should CSF examination for cell count, protein content, and VDRL test result. Other tests, such as bone and chest radiographs, liver function tests, cranial ultrasonography, ophthalmologic examination, and evaluation of auditory brainstem response, should be performed as clinically indicated. These infants are considered to have proven or highly probable disease. Since spirochetemia with invasion of the CNS is likely, it is beneficial for follow-up purposes to establish CNS abnormalities at presentation.

In well-appearing neonates who have normal physical examination findings and a serum quantitative nontreponemal serologic titer that is equal to or less than fourfold the maternal titer, further evaluation and treatment depend on the maternal treatment history (see Fig. 38.4). If the mother has untreated syphilis or the treatment is undocumented or inadequate (<4 weeks before delivery or with any non-penicillin G regimen), a complete evaluation consisting of CSF analysis, long bone radiographs, and complete blood cell and platelet counts should be performed to guide optimal therapy. The evaluation findings must be completely normal if the infant is to be treated with a single intramuscular dose of benzathine penicillin G. Almost none of these infants will have CNS invasion by *T. pallidum* if their complete evaluation findings are normal. Alternatively, a complete evaluation is not necessary if 10 days of parenteral penicillin therapy is provided.

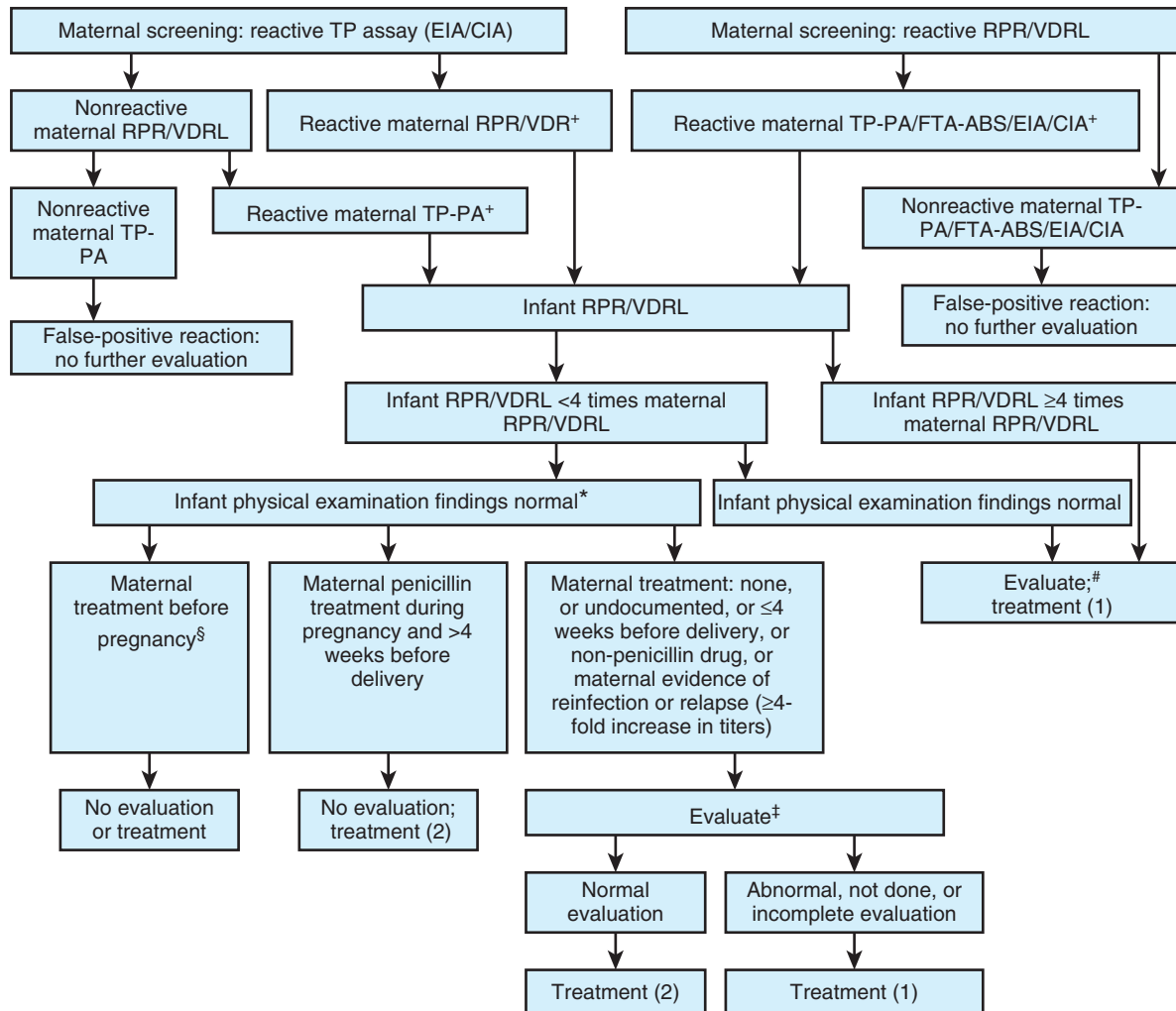
Treatment

Penicillin G is the only known effective antimicrobial agent for prevention of vertical transmission of syphilis and treatment of fetal infection and congenital syphilis. Pregnant women with syphilis should receive the penicillin regimen appropriate for the stage of infection, and if any dose of therapy is missed for latent syphilis, the full course of therapy must be repeated (Table 38.4). Pregnant women who have a history of penicillin allergy should be desensitized and treated with penicillin (Centers for Disease Control and Prevention, 2015b).

The decision to treat an infant for congenital syphilis is based on the clinical presentation, previous serologic test results and treatment of the mother, and the results of serologic testing of the neonate and mother at the time of delivery (Table 38.5, see Fig. 38.4). Infants with proven or highly probable disease, or who have normal physical examination findings but their evaluation findings are abnormal or their evaluation is incomplete, should be treated with either aqueous crystalline penicillin G (50,000 U/kg intravenously every 12 hours for the first week after birth, followed by every 8 hours beyond 7 days of age) or aqueous procaine penicillin G (50,000 U/kg intramuscularly once daily) for 10 days. If more than 1 day of therapy is missed, the entire course should be restarted. Data are insufficient regarding the use of other antimicrobial agents (e.g., ampicillin). When possible, a full 10-day course of penicillin is preferred, even if ampicillin was initially provided for possible sepsis.

Infants who have normal physical examination findings, CSF examination findings, complete blood cell and platelet counts, and long bone radiographs can be treated with a single intramuscular injection of benzathine penicillin G at a dose of 50,000 U/kg. If the risk of infection in these infants is substantial and adequate

Algorithm for Evaluation and Treatment of Infants Born to Mothers With Reactive Serologic Tests for Syphilis



+ Test for HIV antibody. Infants of HIV-infected mothers do not require different evaluation or treatment.

* If the infant's RPR/VDRL is nonreactive AND the mother has had no treatment, undocumented treatment, treatment ≤ 4 weeks before delivery, or no evidence of reinfection or relapse (≥ 4 -fold increase in titers), THEN treat infant with a single IM injection of benzathine penicillin (50,000 U/kg). No additional evaluation is needed.

§ Women who maintain a VDRL titer $\leq 1 : 2$ (RPR $\leq 1 : 4$) beyond 1 year following successful treatment are considered serofast.

Evaluation consists of CBC, platelet count; CSF examination for cell count, protein, and quantitative VDRL. Other tests as clinically indicated: long-bone X-rays, neuroimaging, auditory brainstem response, eye examination, chest X-ray, liver function tests.

‡ CBC, platelet count; CSF examination for cell count, protein, and quantitative VDRL; long-bone X-rays.

TREATMENT:

(1) Aqueous penicillin G 50,000 U/kg IV every 12h (≤ 1 week of age), q8 hr (> 1 week) or procaine penicillin G 50,000 U/kg IM single daily dose, x 10 days

(2) Benzathine penicillin G 50,000 U/kg IM x 1 dose

• **Fig. 38.4** Algorithm for evaluation and treatment of infants born to mothers with reactive serologic test results for syphilis. CBC, Complete blood cell; CIA, chemiluminescence immunoassay; CSF, cerebrospinal fluid; EIA, enzyme immunoassay; FTA-ABS, fluorescent treponemal antibody absorption; HIV, human immunodeficiency virus; IM, intramuscular/intramuscularly; IV, intravenously; RPR, rapid plasma reagin; TP, *Treponema pallidum*; TP-PA, *Treponema pallidum* particle agglutination; VDRL, Venereal Disease Research Laboratory.

TABLE 38.4 Recommended Treatment for Syphilis During Pregnancy

| Stage of Syphilis ^a | Drug (Penicillin) ^b | Route | Dose/Dosage |
|---|--------------------------------------|-------|--|
| Primary and secondary | Benzathine penicillin G ^c | IM | 2.4 million units in a single dose |
| Early latent (<1 year duration) | Benzathine penicillin G ^c | IM | 2.4 million units in a single dose |
| Late latent (>1 year duration) or latent syphilis of unknown duration | Benzathine penicillin G | IM | 2.4 million units weekly × 3 |
| Neurosyphilis and ocular syphilis | Aqueous penicillin G | IV | 3–4 million units every 4 h or continuous infusion for 10–14 days ^d |
| | or procaine penicillin G | IM | 2.4 million units once daily IM |
| | plus probenecid | | plus 500 mg orally four times a day, both for 10–14 days ^d |

^aPersons with human immunodeficiency virus (HIV) infection who have syphilis should be treated as those without HIV infection.

^bPregnant women who have a history of penicillin allergy should be desensitized and treated with penicillin.

^cFor women who have primary, secondary, or early latent syphilis, a second dose of benzathine penicillin G (2.4 million units IM) can be administered 1 week after the initial dose.

^dBenzathine penicillin G, 2.4 million units IM once per week for up to 3 weeks, can be considered after completion of 10–14 days of IV treatment.

IM, Intramuscular; IV, intravenous.

From Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines. <http://www.cdc.gov/std/tg2015/congenital.htm>.

TABLE 38.5 Recommended Treatment of the Neonate (≤28 Days Old) With Syphilis

| Neonate | Maternal Stage/Treatment | Evaluation | Antimicrobial Regimen |
|--|---|---|--|
| <i>Proven or highly probable disease:</i> abnormal physical examination findings; <i>or</i> abnormal evaluation findings ^a ; <i>or</i> serum nontreponemal titer ≥4 times maternal titer; <i>or</i> visualization of spirochetes <i>or</i> detection of <i>Treponema pallidum</i> DNA by PCR in clinical specimen | Any or none | CSF analysis: VDRL test, cell count, and protein; <i>or</i> CBC count and platelet count; <i>or</i> other tests as clinically indicated (e.g., long-bone radiographs, liver function tests, ophthalmologic examination, hearing evaluation, neuroimaging) | Aqueous crystalline penicillin G 50,000 U/kg IV every 12 h (≤1 week old) and every 8 h thereafter for 10 days <i>or</i> penicillin G procaine 50,000 U/kg per dose IM for 10 days |
| <i>Possible congenital syphilis:</i> normal physical examination findings; <i>or</i> serum nontreponemal titer ≤4 times the maternal titer | Any stage of infection <i>and</i> mother was not treated, inadequately treated, or has no documented treatment; <i>or</i> treated with erythromycin or other nonpenicillin regimen; <i>or</i> received appropriate treatment but ≤4 weeks before delivery | CSF analysis (VDRL test, cell count, and protein); <i>and</i> CBC count and platelet count; <i>and</i> long-bone radiographs | If results of complete evaluation are normal: ^b benzathine penicillin G 50,000 U/kg IM once, <i>or</i> aqueous crystalline penicillin G 50,000 U/kg IV every 12 h (≤1 week old) and every 8 hours thereafter for 10 days, <i>or</i> procaine penicillin G 50,000 U/kg per dose IM for 10 days |
| <i>Congenital syphilis less likely:</i> normal physical examination findings; <i>or</i> serum nontreponemal titer ≤4 times the maternal titer; <i>or</i> no evidence of reinfection | Mother was treated during pregnancy, treatment was appropriate for the stage of infection, and treatment was administered >4 weeks before delivery. | None | Benzathine penicillin G 50,000 units/kg IM once |

^aComplete blood cell (CBC), platelet count, cerebrospinal fluid (CSF) examination, bone radiographs.

^bIf complete evaluation was not done, the infant must receive penicillin therapy for 10 days.

IM, intramuscularly; IV, intravenously; PCR, polymerase chain reaction; VDRL, Venereal Disease Research Laboratory.

From Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines. <http://www.cdc.gov/std/tg2015/congenital.htm>.

follow-up cannot be ensured, the 10-day course of aqueous or procaine penicillin is recommended by the CDC and American Academy of Pediatrics, regardless of the results of the CSF and laboratory examination. Failure of a single injection of benzathine penicillin G in the treatment of congenital syphilis has been reported. Treatment failures have been attributed to the inability of penicillin to adequately penetrate certain sites such as the aqueous humor and CNS and achieve treponemicidal concentrations in them.

Normal neonates born to mothers adequately treated during pregnancy and at more than 4 weeks before delivery should be considered as a “close contact” and receive a single intramuscular injection of benzathine penicillin G (50,000 U/kg), although no evaluation is required or recommended. Similarly, normal infants who have a nonreactive serum nontreponemal test result but are born to mothers with untreated or inadequately treated syphilis can receive a single dose of benzathine penicillin G intramuscularly without evaluation—an increasingly common scenario with the use of treponemal tests such as EIAs or CIAs for syphilis screening (“reverse sequence” screening).

During times of penicillin shortage when preparations of penicillin are unavailable, a 10-day course of ceftriaxone can be considered with careful clinical and serologic follow-up, including repeated CSF evaluation ([Centers for Disease Control and Prevention, 2015b](#)). Research efforts are needed to evaluate whether other antibiotics such as ampicillin can effectively treat CNS disease.

Within 24 hours of initiation of penicillin therapy, a small percentage of infants who are treated for congenital syphilis may develop a Jarisch–Herxheimer reaction, an acute inflammatory response likely caused by the rapid killing of spirochetes. It is characterized by fever, tachypnea, tachycardia, hypotension, accentuation of cutaneous lesions, or even death due to cardiovascular collapse. Treatment is supportive care.

Follow-Up

In infants with reactive serologic test results, serial quantitative nontreponemal tests should be performed every 2–3 months until the test results become nonreactive. In infants with congenital syphilis, nontreponemal serologic test values should decline fourfold, and the test results should become nonreactive within 6–12 months after appropriate treatment. Uninfected infants usually become seronegative by 6 months of age. Infants with persistently low, stable titers of nontreponemal tests beyond 1 year of age may require retreatment. A reactive treponemal test result beyond 18 months of age when the infant has lost all maternal IgG antibody confirms the diagnosis of congenital syphilis. Infants with abnormal CSF findings should undergo a repeated lumbar puncture performed 6 months after therapy. A reactive CSF VDRL test result or an abnormal protein content or cell count at that time is an indication for retreatment.

Prevention

Congenital syphilis is effectively prevented by prenatal serologic screening of mothers and penicillin treatment of infected women, their sexual partners, and their newborns ([US Preventive Services Task Force, 2009](#)). In all pregnant women a serologic test for syphilis should be performed at the first prenatal visit in the first trimester, with the test being repeated at 28–32 weeks' gestation and at delivery in areas with a high incidence of syphilis. Serologic screening tests should be performed on mothers and not on infants,

because the infant may have a nonreactive serologic test result, but the mother's test result may be reactive at a low level.

Although nontreponemal antibody testing has been recommended for antepartum syphilis screening, treponemal antibody testing (i.e., EIA or CIA) is being used increasingly by many laboratories as a cost-cutting measure for screening pregnant women (reverse sequence screening). If a treponemal EIA or CIA test is used for antepartum syphilis screening and the result is positive, a quantitative nontreponemal test (rapid plasma reagin test or VDRL test) should then be performed. If the nontreponemal test result is reactive, then a diagnosis of past or present syphilis is made, and the treatment of the mother and infant should be as discussed previously. However, if the nontreponemal test result is negative, then the results are considered discrepant and a second treponemal test (*T. pallidum* particle agglutination test is preferred) should be performed, preferably on the same specimen. If the second treponemal test result is reactive, current or past syphilis infection is confirmed. For women with a history of adequately treated syphilis, no further treatment is necessary. Women without a history of treatment should be staged and treated accordingly with a recommended penicillin regimen. If the second treponemal test result is nonreactive, then the positive EIA/CIA result is likely to represent a false-positive test result. The management of these infants is shown in [Fig. 38.4](#). Infants with suspected or proven congenital syphilis can be treated with standard precautions only. If there are cutaneous lesions or mucous membrane involvement, then gloves should be worn as well until 24 hours of treatment has been completed.

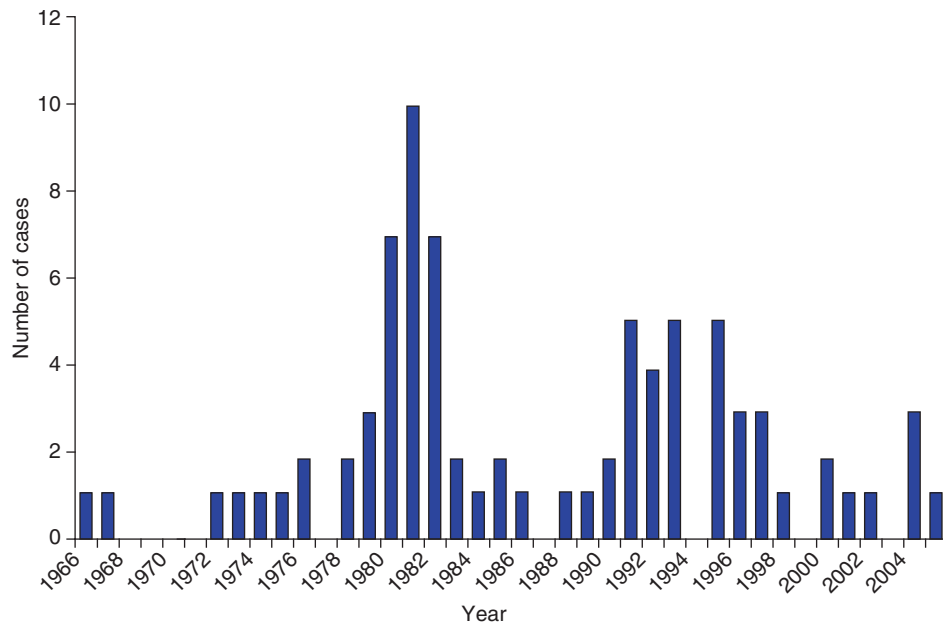
All cases of syphilis must be reported to the local public health department, which performs contact investigation and identifies core environments and populations. The public health impact of syphilis in pregnancy and infancy remains substantial, and only through optimal prenatal healthcare services will elimination of mother-to-child transmission of syphilis become a reality.

Congenital Malaria

Epidemiology

Malaria is a parasitic disease of epidemic proportion. An estimated 214 million new cases of malaria, with 438,000 deaths, were reported in 2015 alone ([World Health Organization, 2015d](#)). The greatest burden of disease occurs in the African region (88%), with Asia (10%) and the eastern Mediterranean areas (2%) also being affected. In areas of high transmission, death is concentrated largely among young children and pregnant women. In sub-Saharan Africa, malaria accounts for 10% of childhood deaths. Malaria is caused by four *Plasmodium* spp.: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium malariae*. Of these, *P. falciparum* is the major cause of morbidity and death. Humans typically acquire infection through the bite of the *Anopheles* spp. mosquito. Transmission may also occur through blood transfusion or vertically from the mother to fetus, resulting in congenital malaria ([Fig. 38.5](#)).

Congenital malaria occurs with all *Plasmodium* species. Among the 107 cases of congenital malaria reported in 1950 (mostly from Africa), 64% were caused by *P. falciparum*, 32% were caused by *P. vivax*, and 2% were caused by *P. malariae* ([Covell, 1950](#)). Although *P. falciparum* remains the predominant pathogen in sub-Saharan Africa, *P. vivax* may account for a larger proportion of cases in Asia. Among 27 cases reported in Thailand between 1981 and 2005, 82% were caused by *P. vivax* ([Wiwanitkit, 2006](#)). *P. malariae*



• **Fig. 38.5** Number of cases of congenital malaria reported to the National Malaria Surveillance System per year, 1966–2005. (From Lesko CR, Arguin PM, Newman RD. Congenital malaria in the United States: a review of cases from 1966 to 2005. *Arch Pediatr Adolesc Med.* 2007;161:1062–1067.)

is less frequently a causative agent, with fewer than 10 cases reported worldwide since 1950 (de Pontual et al., 2006). In China, *P. vivax* accounted for most (92.5%) of the 107 cases of congenital malaria cases reported (Tao et al., 2014). Concurrent infection with *P. malariae* and *P. vivax* has been documented (MacLeod et al., 1982). In the United States the predominant *Plasmodium* spp. causing congenital malaria reflects the countries of origin of the mothers. In Hulbert's (1992) review of 49 cases from 1950–1991, 82% of infections were caused by *P. vivax*. In the updated review in the United States reflecting 1966–2005, the predominant infecting species remained *P. vivax* (81%), although all four species were represented (Lesko et al., 2007).

The true rates of congenital malaria reported in the literature differ significantly depending on the time span reported, the method of reporting, and the clinical definition. Congenital malaria is commonly defined as the presence of *Plasmodium* spp. parasites in the peripheral blood during the first 7 days of life where the transmission occurs from the mother via placental transfer (Covell, 1950; Moran and Couper, 1999; Menendez and Mayor, 2007; Uneke, 2007a; Sotimehin et al., 2008). (There is an important distinction that the parasites are identified in the peripheral blood of the neonate and not the umbilical cord blood or placenta. While some have proposed use of umbilical cord blood as an alternative to peripheral blood, it does not represent active infection as is discussed later in this chapter.) In contrast, neonatal malaria is defined as the presence of *Plasmodium* spp. parasites in the peripheral blood between 7 and 30 days of life where mosquito transmission is the most likely cause. These definitions are most applicable in areas of high malaria transmission, where, among older infants, it would be difficult to distinguish congenitally acquired from mosquito-acquired disease. (This has been suggested by findings from Malawi, where approximately 50% of newborns with umbilical cord blood parasitemia were infected with parasites of a genotype different from that of the parasites that their mothers were infected at the time of delivery; Fischer, 2003.) Outside endemic areas, where postnatal transmission can be reasonably excluded, clinical

onset of disease often does not occur until after the first week of life, and age-specific criteria are not useful for the diagnosis of congenital malaria. It is likely that because of the delay in clinical presentation, many cases of congenital malaria in endemic areas were misclassified as being acquired from mosquitoes. Covell (1950) found the prevalence of congenital malaria among nonimmune populations (i.e., Europeans residing in or visiting endemic areas) to be approximately 7%. In other estimates before the 1970s, the prevalence of congenital malaria, defined as parasitemia detected in the first 7 days of life, was estimated to be 0.3% (16 of 5324 births) among immune mothers. Subsequent reports supported the observed low frequency of congenital malaria, defined as umbilical cord parasitemia or parasitemia in the first 24 hours of life, among indigenous populations (Bruce-Chwatt, 1952; Cannon, 1958; Williams and McFarlane, 1970; McGregor, 1984). These observations have been cited repeatedly in the literature to support the notion that congenital malaria is an uncommon occurrence in endemic areas despite the high prevalence of maternal and placental malaria.

In the last several decades, however, more in-depth studies of congenital malaria have suggested that it is far more common than initially reported, as rates from endemic and nonendemic areas ranged from 0.2%–47% (Obiajunwa et al., 2005; Desai et al., 2007; Menendez and Mayor, 2007; Enweronu-Laryea et al., 2013). Many have suggested that the low prevalence previously reported for congenital malaria was likely due to inadequate recognition and underreporting (Akindele et al., 1993; Ibhanesebhor, 1995; Fischer, 1997; Obiajunwa et al., 2005; Falade et al., 2007; Menendez and Mayor, 2007; Uneke, 2007b; Enweronu-Laryea et al., 2013). In Zambia during a season of heavy malaria transmission, incidence rates for congenital malaria ranged from 4%–15% (Nyirjesy et al., 1993). Congenital malaria, defined as neonatal parasitemia, was detected in 15.3% and 17.4% of neonates born in two sites in Nigeria (Mukhtar et al., 2006; Runsewe-Abiodun et al., 2006). The apparent increase in the frequency of congenital malaria has been attributed to increasing resistance of *P. falciparum* to

antimalarial drugs, resulting in increased maternal parasitemia, increased virulence of the parasite, and reduced transmission of antibody from the mother to the newborn because of malaria chemoprophylaxis administered to pregnant women. Meanwhile a multicenter trial in Nigeria showed an overall prevalence rate of 5% (range of 1.1%–11.5%) (Falade et al., 2007). The inconsistency may also represent true environmental differences with differences in levels of maternal immunity. It is clear that congenital malaria can exist in asymptomatic infants, which can make reporting in the literature even more variable (Mukhtar et al., 2006; Uneke, 2007a; Sotimehin et al., 2008).

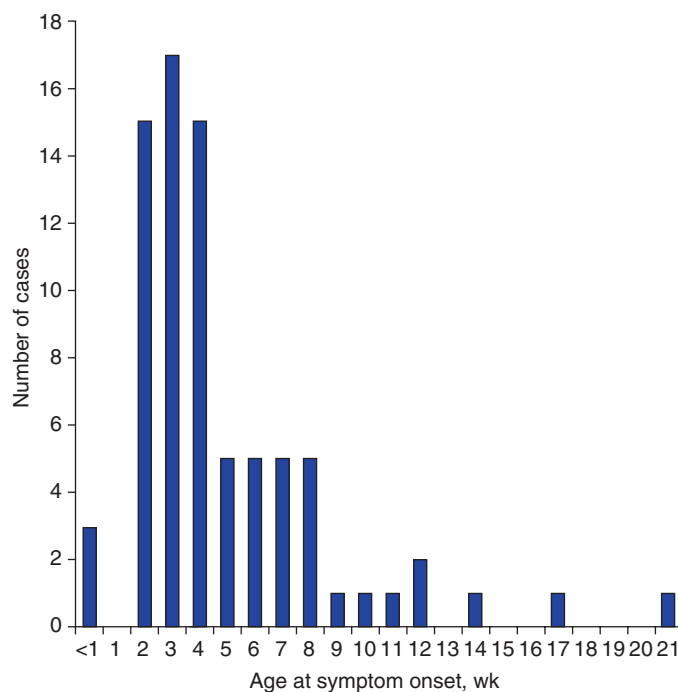
Congenital malaria in nonendemic areas is rare. As of 1995, only 300 cases of congenital malaria had been reported in the literature (Balatbat et al., 1995), largely from outside malaria-endemic areas. In the United States the occurrence of congenital malaria is well documented because the country has been free of indigenous disease since the 1950s. From 1950–1991, 49 cases of congenital malaria were reported in the literature (Hulbert, 1992), and additional cases were reported during the next 15 years (Balatbat et al., 1995; Centers for Disease Control and Prevention, 2002; Baspinar et al., 2006). From 1966–2005, 81 cases of congenital malaria were reported to the National Malaria Surveillance System of the CDC (Gereige and Cimino, 1995; Starr and Wheeler, 1998; Viraraghavan and Jantausch, 2000; Lesko et al., 2007). Almost all the cases were among infants whose mothers were foreign born, suggesting that congenital malaria is primarily a health problem of recent immigrants rather than of US-born travelers to malaria-endemic countries. Forty-four women (54%) had emigrated from Asia, 27 (33%) from South America or Central America, and 7 (9%) from Africa. Until 1979, one to two cases were reported annually (Malviya and Shurin, 1984). An abrupt rise to 16 cases around 1981 (Fig. 38.6) correlated with an increase in the total number of cases of malaria that occurred as a result of a large influx of refugees and immigrants from Southeast Asia, with 15 of the 16 infants being born to mothers from that region (Quinn et al., 1982). In 2013 the CDC reported two cases of congenital malaria among the 1727 reported cases in the United States (Cullen et al., 2016). The lack of familiarity with this disease in the United States renders it a diagnostic and therapeutic challenge for clinicians, with delays in diagnosis potentially leading to significant morbidity and mortality (Griffith et al., 2007).

Natural History, Pathogenesis, and Clinical Presentation

Two key features play a critical role in the pathogenesis and natural history of congenital malaria:

1. Maternal and placental parasitemia
2. Existence of correlation between umbilical cord parasitemia and neonatal parasitemia

Pregnancy itself increases the risk of severe malaria, resulting in maternal–fetal detriment. Malaria also increases the risk of adverse pregnancy outcomes, including prematurity, abortion, and stillbirth. Despite major global health efforts, 28 million pregnant women were at risk of malaria in 2015 worldwide (World Health Organization, 2016). It is well established that both the frequency of disease and the density of parasitemia are higher in pregnant women than in nonpregnant women (Desai et al., 2007; Rogerson et al., 2007; Coll et al., 2008). Among 20 studies conducted between 1985 and 2000, the median prevalence of maternal malaria infection (defined as peripheral or placental infection) was 28% (Steketee et al., 2001). More recent studies of endemic areas within the last



• **Fig. 38.6** Age in weeks at symptom onset of infants with reported congenital malaria, United States, 1966 to 2005. (From Lesko CR, Arguin PM, Newman RD. Congenital malaria in the United States: a review of cases from 1966 to 2005. *Arch Pediatr Adolesc Med.* 2007;161:1062–1067.)

decade have reported significant geographic variation in the prevalence of malaria among pregnant women ranging between 8.1% and 52% (Raimi and Kanu, 2010; Mutagonda et al., 2016; Willilo et al., 2016). For this reason, it is widely contended that one in four pregnant women in areas of stable transmission in Africa have evidence of malaria infection at the time of delivery.

The clinical features of *P. falciparum* malaria in a pregnant woman depend to a large degree on her immune status, which in turn is determined by her prior exposure to malaria. Pregnancy itself suppresses both the humoral and the cell-mediated portions of the immune system, increasing the susceptibility to severe malaria. Impaired T-cell responses reduce systemic control of the infection and the ability to control liver-stage infections, resulting in relapse among some forms of *Plasmodium* spp. malaria (McLean et al., 2015). Primigravid women have a twofold to fourfold increased risk of placental malaria compared with multigravid women (McGregor, 1984; Desai et al., 2007; Uneke, 2008) that may be due to levels of antigen-specific *Plasmodium* spp. antibodies that appear to be boosted with successive pregnancies (McLean et al., 2015). In pregnant women with little or no preexisting immunity, such as women from nonendemic countries or travelers to malaria-endemic areas, infection is associated with high risks of severe disease, with significant maternal and perinatal mortality. In contrast, women residing in areas of stable malaria transmission usually have a high level of immunity to malaria. Infection may be frequently asymptomatic and therefore unsuspected or undetected, but it is associated with placental parasitization, with consequent effects on maternal and fetal outcomes. The most significant consequence of pregnancy-associated malaria is maternal anemia. It is estimated that in sub-Saharan Africa between 200,000 and 500,000 pregnant women develop anemia as a result of malaria and that up to 10,000 maternal anemia-related deaths are a consequence of *P. falciparum* parasitemia (Uneke, 2008). Malaria

in pregnancy also has potentially devastating effects on the fetus and newborn, including spontaneous abortion, stillbirth, premature delivery, congenital infection, and neonatal death (Fischer, 2003; Coll et al., 2008). In areas of high transmission in Africa, the risk of low birth weight approximately doubles if women have placental malaria, with the greatest effect in primigravidae (Desai et al., 2007). Pregnancy-associated malaria is believed to be responsible for 30%–35% of low birth weight infants and for 75,000–200,000 infant deaths each year (Coll et al., 2008).

The timing and mechanism of transmission of *Plasmodium* spp. parasites from the mother to the fetus are not well understood. Postulated mechanisms include maternal transfusion into fetal circulation either during pregnancy or at delivery or direct penetration of parasitized red blood cells through the chorionic villi or through premature separation of the placenta. (Transmission of malaria by breastfeeding is not known to occur.) *Plasmodium*-infected erythrocytes have the unique ability to undergo sequestration to the placenta, which may facilitate transmission to the fetus (Salanti et al., 2003; Rogerson et al., 2007). In utero transmission is supported by the finding of malarial parasites in fetal tissues at autopsy (Centers for Disease Control, 1981), by umbilical cord blood parasitemia (McGregor, 1984; Larkin and Thuma, 1991; Fischer, 1997), and by the onset of clinical signs of malaria within hours of birth (Covell, 1950; Brandenburg and Kenny, 1982; Gereige and Cimino, 1995). Vertical transmission of malaria probably does not occur as a result of transplacental passage of exoerythrocytic parasites. More likely, transmission occurs by transfusion of parasitized maternal erythrocytes through a breach in the placental barrier that may occur either prematurely during pregnancy or during labor.

The fate of the *Plasmodium* spp. parasite is unclear after it is transmitted to the fetus. Reinhardt (1978) found parasites in thick smears of umbilical cord blood in 22% of 19 infants born to women in Ivory Coast, but the peripheral blood smears were negative for parasites for all of the infants. Similarly, 4% of 1009 infants born to Tanzanian women had parasites in umbilical cord blood, but parasitemia was detected on peripheral smear in only 2 of 11 infants (McGregor, 1984). Parasitemia detected shortly after birth may also resolve without evolving into clinically symptomatic disease. The correlation between maternal, placental, umbilical cord, and peripheral parasitemia was evaluated in 1875 mother–baby pairs in Nigeria. Thick and thin blood smears were obtained within 4 hours of birth, and smear-positive (umbilical cord or peripheral) neonates were retested on days 2, 3, and 7 of life. Treatment was provided for those with symptoms or persistent parasitemia. The overall prevalence of congenital malaria was 5.1%, with parasitemia detected in 19% of neonates born to mothers with peripheral parasitemia, 21% of those born to mothers with placental malaria, and 45% of those with positive umbilical cord smear results. Spontaneous clearance of parasitemia occurred in 62% of neonates before day 2, whereas 33% were symptomatic within 3 days of birth (Falade et al., 2007). Depending on the region, spontaneous clearance of peripheral parasitemia has been documented in 87%–100% of neonates (Mukhtar et al., 2006; Lesko et al., 2007). Larkin and Thuma (1991) found peripheral parasitemia within 24 hours of age in 19 of 51 newborns (65%), but only 7 had clinical signs of disease. Because all 19 newborns received antimalarial therapy, it is unknown how many would have manifested disease if they had not been treated. The spontaneous clearance of *Plasmodium* spp. has been attributed to the protective effects of passive maternal antibody and weakened adherence to fetal hemoglobin (Amaratunga et al., 2011).

Infants with congenital malaria may be asymptomatic or can develop symptoms several weeks after birth. The clinical picture of overt congenital malaria is detailed in cases reported outside endemic areas (Harvey et al., 1969; Hindi and Azimi, 1980; Hulbert, 1992; Lesko et al., 2007). The manifestation of disease, although occasionally noted within hours of birth (Brandenburg and Kenny, 1982; Gereige and Cimino, 1995), is typically delayed until the infant is several weeks old. In the classic review of 49 infants with congenital malaria reported in the United States between 1950 and 1992, the mean age at onset of symptoms was 5.5 weeks, with 96% of infants presenting symptoms between 2 and 8 weeks of age (Hulbert, 1992). Among cases reported to the CDC from 1966–2005 (Lesko et al., 2007), the median age of symptom onset for 81 neonates was 21.5 days for all species combined (Table 38.6). Infants infected with *P. malariae* were significantly older at symptom onset (mean 53 days) compared with those infected with *P. vivax* or *P. falciparum*.

The prolonged interval between birth and onset of clinical manifestations may be explained by transmission late in pregnancy

TABLE 38.6 Frequency of Symptoms, Signs, and Laboratory Findings Among 81 Infants With a Diagnosis of Congenital Malaria (United States, 1966–2005)

| Symptoms, Signs, and Laboratory Findings | Infants ^a |
|--|----------------------|
| Fever | 70 (86%) |
| Anemia | 28 (36%) |
| Splenomegaly | 25 (31%) |
| Hepatomegaly | 16 (20%) |
| Thrombocytopenia | 12 (15%) |
| Jaundice | 11 (14%) |
| Irritability | 8 (10%) |
| Anorexia | 8 (10%) |
| Vomiting | 8 (10%) |
| Cough | 6 (7%) |
| Diarrhea | 3 (4%) |
| Lethargy | 3 (4%) |
| Hemolysis | 3 (4%) |
| Pallor | 3 (4%) |
| Hyperbilirubinemia | 2 (3%) |
| Failure to thrive | 2 (3%) |
| Seizures | 2 (3%) |
| Dyspnea | 1 (1%) |
| Purpura | 1 (1%) |
| Tachycardia | 1 (1%) |
| Monocytosis | 1 (1%) |

^aPercentages do not total 100% because each case can have more than one symptom, sign, or laboratory finding.

From Lesko CR, Arguin PM, Newman RD. Congenital malaria in the United States: a review of cases from 1966 to 2005. *Arch Pediatr Adolesc Med.* 2007;161:1062–1067.

TABLE 38.7 Reviews of Congenital Tuberculosis Cases Reported in the English-Language Literature in the Era of Chemotherapy

| Reference | Years Cases Reported | Number of Cases | Age at Clinical Presentation (Days) | Number of Infants With Reactive TST Results | Common Symptoms | Mortality (%) ^a |
|------------------------|----------------------|-----------------|-------------------------------------|---|---|----------------------------|
| Hageman et al. (1980) | 1952–1980 | 26 | NR | 2 of 14 | Respiratory distress, fever, hepatomegaly | 46 (12) |
| Cantwell et al. (1994) | 1980–1994 | 31 | Median 24 (range 1–84) | 0 of 9 | Hepatosplenomegaly, respiratory distress, fever | 38 (22) |
| Abughali et al. (1994) | 1952–1994 | 58 | NR | 1 of 19 | Respiratory distress, hepatomegaly, fever | 45 (14) |
| Laartz et al. (2002) | 1994–2002 | 16 | Mean 17.4 (range 1–60) | 1 of 4 | Respiratory distress, hepatomegaly, fever | 20 |

^aMortality with treatment is given in parentheses.
NR, Not reported; TST, tuberculin skin test.

or at delivery, such that multiple erythrocytic life cycles are required to produce clinically evident disease. Alternatively, the delay may be attributed to the presence of transplacentally acquired maternal antimalarial antibodies. When such antibodies are present in sufficient concentrations, as in infants born to immune mothers, parasitic replication can be prevented or attenuated, and clinical signs can be mild, delayed, or even absent. The presence of a high concentration of fetal hemoglobin in newborns may also promote resistance to multiplication of parasites. Among infants born to mothers with low or nonexistent immunity, parasitic replication is more likely uninhibited, and clinical signs of malaria may supervene. Preterm infants, who do not benefit from passive immunity, can manifest clinical signs earlier than full-term infants. In a review of premature neonates with congenital malaria, four of five neonates received a diagnosis in the first week of life (Ahmed et al., 1998), although the prompt medical evaluation afforded these neonates may have facilitated earlier detection.

The clinical features of congenital malaria are nonspecific and often resemble those of bacterial or viral sepsis and other congenital infections. Fever is almost uniformly present, although without the classic paroxysmal pattern described for malaria beyond the neonatal period. Hulbert (1992) noted fever in all 44 infants for whom clinical information was available. In the cases reported from 1966–2005, fever was reported in 70 of 81 cases (86%) (Lesko et al., 2007). Hepatomegaly and splenomegaly suggestive of a transplacentally acquired infection are found in a substantial portion of infants (Table 38.7). Anemia (often hemolytic), thrombocytopenia, and hyperbilirubinemia are the most commonly reported laboratory findings. Additional signs, symptoms, and laboratory findings are listed in Table 38.8.

In endemic areas the traditional belief has been that congenital malaria is rare and that when it occurs the infant is typically asymptomatic and develops no clinical features. The lack of symptoms has been attributed to transplacentally acquired antibodies from the mother as well as the protective effects of high levels of fetal hemoglobin. Falade et al. (2007) noted spontaneous clearance of parasitemia in 62% of 95 neonates before day 2 of life. Of the remaining neonates, 34% were symptomatic within 3 days of birth, with fever and refusal to eat being the most common signs of disease. When active surveillance for malaria was conducted in newborns being evaluated for possible bacterial sepsis in Nigeria, 16 of 203 neonates (8%) had parasitemia, and 10 (5%) met the definition of congenital malaria (Ibhanesebhor, 1995). Predominant

TABLE 38.8 Clinical Signs and Symptoms of Congenital Tuberculosis in 170 Infants

| Sign | Number of Patients | Percentage of Patients |
|---|--------------------|------------------------|
| Fever | 107 | 64.4 |
| Respiratory distress | 106 | 63.8 |
| Hepatomegaly with or without splenomegaly | 108 | 65.6 |
| Lethargy or irritability | 66 | 39.7 |
| Poor feeding | 65 | 39.1 |
| Failure to thrive | 42 | 25.3 |
| Cough | 59 | 35.5 |
| Cyanosis | 39 | 23.4 |
| Lymphadenopathy | 34 | 20.4 |
| Jaundice | 23 | 13.8 |
| Abdominal distention | 37 | 22.2 |
| Ear discharge | 9 | 15 |
| Skin lesions | 17 | 10.2 |
| Vomiting | 14 | 8.4 |
| Seizure | 6 | 3.6 |

Modified from Peng W, Yang J, Liu E. Analysis of 170 cases of congenital TB reported in the literature between 1946 and 2009. *Pediatric Pulmonol.* 2011;46:1215–1224.

features of disease included fever, respiratory distress, anemia, and hepatomegaly. In another area in Nigeria, of 202 neonates younger than 1 week who were admitted for evaluation of sepsis, 71 (35%) received a diagnosis of congenital malaria (Ekanem et al., 2008). Fever was the most common symptom and was present in 93% of the neonates. Refusal to feed and jaundice were reported in approximately 33%. These observations suggest that, as in infants with diagnosed congenital malaria outside endemic areas, the clinical presentation of congenital malaria in endemic areas does not differ significantly from bacterial sepsis. Because the clinical symptoms

of congenital malaria may be indistinguishable from those of neonatal sepsis, it is suggested that screening for malaria be included as part of routine investigation of newborns with fever in areas of high malaria transmission (Runsewe-Abiodun et al., 2006; Ekanem et al., 2008).

Diagnosis

Diagnostic tests for malaria include blood smears, rapid antigen detection tests, and PCR. Definitive diagnosis of congenital malaria is based on the microscopic demonstration of parasites on stained thick and thin blood films. Thick blood smear films test for the presence of parasites by the concentration of red blood cells, whereas thin blood smear films allow species identification and quantification of parasitemia. In cases of suggested congenital malaria, specimens for smears should be obtained from both the infant and the mother. If the test results from the initial set of smears are negative, additional sets should be obtained every 12–24 hours; three sets are generally considered sufficient for diagnostic evaluation. Response to therapy may also be measured by clearance of parasitemia on blood films.

Rapid diagnostic tests (RDTs) are based on the immunochromatographic detection of parasite-specific antigens circulating in the bloodstream. Many RDTs are commercially available outside the United States, and, depending on the antigens targeted, the tests may detect only *P. falciparum* or all *Plasmodium* spp. RDTs are simple to use, do not require specialized training or facilities, and offer a useful alternative to microscopy in situations where reliable microscopic diagnostics are not readily available. However, the tests have demonstrated mixed results in multiple trials, and sensitivity remains a problem, especially at low parasite densities. To assure standard high-quality control among RDTs used worldwide, the World Health Organization has provided a laboratory-based evaluation to compare the performance of numerous RDTs used among malaria programs. (The minimum performance criteria are >90% sensitive, detection of at least 75% of low-density samples, false-positive rate of <10% and invalid rate of <5%.) In the most recent round of testing in 2014–2015, all products tested could detect high levels of parasitemia, but low parasite density (200 parasites per microliter) detection differed significantly (World Health Organization, 2015c). Information regarding the sensitivity of these tests is limited for neonatal or congenital malaria. Currently the World Health Organization recommends microscopy or malaria RDTs in all patients with suspected malaria before treatment is initiated.

The major advantage of PCR for the diagnosis of malaria is its ability to detect low-level parasitemia with species specification. Currently, PCR is used mainly to confirm positive blood smear tests, particularly when the results of the smear test are not definitive or there is a mixed species infection. PCR may detect DNA from circulating nonviable parasites after treatment, resulting in difficulty differentiating an active infection from a recently cleared infection. Currently, PCR is used only for epidemiologic research or survey mapping in endemic areas. Although PCR is a highly sensitive alternative to microscopy, the infrastructure and expertise required preclude its use in malaria-endemic areas and in many healthcare settings in the United States.

As with malaria in general, the diagnosis of congenital malaria outside endemic areas is often delayed because of nonspecific features and lack of clinical suspicion. Among the 81 cases reviewed by Lesko et al. (2007), a median length of delay of 8.5 days was noted for 15% of the infants. Occasionally the diagnosis is made incidentally. In all four cases of congenital malaria reported by Quinn

et al. (1982), *Plasmodium* spp. parasites were noted by hematology technicians on routine smear tests performed for blood cell counts. Maternal history of recent travel to or emigration from an endemic area may suggest the diagnosis but is often obscured by the lack of clinical or laboratory findings in the mother. Lesko et al. (2007) found that of the mothers for whom history was available, 67% reported having fever during pregnancy, and 26% reported a diagnosis of malaria during pregnancy. Maternal blood film tests were performed after either symptomatic illness or malaria diagnosis in the infant. Overall, parasitemia was detected in only 42% of women, although it is not clear whether an adequate number of smear tests were conducted for each patient. As a result, lack of peripheral parasitemia in the mother of an infant with suspected congenital malaria does not exclude the diagnosis.

Further confounding the early recognition of disease in the infant is the potentially prolonged lapse between malaria exposure in the mother and transmission of infection to the infant. *P. vivax* and *P. ovale* may remain dormant in the liver, especially if the infected individual did not receive therapy for the exoerythrocytic stage, which can cause a delayed relapse of malaria in travelers or immigrants. *P. malariae* can persist for 20–40 years before clinical symptoms or demonstrable parasitemia appear (Centers for Disease Control and Prevention, 2002). Congenital malaria has been reported in an infant whose mother lived in the United States for 5 years before delivery and had no signs or symptoms or malaria for more than 20 years (Harvey et al., 1969). In North Carolina, congenital *P. malariae* infection was reported in a 10-week-old infant who was born to a mother who had emigrated from the Democratic Republic of the Congo 4 years before delivery (Centers for Disease Control and Prevention, 2002). In a review by Lesko et al. (2007), the median duration from the mother's last exposure to delivery was 9.5 months. The time elapsed since exposure was longest for those with *P. malariae* infection, ranging from 2–12 years.

With recognition that congenital malaria is an exceptional occurrence in the United States, it is still important to include malaria in the differential diagnosis of fever in infants born to mothers who have been exposed to malaria, even if the exposure is remote and even if the woman is asymptomatic. Of 11 infants with congenital malaria in the United States born to women known to have parasitemia at or shortly after delivery, only five underwent testing by blood smears, and all five had negative test results at the time of delivery (Lesko et al., 2007). There are insufficient data to determine the overall risk of an infant developing congenital malaria when born to a woman at risk of parasitemia or identified with parasitemia at birth. Consequently, the evaluation of infants born outside endemic areas to women with epidemiologic risk factors for parasitemia should be individualized. In malaria-endemic areas, and as a public health measure, it has been recommended that blood smears should be checked as part of the evaluation of neonates with fever born to mothers who have had fever within a few weeks of delivery (Uneke, 2007a).

Treatment

The treatment of patients with malaria consists of supportive care and antimalarial therapy. Information regarding treatment of congenital malaria is limited, and the recommended chemotherapy is similar to that for noncongenital infections. The treatment regimen is based on the infecting species, the possibility of drug resistance, and the severity of the disease. For mild infections caused by *P. vivax*, *P. ovale* and *P. malariae* or chloroquine-sensitive *P. falciparum*,

chloroquine orally (10 mg base/kg initially followed by 5 mg base/kg 6, 24, and 48 hours later) is recommended. Treatment with primaquine is not necessary for congenitally acquired *P. vivax* or *P. ovale* infection because, like transfusion-associated malaria, congenital infection does not involve the exoerythrocytic phase (Del Punta et al., 2010).

The treatment of congenital malaria caused by chloroquine-resistant *P. falciparum* is poorly defined. In older children, three treatment options currently recommended are (1) orally administered quinine plus either tetracycline, doxycycline, or clindamycin; (2) atovaquone–proguanil; and (3) mefloquine. For the treatment of congenital malaria, oral administration of quinine sulfate and trimethoprim–sulfamethoxazole for 5 days was recommended by Quinn et al. (1982), who used the regimen to treat a 1-month-old infant. Ahmed et al. (1998) used a similar regimen for the treatment of an infant born at 28 weeks' gestation to a mother from Zaire. Quinine and clindamycin have been used for *P. falciparum* congenital malaria (Harrington and Duffy, 2008). Artemisinin-based combination treatments, such as dihydroartemisinin–piperaquine, can also be used in infants for treatment of malaria caused by drug-resistant *Plasmodium* spp. (Enweronu-Laryea et al., 2013; World Health Organization, 2015b). Other regimens used successfully in neonates include oral administration of quinine sulfate and pyrimethamine–sulfadoxine (Gerege and Cimino, 1995) and intravenous administration of quinine hydrochloride followed by oral administration of quinine (Airede, 1991). Intravenously administered quinine is no longer available in the United States. Because of the rarity of congenital malaria in the United States, the changing pattern of resistance, and the potential toxicity associated with drugs used for therapy, current treatment recommendations should be sought from the Malaria Branch of the CDC (<http://www.cdc.gov/malaria>). For healthcare professionals, assistance with management of malaria is also available 24 hours a day through the CDC Malaria Hotline (telephone 855-856-4713).

Severe malaria occurs most commonly with *P. falciparum* infection and is characterized by one or more of the following: (1) parasitemia with more than 5% of red blood cells infected, (2) CNS or other end-organ involvement, (3) shock, (4) acidosis, (5) severe anemia, or (6) hypoglycemia. Management of severe malaria involves parenteral treatment in an intensive care setting. Until recently the only parenteral therapy available in the United States was quinidine gluconate therapy. Quinidine is more cardiotoxic than quinine and should be administered with continuous cardiac monitoring. Artesunates can be given either intravenously or intramuscularly for 24 hours until oral medication can be tolerated, at which time combination therapy should be instituted (World Health Organization, 2015b). Exchange transfusion may be warranted when more than 10% of red blood cells are infected or if there are complications at lower parasite densities.

The efficacy of treatment should be monitored by examination of blood smears (i.e., malaria smears) every 12 hours until they are negative for malaria parasites. Response to therapy with chloroquine for non-*P. falciparum* malaria is usually favorable (Hindi and Azimi, 1980; Brandenburg and Kenny, 1982; Dowell and Musher, 1991). It has been suggested that neonates born to mothers with parasitemia at delivery should be treated presumptively for congenital malaria (Lesko et al., 2007). There are insufficient data to determine the risk of a neonate developing congenital malaria when born to a mother with parasitemia. Although there is evidence from endemic areas that parasitemia detected at or shortly after delivery may clear spontaneously, the clinical relevance

of this observation in nonendemic areas is unclear. It is recommended that physicians judge each case individually, considering factors such as access to medical care and reliability of follow-up in deciding whether to treat neonates presumptively.

Prognosis

Malaria during pregnancy is likely an underappreciated risk factor for increased infant morbidity and mortality in endemic areas. In a review of studies published between 1985 and 2000, a 3%–8% infant mortality rate was calculated on the basis of population-attributable risks for maternal malaria (Steketee et al., 2001). It was estimated that 75,000–200,000 infant deaths annually are associated with malaria during pregnancy, although what proportion of these are related to congenital malaria is unknown. Outside endemic areas, the short-term outcome of congenital malaria has been favorable. Most infants respond rapidly to therapy, with clearance of parasitemia. There were no reports of death or adverse outcomes in the 49 cases reported from 1950–1992 or in the 81 cases reported to the CDC from 1966–2005 (Hulbert, 1992; Lesko et al., 2007). It is unclear, however, whether the outcomes are due to an overall favorable prognosis or reporting bias.

Prevention

The prevention of congenital malaria is based on a pregnant woman's avoidance of exposure and use of chemoprophylaxis. The burden of malaria among pregnant women in endemic areas is well recognized, and prevention and control strategies for areas of high *P. falciparum* transmission aim at reducing maternal and infant mortality. The World Health Organization has proposed a three-pronged approach: (1) long-lasting insecticidal nets, (2) intermittent preventive treatment with an effective antimalarial agent in pregnancy, and (3) prompt diagnosis and effective treatment of malaria infection. In areas of moderate to high transmission of *P. falciparum*, this three-pronged intervention is strongly recommended, including intermittent preventive treatment with sulfadoxine–pyrimethamine. This drug is administered during prenatal care starting in the second trimester, at least 1 month apart, until the time of delivery (World Health Organization, 2014). A metaanalysis of more recent intervention trials suggests that successful prevention of these infections reduces the risk of severe maternal anemia by 38%, low birth weight by 43%, and perinatal mortality by 27% among paucigravid women (Desai et al., 2007). Unfortunately, the full implementation of prenatal malaria prevention efforts is burdened by the challenges associated with healthcare delivery in the developing world. In 2015, 15 million of the 28 million pregnant women at risk of malaria did not receive a dose of preventive medication (World Health Organization, 2015d).

Pregnant women originally from areas where malaria is endemic but who are now living in nonendemic areas may be only partially immune. When they travel to their countries of origin, they should be considered nonimmune and thus should receive the same recommendations as nonimmune women. For women in the United States, the CDC advises women who are pregnant or likely to become pregnant to avoid travel to areas with malaria transmission. If such travel is unavoidable, consultation with an infectious disease or malaria expert is advised. The use of mosquito netting, mesh screens on windows, insecticides, and mosquito repellents can decrease potential exposure to malaria parasites. For pregnant

women traveling to areas where there is no chloroquine-resistant *P. falciparum* malaria, prophylaxis with chloroquine is recommended. The safety of chloroquine for the fetus when used at the recommended doses for malaria prophylaxis is well established (MacLeod et al., 1982). For travel to areas where chloroquine resistance has been reported, mefloquine is the only medication that is currently recommended for prophylaxis during pregnancy. Use of atovaquone–proguanil during pregnancy was not associated with an increased risk of birth defects (Pasternak and Hviid, 2011); however, it is currently not recommended during pregnancy. Doxycycline is contraindicated because of adverse effects on the fetus caused by a related drug, tetracycline, which include dysplasia and discoloration of teeth and inhibition of bone growth. Healthcare professionals caring for women who cannot take the recommended antimalarial agent should contact the CDC Malaria Hotline (telephone 855-856-4713).

Congenital Tuberculosis

Epidemiology

Tuberculosis (TB) now equals HIV infection as a leading cause of death worldwide (World Health Organization, 2015a), and it remains one of the deadliest communicable diseases. One-third of the world's population is infected with *Mycobacterium tuberculosis*. In 2014, almost 10 million new TB cases were identified, and 1.5 million people died of the disease. The greatest burden of disease is in developing countries, where TB remains a major public health threat. While case rates have decreased in the United States and Europe, the rates have increased dramatically in the former Soviet Union, where public health efforts are impeded by political unrest, and in sub-Saharan Africa, where TB has been fueled by the HIV epidemic.

In the United States the incidence of TB declined steadily from 1953 through 1984, reaching a nadir of 9.4 cases per 100,000 people. The resurgence of disease was attributed to multiple factors, including the HIV epidemic, increased immigration, and a decline in public health funding for TB control. With the availability of antiretroviral therapy for HIV and fortification of public health measures, the epidemiologic trend was reversed. The overall incidence of TB in the United States has decreased dramatically since 1992 from 26,673 per 100,000 to only 9421 per 100,000 in 2014 (Centers for Disease Control and Prevention, 2016). Despite the decrease in the total burden of disease, TB continues to disproportionately affect the foreign-born and racial and ethnic minorities. In 2014, 66.5% of all cases of TB in the United States occurred in foreign-born persons (Scott et al., 2015).

The current epidemiology of tuberculosis in pregnancy is not well defined. Historical studies before the 1900s indicated that the severity of the disease was greater during pregnancy, though later reports appeared to contradict these early reports (Hedvall, 1953). With the resurgence of TB in the 1980s, the largest increase in the incidence of the disease occurred in the 25–44-year-old age group, and the number of cases among women of childbearing age rose by 40% (Cantwell et al., 1994). In 2014, nearly 40% of TB cases among women were in those of childbearing age between 15 and 45 years old (Centers for Disease Control and Prevention, 2015a). Thus women of childbearing age, especially those who are foreign born, and their newborns are at continued risk.

An estimated 1 million new cases of TB in children were identified in 2014 worldwide (World Health Organization, 2015a).

The prevalence of congenital TB is likely rare as fewer than 400 case reports have been published in the English-language literature. Most of the published cases were those in the prechemotherapy era (Laartz et al., 2002). Hageman et al. (1980) reported two cases of congenital TB and reviewed another 24 reported in the English-language literature since the introduction of isoniazid (INH) in 1952. In the subsequent decades, more than 34 additional cases of neonates with congenital TB have been described (Abughali et al., 1994; Cantwell et al., 1994; Manji et al., 2001; Mazade et al., 2001; Saitoh et al., 2001; Pejham et al., 2002; Grover et al., 2003; Laartz et al., 2002; Hatzistamatiou et al., 2003; Chen and Shih, 2004; Nicolaidou et al., 2005; Doudier et al., 2008; Mony et al., 2014; Hoyos-Orrego et al., 2015; Rosal et al., 2016; see Table 38.7). Most reports describe infants born in low-burden countries to mothers who have emigrated from high-burden countries. Although some reports originate from countries where TB is endemic, it is likely that congenital TB is underrecognized and underreported in these areas, largely because of the nonspecific clinical features of the disease and the limited diagnostic capability.

With increased global mobility and the epidemiologic trends of TB, it is likely that TB and congenital TB will continue to be observed in developed countries. The nonspecific features of congenital TB and the mortality associated with untreated disease underscore the importance of maintaining a high index of suspicion for TB in pregnant women and young infants.

Pathogenesis, Clinical Presentation, and Natural History

Transmission of *M. tuberculosis* from the mother to the neonate can occur in utero, during birth, or after birth. Although congenital infection is classically considered the result of in utero infection of the fetus, the term *congenital tuberculosis* has historically referred to infection acquired either in utero or during birth. The infection can be transmitted by direct spread to the fetus from the placenta via the umbilical vein or by aspiration/ingestion of infected amniotic fluid, either in utero or during birth.

Historically, congenital TB criteria were described by Beitzke (1935) to distinguish congenital tuberculosis from postnatally acquired TB. The criteria required that the infant have proven TB lesions and one of the following: (1) a primary hepatic complex as evidence of dissemination of the tubercle bacilli via the umbilical vein or (2) in the absence of a primary complex, the presence of tuberculous lesions in the first few days of life or the exclusion of postnatal infection by separation of the neonate at birth from the mother and other potential sources of infection. These criteria were developed before the introduction of chemotherapy, when infant mortality with congenital TB was high and diagnosis was largely based on autopsy findings. The demonstration of a primary hepatic complex with liver and regional node involvement requires an open surgical procedure; a percutaneous liver biopsy may demonstrate caseating granulomas, but the primary complex will seldom be identified. Given the impractical nature of such criteria, Cantwell et al. (1994) proposed a revised set of diagnostic criteria that became more applicable to current practice with increased diagnostic sensitivity. The neonate must have proven tuberculous lesions and at least one of the following: (1) lesions in the first week after birth, (2) a primary hepatic complex or caseating hepatic granulomas, (3) tuberculous infection of the placenta or maternal genital tract, or (4) exclusion or postnatal transmission by

thorough investigation of contacts. While the distinction between congenital TB and postnatally acquired disease may be relevant for academic or epidemiologic purposes, it does not affect the management, treatment, or prognosis of the disease.

The risk of congenital TB in infants born to women with TB is unknown but is likely low. Blackall (1969) reported only three cases among infants born to 100 mothers with TB. Ratner et al. (1951) identified no cases among infants born to 260 mothers with the disease. In a study of 1369 infants separated at birth from their tuberculous mothers and placed in foster care, only 12 became tuberculin positive during 4 years of observation, and in all 12 cases there was a source of infection in the postnatal environment (Cantwell et al., 1994). The low incidence of congenital TB is in part attributable to the high likelihood of infertility in women who have endometrial TB (Balasubramanian et al., 1999). However, in areas with high rates of TB transmission, TB in neonates may be undiagnosed or underreported, and the incidence of congenital infection or vertical transmission remains unknown.

Congenital TB is transmitted in one of three ways: (1) hematogenous spread from the infected placenta via the umbilical vein, (2) in utero aspiration or ingestion of amniotic fluid infected from the placenta or endometrium, or (3) ingestion of infected amniotic fluid or secretions from maternal genital lesions during delivery. In pregnant women, tuberculous bacillema can result in dissemination of infection to the placenta, the endometrium, or the genital tract. Hematogenous seeding and in utero aspiration likely account for most of the congenital TB cases, though actual sources are not well documented (Kaplan et al., 1980; Sosa et al., 2007; Abramowsky et al., 2012; Hoyos-Orrego et al., 2015). Several anecdotal cases of congenital TB have been reported in the literature associated with in vitro fertilization; in retrospect, tuberculous salpingitis was the likely cause of sterility (Zheng et al., 2014; Gleeson et al., 2015; Emiralioglu et al., 2016). While such events are likely to be associated with a very low frequency of congenital infection, they occur in more medically sophisticated areas that are likely to report them in the medical literature.

Tuberculous bacilli have been demonstrated in the decidua, amnion, and chorionic villi of the placenta (Abramowsky et al., 2012). It is unlikely that the fetus can be infected directly from the mother without the presence of a caseous lesion in the placenta, although massive involvement of the placenta does not always result in congenital TB. When a tubercle ruptures into the fetal circulation, bacilli in the umbilical vein can infect the liver, forming a primary focus with involvement of periportal lymph nodes. The bacilli may also pass through the liver and right ventricle and into the lungs, or they can enter the left ventricle via the foramen ovale and pass into the systemic circulation. The organisms in the lung remain dormant until after birth, when oxygenation and circulation result in their multiplication and the subsequent development of a primary pulmonary focus. Alternatively, if the caseous lesion in the placenta ruptures directly into the uterine cavity and bacilli infect the amniotic fluid, the fetus can inhale or ingest the bacilli, leading to primary foci in the lung, intestine, or middle ear. Pathologic examination of TB in the fetus and newborn usually demonstrates disseminated disease, with the liver and lungs being principally involved. Among 38 postmortem cases (Siegel, 1934), the lungs were involved in 97%, the liver in 82%, and the spleen in 76% of the infants. Other sites described are the gastrointestinal tract, kidneys, adrenal glands, and skin (Hageman et al., 1980; Agrawal and Rehman, 1995; Sood et al., 2000). It is not always possible to determine whether sites represent multiple primary

foci or are secondary to primary lesions in the lung or liver. The only lesion in the neonate that is unquestionably associated with congenital infection is a primary complex in the liver; all others may be acquired congenitally or postnatally.

M. tuberculosis infection acquired in utero or perinatally may be indistinguishable from postpartum infection. Postnatal acquisition of *M. tuberculosis* acquired by airborne inoculation, either from the mother or from another contagious adult in the newborn's environment, is the most common route of infection of the neonate. In addition, postnatal infection can occur from ingestion of infected breast milk from a mother with a tuberculous breast abscess. In the absence of a breast abscess, transmission of TB via breast milk has not been documented (Wang and Coonley, 1917).

The clinical manifestations of congenital TB are protean. Complications from TB during pregnancy include stillbirth, recurrent abortion, and infertility (Mittal et al., 2014). A retrospective cohort study from Mexico of neonates born to 35 mothers whose pregnancies were complicated by TB demonstrated an approximately twofold risk of prematurity compared with newborns of mothers without TB (Figueroa-Damian and Arredondo-Garcia, 2001). The manifestations of the disease resemble those of neonatal sepsis or other congenital infections. The affected neonate is commonly born prematurely (Davis et al., 1960; Foo et al., 1993; Amodio et al., 2005; Katumba-Lunyanya et al., 2005; Premkumar et al., 2008; Wanjari et al., 2008). Clinical signs may be evident shortly after birth but typically do not appear until 2–4 weeks of age (see Table 38.8). Among the 29 cases reviewed by Cantwell et al. (1994), the median age of presentation was 24 days. In an updated review of 16 cases reported since 1994, the mean age at presentation was slightly younger at 17.4 days (Laartz et al., 2002).

Before the availability of INH, congenital TB was almost uniformly fatal. Notable signs included failure to thrive, jaundice, and CNS involvement. In the post-INH era, the most commonly described features of the disease are respiratory distress, hepatomegaly with or without splenomegaly, and fever (Hageman et al., 1980; Abughali et al., 1994; Cantwell et al., 1994; Peng et al., 2011). Additional findings are listed in Table 38.8. Although it is important to evaluate an infant with suspected congenital TB for meningitis, CNS involvement occurs in less than 50% of cases (Hageman et al., 1980; Starke, 1997). Otitis media with aural discharge has been described as the presenting sign of congenital TB (Ng et al., 1995; Senbil et al., 1997), accompanied by regional lymphadenopathy (Gordon-Nesbitt and Rajan, 1973; Hatzistamatiou et al., 2003) or facial palsy (Pejham et al., 2002). It is presumed that the infection is due to the accumulation of infected amniotic fluid in the eustachian tube, either in utero or at birth. Cutaneous manifestations of congenital TB include papular, pustular, or vesicular lesions often surrounded by erythema (Loeffler et al., 1996; Sood et al., 2000; Al-Katawee et al., 2007). Biopsy of the lesions is often confirmatory, demonstrating granulomatous inflammation and the presence of acid-fast bacilli (AFB) on tissue stain (Hageman et al., 1980; Loeffler et al., 1996). A unique case of congenital TB involving the spine was reported in India (Grover et al., 2003).

Laboratory abnormalities among infants with congenital TB are also nonspecific but include leukocytosis (63.8%), thrombocytopenia (80%), elevated levels of inflammatory markers such as C-reactive protein (94.7%) or erythrocyte sedimentation rate (60.8%), and elevated liver enzyme levels (76.4%) (Peng et al., 2011).

Diagnosis

The timely diagnosis of congenital TB requires a high index of suspicion. Clinical signs of disease in the neonate are nonspecific, and disease in the mother may be unsuspected (as many cases are not diagnosed until the disease has been diagnosed in the neonate), contributing to further delay in diagnosis. The diagnosis of congenital TB should be considered in any neonate with suspected infection who is unresponsive to conventional antimicrobial therapy, especially when the mother is from a TB-endemic region. Evaluation for suspected disease should include a tuberculin skin test (TST), chest radiography, lumbar puncture, and mycobacterial culture of appropriate specimens. Biopsy specimens of affected tissue, either from the neonate or from the mother, and the placenta have been confirmatory in several case reports (Hageman et al., 1980; Abughali et al., 1994; Cantwell et al., 1994; Loeffler et al., 1996; Chou, 2002; Laartz et al., 2002).

The TST is the most commonly used diagnostic test for TB. The test uses five tuberculin units of purified protein derivative injected intradermally on the volar surface of the forearm. The reaction is measured 48–72 hours later as millimeters of induration. An estimated 10%–40% of immune-competent children with culture-proven TB do not initially react to a TST. Host factors such as young age and immunocompromised state can also decrease the sensitivity of the TST. Thus the TST cannot be used to exclude the diagnosis, and the findings must be interpreted in the context of each patient. Specificity of the TST may be compromised by cross-reactivity with bacille Calmette–Guérin vaccine or with environmental nontuberculous mycobacteria. The TST result is usually negative in neonates with congenital or perinatal TB, either secondary to immature cell-mediated immunity or because of overwhelming disease. Hageman et al. (1980) found that only 2 of 14 infants who underwent skin testing had positive test results; on repeated testing, seven infants subsequently demonstrated positive TST findings, the earliest being at 6 weeks of age, almost 4 weeks after presentation with clinical signs. Similarly, the results of TSTs performed in 9 of 29 patients described by Cantwell et al. (1994) were all negative, with the results of subsequent testing being positive in two of the nine infants. Among the 16 infants with congenital TB reviewed by Laartz et al. (2002), three of four infants tested had nonreactive TST results.

Recent advances in diagnostic tools for TB include whole-blood IGRAs, which are immunologically based tests that measure interferon γ production from lymphocytes in response to antigens that are fairly specific to *M. tuberculosis*. The two types of assays currently available are the Quantiferon Gold (Cellestis, Valencia, CA, USA) and the enzyme-linked immunosorbent spot assay commercially known as the T-Spot.TB (Oxford Immunotec, Abington, United Kingdom). The advantages of these tests include lack of cross-reactivity with bacille Calmette–Guérin vaccine and most nontuberculous mycobacteria. However, the correlation between the findings of IGRAs and TSTs is variable, and negative results do not definitively exclude TB. Moreover, published experience with the use of IGRAs in children is limited, and the negative predictive value of these tests in this population is unclear. Although IGRAs are endorsed by the CDC for use in circumstances in which a TST is indicated (Mazurek et al., 2010), the tests are not approved for use in children younger than 5 years (Starke and Committee on Infectious Diseases, 2014). There is, however, growing experience with these assays, and many experts in the pediatric infectious disease field have used the IGRAs in children aged between 2 and 4 years (Starke and Committee on Infectious Diseases, 2014). The

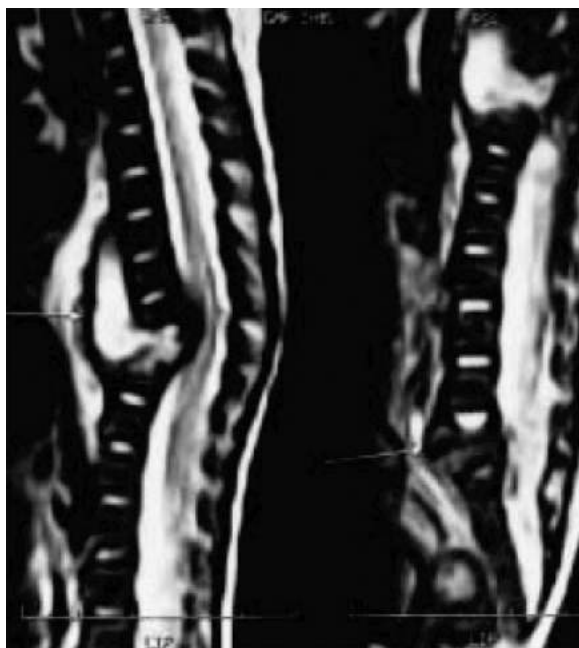


• **Fig. 38.7** Miliary Tuberculosis in a Neonate With Congenital Tuberculosis. (From Singh M, Kothur K, Dayal D, Kusuma S. Perinatal tuberculosis a case series. *J Trop Pediatr.* 2007;53:135–138.)

sensitivity of IGRAs in children younger than 1 year is reduced compared with that in adults (Nicol et al., 2009), and data on the use of IGRAs in newborns are limited to case reports (Connell et al., 2006; Zheng et al., 2014; Sun et al., 2015). While more immunologically advanced IGRAs are being tested and should overcome these limitations, these assays should not yet be substituted for TSTs in the evaluation of congenital TB.

Given the frequency of respiratory distress in infants with congenital TB, it is not surprising that chest radiographs are frequently abnormal at the first examination. In a metaanalysis of 170 infants with congenital TB between 1946 and 2009, 93.1% of all infants had an abnormality on the chest radiograph. Among the 29 cases reviewed by Cantwell et al. (1994), 23 infants (79%) had chest radiograph abnormalities, most being nonspecific infiltrates. Miliary disease was the most common specific radiographic characteristic (46.8%) (Fig. 38.7), followed by multiple pulmonary nodules (11.1%), lobar pneumonia (11.8%), bronchopneumonia (9.7%), and interstitial pneumonia (9%). Cavitation secondary to progressive pulmonary involvement has been reported (Cunningham et al., 1982). Mediastinal adenopathy was also reported in 9.7% of infants. CT of the chest may demonstrate adenopathy suggestive of TB or confirm miliary disease (Singh et al., 2007; Das et al., 2008). An ultrasound or CT image of the abdomen may reveal enlargement of the liver, spleen, or both, possibly with areas of abscesses (Senbil et al., 1997; Grover et al., 2003; Berk and Sylvester, 2004; Amodio et al., 2005), necrotic retroperitoneal or intra-abdominal lymphadenopathy, or ascites (Peng et al., 2011). Spinal disease associated with congenital TB was identified by radiograph and confirmed by magnetic resonance imaging in India (Grover et al., 2003; Fig. 38.8).

Microbiologic confirmation of disease in the neonate should be sought using specimens from multiple sites. For infants and children unable to expectorate sputum, gastric aspirates are considered the specimens of choice. Additional sources for culture include endotracheal aspirate, bronchial washing, middle-ear



• **Fig. 38.8** The sagittal magnetic resonance image on the *left* demonstrates destruction of the T9 to T11 vertebral bodies with the collapse of the T10 vertebral body, leading to a kyphotic deformity causing cord compression. A large prevertebral collection is also seen. The image on the *right* demonstrates destruction with collapse of the L5 and S1 vertebral bodies. (From Grover SB, Pati NK, Mehta R, Mahaian H. Congenital spine tuberculosis: early diagnosis by imaging studies. *Am J Perinatol.* 2003;20:147–152.)

discharge, bone marrow, lymph node tissue, peritoneal fluid, or other suspected sites of disease. CSF should be analyzed and cultured, although isolation of *M. tuberculosis* from CSF is uncommon (Hageman et al., 1980; Abughali et al., 1994). Traditionally the detection of mycobacterial organisms by smear test or culture has been considered difficult because children have paucibacillary disease relative to adults. With three morning gastric aspirates collected appropriately in hospitalized children with a clinical diagnosis of TB, only 40% of children had positive culture results (Starke and Taylor-Watts, 1989). Only 5%–12% of samples are actually acid-fast smear positive (Gomez-Pastrana et al., 2001), though fluorescent staining methods are more sensitive than AFB smear tests (Ryan et al., 2014). In comparison, cultures of aspirates from infants (younger than those in the childhood studies discussed before) evaluated at the same institution had a 75% yield (Starke and Taylor-Watts, 1989; Vallejo et al., 1994).

The improved diagnostic yield in infants likely reflects more widely disseminated and progressive disease, with higher bacillary loads. Hageman et al. (1980) found positive cultures of *M. tuberculosis* in 10 of 12 gastric aspirates, 3 of 3 liver biopsy specimens, 3 of 3 lymph node specimens, and 2 of 4 bone marrow biopsy specimens. Among the 31 cases reviewed by Cantwell et al. (1994), noninvasive procedures and biopsy were useful for the diagnosis of congenital TB in most infants (Table 38.9). More recent reports confirm the high yield of cultures from a variety of specimens in neonates (Mazade et al., 2001; Chou, 2002; Berk and Sylvester, 2004; Premkumar et al., 2008; Wanjari et al., 2008). Histologic examination of tissue may suggest the diagnosis before culture results are available. For example, histopathologic evidence

TABLE 38.9 Results of Diagnostic Procedures Performed on 29 Infants With Congenital Tuberculosis Reported From 1980–1994

| Type of Specimen | Acid-Fast Smear Test ^a | Mycobacterial Culture | Smear Test or Culture |
|------------------------|-----------------------------------|-----------------------|-----------------------|
| Gastric aspirate | 8/9 | 8/9 | 9/11 |
| Endotracheal aspirate | 7/7 | 7/7 | 7/7 |
| Ear discharge | 2/2 | 1/1 | 2/2 |
| Cerebrospinal fluid | 1/2 | 1/2 | 1/2 |
| Urine | 0/2 | 0/2 | 0/2 |
| Peritoneal fluid | 1/1 | 1/1 | 1/1 |
| Bronchoscopic specimen | 1/1 | 1/1 | 1/1 |
| Biopsy specimen | 14/19 | 11/12 | 16/21 |
| Lymph node | 7/8 | 6/6 | 7/8 ^b |
| Liver | 4/6 | 1/2 | 4/6 ^b |
| Skin | 1/3 | 1/1 | 1/3 |
| Lung | 1/1 | 1/1 | 2/2 |
| Bone marrow | — | 1/1 | 1/1 |
| Ear | 1/1 | 1/1 | 1/1 |

^aResults are expressed as the number of positive results per number of patients tested.

^bAll biopsy specimens of lymph nodes and liver that tested negative on the smear test and culture showed histopathologic changes consistent with tuberculosis (i.e., giant cell transformation of granulomas, with or without caseation).

Modified from Cantwell MF, Shehab ZM, Costello AM, et al. Brief report: congenital tuberculosis. *N Engl J Med.* 1994;330:1051–1054.

of granulomas or AFB on stained tissue samples of skin lesions, lymph nodes, and the liver has suggested the diagnosis before culture results were available (Davis et al., 1960; Hageman et al., 1980; Berk and Sylvester, 2004).

Whereas *M. tuberculosis* can require 7–42 days for growth by the standard culture technique, results from techniques such as PCR may be available within 48 hours. A comparison of PCR, AFB smear tests, and culture with clinical diagnosis in children found a sensitivity of 60% and a specificity of 97% (Smith, 2002). Although PCR has been useful for diagnosing congenital TB in a few case reports, it is not sensitive enough to preclude the culture of specimens. Moreover, isolation of *M. tuberculosis* by culture is still important to determine susceptibilities and to optimize treatment.

The mother of a newborn in whom congenital TB is suspected is often asymptomatic or has subclinical disease. In the series of congenitally infected infants reported by Hageman et al. (1980), most of the mothers (16 of 26) did not have a diagnosis until after the disease became apparent in their infants. Cantwell et al. (1994) found that 50% of the mothers of infected infants were not ill at the time their newborns exhibited clinical signs of disease. Evaluation of the mother should include a TST, a chest radiograph, and, if the radiograph is consistent with TB, collection of sputum for microbiologic confirmation. Extrapulmonary disease such as meningitis or peritonitis occurs with some regularity among mothers

with children born with congenital TB (Laartz et al., 2002; Centers for Disease Control and Prevention, 2005), and evaluation may need to be extended to identify such sites if pulmonary disease is not discovered. In mothers with no clinical evidence of disease, endometritis should be considered (Nemir and O'Hare, 1985). Pathologic examination and culture of the placenta (if available) or endometrial biopsy can confirm the diagnosis of genital transmission (Niles, 1982; Cooper et al., 1985; Asensi et al., 1990; Cantwell et al., 1994; Balasubramanian et al., 1999; Surve et al., 2006). In several case reports the diagnosis of maternal TB was ultimately made by endometrial biopsy and culture (Pejham et al., 2002). Culture of amniotic fluid should be performed when genital involvement is suspected. All mothers with TB should be tested for HIV infection, and if the mother is seropositive, the infant should be evaluated for perinatally acquired HIV infection.

Treatment and Management

The successful management of congenital TB depends on early recognition and treatment of disease. In suspected cases, treatment should not be delayed while culture results or results of other diagnostic tests are awaited. Multiple drug therapy for an extended duration has long been recognized as the standard of care for TB. Because of the rarity of the condition, clinical trials have not been conducted to establish the optimal treatment regimen for congenital TB. It is assumed that the regimens used for older infants and children are safe and effective for the treatment of neonates with congenital TB. Consultation with a pediatric infectious disease specialist or TB expert is advised.

Until susceptibility results are known, infants with proven or suspected TB should be treated with a four-drug regimen consisting of INH, rifampin, pyrazinamide, and ethambutol (Table 38.10). Some experts recommend administration of three drugs (INH, rifampin, and pyrazinamide) if antimicrobial resistance is not suspected in the mother (e.g., known susceptible strain in either the mother or the source case or the mother has no risk factors

for resistant *M. tuberculosis*). Supplementation with pyridoxine, although not routinely recommended for otherwise healthy older children, should be provided to breastfeeding infants receiving INH. If the *M. tuberculosis* isolate is determined to be susceptible, the regimen can be narrowed to three drugs (INH, rifampin, and pyrazinamide) for the first 2 months of initial treatment, and subsequently to two drugs (INH and rifampin) to complete the continuation phase of treatment. The adjunctive use of corticosteroids is recommended for the treatment of TB meningitis on the basis of decreased mortality and morbidity demonstrated in adults and children (Girgis et al., 1991) and has been used (though controversial) in cases of endobronchial obstruction, pericardial/pleural disease. Once the infant has discharged to home, directly observed therapy is recommended to ensure adherence and to prevent relapse.

The optimal duration of treatment for infants with congenital TB is unknown. The typical duration of treatment for susceptible *M. tuberculosis* is 6 months for pulmonary disease, pulmonary disease with hilar adenopathy, or hilar adenopathy alone (American Academy of Pediatrics Committee on Infectious Diseases, 2015). With the exception of meningitis, extrapulmonary disease can be treated for the same duration as pulmonary disease. For meningitis, the duration is extended to 9–12 months. The duration of treatment for drug-resistant disease can be prolonged for up to 24 months or longer depending on the clinical course (American Academy of Pediatrics Committee on Infectious Diseases, 2015). Most experts would treat neonates with congenital TB for 9–12 months because of the decreased immunocompetence of neonates (Starke, 1997).

Although there is a substantial amount of data to support the safety of INH, data on the safety and pharmacokinetics of other agents are limited. While INH toxicity is rare in otherwise healthy infants and children, for which monitoring is not required, routine determination of serum transaminase levels and careful monitoring of hepatitis symptoms are indicated in children with severe TB (e.g., miliary or meningitis), those with concurrent liver or biliary

TABLE 38.10

Drugs Commonly Used for Treatment of Tuberculosis in Infants, Children, and Adolescents

| Drug | Dose Forms | Daily Dose (mg/kg) | Twice per Week Dose (mg/kg) | Maximum Dose/ Dosage | Adverse Reactions |
|--------------|--|--------------------|-----------------------------|-------------------------------------|---|
| Ethambutol | Tablets (100 mg, 400 mg) | 20 (15–25) | 50 | 2.5 g | Optic neuritis (usually reversible), decreased red–green color discrimination, gastrointestinal tract disturbances, hypersensitivity |
| Isoniazid | Scored tablets (100 mg, 300 mg) Syrup 10 mg/mL | 10–15 ^a | 20–30 | 300 mg daily, 900 mg twice per week | Mild hepatic enzyme level elevation, hepatitis, ^a peripheral neuritis, hypersensitivity Diarrhea and gastric irritation caused by vehicle in the syrup |
| Pyrazinamide | Scored tablets (500 mg) | 30–40 | 50 | 2 g | Hepatotoxic effects, hyperuricemia, arthralgia, gastrointestinal tract upset |
| Rifampin | Capsules (150 mg, 300 mg) Syrup-formulated capsules | 10–20 | 10–20 | 600 mg | Orange discoloration of secretions or urine, staining of contact lenses, vomiting, hepatitis, influenza-like reaction, thrombocytopenia, pruritus; oral contraceptives may be ineffective |

^aWhen isoniazid in a dosage exceeding 10 mg/kg per day is used in combination with rifampin, the incidence of hepatotoxic effects may be increased.

From American Academy of Pediatrics Committee on Infectious Diseases. Section 3: summaries of infectious diseases, tuberculosis. In: Kimberlin DW, Brady MT, Jackson MA, Long SS, eds. 2015 Red Book: Report of the Committee on Infectious Diseases. 30th ed. Elk Grove Village, IL: American Academy of Pediatrics; 2015:805–831.

disease, or those receiving other potentially hepatotoxic drugs. Routine monitoring of liver function should also be considered for neonates with congenital TB given the paucity of data on adverse effects of anti-TB agents in this age group (Patel and DeSantis, 2008). The risks of optic neuritis with ethambutol should be considered when this agent is used, and vision should be monitored periodically.

The prognosis for congenital TB was dismal in the prechemotherapy era, the diagnosis often being only made at autopsy. Although the survival rate subsequently increased, mortality remained approximately 50% secondary to delayed diagnosis. In a review of 26 cases reported between 1952 and 1980, 12 patients (46%) died, of which nine were untreated but diagnosed at autopsy. Subsequent reviews demonstrated a decrease in case fatality (see Table 38.8) with earlier diagnosis and treatment. Timely diagnosis and initiation of anti-TB therapy are critical for a favorable outcome.

Prevention

Early diagnosis and treatment of *M. tuberculosis* infection in women of childbearing age are the optimal methods of preventing congenital TB. Risk factors for acquiring *M. tuberculosis* infection or progressing to disease should be assessed at prenatal visits, and women with high risk of *M. tuberculosis* infection should undergo tuberculin skin testing or IGRA as recommended by the CDC (Centers for Disease Control and Prevention, 2000). More recent studies indicate that the performance of IGRA is not altered during pregnancy, with better test completion rates compared with TST (Lighter-Fisher and Surette, 2012; Molina et al., 2016). Studies have shown the greatest discordance with IGRAs and TSTs, especially among those with prior bacille Calmette–Guérin vaccination (reviewed in Mathad and Gupta, 2012). Women who test positive for *M. tuberculosis* infection either by a TST or IGRA should undergo evaluation for active disease. The treatment of active TB during pregnancy is considered standard, and early treatment has been shown to improve maternal and neonatal outcome (Figueroa-Damian and Arredondo-Garcia, 1998). The benefit of treating TB during pregnancy far outweighs the potential risk to the pregnant woman and her fetus. The treatment of latent tuberculosis infection (LTBI) during pregnancy is somewhat more controversial. It is recommended that pregnant women at high risk of active disease such as those with HIV infection or those recently infected should start immediate treatment of LTBI. Although there is no demonstrated teratogenic potential for the use of INH, there is concern that pregnant and postpartum women are more vulnerable to INH-related hepatotoxicity on the basis of retrospective analysis (Franks et al., 1989; Malhamme et al., 2016). Prospective data on the risks of INH-induced hepatotoxicity are currently being acquired in a large clinical trial of HIV-infected pregnant women undergoing treatment of LTBI and will provide more information on the true risks of INH (IMPAACT Trial P1078/ NCT0149038 <http://impactnetwork.org/studies/index.asp#ongoing>). More data are needed on the safety in pregnant women not infected with HIV. For those at less risk, treatment can be deferred until weeks to months after delivery. However, treatment should not be delayed, as some suggest, such that the postpartum period may be associated

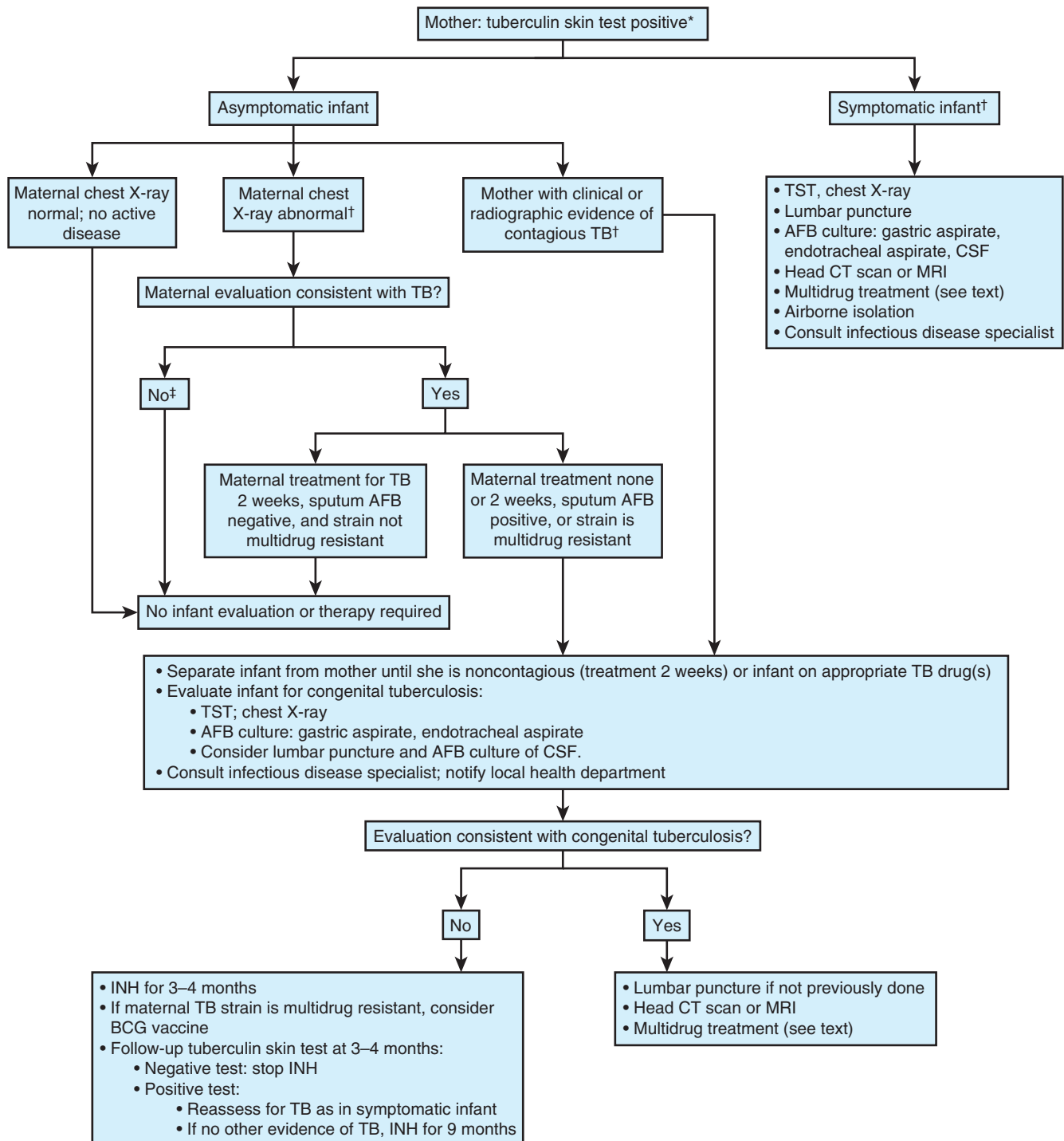
with an increased risk of more severe forms of active disease (Cheng et al., 2003).

The treatment of neonates born to mothers who have LTBI or TB is outlined in Fig. 38.9. Recommendations are based on the categorization of infection in the mother and the potential risk of transmission of TB to the neonate (American Academy of Pediatrics Committee on Infectious Diseases, 2015). Neonates born to mothers with potentially contagious TB should be evaluated for congenital TB. Separation of the neonate and the mother is necessary only in cases in which the mother is highly infectious at the time of delivery, is nonadherent to medication, has drug-resistant disease, or has not been treated for at least 2 weeks (reviewed in Mittal et al., 2014). The mother with latent TB is not contagious. To prevent reactivation disease in the mother and subsequent exposure of the neonate, the mother should receive treatment with INH for latent TB. In addition, latent TB in the mother may be a marker for contagious TB within the household, and it is recommended that all household members and close contacts of the mother be evaluated for TB.

Breastfeeding is not contraindicated in women with LTBI. The breast milk of a woman with TB does not contain tubercle bacilli. For women with TB who are potentially infectious and separated from the newborn, breast milk may be manually expressed and fed to the newborn. Once the mother is noninfectious or the newborn is receiving therapy, breastfeeding can be resumed. The exception, however, is the mother with an active tuberculous breast lesion. In this situation the breast milk may be pumped and discarded until resolution of the lesion (Efferen, 2007).

With regard to isolation practices, airborne precautions are recommended for the following pediatric patients: (1) children and adolescents with adult-type cavitary disease, (2) those with extensive pulmonary involvement, (3) those with laryngeal involvement, (4) those with smears positive for AFB, and (5) congenitally infected neonates undergoing oropharyngeal procedures (e.g., endotracheal intubation) (American Academy of Pediatrics Committee on Infectious Diseases, 2015). In general, children younger than 10 years are not considered contagious given the lack of tussive force they are able to generate and the paucibacillary load. However, the adult contacts of that child may be the source case and potentially contagious. Thus visitation of the hospitalized pediatric patient should be restricted to adults in whom contagious TB has been excluded. Hospitalized children with sputum smears (if obtained) negative for ABS require standard precautions, assuming that contagious TB has been excluded in the visitors.

Compared with older children, neonates likely have a higher concentration of bacilli in their sputum. As noted previously, AFB smear tests on tracheal aspirates and other specimens are frequently positive in this population compared with older children. Transmission of TB from congenitally infected neonates to other hospitalized infants and healthcare workers has been reported and is likely related to aerosolization of bacilli during respiratory manipulation (Lee et al., 1998; Laartz et al., 2002; Crockett et al., 2004; Mouchet et al., 2004). Neonates suspected of having congenital TB should be placed in respiratory isolation if they are intubated or if they are undergoing any procedure with the potential for aerosolization of infected sputum. Exposed infants, visitors, and healthcare workers should undergo evaluation for *M. tuberculosis* infection or disease.



*Acid-fast bacillus culture of amniotic fluid and placenta, if available; placenta for histopathologic examination.

†Mother with chest radiographic findings consistent with old, healed tuberculosis.

• **Fig. 38.9** Management of Infants Born to Mothers With a Positive Tuberculin Skin Test Result. An asterisk indicates that household contacts should have a tuberculin skin test and further evaluation for contagious tuberculosis. The local health department should be consulted. The mother should receive treatment for latent tuberculosis. All persons with tuberculosis should be tested for human immunodeficiency virus infection. AFB, Acid-fast bacilli; BCG, bacille Calmette-Guérin; CSF, cerebrospinal fluid; CT, computed tomography; INH, isoniazid; MRI, magnetic resonance imaging; TB, tuberculosis; TST, tuberculin skin test. (Modified from American Academy of Pediatrics: Tuberculosis. In Pickering LK, ed: 2003 Red Book: Report of the Committee on Infectious Diseases. 26th ed, Elk Grove Village, IL: American Academy of Pediatrics; 2003:642.)

Suggested Readings

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Newborn Sepsis and Meningitis

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KEY POINTS

- Group B streptococcus and *Escherichia coli* account for most of the cases of neonatal early-onset bacterial sepsis.
- Prevention of infection by maternal treatment is the main factor accounting for the decreased incidence of early-onset group B streptococcus sepsis.
- Microbiologic cultures represent the mainstay for diagnosis of infection.
- Management of the asymptomatic newborn at risk of infection because of maternal risk factors is in transition, with recent evidence supporting evaluation and treatment of a small proportion of asymptomatic infants.
- Ampicillin and gentamicin are recommended as initial therapy in neonates with suspected bacterial sepsis. Treatment can then be narrowed appropriately on the basis of the results of antibiotic susceptibility studies.

Throughout pregnancy the fetus is protected from bacterial and viral infections by the chorioamniotic membranes, the placenta, and various antibacterial factors that are poorly described in amniotic fluid. There are several mechanisms by which bacteria can reach the fetus or newborn and initiate infection. Maternal bloodstream infections, caused by bacteria such as *Listeria monocytogenes* and *Mycobacterium tuberculosis*, can reach the fetus and cause infection. Bacteria such as group B streptococcus (GBS) can be acquired from the vagina, cervix, or fecal contamination of the birth canal through either ruptured or intact membranes, leading to amnionitis, intrauterine pneumonitis, and premature delivery (Ancona et al., 1980; Payne et al., 1988; Ferrieri, 1990; Larsen and Sever, 2008). It is thought that subclinical infections of the fetus, amniotic fluid, membranes, or placenta may contribute to the onset of preterm labor and the delivery of preterm neonates. Finally, infection can occur via aspiration of birth canal contents or colonization of mucosal surfaces during passage through the birth canal, leading to pneumonia, followed by bacteremia and sepsis on day 1 or later; this may be the mode by which *Neisseria gonorrhoeae*, *Escherichia coli*, and GBS are acquired.

Early-onset bacterial sepsis remains a major cause of neonatal morbidity and death, although the sepsis-associated death rates per 100,000 live births declined significantly from 2001–2011. Much of this decline in mortality was because of the introduction of intrapartum antibiotic prophylaxis in pregnant women during

labor and delivery (Schrag et al., 2002; Schrag and Stoll, 2006; Centers for Disease Control and Prevention, 2007, 2009). Mortality rates in infected premature neonates and very immature neonates are significantly higher than in term neonates. Major improvements in neonatal intensive care and early identification and recognition of infected neonates have contributed to reduced mortality rates in the newborn period.

Pathogenesis of Early-Onset Neonatal Bacterial Infections

There are multiple portals through which bacteria can enter and infect the newborn. The primary portals of entry appear to be the respiratory tract, as suggested by the high frequency of acute respiratory distress and pneumonia, which occurs in newborns with early-onset disease. Acquisition via the placenta is suggested in some instances by the presentation of high-grade bacteremia and severe sepsis clinically apparent at the time of birth in the presence of intact membranes in neonates born by cesarean delivery. The primary maternal event in this sequence, leading to infection of the fetus and newborn, is colonization of the maternal genital tract with organisms such as GBS. Bacteria that reside in the cervix, vagina, or rectum can ascend into the amniotic cavity through intact or ruptured membranes and lead to chorioamnionitis. Bacteria can initially spread into the choriodecidual space and can occasionally cross intact chorioamniotic membranes. Although organisms recovered from the amniotic sac in the mother are usually polymicrobial and include organisms such as GBS, group D enterococcus, aerobic gram-negative bacteria, and anaerobes such as *Bacteroides* spp. (Gibbs et al., 1980), a single organism causing bacterial sepsis is the rule in de novo sepsis of the newborn. Genital microplasmas are at times recovered from women as well as *Chlamydia* spp., but their precise pathogenic role is unclear (Pankuch et al., 1984; Krohn et al., 1995). *Ureaplasma* spp. and *Chlamydia* spp. can be isolated from neonates' respiratory tract after birth; these plus *Mycoplasma hominis* can be recovered from the respiratory tract after birth, but they are not associated with sepsis syndrome.

Although many microorganisms recovered from the amniotic cavity are thought to induce spontaneous preterm labor, and possibly premature rupture of membranes, the exact mechanisms by which this may occur are debatable. Clinical or subclinical chorioamnionitis

can incite a marked inflammatory response with the release of cytokines that can contribute to the onset of preterm labor and premature rupture of membranes. Other risk factors for clinical intra-amniotic infection include young maternal age, prolonged labor, prolonged rupture of membranes (≥ 18 hours), internal scalp fetal monitoring, the presence of urinary tract infections, and a history of bacterial vaginosis (Newton et al., 1989; Soper et al., 1989). Despite inherent antibacterial properties in amniotic fluid, these may not be sufficient to overcome a large bacterial inoculum, because of rapid multiplication of bacteria during a prolonged labor or the absence of type-specific maternal antibodies for various pathogens (Ferrieri, 1990).

Newborns who immediately display signs of respiratory distress and after birth undoubtedly have onset of infection before or during labor and delivery. With hypoxia and acidosis in utero, the newborn may gasp and inhale contaminated amniotic fluid, leading to pneumonia, bloodstream infection, sepsis, and a severe systemic response syndrome. Newborns who display such signs at birth or within a short time after birth and very low birth weight (VLBW) newborns have the highest mortality rates. Newborns who have an initial asymptomatic period after birth may display symptoms gradually as the organisms multiply in the lungs and in the blood. An example of another invasive site of entry is the scalp lesion created by a monitoring device, which becomes contaminated in the setting of amniotic fluid infected with GBS. An overarching mechanism for continued bacteremia is the absence of sufficient local and systemic host defenses, such as adequate complement levels or type-specific immunity against the invading microorganism (Ferrieri, 1990).

The inflammatory cascade is initiated by activation of macrophages by bacterial cell wall constituents, toxins, or enzymes. A number of proinflammatory cytokines can be released, such as interleukin (IL)-6, IL-8, and tumor necrosis factor- α (TNF- α). These cytokines can alter vascular permeability and vascular tone, decrease myocardial contractility, activate clotting systems, increase pulmonary vascular resistance, and activate other phagocytic cells, such as polymorphonucleocytes (PMNs). Ideally, proinflammatory and antiinflammatory cytokines would be balanced; however, this is usually not the case, and the bacteria persist, with subsequent consequences. It is common in newborns, and particularly in preterm neonates, to have dissemination of bacteria to other organs such as the meninges, kidneys, and bone.

Epidemiology of Early-Onset Bacterial Infections

Before the availability and use of antibiotics in the late 1940s and 1950s, there were few survivors of neonatal bacterial sepsis, contributing to the high perinatal mortality rate of that period. There have been changes in the types of bacteria responsible for neonatal infection over the years. In the 1930s and 1940s, group A streptococcus was a prominent cause of neonatal sepsis; this organism is now rather rare (Bizzarro et al., 2005). In the 1950s, nursery outbreaks of *Staphylococcus aureus* infections appeared across North America and Europe, prompting changes in techniques of hygiene and encouraging the development and use of penicillinase-resistant antibiotics (Bizzarro et al., 2005). In the 1960s, *E. coli* became the most common cause of bacterial sepsis, followed in the 1970s by GBS. Even in an era of intrapartum antibiotic prophylaxis of GBS-colonized mothers, GBS remains the most common bacterial pathogen in neonatal centers of North America and Europe, followed by *E. coli* (Schrag et al., 2006). An update on neonatal sepsis at

Yale, between 1989 and 2003, revealed an overall decrease in the rate of sepsis caused by both GBS and *E. coli* (Bizzarro et al., 2005). Regional differences exist, however, and must be considered before an attempt is made to apply epidemiologic data to individual perinatal units. For example, *L. monocytogenes* is a frequent isolate in some western European countries, and *S. aureus* is found commonly in Germany and Scandinavia (Posfay-Barbe and Wald, 2009).

The incidence of early-onset bacterial infection is variable and ranges from 1–5 per 1000 live births; however, it is clear that the incidence has declined as a result of intrapartum antibiotic therapy (Centers for Disease Control and Prevention, 2007, 2009). Data from the Centers for Disease Control and Prevention (CDC) revealed a downward trend from 2000–2003 (from 0.52–0.31 case per 1000 live births), followed by an increase from 2003–2006 (from 0.31–0.40 case per 1000 live births) (Centers for Disease Control and Prevention, 2009). Stratified by race, the incidence increased significantly among black infants from 2003–2006 (from 0.53–0.86 case per 1000 live births), whereas the incidence among white infants did not change significantly (from 0.26–0.29 case per 1000 live births). When stratified by gestational age, the average incidence of early-onset GBS disease among preterm infants during 2003–2006 was 2.8-fold higher among black infants than among white infants (1.79 cases versus 0.67 case per 1000 live births). In both preterm black and preterm white infants there were increases in the incidence of early-onset disease from 2003–2006 that were not statistically significant. Early-onset disease among term white infants was stable from 2003–2006, whereas in term black infants there was a significant increase in the incidence during this period, from 0.33–0.7 case per 1000 live births.

The overall rates of late-onset GBS disease remained stable from 2000–2006 (0.36 case versus 0.30 case per 1000 live births) (Centers for Disease Control and Prevention, 2009). When stratified by race, late-onset disease incidence among black infants decreased significantly by 42% from 2005–2006 (0.95 case versus 0.55 case per 1000 live births). Between 2003 and 2006 there were no significant trends among black or white infants.

Infants described with early-onset sepsis frequently have one or more identifiable risk factors (Dutta et al., 2010; Puopolo et al., 2011). Prematurity is considered the single greatest risk factor for early-onset bacterial infections. Because it is accepted that extremely low birth weight infants have impairment of host defenses, and since preterm birth may be associated with low-grade chorioamnionitis, it is not surprising that the attack rates for infection by pathogens such as GBS are 26–30-fold higher in preterm infants than in term newborns, with an associated high mortality. Other risk factors for early-onset sepsis are maternal age, health and nutrition, colonization with well-known pathogens (e.g., GBS), maternal fever, and longer duration of rupture of membranes (Schuchat et al., 2000; Puopolo et al., 2011). Neonatal susceptibility to GBS infection is increased with deficiencies in circulating levels of GBS type-specific antibody and complement, which is further heightened by any element of neutrophil dysfunction, as may be seen in the more premature infants (Ferrieri, 1990; Foxman, 2007; Nandyal, 2008; Makhoul et al., 2009).

Bacterial Pathogens in Early-Onset Infections

Group B Streptococcal Infections

Since the early 1930s when Rebecca Lancefield reported her grouping system for hemolytic streptococci, group A streptococcus

(*Streptococcus pyogenes*) was widely acknowledged as the major pathogen associated with puerperal sepsis. GBS was initially thought to be a commensal until 1938, when Frye reported seven cases of GBS-associated puerperal fever with three deaths (Eickhoff et al., 1964). Before the 1960s, GBS was not recognized frequently as a cause of human disease. However, in the late 1960s GBS emerged as the leading cause of neonatal sepsis in newborns (Zaleznik et al., 2000; Bizzarro et al., 2005). Before the era of maternal intrapartum prophylaxis, GBS had a reported national incidence of approximately 2 per 1000 live births and was associated with approximately 50% mortality in newborns. As mentioned previously, in the past decade with the introduction of antibiotic maternal prophylaxis there has been a significant decrease in the incidence of GBS to its current rate of approximately 0.32 per 1000 live births for early-onset disease.

Transmission of Group B *Streptococcus* From Mothers to Infants

In the United States, approximately 20%–35% of pregnant women are asymptomatic carriers of GBS in the genital tract and gastrointestinal tract during pregnancy and at the time of delivery (Ferrieri, 1990; Zaleznik et al., 2000; Ferrieri et al., 2004b). The prevalence of GBS colonization during pregnancy differs. Among women who were positive for GBS between 26 and 28 weeks' gestation, only 65% remain colonized at term, whereas 8% of those with negative prenatal culture results were positive for GBS at term (Ancona et al., 1980; Zaleznik et al., 2000). Treatment of GBS-colonized women during pregnancy only temporarily eradicates the organism, and most women are recolonized within several weeks. At birth, 50%–65% of neonates who were born to GBS-colonized mothers have positive GBS cultures from mucous membranes and skin (external ear canal, throat, umbilicus, and anal or rectal sites) (Shet and Ferrieri, 2004). Before the introduction of intrapartum antibiotic prophylaxis, approximately 1%–2% of colonized neonates developed GBS infection, and the overall incidence of neonatal GBS infection was approximately 2 per 1000 live births in the United States. With intrapartum prophylaxis, approximately 60%–80% of GBS cases occur in infants born to women with a negative prenatal GBS screening result (Centers for Disease Control and Prevention, 2010). A small number of GBS-infected infants acquired their bacteremia because of hematogenous transmission through the placenta. In these situations the mother commonly displays signs and symptoms of chorioamnionitis, although it may occur in the absence of maternal symptoms (Baker and Edwards, 1995).

Detection of GBS colonization has been emphasized since approximately 1996; studies to determine the optimal sites of sampling have been key to the effectiveness of intrapartum prophylaxis. GBS resides in the genitourinary and gastrointestinal tracts, where large numbers of gram-negative bacteria are also present. Most colonization studies have revealed high rates of both rectal and vaginal colonization with GBS (Ancona et al., 1980; Hickman et al., 1999; Zaleznik et al., 2000). The use of the selective broth enrichment medium that inhibits the growth of gram-negative enteric bacilli and other normal flora can increase culture sensitivity for GBS to greater than 90%. The most widely used selective medium is Todd–Hewitt broth with either gentamicin or colistin and nalidixic acid. As recommended in a 2010 publication from the CDC, the optimal time for performing prenatal cultures is between 35 and 37 weeks' gestation, and the highest culture yield is obtained when both the lower vaginal area and anal or rectal sites are sampled (Schrag et al., 2002; Centers for Disease Control and Prevention, 2010).

Epidemiologic studies of GBS-colonized women have shown that those with heavy colonization (3+ to 4+) are more likely to transmit GBS to their infants (Ancona et al., 1980). The colonization of GBS in pregnant women may be long-standing, intermittent, or transient. There is a definite association between GBS colonization and other risk factors for neonatal sepsis; these include preterm labor, preterm delivery, premature rupture of membranes, prolonged rupture of membranes, and maternal fever.

In the past few years, rapid diagnostic tests to detect GBS colonization in pregnant women have included real-time polymerase chain reaction (PCR); compared with broth enrichment cultures, there is an approximately 10%–15% increased sensitivity (P. Ferrieri, unpublished data). The advantages of PCR detection of maternal vaginal or rectal colonization are the rapid turnaround time and the increased sensitivity. Although it is more expensive than traditional culture-based detection assays, the results are available 1–2 days sooner. The argument that this does not provide semiquantitative data on the degree of GBS colonization in the mother is moot, because even low-grade colonization in pregnant women is a risk factor for neonatal GBS sepsis. However, women with heavy (3+ to 4+) colonization, determined by semiquantitative assessment of vaginal or rectal cultures, are more likely to pass the microorganism to their infants (Ancona et al., 1980).

Chemoprophylaxis and Intrapartum Antibiotic Therapy

Prevention is of key importance in decreasing the incidence of invasive GBS disease. The challenge has been to widely promulgate screening cultures in pregnant women. Revised guidelines from the CDC were published in 2002 and presented only the culture screening–based approach for prevention and chemoprophylaxis rather than the two preventive approaches published in 1996: a culture screening–based and a risk-based approach (Schrag et al., 2002). The risk-based approach involved the use of antibiotics solely on the basis of the presence of prenatal or intrapartum risk factors such as maternal fever, preterm labor, or premature rupture of membranes (<37 weeks' gestation); prolonged rupture of membranes (≥18 hours); history of a newborn with GBS disease; and GBS bacteruria during pregnancy. Challenges to the implementation of the CDC guidelines, such as failure to seek prenatal care and the use of suboptimal laboratory culture techniques, continue in certain populations. Data for the United States as a whole show a decrease in the incidence of early-onset GBS disease concurrent with the implementation of maternal GBS screen and intrapartum antibiotic prophylaxis guidelines. The current estimate for the overall US population for early-onset GBS disease is 0.32 per 1000 live births (Van Dyke et al., 2009).

Intrapartum Antibiotic Prophylaxis

GBS is sensitive to penicillin, which is the drug of choice because of its narrow spectrum; the alternative is ampicillin. If a mother is allergic to penicillin but not at high risk of anaphylaxis, the use of cefazolin is recommended (Centers for Disease Control and Prevention, 2010). When patients are at high risk of anaphylaxis, tests for antimicrobial susceptibility of prenatal GBS to clindamycin and erythromycin should be performed. If the prenatal GBS is sensitive to clindamycin and erythromycin, patients can receive either of these drugs intravenously (IV) until delivery. When GBS is resistant to clindamycin or erythromycin or the antibiotic susceptibility is unknown, IV vancomycin given every 12 hours until delivery is the current recommendation (Centers for Disease Control and Prevention, 2010). In the United States, GBS exhibits considerable resistance to erythromycin (5%–32%) and clindamycin

TABLE 39.1 Manifestations of Early-Onset and Late-Onset Group B Streptococcal Disease

| Characteristic | Early-Onset Disease | Late-Onset Disease |
|--|------------------------------------|------------------------------------|
| Age at onset | Birth through day 6 of life | Day 7 to 3 months |
| Symptoms | Respiratory distress, apnea | Irritability, fever, poor feeding |
| Findings | Pneumonia, sepsis | Sepsis, meningitis, osteoarthritis |
| Maternal obstetric complications | Frequent | Uncommon |
| Mode of transmission | Vertical, in utero, or intrapartum | Nosocomial, horizontal |
| Predominant serotypes | Ia, III, V ^a | III, Ia, V ^a |
| Effect of intrapartum antibiotic prophylaxis recommended by the Centers for Disease Control and Prevention | Reduces incidence by 85%–90% | No effect |

^aIn decreasing order of frequency.

(3%–21%) (Castor et al., 2008). It is therefore important for antibiotic testing to be done on the group B streptococcal isolates from pregnant women. For laboratories performing PCR on maternal vaginal or rectal cultures, it is recommended that the swabs be placed in a selective enrichment broth containing inhibitory antibiotics (either colistin and nalidixic acid or gentamicin and nalidixic acid) against gram-negative bacteria. If the PCR result is positive, the broth culture can be subcultured, and antibiotic testing can be pursued. Because of the higher sensitivity of PCR compared with the selective broth enrichment culture, the organism will not grow on 10%–15% of occasions.

Group B Streptococcal Sepsis in Neonates

Most infections in newborns occur within the first week of life and are designated as *early-onset disease* (Table 39.1; Centers for Disease Control and Prevention, 2010). In the intensive care unit setting, a common definition of early-onset sepsis is onset of sepsis within 72 hours of birth. Late-onset infections occur in newborns 7 days or older, with most of these infections appearing in the first 3 months of life. Although chemoprophylaxis has led to a significant decrease in the incidence of early-onset GBS disease, there is no evidence that chemoprophylaxis prevents late-onset disease (Hamada et al., 2008; Jordan et al., 2008; Cohen-Wolkowicz et al., 2009). Young infants with early-onset invasive GBS disease usually have pneumonia, sepsis, and less often meningitis, osteomyelitis, or septic arthritis (Koenig and Keenan, 2009). The frequency of meningitis, osteomyelitis, or septic arthritis is higher among infants with late-onset disease.

There are nine antigenically distinct GBS serotypes, based on their capsular polysaccharide analysis (types Ia, Ib, and II–VIII) and a proposed new type, IX (Henrichsen et al., 1984; Slotved et al., 2007; Diedrick et al., 2010). In the United States and western Europe, types Ia, II, and III account for most of the isolates from infants with early-onset disease (Zaleznik et al., 2000; Diedrick

et al., 2010). However, recent studies in the United States have demonstrated that serotypes Ia, III, and V, the latter emerging in recent years (Elliott et al., 1998; Harrison et al., 1998; Diedrick et al., 2010), account for most cases (70%–75%) of early-onset invasive GBS disease in newborns and parturient women (Zaleznik et al., 2000; Diedrick et al., 2010). Late-onset GBS disease in infants is dominated by serotype III, followed by serotypes Ia and V (Shet and Ferrieri, 2004). A polysaccharide capsule is considered the most important virulence factor (Kasper et al., 1996; Paoletti et al., 1997, 2001; Cieslewicz et al., 2005); however, the role of surface-localized GBS proteins (Johnson and Ferrieri, 1984; Ferrieri et al., 2004a; Smith et al., 2004; Lindahl et al., 2005) in pathogenesis and immune protection has gained favor (Maione et al., 2005; Tettelin et al., 2005).

The remaining GBS isolates from invasive disease consist primarily of types Ib and II, but types IV, VI, VII, and VIII compose a small fraction. Type IV GBS represented between 0.4% and 0.6% of colonizing GBS isolates (Diedrick et al., 2010), but it was relatively uncommon for type IV isolates to be found in invasive GBS (Puopolo and Madoff, 2007; Ferrieri et al., 2008). Recent studies in the United Arab Emirates, Turkey, and Zimbabwe showed large proportions of type IV GBS among their isolates (Amin et al., 2002; Moyo et al., 2002; Ekin and Gurturk, 2006; Diedrick et al., 2010). In Zimbabwe it was the fourth most common serotype, accounting for 4.6% of the colonizing and invasive isolates (Moyo et al., 2002). Serotypes VII and VIII are uncommon in Western countries.

Infected infants have low levels of type-specific antibody to the infecting GBS serotype (Baker and Edwards, 2003). Vaccines against the common GBS serotypes have been shown to elicit a specific antibody response in humans (Kasper et al., 1996; Paoletti et al., 1997, 2001; Baker and Edwards, 2003). Sera from these vaccinated humans protected neonatal mice against GBS challenge, suggesting that high-titer GBS immunoglobulin may have the potential to prevent or modulate invasive neonatal GBS disease in infants (Paoletti et al., 1997). The prospect of a multivalent GBS vaccine, with or without conjugated GBS surface-localized proteins, makes the study of the common GBS serotypes important because of the possibility of serotype replacement or capsular switch (Lipsitch, 1999; Cieslewicz et al., 2005; Maione et al., 2005; Tettelin et al., 2005).

Escherichia Coli Infections

Historically, *E. coli* has been the second most common pathogen causing sepsis and meningitis in newborns. The antigenic structure of *E. coli* is complex and is composed of approximately 150 somatic or cell wall O antigens, 50 flagellar H antigens, and approximately 80 capsular K antigens. However, a limited number of K antigen *E. coli* strains cause meningitis, and approximately 80% of the strains causing meningitis and 40% of the strains causing bacteremia or sepsis express K1 (Mulder et al., 1984). The capsular K1 polysaccharide antigen is highly homologous to the capsular antigen of group B *Neisseria meningitidis*. Because a high percentage of women may have bacteriuria with strains of *E. coli* that express the K1 antigen or are colonized with it at the time of delivery, it is surprising that *E. coli* sepsis or meningitis is not more common. It has been estimated that disease occurs in 1 in 100–200 infants colonized by K1 *E. coli*. Surveillance data from the National Institute of Child Health and Human Development Neonatal Research Network, a consortium of 16 US academic neonatal centers, revealed that in the era of widespread implementation of antibiotic prophylaxis, the rate of *E. coli* sepsis increased from 3.2–6.8 cases per 1000 live births. This increase was observed in the 1998–2000 era

and persisted from 2002–2003. Approximately 85% of *E. coli* infections in VLBW infants were ampicillin resistant (Stoll et al., 2002). However, most evidence suggests that intrapartum antibiotic prophylaxis has not been associated with a concomitant adverse impact of increasing the incidence of *E. coli* or other non-GBS bacterial causes. Among preterm infants, however, the incidence of *E. coli* and ampicillin-resistant *E. coli* infections increased significantly (Bizzarro et al., 2005). A retrospective case–control study performed between 1997 and 2001 concluded that exposure to intrapartum antibiotic prophylaxis in mothers did not increase the odds of invasive, early-onset *E. coli* infection. In fact, among term infants, exposure to 4 hours or more of intrapartum antibiotic therapy was associated with decreased odds of early-onset *E. coli* infection (Schrag et al., 2006).

Listeria Monocytogenes Infections

L. monocytogenes is a small, facultative anaerobic, gram-positive motile bacillus that produces a narrow zone of beta hemolysis on blood agar plates and can be confused with GBS unless a careful Gram stain, a catalase reaction, and other tests are performed. Most disease is due to three primary serotypes: 1a, 1b, and 4b. The last serotype has been described in most outbreaks of listeriosis (Posfay-Barbe and Wald, 2009). Most cases of listeriosis appear to be food-borne, including those acquired by pregnant women.

Foods that can be contaminated by *L. monocytogenes* include raw vegetables such as cabbage, raw milk, fish, poultry, processed chicken, beef, and hot dogs (Schlech, 2000). Transmission to the fetus occurs through either a hematogenous (transplacental) route or via an ascending infection through the birth canal. Frequently, infections with *Listeria* spp. early in gestation result in abortion; later in pregnancy, infection with *Listeria* spp. can result in premature delivery of a stillborn or infected newborn. Approximately 70% of *Listeria*-infected women deliver before 35 weeks' gestation. Illness in the mother may be undetected because of vague influenza-like illnesses that may not come to medical attention. In approximately half of perinatal cases, illness in the mother has preceded delivery by 2 days to 2 weeks. At autopsy of stillborn neonates or of those who die in the perinatal period, granulomas may be found throughout organs such as the liver and lungs, and infection is widely disseminated, including involvement of the meninges. Treatment of *Listeria* spp. infection or bacteremia during pregnancy can prevent infection in the fetus (Kalstone, 1991). Like GBS infection, *Listeria* spp. infection may have either an early-onset or a late-onset presentation. Epidemics of neonatal *Listeria* spp. infection have been described after ingestion of contaminated foods such as cheese or coleslaw. The first clearly documented food-borne (coleslaw) outbreak of listeriosis was in 1981 from the Maritimes in Canada (Schlech, 2000); it was associated with a fatality rate of 27%. There are reports of repeated abortions in women with colonization in the gastrointestinal tract, and cold-enrichment cultures can be performed to try to detect fecal carriage in such women. However, cold enrichment cultures are inferior to selective media for *Listeria* spp. in isolating the organism from various foods or stool specimens. Rapid antigen tests based on nucleic acid amplification are not in common use in clinical diagnostic laboratories. There is no current vaccine for *Listeria* spp. infection, but preventive measures have included the surveillance programs from the US Department of Agriculture, prohibiting the sale of contaminated meats. Between 1996 and 2006, the incidence of *Listeria* spp. infections declined by 36%; however, an outbreak of disease in 2002 related to contaminated

turkey meat led to 54 illnesses, 8 deaths, and 3 fetal deaths in 9 states (Posfay-Barbe and Wald, 2009).

Miscellaneous Bacterial Pathogens

The bacteria responsible for early-onset neonatal sepsis have changed dramatically over time. There are regional differences in the organisms commonly responsible for early-onset sepsis. In addition to the organisms mentioned previously, other bacterial pathogens associated with early-onset bacteremia or sepsis in newborns include *Enterococcus* spp., viridans group *Streptococcus* spp., *Klebsiella* spp., *Enterobacter* spp., *Haemophilus influenzae* (typeable and nontypeable), *S. aureus*, *Streptococcus pneumoniae*, group A streptococcus and other beta-hemolytic streptococci, and coagulase-negative staphylococci.

Clinical Signs of Bacterial Sepsis

There is great variability in the clinical presentations of neonates with early-onset bacterial sepsis (Box 39.1). Most neonates exhibit respiratory distress in the first 12 hours of life, frequently immediately after birth. In these neonates the progression may be rapid, with cardiovascular instability, shock, and death. Presentation within the first 12 hours of life suggests that the infection with pneumonia and bacteremia occurred at or near the time of birth or during the immediate postnatal period. Neonates with hypoxia in utero may gasp, inhaling contaminated amniotic fluid and setting the stage for early-onset pneumonia, bacteremia, and sepsis.

The signs of early-onset infection may be subtle, with tachypnea suggesting “wet lung disease,” or may be more overt, with respiratory distress and hypotension. Because the signs of sepsis can be relatively nonspecific, such as poor feeding and increased sleepiness, they can be overlooked. In newborn, intermediate care, or intensive care nurseries, one must be attuned to subtle abnormal findings in newborns. The clinical signs of neonatal sepsis include hyperthermia or hypothermia, respiratory distress, apnea, cyanosis, jaundice, hepatomegaly, abdominal distention, feeding abnormalities, and neurologic abnormalities. Autopsy findings in preterm infants with fatal early-onset GBS infection suggested that surfactant-deficiency respiratory distress syndrome was common (Payne et al., 1988).

Laboratory Testing

Many laboratory tests have been evaluated for infants with possible sepsis, and the results must be interpreted with caution, assessing

• BOX 39.1 Common Clinical Signs of Neonatal Sepsis

- Abnormal neurologic status: irritability, lethargy, poor feeding
- Abnormal temperature: hyperthermia or hypothermia
- Apnea
- Bleeding problems: petechiae, purpura, oozing
- Cardiovascular compromise: tachycardia, hypotension, poor perfusion
- Cyanosis
- Gastrointestinal symptoms: abdominal distention, emesis, diarrhea
- Jaundice
- Respiratory distress: tachypnea, increased work of breathing, hypoxemia
- Seizures

| | | Bacterial Infection Present | | |
|------------------------|----------|-----------------------------|----------------------------|--|
| | | YES | NO | |
| Laboratory Test Result | POSITIVE | True Positive TP | False Positive FP | Positive Predictive Value = (TP)/(TP+FP) |
| | NEGATIVE | False Negative FN | True Negative TN | Negative Predictive Value = (TN)/(FN+TN) |
| | | Sensitivity = (TP)/(TP+FN) | Specificity = (TN)/(FP+TN) | |

• **Fig. 39.1** The relationships among sensitivity, specificity, positive predictive value, and negative predictive value in bacterial infections. *FN*, Number of infants with infection with an incorrect diagnosis by the test of being healthy; *FP*, number of healthy infants with an incorrect diagnosis by the test of being infected; *TN*, number of healthy infants with a correct diagnosis by the test of not being infected; *TP*, number of infants with infection correctly diagnosed by the test.

the sensitivity and specificity of a particular test as well its positive and negative predictive accuracy (Fig. 39.1). The sensitivity of a test is defined as the proportion of individuals with proven or probable sepsis in whom the result is abnormal; the specificity is the proportion of healthy or uninfected infants in whom the result is normal. Ideally, a test would have a high sensitivity and a high specificity, but this is rarely achievable. High sensitivity is the most desirable characteristic when dealing with serious and treatable diseases such as neonatal sepsis. Because sepsis is generally treated with antibiotic agents that have a low toxicity, diagnostic tests do not need to have a high specificity but should have a high sensitivity, which will allow sepsis to be excluded. A positive predictive accuracy is the probability that an infant with an abnormal laboratory result is infected; a negative predictive value is the probability that infection with a normal or negative result is free of infection. The more sensitive the test, the greater its negative predictive value; the more specific a test, the greater its positive predictive value.

Microbiologic Cultures

Previously, cultures of superficial body sites in newborns (external auditory canal, gastric aspirate, umbilicus, and nasopharynx) were used to identify bacterial pathogens when more specific blood culture results were negative. Surface cultures are no longer used to make clinical decisions, because they are of limited value in predicting the cause of bacterial sepsis in newborns (Evans et al., 1988; Shenoy et al., 2000). Examination of gastric aspirates by Gram stain as a screening mechanism has also lost favor, as has examination for PMNs as a representation of amnionitis because PMNs in the gastric acid have no specificity in predicting bacterial sepsis for the infants (Vasan et al., 1977).

Blood Cultures

The gold standard for detection of bacteremia in newborns with suspected sepsis is a positive blood culture result. With the introduction of newer blood culture detection instruments that are semiautomated and determine the presence of growth from bacterial CO₂ production that is detected by the internal computer of the instrument every minute, the sensitivity of detecting positive blood culture results has increased. Another variable that influences the sensitivity of detection of bacteremia is the volume of blood obtained and placed in the culture bottles. Ideally 1–3 mL of blood from infants should be obtained, but this is not always possible in very small infants.

Most positive blood culture results are detected within 24–48 hours with use of the new technology (Garcia-Prats et al., 2000; Jardine et al., 2006). However, the use of intrapartum antibiotic prophylaxis in mothers with either GBS colonization or suspected amnionitis originating from any cause can reduce the ability to detect bacteremia in newborns. In a term infant who remains asymptomatic at the initiation of antibiotic therapy, stopping antibiotic administration is recommended if the blood culture results remain negative after 48 hours (Polin et al., 2014). However, the decision to discontinue treatment with antibiotics should include the assessment of the infant's clinical condition and should not rely solely on a negative blood culture result. When the suspicion of sepsis is high, clinicians should consider continuing antibiotic therapy for a complete course despite negative blood culture results (Ottolini et al., 2003).

Urine Cultures

The frequency of positive urine culture results in infants with early-onset sepsis is relatively low, and it is rare to find bacteriuria in infants with negative blood culture results (DiGeronimo, 1992). Infants with late-onset sepsis tend to have a higher rate of positive urine culture results (Visser and Hall, 1979). In the era of widespread intrapartum antibiotic prophylaxis in the mother, positive urine culture results may be obscured because of excretion of antibiotics in the urine of newborns. When pyelonephritis is found in newborns, it likely represents metastatic seeding of the kidney during a bout of bacteremia. In the first 72 hours of life, because the yield from urine cultures is low, it is not generally recommended to obtain urine specimens. However, in the newborn older than 72 hours, a urine sample collected by an aseptic technique (urinary catheter or suprapubic bladder aspiration) is an essential part of the sepsis work-up.

Cerebrospinal Fluid

Lumbar punctures are deferred in infants with any instability or uncorrected bleeding disorders. The details of examination of cerebrospinal fluid (CSF) and the diagnostic approach for examining CSF will be discussed in the subsection on Diagnosis in the Neonatal Bacterial Meningitis section.

White Blood Cell Count and Neutrophil Indices

Normal white blood cell (WBC) counts range from 9000–32,000 cells per microliter at the time of birth, and differences in the site

of sampling can affect these values. The absolute neutrophil count (ANC), the absolute band count of immature neutrophils, and the ratio of immature neutrophils to total neutrophils (I/T) are regarded as more useful than total leukocyte counts in the diagnosis of neonatal sepsis.

The lower limit of total neutrophil count rises to 7200 cells per microliter by 12 hours of age, and then declines to approximately 1720 cells per microliter by 72 hours of age (Manroe et al., 1979; Schmutz et al., 2008). Postnatally, the absolute band count also undergoes similar changes, with peak values of 1400 cells per microliter at 12 hours of age, and then declines. In contrast, the I/T ratio is maximum at birth and then declines to 0.12 beyond 72 hours of age (Manroe et al., 1979; Schelonka et al., 1994). The optimal time to obtain WBC counts is after 4 hours of age, and most recommendations are to obtain the first counts at 6–12 hours of age (Centers for Disease Control and Prevention, 2010; Newman et al., 2010). In VLBW infants there is a greater reference range for the total neutrophil counts (Manroe et al., 1979; Mouzinho et al., 1994; Christensen et al., 2009). There are no significant differences in the I/T ratio or absolute immature neutrophil counts in VLBW infants. There is considerable overlap of the cutoff values of the ANC, absolute band count, and I/T ratio between healthy and infected newborns.

There are a number of clinical conditions that affect the total neutrophil count. Prolonged crying, meconium aspiration syndrome, maternal fever, and asphyxia are all associated with an increase in the total neutrophil count, and there may be an increase in the total immature neutrophil forms, as well as an increased I/T ratio. Maternal hypertension is associated with a decrease in the total neutrophil count. At high altitudes, a higher upper limit of neutrophil values occurs (Schmutz et al., 2008).

In approximately two-thirds of infants with sepsis, the total neutrophil count is abnormal. Neutropenia is the best predictor of sepsis, whereas neutrophilia does not correlate well. The absolute neutrophil band count is not a sensitive marker of sepsis but has a relatively good predictive value and specificity. The I/T ratio is considered to have the best sensitivity of all of the neutrophil indices (Table 39.2; Gerdes, 2004).

Platelet Counts

Approximately 25%–30% of infants exhibit thrombocytopenia at the time of diagnosis of sepsis, and the frequency increases during the course of infection. Accelerated platelet destruction and possibly

depressed production caused by bacterial products on the bone marrow are the underlying mechanisms for thrombocytopenia in infected infants. Disseminated intravascular coagulation may be seen in some infants with severe sepsis.

Acute-Phase Reactants and Erythrocyte Sedimentation Rate

A number of acute-phase reactants have been studied to help identify infants with likely sepsis. Most biochemical markers currently in use are derived from components of the complex inflammatory response to an invading pathogen. These markers have been studied during the past 20 years and continue to be investigated. The availability of tests for these inflammatory mediators and the value of these tests in assisting with the early diagnosis of neonatal sepsis are both concerns. It is known that the levels of some proinflammatory cytokines peak rapidly, within 1–4 hours after a sepsis stimulus (Lam and Ng, 2008). Among the early markers are the proinflammatory mediators IL-1 β , IL-6, the chemokine IL-8, TNF- α , and interferon gamma; these activate host defenses against bacterial and other infecting agents, whereas antiinflammatory mediators such as IL-4, IL-10, and transforming growth factor β 1 are important in regulating and limiting the inflammatory response, thus preventing an excessive reaction that could lead to organ damage and tissue cell death (Mehr and Doyle, 2000). C-reactive protein (CRP) is induced by proinflammatory cytokines (Hofer et al., 2012), and its level rises to a maximum at 12–24 hours. Procalcitonin (PCT) concentration rises at 4 hours, peaks at 6 hours, and plateaus 8–24 hours after a stimulus. The advantages of having a readily available surrogate marker for sepsis are apparent because of the limitations of WBC counts in predicting neonatal sepsis.

CRP is probably the most studied acute-phase reactant in neonatal sepsis (Weitkamp and Aschner, 2005; Lam and Ng, 2008; Hofer et al., 2012). Monitoring CRP levels has been widely promulgated as a way to diagnose neonatal infection and to adjust the duration of antibiotic therapy in infants with suspected versus proven sepsis (Philip and Mills, 2000; Gerdes, 2004). Depending on the laboratory, a CRP value of 1–8 mg/dL is considered the upper limit of normal; it is important to know the cutoff values in the laboratory supporting one's neonatal units. However, CRP concentration also rises physiologically during the 3 days after birth, and therefore use of serial CRP or higher than normal cutoff values may be more appropriate (Weitkamp and Aschner, 2005;

TABLE 39.2

Predictive Values of Components of the White Blood Cell Count and of C-Reactive Protein Concentration

| Laboratory Result | Sensitivity (%) | Specificity (%) | PREDICTIVE VALUE (%) | |
|--|-----------------|-----------------|----------------------|----------|
| | | | Positive | Negative |
| ANC <1750 cells/ μ L | 38–96 | 61–92 | 20–77 | 96–99 |
| ANC <10% (\approx 5580 cells/ μ L for term) | 48 | 73 | 4 | 98 |
| I/T ratio \geq 0.2 | 90–100 | 30–78 | 11–51 | 98 |
| I/T ratio \geq 0.3 | 35 | 89 | 7 | 98 |
| CRP level >1 mg/dL | 70–93 | 78–94 | 27 | 100 |

ANC, Absolute neutrophil count; CRP, C-reactive protein; I/T, immature neutrophil to total neutrophil ratio.

Modified from Gerdes JS. Diagnosis and management of bacterial infections in the neonate. *Pediatr Clin North Am.* 2004;51:939–959.

Hofer et al., 2012). Theoretically, results can be available within 30 minutes.

CRP is produced by the liver in response to stimulation by the proinflammatory cytokine IL-6, which is produced by both T and B cells (Weitekamp and Aschner, 2005; Lam and Ng, 2008). Because exposure of the host to bacterial products results in a substantial and rapid increase in IL-6 concentrations, it appears that IL-6 is potentially a more useful marker than CRP during the early phase of an infection. In one study, the IL-6 concentration had a sensitivity of 89%, versus 60% for CRP, at the onset of clinical suspicion of a neonatal infection (Lam and Ng, 2008). The negative predictive values for IL-6 are much higher than those for CRP (Lam and Ng, 2008), and this was also found for umbilical cord blood IL-6 levels, where the sensitivity of detection was high. This finding could be useful in deciding which infants do not require antibiotic therapy and those in whom antibiotic therapy can be discontinued after a relatively short course.

High-sensitivity CRP (hsCRP) measurement has been shown to provide increased sensitivity for detecting neonatal infection (Edgar et al., 2010). Not all diagnostic laboratories can provide hsCRP values in a timely fashion. In addition, the optimum diagnostic cutoff levels for CRP and hsCRP are debatable. Serum soluble intercellular adhesion molecule 1 (sICAM-1), hsCRP, soluble E-selectin (sE-selectin), and serum amyloid A, individually and in combination, have been studied for the diagnosis of sepsis in a neonatal intensive care unit (Edgar et al., 2010). In that study, all four measurements had some diagnostic value for neonatal infection; however, sICAM-1, hsCRP, and sE-selectin demonstrated the highest negative predictive value individually (sICAM-1, 84%; hsCRP, 79%; and sE-selectin, 74%) (Edgar et al., 2010). Use of a combination of these measurements enhanced the diagnostic value, with sensitivities of 90.3% and a negative predictive value of 91.3% (Edgar et al., 2010). However, the application of this set of diagnostic markers is not available for most facilities, and more investigative work is needed to confirm their role in excluding early-onset infection.

In another study, early markers (IL-6, TNF- α , IL-8, interferon gamma, CRP, IL-18, the antiinflammatory cytokine IL-10, the I/T ratio, and PCT, a later marker of infection) were studied for use in detection of early-onset sepsis in 123 newborns (Bender et al., 2008). The study concluded that IL-6 combined with PCT was a fair measure for evaluating early-onset infection and that the traditional I/T ratio was almost as efficient as IL-6. Combining an early marker such as IL-6 and an I/T ratio may reduce the number of diagnostic tests and inconclusive values. The combined value of IL-6 and PCT at the first blood drawing had a sensitivity of 71% and a specificity of 88% (Bender et al., 2008).

There are many studies of PCT in the literature, and most have concluded that PCT levels are superior to CRP levels in the early diagnosis of neonatal sepsis (Lam and Ng, 2008). PCT is the precursor of calcitonin, normally synthesized in the C cells of the thyroid gland. PCT is induced by systemic inflammation and bacterial sepsis and is produced by cells such as hepatocytes, nephrons, and monocytes. The physiologic function of PCT is unknown. In bacterial infections, plasma PCT concentrations increase from 0.001–0.01 ng/mL at the baseline to values ranging from 1–1000 ng/mL. PCT concentrations rise much faster than CRP concentrations (6–8 hours versus 48 hours for maximum levels). In healthy newborns, plasma PCT concentrations increase gradually after birth, reaching peak levels at approximately 24 hours of age (range 0.1–20 ng/mL) and then decrease to normal values of less than 0.5 ng/mL by 48–72 hours of age (Stocker

et al., 2010). Various studies on the use of PCT as a marker of neonatal sepsis have yielded contradictory results regarding its application to clinical decision making for both diagnosis and adjustment of the length of antibiotic therapy. In a study of 121 newborns with suspected early-onset sepsis, serial PCT concentration determinations allowed the duration of antibiotic therapy to be shortened (Stocker et al., 2010). PCT and IL-6 were also reported to be the best markers for sepsis in umbilical cord samples (Su et al., 2014). Larger studies are needed to determine the value of PCT in diagnosis and therapy.

To date, studies on the role of various cytokine determinations in assisting with diagnosis and treatment of early-onset sepsis are intriguing, but there has not been translation to widespread use because of a lack of clear efficacy. Other molecular technologies are also being studied to diagnose neonatal sepsis by the rapid identification and differentiation of gram-negative and gram-positive bacterial bloodstream infections. PCR, using universal bacterial primers, targets conserved regions of the 16S ribosomal RNA gene common to all bacteria but not found in other organisms (Dutta et al., 2009). In one study, universal primer PCR was performed in newborns with clinically suspected sepsis. PCR was performed before antibiotic therapy was started and was repeated at 12, 24, and 48 hours after drug therapy had started (Dutta et al., 2009). The sensitivity, specificity, and positive and negative predictive values of universal primer PCR were 96.2%, 96.3%, 87.7%, and 98.8%, respectively. The results of testing in two patients were blood culture positive but 0-hour PCR negative, and the results in seven patients were 0-hour PCR positive, but the blood culture result was negative. Of the patients with a 0-hour PCR-positive result, seven remained positive at 12 hours but none remained positive at 24 and 48 hours after starting antibiotic therapy. Although universal bacterial primer PCR may be a useful test for diagnosing an early episode of culture-proven sepsis, it cannot be used for diagnosis if the patient has been exposed to antibiotic therapy for 12 hours or more (Dutta et al., 2009). Again, larger studies are required before this assay can be recommended for routine clinical use in newborns suspected of having sepsis.

Prevention

Intrapartum Management of Parturients

The universal GBS screening strategy recommended by the CDC is performed at 35–37 weeks' gestation. Antepartum antibiotic therapy is recommended for women with a previous infant with invasive GBS disease, a positive GBS screening culture result during pregnancy, unknown GBS status (culture either not performed or incomplete or results unknown), and any of the following features: delivery before 37 weeks' gestation, rupture of chorioamniotic membranes for more than 18 hours, intrapartum temperature of 38°C or higher, and GBS bacteriuria during pregnancy.

Women who are not allergic to penicillin can be given penicillin G (5,000,000 units as a loading IV dose followed by 2.5 million units every 4 hours until delivery) or ampicillin (2 g IV as a loading dose followed by 1 g every 4 hours until delivery). For women who are allergic to penicillin, the recommendation is to determine, ideally during prenatal care, whether the patient is at high risk of anaphylaxis (i.e., history of immediate hypersensitivity reactions). Women who are not at high risk of anaphylaxis can receive cefazolin (2 g IV and 1 g IV every 8 hours until delivery). Women who are at high risk of anaphylaxis whose GBS isolate is not resistant to clindamycin or erythromycin can receive clindamycin (900 mg

IV every 8 hours until delivery) or erythromycin (500 mg IV every 6 hours until delivery) (Centers for Disease Control and Prevention, 2010). Women with clindamycin–erythromycin–resistant GBS isolates can receive vancomycin (1 g IV every 12 hours until delivery).

There were initial concerns about adverse effects of implementing the CDC consensus strategies, but these have proved unwarranted. The primary risks considered were maternal anaphylaxis from administered antibiotics, and these were unfounded. The possibility of the emergence of infections in mothers and infants caused by antibiotic-resistant organisms (e.g., *E. coli*) has also been addressed. In 2002 the Neonatal Network of the National Institute of Child Health and Human Development reported a change in the pathogens causing early-onset sepsis, with an increase in sepsis caused by *E. coli* from 3.2–6.8 per 1000 live births (Stoll et al., 2002). Most of the *E. coli* isolates were resistant to ampicillin. In another study comparing a period of no prophylaxis with periods of risk-based and universal screening–based prophylaxis, no change in the incidence of infection with ampicillin-resistant organisms was observed overall or among VLBW infants (Puopolo and Eichenwald, 2010). However, an increased proportion of infections was caused by ampicillin-resistant organisms. Mothers of infants with ampicillin-resistant infections were also more likely to have been treated with ampicillin (Stoll et al., 2002; Chen et al., 2005). A 10-year study of the effect of intrapartum antibiotic prophylaxis on GBS and *E. coli* sepsis in Australasia revealed a steady decline in the rate of early-onset GBS infection and a trend to decreasing incidence of early-onset *E. coli* sepsis in all infants and a stable rate for this infection in VLBW infants (Daley et al., 2004).

Intravenous Immune Globulin for Prevention of Early-Onset Sepsis

Premature infants are susceptible to early-onset sepsis because of diminished transplacental transfer of immunoglobulins and decreased synthesis of immunoglobulin G. An IV infusion of immune globulin will increase the typically low levels of serum immunoglobulin in preterm infants, and it has been proposed to improve immune function, which may lead to improved clinical outcome (Wynn et al., 2009). Clinical trials examining the possible benefit of intravenous immune globulin (IVIG) began more than 15 years ago (Haque et al., 1986; Chirico et al., 1987; Conway et al., 1990). A Cochrane metaanalysis showed no reduction in mortality or major disability at 2 years of age for newborns treated with IVIG for what were subsequently proved to be bacterial infections (mortality relative risk 0.95, 95% confidence interval 0.74–1.21; Ohlsson and Lacy, 2015). Currently available data do not support the suggested immunologic enhancements expected for IVIG in premature neonates.

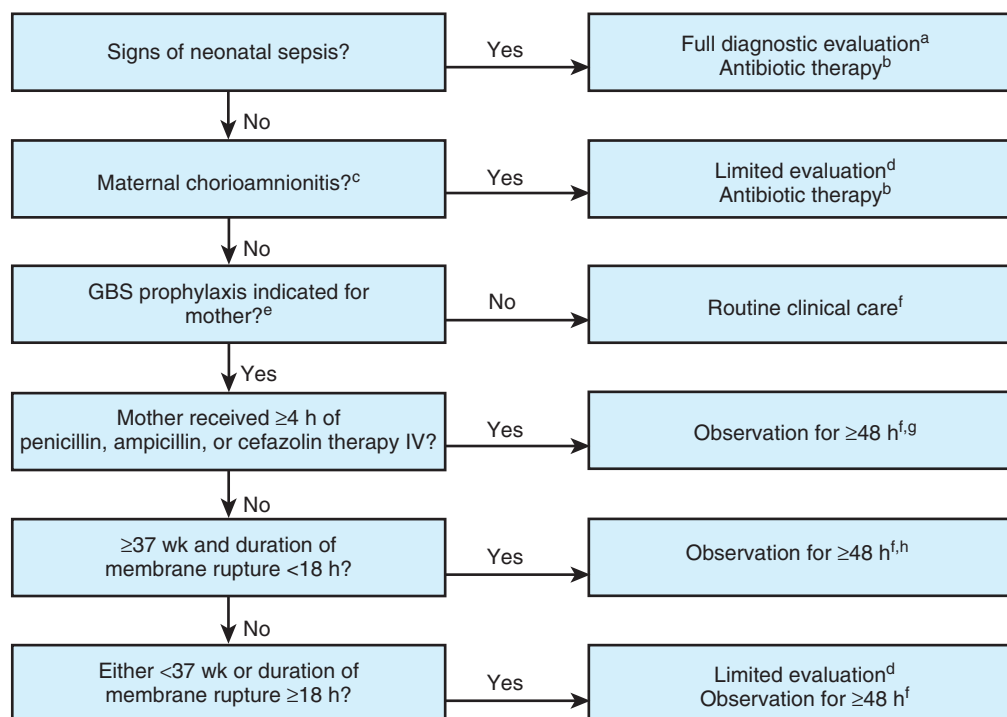
Other immunomodulating factors such as granulocyte colony-stimulating factor (G-CSF) have been studied to both prevent and treat neonatal sepsis. A Cochrane metaanalysis concluded that prophylaxis with G-CSF does not significantly reduce mortality in all infants, although in premature infants with neutropenia (ANC <1750) mortality may be reduced (Carr et al., 2009). It is possible that combination therapies using IVIG and other immunomodulating factors, such as granulocyte–macrophage colony-stimulating factor (GM-CSF), G-CSF, and complement-containing blood products, may lead to improved immune function in these highly susceptible infants; however, such studies are not currently being conducted.

Diagnostic Approach to Neonates With Suspected Sepsis

Obviously, all symptomatic newborns must be carefully evaluated for the possibility of bacterial sepsis and treated with antibiotics, if necessary. Although the presence of various risk factors should increase the suspicion of sepsis, the absence of risk factors in the symptomatic infant does not indicate that sepsis risk can be dismissed. In adjusting to postnatal life, some newborns exhibit abnormal signs transiently, such as tachypnea, before becoming asymptomatic. However, in any newborn who has other findings or is still symptomatic 6 hours after birth, a diagnostic evaluation with a complete blood cell count and differential, a blood culture, and, as appropriate, a lumbar puncture and a chest radiograph should be strongly considered. Antibiotic therapy can be stopped when the physical findings are normal, the clinical suspicion of sepsis is low, and the screening results for sepsis, including the blood culture results, remain negative. If the blood culture result is positive, the lumbar puncture findings abnormal, or there are clinical signs of sepsis, then the newborn should be treated with an appropriate course of antibiotics.

Treatment of the asymptomatic infant with risk factors for sepsis is much more controversial. The 2010 CDC guideline recommended that all infants born to mothers with a diagnosis of chorioamnionitis be evaluated for sepsis and treated with antibiotics for 48 hours (Fig. 39.2; Polin and Committee on Fetus and Newborn, 2012; Brady and Polin, 2013). One of the major difficulties in implementing this recommendation is a lack of standard definition of chorioamnionitis (Higgins et al., 2016). In addition, a number of analyses suggested that fewer infants would be treated with antibiotics if specific risk factors and the infant's clinical status are considered. Escobar et al. (2000) retrospectively reviewed 2785 newborns with birth weight greater than 2000 g and who were evaluated for sepsis after birth. Asymptomatic status was associated with a significantly decreased odds ratio for infection. Further analysis identified the most predictive factors for early-onset sepsis to be highest intrapartum maternal temperature, duration of rupture of membranes, gestational age, maternal GBS status, and intrapartum antibiotic use (Puopolo et al., 2011). Subsequent studies showed that following the CDC guidelines resulted in asymptomatic infants being treated with antibiotics for 48 hours or more with no clear evidence of benefit (Benitz et al., 2015). In addition, the use of ancillary tests such as the complete blood cell count and CRP concentration as predictors of sepsis in these asymptomatic infants resulted in many infants with negative culture results being treated with antibiotics for 5–7 days (Brady and Polin, 2013; Kiser et al., 2014; Polin et al., 2014).

Combining the objective risk factors found to be most highly associated with neonatal sepsis (Puopolo et al., 2011) with the newborn physical examination over the first 12 hours led to the development of a neonatal sepsis risk calculator to guide evaluation and therapy of the late preterm and term infant who has maternal risk factors for sepsis (Escobar et al., 2014). The current version of the neonatal early-onset sepsis calculator (<https://neonatalesepsiscalculator.kaiserpermanente.org/>) includes definitions of clinical status, including clinical illness, equivocal symptoms, and well appearing. Combining clinical status with maternal risk factors, the calculator provides a numerical estimate of the risk of sepsis and a clinical recommendation for management and monitoring. Use of the neonatal sepsis calculator has the benefit of reducing unnecessary antibiotic exposure and provides a model



• **Fig. 39.2** Secondary Prevention of Group B Streptococcus Disease. Algorithm for the prevention of early-onset group B streptococcus (GBS) infection in the newborn. *Superscript a* indicates full diagnostic evaluation including a blood culture; complete blood cell (CBC) count, including white blood cell differential and platelet counts; chest radiograph (if respiratory abnormalities are present); and lumbar puncture (if the patient is stable enough to tolerate the procedure and sepsis is suspected). *Superscript b* indicates antibiotic therapy should be directed toward the most common causes of neonatal sepsis, including intravenous ampicillin therapy for GBS infection and coverage for infection by other organisms (including *Escherichia coli* and other gram-negative pathogens) and should take into account local antibiotic-resistance patterns. *Superscript c* indicates consultation with obstetric providers is important in determining the level of clinical suspicion for chorioamnionitis. Chorioamnionitis is diagnosed clinically, and some of the signs are nonspecific. *Superscript d* indicates limited evaluation, including blood culture (at birth) and CBC count with differential and platelet counts (at birth and/or at 6–12 hours after birth). *Superscript e* indicates GBS prophylaxis is indicated if one or more of the following are true: (1) the mother is GBS-positive late in gestation and is not undergoing cesarean delivery before labor onset with intact amniotic membranes; (2) GBS status is unknown and there are one or more intrapartum risk factors, including less than 37 weeks' gestation, rupture of membranes for 18 hours or more, or temperature of 38.0°C or greater; (3) GBS bacteriuria during current pregnancy; or (4) history of an infant with GBS disease. *Superscript f* indicates that if signs of sepsis develop, a full diagnostic evaluation should be performed, and antibiotic therapy should be initiated. *Superscript g* indicates that if the stage is 37 weeks' gestation or later, observation may occur at home after 24 hours if other discharge criteria have been met, there is ready access to medical care, and a person who is able to comply fully with instructions for home observation will be present. If any of these conditions are not met, the newborn should be observed in the hospital for at least 48 hours and until discharge criteria have been achieved. *Superscript h* indicates that some experts recommend a CBC count with differential and platelet counts at 6–12 hours of age. IV, Intravenously; wk, weeks. (From Centers for Disease Control and Prevention. Prevention of perinatal group B streptococcal disease – revised guidelines from CDC. *MMWR Recomm Rep*. 2010;59(RR-10):1–36. Reproduced with permission from Brady MT, Polin RA. Prevention and management of infants with suspected or proven neonatal sepsis. *Pediatrics*. 2013;132:166–168. Copyright 2013 American Academy of Pediatrics.)

that can be updated as new data become available (Kuzniewicz et al., 2016).

In addition to the neonatal sepsis calculator, the National Institute of Child Health and Human Development has recently published the results of a workshop to provide evidence-based guidelines for diagnosis and management of pregnant women and their neonates at risk of infection because of the condition commonly called chorioamnionitis (Higgins et al., 2016). The workshop summary explicitly addressed the lack of a clinical definition of chorioamnionitis that caused wide variability in the use of this

diagnosis to encompass heterogeneous conditions involving both inflammation and infection. The panel recommends replacing the diagnosis of chorioamnionitis with a classification of “intrauterine infection or inflammation or both,” which it calls “Triple I.” The summary provides detailed definitions of maternal fever and other parameters and recommends dividing maternal fever into three categories: isolated fever, suspected Triple I, and confirmed Triple I. Suspected Triple I is defined as fever without a source combined with baseline fetal tachycardia, maternal WBC count greater than 15,000, and/or purulent fluid from the cervical os. Confirmed Triple

It requires symptoms compatible with suspected Triple I plus biologic or microbiologic amniotic fluid results consistent with microbial invasion of the amniotic cavity. The final recommendations of the workshop included no empiric treatment of the “well-appearing” neonate born at more than 33 completed weeks of gestation, and use of the neonatal sepsis calculator to help with decisions about treatment is mentioned (Higgins et al., 2016).

Recent CDC and American Academy of Pediatrics (AAP) guidelines for the prevention of early-onset neonatal GBS sepsis have a different approach to management of the at-risk asymptomatic infant (see Fig. 39.2; Centers for Disease Control and Prevention, 2010; Brady and Polin, 2013). If there are signs of sepsis in the newborn, then a full sepsis work-up and antibiotic therapy are recommended. If there is maternal chorioamnionitis, a limited sepsis evaluation and antibiotic therapy are recommended. In the asymptomatic infant with a negative blood culture result, consideration should be given to stopping antibiotic therapy after 48–72 hours, even if screening test results were abnormal (Polin et al., 2014). For infants whose mother received antibiotic therapy for GBS positive status for more than 4 hours, it is recommended that the infant be observed in the hospital for 24–48 hours. For infants whose mother did not receive antibiotic prophylaxis for more than 4 hours, a 48-hour in-hospital observation is recommended. If the infant was born at less than 37 weeks’ gestation, or the duration of rupture of membranes is greater than or equal to 18 hours, then a limited evaluation and observation in the hospital for approximately 48 hours are recommended. The CDC and AAP guidelines advocate wider antibiotic use and longer in-hospital observation for asymptomatic newborns than the risk-based neonatal sepsis calculator described earlier and are likely to be revised soon. The reader is advised to look for the most up-to-date CDC and AAP guidelines.

Treatment

Antimicrobial Therapy

The choice of antibiotic for an infant with suspected early-onset sepsis depends on the predominant bacterial pathogens and the antibiotic susceptibility profiles for the microorganisms causing early-onset disease in a particular geographic region. Any decision to discontinue antimicrobial therapy should be based on the level of suspicion for sepsis at the time treatment was begun, the culture results, laboratory test results, and the clinical behavior and course of the infant. If sepsis is highly suspected in an infant, antibiotics should be considered for a full course even if the culture results are negative.

Empiric therapy for early-onset sepsis generally consists of combinations of antibiotics effective against gram-positive pathogens (e.g., GBS, *L. monocytogenes*) and gram-negative pathogens (e.g., *E. coli*). The two most commonly used combinations are (1) ampicillin with an aminoglycoside, usually gentamicin, and (2) ampicillin with a third-generation cephalosporin, usually cefotaxime. Cefotaxime has minimal toxicity and is well tolerated by newborns. However, the third-generation cephalosporins, as well as vancomycin, a glycopeptide antibiotic, have been associated with the development of vancomycin-resistant enterococci and, in the case of cephalosporins, with the induction of various β -lactamase-producing gram-negative bacteria, including extended-spectrum β -lactamase-producing organisms (de Man et al., 2000). These latter bacteria are resistant to all β -lactam antibiotics and are frequently resistant to other antibiotics, but not to meropenem. Another disadvantage

of the cephalosporin antibiotics is the lack of effectiveness against enterococci or *L. monocytogenes*. *L. monocytogenes* is usually treated with ampicillin and an aminoglycoside until the blood culture result is negative and the infant has shown an improved outcome. In infants with bacteremia and sepsis caused by GBS, gentamicin is frequently combined with ampicillin or penicillin, although there are no data to suggest that the addition of the aminoglycoside improves outcome. However, it is common practice to use the combination of these two drugs during the first few days of therapy and then to continue the full course of therapy with ampicillin or penicillin alone.

When the likelihood of infection is very low, the antibiotic therapy should be stopped. In most hospitals using modern blood culture instrumentation, 48 hours is sufficient to determine whether a blood culture result is negative, assuming that no antibiotics were being given when the culture was obtained (García-Prats et al., 2000; Jardine et al., 2006). Blood culture bottles with antimicrobial binding resins are in common use in microbiology laboratories, enhancing the ability to demonstrate positive blood culture results. Infants with proven bacteremia, but without meningitis, are commonly treated for 7–10 days. The use of antibiotics with nephrotoxicity (i.e., aminoglycosides) should be monitored with the use of appropriate drug levels.

Immunologic Therapies for Early-Onset Sepsis

Various adjunctive therapies have been proposed to improve the immune status of the newborn in an attempt to reduce neonatal sepsis mortality. These included IVIG, granulocyte transfusions, and G-CSF or GM-CSF treatment. Overall there are insufficient data to recommend the routine use of any of these therapies for the treatment of sepsis in newborns. Mortality in cases of subsequently proven infection was not reduced in the IVIG-treated newborns (Ohlsson and Lacy, 2015), and clinical studies also did not show that granulocyte transfusions provided a significant benefit to neonates with culture-proven early-onset infections (Vamvakas and Pineda, 1996).

Use of GM-CSF and G-CSF to enhance the quantity and quality of neutrophils has also been studied in human neonates (Carr et al., 2009). Although these agents have induced circulating numbers of neutrophils and appeared to be relatively safe, they have not significantly reduced neonatal sepsis mortality (Wynn et al., 2009). In two metaanalyses, subgroup analysis showed a significant reduction in mortality in the group of infants with systemic infection and neutropenia (Bernstein et al., 2001; Carr et al., 2009). Future studies may prove that selective use of these therapies is beneficial in specific subgroups of septic infants.

Neonatal Bacterial Meningitis

Neonatal bacterial meningitis is ominous because of the associated mortality and morbidity. Meningitis is associated with the same pathogens that cause bacterial sepsis, with GBS and *E. coli* accounting for approximately 70% of all cases and *L. monocytogenes* accounting for an additional 5% in the first week of life. On occasion, it is possible to isolate *S. pneumoniae* and *H. influenzae*, and in newborns who are older than 1 week residing in neonatal intensive care units, coagulase-negative staphylococci are the most common isolates. The underlying pathogenesis of bacterial meningitis is a seeding of the meninges during a bacteremic phase in the newborn. Studies in neonatal rats have shown that high-grade

bacteremia is more likely than low-grade bacteremia to lead to bacterial meningitis.

GBS meningitis (with mortality approaching 30% and morbidity of 50%) usually presents as late-onset disease, and the most common GBS serotype identified is III (Levent et al., 2010). Ansong et al. (2009) reported that GBS meningitis complicated 22 of 145 episodes (15%) of early-onset GBS sepsis and 13 of 23 episodes (57%) of late-onset GBS sepsis. GBS meningitis can occur in the presence of negative blood culture results, and 20% of infants in the study had negative blood culture results (Ansong et al., 2009).

Approximately 80% of all serotypes of *E. coli* that cause meningitis in newborns possess the K1 or capsular antigen. The K1 capsular polysaccharide antigen is considered one of the primary virulence factors of this capsular type of *E. coli*, because antibody against K1 antigen has been shown to be protective in neonatal rat models of infection. Mortality rates for neonatal *E. coli* meningitis range from 20%–30% in some centers and from 50%–60% in others (Dodge, 1994).

Pathology and Clinical Manifestations

At autopsy, infants who die of meningitis have purulent exudates of the meninges and the surfaces of the ventricles associated with inflammation. Historically, hydrocephalus and a noninfectious encephalopathy were demonstrated in approximately 50% of infants who died of bacterial meningitis.

The signs and symptoms of neonatal meningitis are not easy to distinguish from those of sepsis. The most common presenting symptoms are lethargy, feeding problems, instability of temperature regulation, vomiting, respiratory distress, and apnea. A bulging fontanel may be seen, but this is usually a late manifestation. Seizures are frequently observed and can be caused by either direct central nervous system inflammation or by metabolic abnormalities such as hypoglycemia or hyponatremia.

Diagnosis

The gold standard for diagnosis of meningitis is the analysis of the CSF, including the WBC count, glucose and protein levels, Gram stain, and culture. The interpretation of CSF cell counts in newborns may be difficult (Garges et al., 2006; Greenberg et al., 2008). During the first week of life the CSF WBC count slowly decreases in term newborns but may remain high or even increase in premature newborns. There is no change in CSF WBC counts or protein content with gestational age, but there is a significant decrease with postnatal age (Mhanna et al., 2008). The 95th percentile for CSF WBC count is reported to be 19/ μ L, but individual infants with no proven infection had higher WBC counts ranging from 75–100/ μ L (Ahmed et al., 1996; Kestenbaum et al., 2010). The cell counts, protein concentrations, and glucose concentrations from healthy infants may overlap with those from infants with meningitis, and 1%–10% of infants with proven meningitis have normal results on CSF analysis (Hristeva et al., 1993; Garges et al., 2006; Greenberg et al., 2008). Finally, approximately 30% of all infants with positive results of CSF cultures for bacteria have negative blood culture results (Wiswell et al., 1995; Garges et al., 2006).

A Gram stain of CSF must be examined carefully for every infant with suspected meningitis. The stains for approximately 20% of newborns with proven meningitis are reported as showing “no bacteria seen.” Although an increase is expected in the number of neutrophils with bacterial meningitis, one may see a predominance

of lymphocytes within a conversion to PMNs. With *L. monocytogenes*, a mononuclear cellular response is found on examination of the CSF. In clinical care units, it is routine to repeat the CSF examination and culture 2–3 days after the initiation of antibiotic therapy. This examination is especially important if the patient has not responded clinically and is experiencing seizures or continued fever. At times it is difficult to eradicate the organism from the CSF, and consideration can be given to examining the inhibitory and bactericidal concentrations in CSF. It is especially important to repeat the CSF examination before antibiotic therapy is stopped in patients with more complicated courses and for enteric gram-negative bacterial meningitis.

Therapy

Infants with bacterial meningitis are frequently ill and should be monitored in intensive care units, where the critical needs can be met with aggressive management. These patients may require mechanical ventilation, complex fluid management to attenuate the effects of cerebral edema and effects of secretion of inappropriate antidiuretic hormone, seizure control, vasopressor support, and cardiopulmonary monitoring. The choice of appropriate antibiotic therapy is based on several factors, including the achievable CSF levels of drugs that have in vitro efficacy against the microorganism. In the case of gram-positive bacteria, the use of penicillin and ampicillin will achieve 10–100-fold higher concentrations than the minimal inhibitory concentrations needed to inhibit the bacteria, and there is rapid sterilization of the CSF. In contrast, aminoglycosides, such as gentamicin and tobramycin, achieve only 40% of peak serum levels and may not achieve minimal inhibitory concentrations more than those equal to or slightly greater than found in vitro for gram-negative bacteria.

In many intensive care units, ampicillin and gentamicin are recommended as initial therapy for neonatal meningitis. An alternative regimen of ampicillin and cefotaxime can also be used, recognizing the potential for the introduction of cephalosporin-resistant gram-negative isolates in the unit. Although used in the past, neither intrathecal nor intraventricular administration of antibiotics has been found to reduce the morbidity or mortality associated with gram-negative meningitis (Shah et al., 2008). Once the microorganism has been identified and the antibiotic susceptibility results are available, either a single drug or a combination of drugs found to be effective in vitro should be used. Usually penicillin or ampicillin is used for GBS meningitis; in the first few days of therapy, dual therapy with an aminoglycoside may be used. It is common to treat *L. monocytogenes* infection with ampicillin with or without gentamicin. Ampicillin or cefotaxime with or without an aminoglycoside can be used for infection with gram-negative enteric bacteria. The precise length of antibiotic therapy depends on the rapidity of response and sterilization of CSF. In general, therapy should be continued for approximately 2 weeks after sterilization of the CSF or a minimum of 2 weeks for gram-positive meningitis and a minimum of 3 weeks for gram-negative meningitis. In difficult situations, therapy may be required for as long as 4–6 weeks. A Cochrane review of two trials of adjuvant corticosteroids in neonatal bacterial meningitis suggested a reduction in death and hearing loss with steroid therapy, but there was no reduction in neurologic sequelae at 10 weeks. Both trials were small, and further study is necessary before steroid use can be routinely recommended (Ogunlesi et al., 2015).

It may be prudent to repeat a CSF examination and culture after initiation of therapy, especially if the clinical response is less

than satisfactory. If organisms are seen on gram-stained smears of the fluid, modification of the therapeutic regimen should be considered. In general, approximately 3 days or more is required for an antibiotic regimen to sterilize the CSF in infants with gram-negative meningitis. In infants with gram-positive meningitis, sterilization is usually seen within 36–48 hours. Neuroimaging should be considered to exclude parameningeal foci and abscess formation and to assist in assessing the infant's prognosis.

Prognosis

Complications from neonatal meningitis include brain abscess, communicating or noncommunicating hydrocephalus, subdural effusions, ventriculitis, deafness, and blindness. Generally, the severity of complications is related to the severity of the disease during the early neonatal period. It is imperative to follow hearing competency and to examine these infants for prolonged periods after recovery. The infant who has experienced meningitis may appear relatively healthy at the time of discharge, and only after careful follow-up do perceptual difficulties, reading problems, or signs of brain damage become apparent. Approximately 40%–50% of survivors have some evidence of neurologic damage, with severe damage being obvious in 11%. Infants who survive neonatal meningitis should have regular audiology, language, and neurologic evaluations until they enter school (Edwards et al., 1985; Stevens et al., 2003).

Suggested Readings

- Ansong AK, Smith PB, Benjamin DK, et al. Group B streptococcal meningitis: cerebrospinal fluid parameters in the era of intrapartum antibiotic prophylaxis. *Early Hum Dev.* 2009;85:S5-S7.
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Health Care-Associated Infections

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KEY POINTS

- Health care–associated infections (HAIs) are an important preventable cause of morbidity and death in neonates.
- Central line–associated bloodstream infections cause the bulk of neonatal intensive care infections; other HAIs include ventilator-associated pneumonias, urinary tract infections, skin and soft tissue infections, and nosocomial viral infections.
- Several modifiable risk factors have been identified for these conditions. Preventive strategies must focus on basic hand hygiene and infection control measures, along with specific measures for the infection.
- In recent years quality improvement collaboratives have achieved great strides in infection reduction by developing improved surveillance and benchmarking strategies and implementation of bundles of preventive measures.

Health care–associated infections (HAIs) are infections acquired by patients during hospitalization. For decades HAIs have been considered an unavoidable problem in the neonatal intensive care unit (NICU). Several factors converge to create a high risk of infection in the NICU patient: immature innate and adaptive immune defenses, the need for life-sustaining invasive interventions, broad-spectrum antibiotic exposure, and prolonged hospital stays associated with colonization with potentially pathogenic microorganisms (Stoll et al., 2002; Edwards et al., 2009; Polin et al., 2012b). The growing frequency of surviving very low birth weight (VLBW) infants and the use of invasive technologies magnify the impact of this problem. HAIs have been shown to significantly and independently impact neonatal outcomes, including death, short-term and long-term morbidities, hospital length of stay, and healthcare costs (Payne et al., 2004; Stoll et al., 2004a; Adams-Chapman et al., 2006; Shah et al., 2008; Bassler et al., 2009; Schlapbach et al., 2011; de Haan et al., 2013; Donovan et al., 2013; Mitha et al., 2013).

Clinicians can minimize HAIs by consistently and reliably following best practices for infection prevention and minimizing invasive procedures to the extent possible. Continuous surveillance

and monitoring of HAI rates and the patterns of the pathogens responsible are necessary to establish reference points in each nursery and facilitate early identification of epidemics. Prevention of infections in neonates requires a modified and expanded set of measures in addition to organized hospital-wide initiatives. Quality improvement strategies, standardization of practice, and hospital programs such as antimicrobial stewardship are all essential to preventing harm. In recent years many centers have used these strategies to reduce the burden of HAIs, some with great success (Andersen et al., 2005; Schulman et al., 2011; Wirtschafter et al., 2011; Payne et al., 2012; Fisher et al., 2013; Shepherd et al., 2015). In this chapter we will review the data sources, definitions, epidemiology, and adverse outcomes related to HAI, as well as current concepts and evidence surrounding strategies for prevention of HAI in NICUs.

Health Care–Associated Infection Surveillance and Data Sources

Sources of data on neonatal HAIs in the United States include the Centers for Disease Control and Prevention (CDC), the National Healthcare Safety Network (NHSN), the Vermont Oxford Network (VON), the National Institute for Child Health and Human Development (NICHD), Neonatal Research Network (NRN), and the Children's Hospitals Neonatal Database (Polin et al., 2012b). Reporting overall incidence rates may be misleading because of wide variations in practice and patient populations; therefore the CDC NHSN system, developed in 2005, monitors device-associated HAI rates by using an approach that accounts for variability in device use and length of hospital stay (Emori et al., 1991; Gaynes et al., 1996; Edwards et al., 2008). These data are also stratified by birthweight categories and expressed as incidence density per 100 or 1000 patient or device days. These adjustments modify the relative risk (RR) on the basis of the severity of the illness and the duration of exposure to the risk factor. NHSN tracks HAIs in more than 17,000 medical facilities and shares the data with the facilities, the CDC, and other partners and quality improvement organizations to help guide efforts at infection prevention. Outside of the United States the International Nosocomial Infection Control Consortium runs a surveillance network for HAIs, using CDC

NHSN definitions, in more than 1000 hospitals around the world, and provides aggregate data on HAI from several countries (Rosenthal, 2016).

Defining and Diagnosing Neonatal Health Care–Associated Infection

The CDC provides the following surveillance definition for HAI: illness associated with a pathogen or its toxins that is not present or incubating at the time of admission (Horan et al., 2008). Although this definition appears straightforward, it leaves some room for confusion in neonates. Infections that manifest themselves early in the first week of life are typically related to perinatal risk factors and vertical transmission from the mother. HAIs are more often related to patient colonization and environmental risk factors. While most sources define HAIs in neonates as infections occurring after 3 days of postnatal life (Baltimore, 1998; Stoll et al., 2002), there is no specific age that clearly distinguishes maternally transmitted infections from HAIs (Baltimore, 1998); there may be substantial temporal overlap between HAIs and late-onset, perinatally acquired infections, such as group B streptococcus, for example. The CDC NHSN currently defines HAIs in neonates as those infections initially identified on day 3 or later and thus includes infections

that may have been acquired perinatally (Horan et al., 2008). Therefore neonatal HAI rates may be slightly overestimated during the first few weeks of life.

Definitions of Central Line–Associated Bloodstream Infection, Ventilator–Associated Pneumonia, and Catheter–Associated Urinary Tract Infection

Definitions of central line–associated bloodstream infection (CLABSI), ventilator-associated pneumonia (VAP), and catheter-associated urinary tract infection (UTI) are given in Box 40.1.

Challenges With Central Line–Associated Bloodstream Infection Diagnosis

While coagulase-negative staphylococci (CoNS) are common skin commensals, they are also the most common cause of CLABSIs in the NICU. Differentiating contamination from true infections with these pathogens is one of the greatest challenges surrounding neonatal CLABSI definitions. The CDC introduced a change to its definition in 2008, namely, the requirement for two blood cultures positive for skin commensals to fulfill the definition of a CLABSI

• BOX 40.1 Definitions of Health Care–Acquired Infections for Patients Younger Than 12 Months (Centers for Disease Control and Prevention National Healthcare Safety Network)

Nosocomial Bloodstream Infections

Laboratory-confirmed bloodstream infection (LCBI). Must meet one of the following definitions:

- Recognized pathogen in one or more blood specimens (culture or nonculture based microbiologic methods), performed for clinical diagnostic or therapeutic purposes *and* not related to infection at another site
- Commensal organism (e.g., coagulase-negative staphylococci, diphtheroids, bacillus, viridans streptococci, aerococcus, micrococcus, propionibacterium), identified from two or more blood specimens obtained on separate instances (culture or nonculture based microbiologic methods), performed for clinical diagnostic or therapeutic purposes *and* not related to infection at another site *and* at least one of the following signs: (1) fever (temperature $>38.0^{\circ}\text{C}$), (2) hypothermia (temperature $<36.0^{\circ}\text{C}$), or (3) apnea or bradycardia

Central line–associated bloodstream infection (CLABSI):

- LCBI (as defined above) *and*
- Central line or umbilical catheter in place for more than 2 days *and*
- Central line in place on day of or day before CLABSI diagnosis

Pneumonia:

- Two or more serial chest radiographs with new/progressive and persistent infiltrate, cavitation, consolidation, or pneumatoceles for patients with underlying pulmonary or cardiac disease (respiratory distress syndrome, bronchopulmonary dysplasia, pulmonary edema) *or* one chest radiograph with the aforementioned abnormalities for patients without underlying pulmonary or cardiac disease *and*
- Worsening gas exchange *and*
- At least three of the following: (1) temperature instability, (2) white blood cell count less than $4000/\mu\text{L}$ or greater than $15,000/\mu\text{L}$ with

10% or more bands, (3) new-onset purulent sputum, change in character of sputum, increased respiratory secretions, or increased suctioning requirements, (4) physical examination findings consistent with increased work of breathing or apnea, wheezing, rales, or rhonchi, (5) cough, (6) bradycardia (<100 bpm), and (7) tachycardia (>170 bpm)

Ventilator-associated pneumonia (VAP):

- Pneumonia (as defined above) *and*
- Patient on ventilator for more than 2 days *and*
- Ventilator in place on day of or day before VAP diagnosis

Urinary Tract Infection

Symptomatic urinary tract infection (SUTI):

- At least one of the following symptoms: (1) fever (temperature $>38.0^{\circ}\text{C}$), (2) hypothermia (temperature $<36.0^{\circ}\text{C}$), (3) apnea, (4) bradycardia, (5) lethargy, (6) vomiting, or (7) suprapubic tenderness *and*
- Urine culture with no more than two species identified, at least one of which is present at more than 10^5 CFU/mL

Asymptomatic bacteremic urinary tract infection (ABUTI):

- Urine culture with no more than two species identified, at least one of which is present at more than 10^5 CFU/mL *and*
- Bacteria identified in blood (culture-based or nonculture-based microbiologic method) that matches at least one of the bacteria present at more than 10^5 CFU/mL in urine

Catheter–associated urinary tract infection:

- Urinary tract infection (as defined above, either SUTI or ABUTI) *and*
- Indwelling urinary catheter for more than 2 days *and*
- Urinary catheter in place on day of or day before urinary tract infection diagnosis

bpm, Beats per minute; CFU, colony-forming unit.

Modified from Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control. 2008;36:309–332.

(Horan et al., 2008). However, the Children's Hospitals Neonatal Database and the VON and NICHD databases include infants with only one culture positive for CoNS in the presence of clinical signs of infection (Polin et al., 2012b). Further, despite clinical practice guidelines recommending the drawing of peripheral and central line cultures, clinicians in the NICU often only obtain a single culture because of various constraints. Also, further refinements of CLABSI definitions (in terms of the timing of infection following hospital admission, line placement, etc.) may impact the number of CLABSIs reported (Farrell et al., 2015; Hazamy et al., 2015). Another area of uncertainty surrounds whether some CLABSIs in infants with underlying gastrointestinal (GI) conditions may result from translocation caused by mucosal barrier injury; studies to date have not fully borne out this hypothesis (see [Risk Factors for Development of Health Care–Associated Infection](#)) (Coffin et al., 2014). Recent studies have demonstrated that International Classification of Diseases, Ninth Revision (ICD-9) coding of hospital administrative data has low concordance with NHSN definitions of CLABSI (Moehring et al., 2013; Patrick et al., 2013). As the Centers for Medicare and Medicaid Services uses ICD-9 codes for case finding, this discrepancy highlights definitional challenges surrounding the policy of the Centers for Medicare and Medicaid Services of reduced payment or nonpayment for hospital-acquired conditions such as CLABSI.

Challenges With Ventilator-Associated Pneumonia Diagnosis

VAP definitions were developed for surveillance purposes, but they are problematic to apply in neonates, as they have not truly been validated as clinical diagnostic criteria (Baltimore, 2003; Garland, 2010; Polin et al., 2012b). Overlap of signs and symptoms and radiographic findings with underlying respiratory conditions poses significant challenges to the diagnosis of VAP in neonates and may lead to overdiagnosis (Baltimore, 2003; Garland, 2010; Polin et al., 2012b). Moreover, microbiologic testing such as respiratory cultures does not reliably distinguish bacteria colonizing the respiratory tract from true infections. Gram stain of respiratory secretions may demonstrate an inflammatory infiltrate with neutrophils, but this may indicate a tracheitis or pneumonia (Salata et al., 1987). If the Gram stain and culture identify the same organism, this strengthens the likelihood of its causal role in the VAP (Salata et al., 1987). Finally, it is challenging to obtain true samples of lower respiratory tract secretions from infants (Barzilay et al., 1988; Allen et al., 1994). Because of these challenges with defining VAP accurately in the neonatal population, in 2014 the NHSN discontinued accepting and analyzing VAP identified in the NICU. However, many NICUs and collaboratives continue surveillance and internal benchmarking of this condition.

Challenges With Urinary Tract Infection Diagnosis

Clinicians often use less stringent microbiologic criteria (ranging from 100 to 10,000 colony-forming units [CFUs] of bacteria per milliliter in urine) for neonatal UTI diagnosis compared with CDC definitions (Bauer et al., 2003; Levy et al., 2009; Clarke et al., 2010). CoNS may be isolated in urine samples, raising the question (similar to bloodstream infections) of whether they represent contamination or true infection (Clarke et al., 2010; Foglia et al., 2012).

Epidemiology of Health Care–Associated Infection

Health Care–Associated Infection Epidemiology in the Newborn Nursery

Accurate rates of HAI in the “well baby” nursery are difficult to ascertain because there is no systematic reporting or surveillance network for this location; however, the incidence appears to be low. Some researchers estimate rates of less than 1 per 100 patients discharged (Baltimore, 1998). Typical risk factors for acquiring an HAI are uncommon in this population. Discharge from the hospital within 48 hours of birth, “baby-friendly” hospital policies, and rooming-in practices have helped to further decrease the risk of exposure in modern mother–baby units. However, early discharge may also limit surveillance efforts and documented rates of HAI, as patients may be discharged home before they become symptomatic from HAIs (Goldmann, 1989).

HAIs in the well baby nursery are commonly superficial, involving the skin, mouth, or eyes, and include omphalitis, pustules, abscesses, and bullous impetigo (Goldmann, 1989). In recent years, community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) has been implicated in outbreaks of skin and soft tissue infections (SSTIs) and pneumonia in newborn nurseries (Alsubaie et al., 2012; Huang et al., 2012; Filleron et al., 2013; Sanchini et al., 2013; Lee et al., 2014). Legionella outbreaks have been reported in term neonates, because of nosocomial contamination from water sources (water births, humidifiers) (Franzin et al., 2001; Yiallourous et al., 2013). Nursery epidemics of other bacterial enteropathogens and viral enteropathogens have been reported but occur infrequently in the United States in the current era (Goldmann, 1989; Syriopoulou et al., 2002; Fuchs et al., 2013).

Health Care–Associated Infection Epidemiology in the Neonatal Intensive Care Unit

Most neonatal HAIs occur in term and preterm infants hospitalized in NICUs. Comparison of surveillance data between institutions needs to be carefully performed because of differences in patient demographics and the use of different definitions for HAI.

Data from the NICHD NRN in 2002 demonstrated that 21% of VLBW infants who survived beyond 3 days postnatally had at least one episode of late-onset sepsis (Stoll et al., 2002). There was significant variability among the participating centers, with rates ranging from 10.6% to 31.7% at individual sites (Stoll et al., 2002). In another study, 19% of VLBW infants had HAIs, with the highest rates in infants with the lowest birthweights (Brodie et al., 2000). A point prevalence survey conducted by 29 level II to level IV nurseries participating in the Pediatric Prevention Network revealed a prevalence of 11.4% for HAI (Sohn et al., 2001). Recent data from the NICHD NRN showed that the rates of late-onset sepsis declined from 2005 to 2012 among VLBW infants of all gestational ages: the rates for infants born at 24 weeks' gestation declined from 54% to 40% (adjusted RR 0.94, 95% confidence interval [CI] 0.93–0.95), for infants born at 26 weeks' gestation the rates declined from 37% to 27% (adjusted RR 0.93, 95% CI 0.92–0.94), and for infants born at 28 weeks' gestation the rates declined from 20% to 8% (adjusted RR 0.91, 95% CI 0.90–0.92) (Stoll et al., 2015).

CLABSIs constitute most of the HAIs in the NICU (Gaynes et al., 1996; Sohn et al., 2001; Dudeck et al., 2013). The remaining cases involve the respiratory tract, eye, ear, nose, throat, GI tract, or urinary tract or may be SSTIs. VAPs are difficult to diagnose in the NICU but can contribute to morbidity, especially in patients with evolving lung disease. Although the use of bladder catheters is low in the NICU compared with other intensive care units, the acquisition of UTIs while the neonate is in the NICU is still a problem. Surveillance data for meningitis are limited. There are widespread differences in clinical practice regarding the inclusion of a lumbar puncture with cerebrospinal fluid analysis in the evaluation for possible neonatal sepsis. While early-onset meningitis is rare, studies suggest that late-onset meningitis may be underdiagnosed in the high-risk population of VLBW infants (Stoll et al., 2004b, 2011).

Rates of device-associated infections in NICUs in the United States are presented in Table 40.1. These rates remained constant in the 1990s (Gaynes et al., 1996; Centers for Disease Control and Prevention, 2000) but appear to have dropped in the late years of the first decade of this century, likely because of the impact of HAI preventive collaborative quality improvement efforts (see Prevention of Health Care–Associated Infection) (Edwards et al., 2009; Schulman et al., 2011; Wirtschafter et al., 2011; Hocevar et al., 2012; Payne et al., 2012; Dudeck et al., 2013; Patrick et al., 2014). While it is encouraging to note that the CLABSI rate decreased from 4.9 to 1.5 per 1000 central line days across 173

US hospitals that provided data to NHSN from 2007 to 2012 (Fig. 40.1), CLABSIs continue to contribute to 25%–60% of HAIs in the neonatal population (Dudeck et al., 2013; Patrick et al., 2014). In addition to the impact of collaborative quality improvement work in NICUs across the United States, mandated reporting of HAIs (which has been implemented in several states) may be influencing HAI rates. A study examining the impact of these mandates found that CLABSI rates decreased in infants weighing less than 750 g in states with mandated reporting but revealed no impact on overall NICU CLABSI rates (Zachariah et al., 2014). VAP rates also decreased from 1.6 to 0.6 per 1000 ventilator days from 2007 to 2012 (see Fig. 40.1) (Patrick et al., 2014). While catheter-associated UTIs are not very frequent in the NICU, non-catheter-associated UTIs remain a common cause of infections and need for antibiotic treatment in the NICU (Shaikh et al., 2008). SSTIs, on the other hand, appear to be on an increasing trend in both inpatient and emergency settings, likely because of the increasing prevalence in both healthcare and community settings of MRSA, the most common etiologic agent of SSTIs (Hester et al., 2015).

The International Nosocomial Infection Control Consortium published surveillance data from 2007 to 2012 from 503 NICUs in Africa, Asia, Europe, and Latin America (Rosenthal et al., 2014). On examination of the available NICU data, it was concluded that the rates of CLABSI and VAP were generally higher than the corresponding CDC NHSN rates in the United States (Table 40.1;

TABLE 40.1

Health Care–Associated Infection Rates in the United States and Internationally

| CLABSI RATES FOR LEVEL III NICUS IN THE UNITED STATES, 2012 (CDC NHSN) | | | | | |
|--|-------------------------------|------------------|-------------------|-------------------------|--------------------------------------|
| Birthweight Category (g) | No. of Locations ^b | No. of CLABSIs | Central Line Days | Pooled Mean CLABSI Rate | |
| ≤750 | 380 (334) | 420 | 185,851 | 2.3 | |
| 751–1000 | 401 (339) | 256 | 160,230 | 1.6 | |
| 1001–1500 | 418 (370) | 195 | 172,732 | 1.1 | |
| 1501–2500 | 415 (338) | 104 | 161,361 | 0.6 | |
| >2500 | 422 (322) | 136 | 176,853 | 0.8 | |
| VAP RATES FOR LEVEL III NICUS IN THE UNITED STATES, 2012 (CDC NHSN) | | | | | |
| Birthweight Category (g) | No. of Locations ^b | No. of VAP Cases | Ventilator Days | Pooled Mean VAP Rate | |
| ≤750 | 157 (133) | 97 | 73,987 | 1.3 | |
| 751–1000 | 163 (123) | 47 | 39,689 | 1.2 | |
| 1001–1500 | 167 (95) | 14 | 22,701 | 0.6 | |
| 1501–2500 | 165 (83) | 4 | 20,945 | 0.2 | |
| >2500 | 167 (87) | 10 | 30,305 | 0.3 | |
| CLABSI RATES FOR LEVEL III NICUS INTERNATIONALLY, 2007–12 (INICC) | | | | | |
| Birthweight Category (g) | No. of NICUs | No. of Patients | No. of CLABSI | Central Line Days | Pooled Mean CLABSI Rate ^a |
| ≤750 | 17 | 268 | 7 | 1744 | 4.01 (1.6–8.3) |
| 750–1000 | 31 | 1295 | 60 | 8493 | 7.06 (5.4–9.1) |
| 1001–1500 | 36 | 2408 | 65 | 12,435 | 5.23 (4.0–6.7) |
| 1501–2500 | 37 | 5849 | 67 | 13,923 | 4.81 (3.7–6.1) |
| >2500 | 37 | 6453 | 45 | 10,563 | 4.26 (3.1–5.7) |
| Pooled | 38 | 16,273 | 244 | 47,158 | 5.17 (4.5–5.9) |

Continued

TABLE 40.1**Health Care–Associated Infection Rates in the United States and Internationally—cont'd**

| VAP RATES FOR LEVEL III NICUS INTERNATIONALLY, 2007–12 (INICC) | | | | | |
|--|--------------|-----------------|------------------|-----------------|-----------------------------------|
| Birthweight Category (g) | No. of NICUs | No. of Patients | No. of VAP Cases | Ventilator Days | Pooled Mean VAP Rate ^a |
| ≤750 | 17 | 268 | 10 | 2057 | 4.86 (2.3–8.9) |
| 750–1000 | 31 | 1295 | 56 | 6398 | 8.75 (6.6–11.4) |
| 1001–1500 | 36 | 2408 | 47 | 5523 | 8.51 (6.3–11.3) |
| 1501–2500 | 37 | 5849 | 74 | 6915 | 10.70 (8.4–13.4) |
| >2500 | 37 | 6453 | 95 | 8681 | 10.9 (8.9–13.4) |
| Pooled | 38 | 16,273 | 282 | 29,574 | 9.54 (8.5–10.7) |

| COMPARISON OF HAI RATES PER 1000 DEVICE DAYS IN THE NICUS OF THE INICC (2007–12) AND THE US NHSN (2012) | | |
|---|--|---------------------------------------|
| HAI in Infants Weighing 1501–2500 g | INICC 2007–12 Pooled Mean ^a | US NHSN 2012 Pooled Mean ^a |
| CLABSI | 4.8 (3.7–6.1) | 0.6 (0.5–0.8) |
| VAP | 10.7 (8.4–13.4) | 0.2 (0.1–0.5) |

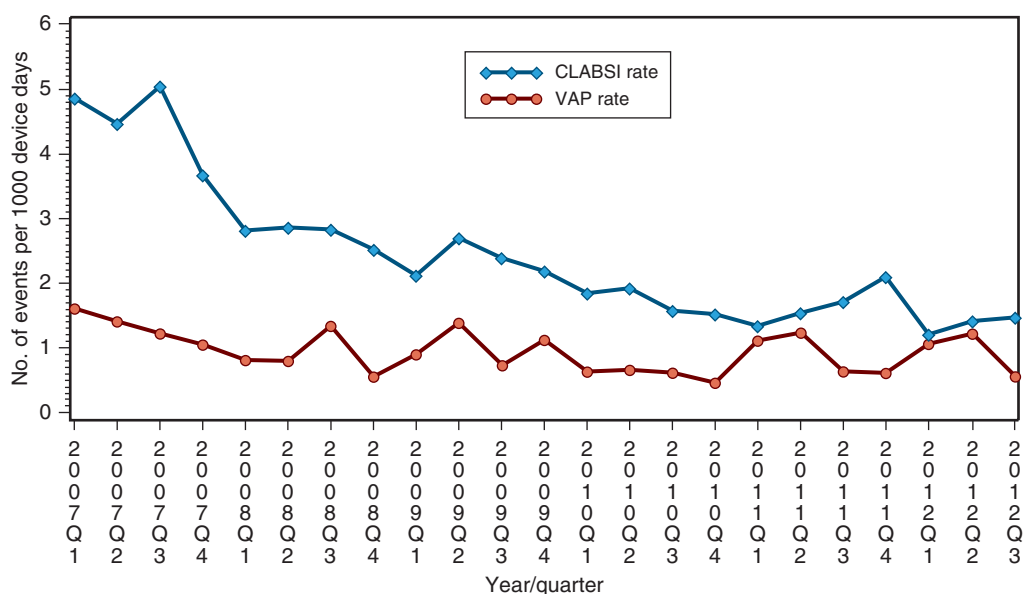
| COMPARISON OF HAI RATES PER 1000 DEVICE DAYS IN THE NICUS (1501–2500 G INFANTS) OF THE INICC IN 2008, 2010, 2012, AND 2014 REPORTS (POOLED MEANS) ^a | | | | |
|--|------------------|------------------|------------------|-----------------|
| Details | 2002–07 | 2003–08 | 2004–09 | 2007–12 |
| No. of countries | 18 | 25 | 36 | 43 |
| CLABSI | 15.2 (10.3–21.5) | 13.9 (12.4–15.6) | 11.9 (10.2–13.9) | 4.8 (3.7–6.1) |
| VAP | 6.68 (3.0–12.7) | 9.50 (7.9–11.3) | 10.1 (7.9–12.8) | 10.7 (8.4–13.4) |

^aThe 95% confidence interval is given in parentheses.

^bThe number in parentheses is the number of locations meeting minimum requirements for percentile distributions (ie, ≥50 device days for rate distributions, ≥50 patient days for device utilization ratios) if less than the total number of locations. If this number is <20, percentile distributions are not calculated.

CDC, Centers for Disease Control and Prevention; CLABSI, central line–associated bloodstream infection; HAI, health care–associated infection; INICC, International Nosocomial Infection Control Consortium; NHSN, National Healthcare Safety Network; NICU, neonatal intensive care unit; VAP, ventilator-associated pneumonia.

Data from Dudeck MA, Weiner LM, Allen-Bridson K, et al. National Healthcare Safety Network (NHSN) report, data summary for 2012, device-associated module. *Am J Infect Control.* 2013;41:1148–1166; and Rosenthal VD, Maki DG, Mehta Y, et al. International Nosocomial Infection Control Consortium (INICC) report, data summary of 43 countries for 2007–2012. Device-associated module. *Am J Infect Control.* 2014;42:942–956.



• **Fig. 40.1** Trends in Rates of CLABSI and VAP in US NICUs. CLABSI, Central line–associated bloodstream infection; VAP, ventilator-associated pneumonia. (Modified from Patrick SW, Kawai AT, Kleinman K, et al. Health care-associated infections among critically ill children in the US, 2007–2012. *Pediatrics.* 2014;134:705–712.)

Rosenthal et al., 2014). Recent advances in resources, technology, and regulations in health care in many countries have led to reductions in infection rates, which are reflected in the reductions in NICU CLABSI and VAP rates seen in the later years of surveillance (Table 40.1; Rosenthal et al., 2014).

Risk Factors for Development of Health Care–Associated Infection

Minimizing exposure to known risk factors for infection is important to reduce the rate of HAIs in the nursery. Experience in the past decade in the NICU has demonstrated that many HAIs previously thought to be not preventable can in fact be substantially decreased in frequency with appropriate preventive interventions.

Risk Factors Related to Patient Characteristics

The risk of developing an HAI is inversely related to gestational age and birthweight (Gaynes et al., 1996; Stoll et al., 1996; Suara et al., 2000; Stover et al., 2001; Stoll et al., 2002; Patrick et al., 2014). Infants with birthweights less than 1000 g have been shown to experience twice the rate of nosocomial bloodstream infections than infants with birthweights greater than 1000 g (Gaynes et al., 1996; Brodie et al., 2000; Stover et al., 2001; Stoll et al., 2002; Geffers et al., 2008). This increased risk to premature infants continues to persist, as demonstrated by an analysis of CDC NHSN data from 2007 to 2012 showing that VLBW infants have a twofold increased risk of developing CLABSI and a 3.5-fold increased risk of VAP compared with normal-weight infants (Patrick et al., 2014). VAPs are more common in infants with underlying cardiopulmonary disease such as bronchopulmonary dysplasia (BPD), as well as infants who have experienced previous thoracoabdominal surgery. UTIs are more prevalent in ex-preterm infants; neonatal males are at higher risk than neonatal females, as are infants with underlying renal anomalies (Shaikh et al., 2008; Foglia and Lorch, 2012). Overall, VLBW infants are more vulnerable to infection, because of both the immaturity of their immune response and their greater need for invasive devices. Additionally, severity of illness scores may serve as an important proxy for physiologic instability and the need for intervention and invasive therapies (Goldmann, 1989; Gray et al., 1995). Infants with an underlying GI condition such as necrotizing enterocolitis, gastroschisis, or omphalocele may be predisposed to mucosal barrier injury and at heightened risk of CLABSI. In a multicenter cohort study in 14 NICUs, 40% of infants who developed CLABSIs had underlying GI conditions associated with loss of mucosal integrity or impaired gut motility or both (Coffin et al., 2014). However, the proportion caused by “enteric pathogens” was not different in the overall group, except for infants with intestinal failure who developed multiple CLABSIs; this latter group experienced higher rates of infections with enteric pathogens (Coffin et al., 2014). A study from the NICHD NRN also demonstrated that VLBW infants with intestinal failure were at increased risk of recurrent bloodstream infections; however, gram-positive organisms were the most frequently implicated pathogens (Cole et al., 2012).

Risk Factors Caused by Necessary Medical Interventions

The use of any type of invasive device increases the risk of infection. The most common invasive devices used in the nursery are intravascular catheters, mechanical ventilators, ventriculoperitoneal shunts, and urinary catheters. In general, risk rises as the duration of exposure lengthens. Compared with adult patients, neonates are at higher risk of CLABSI and at lower risk of VAP and UTI

(Langley et al., 2001; Dudeck et al., 2013). These patterns correlate with the frequency with which these invasive devices are used in the neonatal population.

Prolonged duration of mechanical ventilation is the primary risk factor for development of hospital-acquired pneumonia. Endotracheal tubes circumvent normal airway protective and clearance mechanisms and become coated with biofilms of bacteria, which have the potential to enter the lower airways and cause infections (Garland, 2010). Contamination of respiratory equipment, especially with gram-negative organisms that thrive in moist environments, such as *Acinetobacter*, *Pseudomonas*, and *Flavobacterium* species (spp.), frequently leads to colonization of the respiratory tract (Sole et al., 2002). Aspiration of gastric and oropharyngeal secretions around uncuffed endotracheal tubes could be a mechanism for VAP (Goodwin et al., 1985; Farhath et al., 2008; Garland, 2010). The various closed-system suctioning devices may decrease the risk of environmental contamination during suctioning; however, there is a theoretical risk that they could reintroduce pathogens suctioned from the secretions from major airways into smaller lower airways. While there is a paucity of NICU evidence on the subject, adult studies have demonstrated higher colonization rates but lower VAP rates with closed suctioning systems (Deppe et al., 1990; Johnson et al., 1994; Combes et al., 2000; Cordero et al., 2000; Woodgate and Flenady, 2001; Tablan et al., 2004). Supine positioning and dependent position of the endotracheal tube in relation to the ventilator circuit may increase the risk of VAP by increasing the risk of aspiration (Torres et al., 1992; Drakulovic et al., 1999; Aly et al., 2008). Data on nasal continuous positive airway pressure suggest that its lack of disruption of normal airway protective mechanisms compared with endotracheal tubes may be responsible for lower rates of nosocomial pneumonia; however, the association of nasal continuous positive airway pressure with greater rates of gram-negative sepsis needs further elucidation (Hentschel et al., 2005; Graham et al., 2006).

The intravascular devices commonly used in the neonatal population are peripheral intravenous catheters (PIVCs), umbilical catheters, peripherally inserted central catheters (PICCs), surgically placed central venous catheters (CVCs), and percutaneous arterial catheters. Regardless of the type of device used, the rate of CLABSI appears to be related to the number of days the catheter is in place (Gaynes et al., 1996; Suara et al., 2000; Mahieu et al., 2001; Stoll et al., 2002). Table 40.2 summarizes evidence regarding several of the interventions associated with increased CLABSI risk (Cantey and Milstone, 2015).

PIVCs are the most commonly used device for vascular access in neonates. In adults, the removal of such catheters after 72 hours is recommended. Data in neonates are insufficient to recommend elective removal of PIVCs after 72 hours, because studies have not shown a clear correlation between the higher colonization rate noted after 72 hours and an increased CLABSI rate (Pearson, 1996; Oishi, 2001). However, risks of PIVC infiltration and associated complications, as well as the likely duration of intravenous (IV) access and physiologic disruption often associated with IV insertion, need to be carefully evaluated by clinicians while making decisions around PIVC use and retention.

Data comparing the infection rates of the various types of intravascular catheters are limited. A 2015 Cochrane review of trials comparing PICC lines with PIVCs found that use of a PICC line decreases the risk of complications associated with PIVCs, without increasing the rate of infection (Ainsworth and McGuire, 2015). For central lines, theoretically the risk should be lower for tunneled catheters, because the Dacron cuff proximal to the exit site of a

TABLE 40.2 Studies Identifying Interventions With Increased Adjusted Risk of Bloodstream Infections

| Intervention | Adjusted Risk for Each Intervention ^a | | | |
|----------------------------|--|---------------------------------|--------------------------------|--------------------------------|
| MV | 6.8 (5.9–7.8) ^b | 1.7 (1.4–2.1) ^g | 4.2 (1.4–12.4) ^d | |
| CL | 6.1 (5.0–7.4) ^b | 9.3 (5.9–14.8) ^h | | |
| CL >7 days | 6.2 (5.0–7.6) ^b | 3.5 (1.3–9.2) ⁱ | | |
| CL >21 days | 6.1 (4.6–8.0) ^b | 80.6 (6.9–945) ^j | | |
| UC >7 days | 1.9 (1.7–2.1) ^b | | | |
| PICC >7 days | 2.9 (2.5–3.3) ^b | | | |
| PAL >7 days | 3.7 (3.0–4.6) ^b | | | |
| PN > 7 days | 14.2 (8.8–22.9) ^c | 12.9 (9.7–17.2) ^b | 7.1 (2.8–18.1) ⁱ | 4.7 (2.2–9.9) ^h |
| Vancomycin | 6.1 (1.9–20.1) ^d | | | |
| Steroids | 1.8 (1.0–3.3) ^e | 4.8 (1.7–13.2) ⁱ | | |
| H ₂ blocker/PPI | 6.7 (3.8–12.9) ^f | 3.1 (1.3–7.6) ^e | 7.9 (2.8–21.1) ^d | 3.1 (1.0–10.2) ⁱ |

^aThe 95% confidence interval is given in parentheses.

^bFrom Stoll et al. (2002).

^cFrom Holmes (2008).

^dFrom Smith et al. (2010).

^eFrom Stoll et al. (1999).

^fFrom Bianconi (2007).

^gFrom Makhoul et al. (2002).

^hFrom Perlman (2007).

ⁱFrom Mahieu et al. (2001).

^jFrom Graham et al. (2006).

CL, Central line; H₂ blocker, histamine H₂ receptor antagonist; MV, mechanical ventilation; PAL, peripheral arterial line; PICC, peripherally inserted central catheter; PN, parenteral nutrition; PPI, proton pump inhibitor; UC, umbilical catheter.

Modified from Cantey JB, Milstone AM. Bloodstream infections: epidemiology and resistance. *Clin Perinatol*. 2015;42:1–16.

surgically placed catheter can inhibit the migration of organisms into the catheter tract (Mermel et al., 2001). Adult data suggest that tunneled catheters have lower infection rates than nontunneled catheters; however, studies of neonates requiring tunneled lines show rates of infection comparable with or worse than the reported rates of PICC line infections in other NICU populations (Klein et al., 2003; Freeman et al., 2015). Another recent study from the Netherlands found that umbilical catheters confer the greatest risk of CLABSI (Yumani et al., 2013). This finding is in contrast to the findings of previous studies that found no differences in rates from PICC lines and yet others that found a higher risk with PICC lines (Mahieu et al., 2001; Chien et al., 2002; de Brito et al., 2010; Sannoh et al., 2010). Findings regarding catheter dwell time on CLABSI rates are also mixed. Some studies have found no relationship between catheter dwell time and CLABSI

risk (Mahieu et al., 2001; Smith et al., 2008). However, several others have suggested that increasing risk of CLABSI is associated with PICC and umbilical lines with increased dwell time (Sengupta et al., 2010; Yumani et al., 2013). Recently, a large retrospective review of more than 13,000 infants found that while dwell time did not affect CLABSI risk for PICCs, it did impact CLABSI risk with tunneled catheters, with increased CLABSI risk beyond week 7 from placement (Greenberg et al., 2015). There continues to be much work to be done to determine the ideal IV access and ideal catheter maintenance procedures for the different populations cared for in the NICU.

Exposure to parenteral nutrition has been shown to be associated with increased risk of bloodstream infections, which may in part be mediated by increased use of central lines for delivery of parenteral nutrition (Johnson-Robbins et al., 1996; Padula et al., 2014). Lipid emulsions may decrease the flow rate through the IV catheter, potentiate growth and proliferation of some microorganisms, and interfere with host defense mechanisms by impairing the function of neutrophils and reticuloendothelial cells (Nugent, 1984; Freeman et al., 1990; Langevin et al., 1999). Use of lipid emulsions was independently predictive for the development of CoNS bacteremia (Freeman et al., 1990). Administration of such emulsions has also been linked to a higher risk of HAI with *Candida* and *Malassezia* spp. in neonates (Long and Keyserling, 1985; Redline et al., 1985; Saiman et al., 2000). A recent metaanalysis of predictive factors for neonatal HAIs identified parenteral nutrition and lipid infusions as independent predictors of bloodstream infections (Verstraete et al., 2015).

Histamine-blocking agents, proton pump inhibitors, and postnatally administered corticosteroids are the medications most commonly associated with an increased risk of HAIs among newborns (Graham et al., 2006; Smith et al., 2010; Verstraete et al., 2015). It is hypothesized that the reduced gastric pH associated with the use of histamine-blocking agents promotes bacterial overgrowth and invasion of pathogenic bacteria (Beck-Sague et al., 1994). With greater understanding of the adverse neurodevelopmental consequences of prolonged ventilator need and consequent BPD, there is renewed interest in judicious use of lower-dose steroids after the first 2 weeks postnatally, especially in infants at high risk of developing BPD (Schmidt et al., 2003; Doyle et al., 2005, 2006, 2007; Ambalavanan et al., 2012; Schmidt et al., 2015). However, steroid use has been associated with an increased risk of infection in VLBW infants (Yeh et al., 1997; Stoll et al., 1999), and therefore neonatologists will need to include this concern in any risk–benefit analysis of the use of steroids in their patients.

Risk Factors Associated With the Neonatal Intensive Care Unit and Hospital Environment

Nursery design and staffing influence the risk of infection. Overcrowding and larger workloads decrease compliance with hand washing and raise the risk of HAI (Fridkin et al., 1996; Archibald et al., 1997; Harbarth et al., 1999; Vicca, 1999; Robert et al., 2000). Inadequate numbers of staff and the use of temporary or inexperienced staff members both adversely affect the rate of infection. Studies have shown a relationship between nurse-to-patient ratio and colonization of patients with MRSA and CLABSI rates (Fridkin et al., 1996; Vicca, 1999). Furthermore, strategic nursery design and improvement in nursing staffing correlate with lower rates of HAIs (Gladstone et al., 1990). However, there continue to be nurse staffing challenges, as exemplified by a VON study that identified rates of understaffing and infections in NICUs across the United States (Rogowski et al., 2013).

Many of these HAI risk factors hold true globally. Nosocomial infection rates are much higher in lower-income countries than middle-income and higher-income countries (37.0 vs 11.9 [$P < .02$] vs 17.6 [$P < .05$] CLABSI per 1000 catheter days respectively; Table 40.2), likely reflecting a combination of personnel and resource limitations, overcrowding, lack of infection control regulatory and auditing mechanisms, hospital accreditation, and healthcare workers inexperienced in infection prevention standard practices (Rosenthal et al., 2014).

Health Care–Associated Infection: Distribution by Pathogen

The predominant pathogens responsible for nosocomial bloodstream infections have changed over time. Goldmann (1989) proposed that these trends are explained by changes in the neonatal intensive care patient population and advancing technology. *S. aureus* was the most common nosocomial pathogen in the 1950s and 1960s. In the 1960s and 1970s, gram-negative organisms emerged as the predominant pathogens; globally, these organisms are the most important pathogens responsible for HAIs in the nursery (Stoll et al., 2001). In the United States, CoNS was the most common nosocomial pathogen in the 1990s and early years of the first decade of this century (Gaynes et al., 1996; Stoll et al., 2002). Among the cohort of VLBW infants born between 2002 and 2008 with late-onset infections, the NICHD reported that in singleton infants, gram-positive organisms were responsible for 77% of cases, gram-negative organisms were responsible for 16%, and fungi were responsible for 8% of cases (Table 40.3; Boghossian et al., 2013). These findings are similar to those of studies from the previous decade, suggesting that pathogen distributions have stayed the same in recent times, with CoNS being the most frequent causative pathogen (Stoll et al., 2002; Garland et al., 2008); however, reports from at least one center have noted dramatic declines in CoNS as causative pathogens following the introduction of standardized central line insertion and maintenance bundles and checklists (Bizzarro et al., 2015).

Microbial Resistance

For the clinician, understanding the specific colonization and resistance patterns in the individual NICU is critical. There is clear evidence that antimicrobial resistance is increasing across NICUs. Drug-resistant gram-negative pathogens, in particular, are associated with the highest attributable mortality rates (Makhoul et al., 2005; Shah et al., 2015; Tsai et al., 2016). Cantey and Milstone (2015) identified a marked increase in the number of NICU publications associated with MRSA, vancomycin-resistant enterococcus (VRE), extended-spectrum β -lactamase and carbapenemase producing organisms between 1993 and 2013. NHSN data show that MRSA infection rates tripled in NICUs between 1995 and 2004 (Lessa et al., 2009). Resistant bacteria have been implicated in more than 15% of NICU outbreaks worldwide (Cantey and Milstone, 2015). Colonization with resistant bacteria has been associated with approximately a 33% infection risk with the same pathogen (Singh et al., 2002; Carey et al., 2010; Smith et al., 2010). Many NICUs have adopted periodic surveillance strategies to detect resistant bacteria such as MRSA or VRE (Macnow et al., 2013).

Gram-Positive Bacteria

Coagulase-Negative Staphylococci

CoNS (such as *S. epidermidis*, *S. capitis*, *S. hominis*, *S. warneri*, and *S. haemolyticus*), while commonly thought of as skin

TABLE 40.3 Pathogens Associated With Late-Onset Sepsis (2002–2008)

| Organism | Singletons | Multiples |
|---|--------------|-------------|
| Gram-positive bacteria ^a | 2916 (76.8%) | 905 (75.7%) |
| • <i>Staphylococcus</i> , coagulase negative ^a | 2020 (53.2%) | 588 (49.2%) |
| • <i>Staphylococcus aureus</i> | 408 (10.7%) | 137 (11.5%) |
| • Group B streptococcus | 69 (1.8%) | 25 (2.1%) |
| • Other streptococci ^a | 138 (3.6%) | 68 (5.7%) |
| • Other gram-positive bacteria | 281 (7.4%) | 87 (7.3%) |
| Gram-negative bacteria ^a | 597 (15.7%) | 222 (18.6%) |
| • <i>Escherichia coli</i> | 171 (4.5%) | 61 (5.1%) |
| • <i>Klebsiella</i> species | 151 (4.0%) | 63 (5.3%) |
| • <i>Enterobacter</i> species | 102 (2.7%) | 42 (3.5%) |
| • <i>Pseudomonas</i> species | 85 (2.2%) | 27 (2.3%) |
| • <i>Serratia</i> species | 44 (1.2%) | 13 (1.1%) |
| • Other gram-negative bacteria | 44 (1.2%) | 16 (1.3%) |
| Fungi ^a | 284 (7.5%) | 69 (5.8%) |
| • <i>Candida albicans</i> ^a | 172 (4.5%) | 35 (2.9%) |
| • <i>Candida parapsilosis</i> | 73 (1.9%) | 23 (1.9%) |
| • Other fungi | 39 (1.0%) | 11 (0.9%) |

^aThere is a significant difference in the rate between singletons and multiples ($P < .05$). Data from Boghossian NS, Page GP, Bell EF, et al. Late-onset sepsis in very low birth weight infants from singleton and multiple-gestation births. *J Pediatr*. 2013;162:1120–1124.

commensals, are the most common endemic nosocomial pathogen in neonates (Gray et al., 1995; Brodie et al., 2000; Stoll et al., 2002; Garland et al., 2008; Boghossian et al., 2013). Most CoNS infections are bloodstream infections, with a reported incidence of 51%–78% among VLBW infants (Gray et al., 1995; Isaacs et al., 1996; Stoll et al., 1996, 2002; Boghossian et al., 2013). CoNS are lower-virulence pathogens, with low mortality rates noted (Bizzarro et al., 2015). Known risk factors for CoNS infection are low birth weight, lower gestational age, use of CVCs, prolonged parenteral nutrition, use of IV lipid emulsions, postnatal administration of corticosteroids, and prolonged hospital stay (Freeman et al., 1990; Goldmann, 1989; Johnson-Robbins et al., 1996; Brodie et al., 2000). CoNS produce a capsular polysaccharide adhesin—poly(*N*-succinyl glucosamine)—which forms a “biofilm,” enhancing its ability to adhere to intravascular devices (Otto, 2004). Although some studies suggest that prophylactic use of vancomycin or vancomycin locks reduces the risk of CoNS catheter-related infections, this practice is not recommended because of the serious risk of encouraging antibiotic-resistant organisms, especially VRE and staphylococci (Garland et al., 2005).

Staphylococcus aureus

S. aureus has caused epidemics of SSTIs in well baby nurseries and in NICUs and causes up to 10% of CLABSI (Huang et al., 2012; Shane et al., 2012; Filleron et al., 2013; Sanchini et al., 2013; Lee et al., 2014; Blanchard et al., 2015). The skin, nares, and umbilicus

are the most common sites of colonization. MRSA rates differ greatly between institutions but can account for 50%–55% of staphylococcal infections (Lessa et al., 2009; Dolapo et al., 2014; Shane et al., 2012). MRSA colonization increases the risk of MRSA infection (Huang et al., 2006). Consequently, when one is covering for a possible *S. aureus* infection, it is critical to select an antibiotic that is effective against methicillin-resistant strains. Attributable mortality due to *S. aureus* HAI is between 5% and 18%, with rates as high as 25% in VLBW infants, irrespective of whether the strain is methicillin resistant or not (Cohen-Wolkowicz et al., 2007; Shane et al., 2012; Dolapo et al., 2014). *S. aureus* HAI has been found to be associated with increased rates of adverse neurodevelopmental outcomes (Cohen-Wolkowicz et al., 2007).

Enterococcus

Enterococci (*Enterococcus faecalis*, *Enterococcus faecium*) are responsible for both endemic and epidemic HAIs in the NICU and are responsible for approximately 3% of NICU bloodstream infections (Stoll et al., 2002; Boghossian et al., 2013). Use of CVCs, prolonged hospital stay, and prior antibiotic use are recognized risk factors for colonization with these organisms. The GI tract is often the primary source of infection; however, the pathogens are typically spread via the hands of healthcare workers or through environmental contamination. The widespread use of antibiotics has led to the emergence of VRE (Mascini et al., 2005). There are published guidelines to prevent the spread of VRE, which include hand washing, isolation, barrier precautions, and cohorting of infected patients (Gross and Pujat, 2001). Educational programs to limit the indiscriminate use of antibiotics have been effective in decreasing the spread of VRE (Goldmann, 1989; Isaacs, 2000).

Group B Streptococcus

Group B streptococcus remains an important cause of early-onset and late-onset infection in neonates, but neither has a clear role as an HAI.

Gram-Negative Bacteria

Gram-negative organisms are a particularly important cause of nosocomial bloodstream infections, pneumonia, and meningitis because they generally cause severe disease. *Escherichia coli* is the most common gram-negative pathogen (Boghossian et al., 2013). Other gram-negative organisms responsible for HAI include *Klebsiella*, *Pseudomonas*, *Enterobacter*, *Acinetobacter*, *Serratia*, *Haemophilus*, *Citrobacter*, and *Salmonella* spp. (Table 40.3; Boghossian et al., 2013). Gram-negative infections are currently responsible for approximately 15% of infections in the NICU (Boghossian et al., 2013). The GI tract is thought to serve as the reservoir for these bacteria, and prolonged antibiotic therapy may promote selection of these bacteria (Graham et al., 2007; Smith et al., 2010). The attributable mortality is much higher for gram-negative infections than for gram-positive infections (Makhoul et al., 2005; Shah et al., 2015; Tsai et al., 2016). The occurrence of a gram-negative infection in an NICU patient has been found to be associated with a 3.5-fold higher risk of death (Stoll et al., 2002). *Pseudomonas* spp. appear to be particularly virulent, causing death in 42%–75% of infected neonates (Leigh et al., 1995; Karlowicz et al., 2000; Stoll et al., 2002; Tsai et al., 2016).

Fungi

Fungal infections are discussed in detail in Chapter 41. Invasive fungal infection is estimated to occur in 1%–4% of VLBW infants and up to 10% of extremely low birth weight (ELBW) infants,

with the highest risk in infants weighing less than 750 g (Makhoul et al., 2002; Benjamin et al., 2006; Clerihew et al., 2006; Fridkin et al., 2006; Bartels et al., 2007; Benjamin et al., 2010). The rates and predominant fungal species differ considerably between centers. The smallest and most premature infants appear to be at the highest risk, particularly when they are exposed to broad-spectrum antibiotics and long courses of antibiotics. Other identified risk factors are prolonged mechanical ventilation, prolonged use of CVCs, use of lipid emulsions, antenatal antibiotics, and the use of histamine H₂-receptor antagonists (Saiman et al., 2000; Makhoul et al., 2002; Benjamin et al., 2010). Prophylactic fluconazole therapy has shown promise in reducing the rates of colonization and infection with candida; the impact appears greatest in NICUs with high rates of candida infection (Kaufman et al., 2001; Bertini et al., 2005; Dutta et al., 2005; Healy et al., 2005; Aghai et al., 2006; Manzoni et al., 2006; Uko et al., 2006; Manzoni et al., 2007). However, a large randomized controlled trial of prophylactic fluconazole did not show reductions in mortality or the incidence of invasive fungal disease (Benjamin et al., 2014). Although no study has found an increase in the incidence of fluconazole-resistant fungus, resistance remains a concern with any prophylactic strategy. Limiting a prophylactic strategy to NICUs with high rates of invasive candidal disease and to patients with the highest risk will likely provide the greatest benefit with the lowest risk.

Viruses

Viral organisms that commonly cause HAI in the NICU include respiratory syncytial virus (RSV), rhinovirus, metapneumovirus, influenza virus, rotavirus, and enterovirus. Isolated infections generally result from contact with infected caregivers or family members. Nursery epidemics may occur in addition to isolated individual cases. Viral infections have only recently gained attention as causes of HAI and adverse outcomes, in part because of recent advances in the ability to diagnose viral infections. An analysis of infectious outbreaks in NICUs globally found that approximately 10% of outbreaks affecting neonates were attributed to viral causes, with the most common etiologic agents being rotavirus, RSV, enterovirus, hepatitis A virus, and adenovirus (Civardi et al., 2013). Measures to prevent and contain viral infections include standard precautions such as isolation and cohorting of affected patients, meticulous hand hygiene and use of personal protective equipment, and surveillance of patients and healthcare personnel during outbreaks. Alarming, mortality rates for viral outbreaks appear to be similar to those for bacterial outbreaks (7.2% vs 6.4%) (Civardi et al., 2013).

Respiratory Syncytial Virus

RSV is a fastidious organism capable of surviving on inanimate objects for prolonged periods and which can cause severe disease in neonates, particularly those who are premature or who have cardiopulmonary disease. Rapid testing to detect RSV in nasal washings facilitates efforts to cohort infected patients (Madge et al., 1992). The most recent guidelines from the American Academy of Pediatrics (AAP) recommend that all high-risk infants born at less than 29 weeks' gestation, or with chronic lung disease, or with hemodynamically significant congenital heart disease receive up to five doses of palivizumab (an RSV monoclonal antibody) during the RSV season but starting only at hospital discharge (American Academy of Pediatrics et al., 2014). Although current guidelines do not list prophylactic palivizumab for prevention of nosocomial transmission of RSV, some centers choose to provide RSV prophylaxis to at-risk inpatient neonates because of sporadic occurrences

of inpatient nosocomial deaths from RSV (Abadesso et al., 2004; Kurz et al., 2008; Katz and Sullivan, 2009; Berger et al., 2010; Ohler et al., 2013).

Influenza

Influenza is spread primarily via airborne transmission. Hand washing and immunization of healthcare workers are the primary tools to prevent nosocomial spread (Nichol and Hauge, 1997). Some states and institutions mandate yearly immunization among healthcare workers. Infection control guidelines recommend that every healthcare worker wear a mask during contact with infected patients (Nichol and Hauge, 1997). At-risk infants should receive the influenza vaccine during the winter months once they reach the age of 6 months (Committee on Infectious Diseases, 2015). Parents and other close contacts of at-risk infants should also receive influenza vaccination. Oseltamivir is approved by the Food and Drug Administration for treatment (within the first 48 hours of symptoms) of patients older than 2 weeks infected with influenza A virus or influenza B virus; the AAP and CDC recommend its use for treatment of infected infants of any age (Committee on Infectious Diseases, 2015). While safety and efficacy of oseltamivir prophylaxis have not been established in infants younger than 1 year, the CDC recommends its use for this purpose from 3 months of age onward (Committee on Infectious Diseases, 2015). Guidelines for minimizing risk to infants born to mothers who are actively infected have been provided by the CDC (Williams et al., 2013). Pregnant women should receive treatment as soon as possible (Williams et al., 2013). If tolerated, the mother should wear a mask during labor and delivery. After delivery, the newborn should be cared for away from the mother until she has received treatment for 48 hours, has become afebrile, and is able to control cough and secretions (Committee on Infectious Diseases, 2015). If desired, lactation should be facilitated, because the breast milk itself is not thought to be a means of viral transmission (Williams et al., 2013).

Rotavirus

Although rare, epidemics of rotavirus diarrhea may occur in the nursery (Widdowson et al., 2000; Jain et al., 2001; Lee et al., 2001; Herruzo et al., 2009); they are primarily caused by inadequate hand washing and cross-contamination between patients (Bruijning-Verhagen et al., 2012). Standard and contact precautions should be followed throughout the duration of the illness. Some patients have prolonged fecal shedding of low concentrations of the virus; therefore some infection control experts recommend contact precautions for the duration of the hospitalization of such patients. Rotavirus is also an important cause of diarrhea in older infants. Live virus vaccines are now available for the prevention of rotavirus infection. While the 2009 AAP statement recommended administration of the first dose of rotavirus vaccine at discharge from the NICU, several recent studies have documented the safety of pentavalent rotavirus vaccine administration during the hospital stay of preterm infants, with no concern for significant adverse events or symptomatic transmission from shedding (Committee on Infectious Diseases, 2009; Monk et al., 2014; Jaques et al., 2015). Further, one study noted that delaying administration of the first dose until discharge led to more than half of ELBW infants not receiving the rotavirus vaccine, as they missed the window of postnatal age for vaccine administration (Stumpf et al., 2013).

Enterovirus

There are numerous serotypes of enteroviruses, including polioviruses, Coxsackie viruses A and B, echovirus, and nonassigned

subtypes (Chambon et al., 1999). Enterovirus infections have been described among neonates in the well baby nursery and the NICU setting (Isaacs et al., 1989; Wreghitt et al., 1989; Chambon et al., 1999; Sizun et al., 2000; Takami et al., 2000). Both isolated cases and epidemics can occur. The clinical presentation associated with enteroviral infection is variable, ranging from asymptomatic to overwhelming multisystem organ dysfunction with poor prognosis (Isaacs et al., 1989; Wreghitt et al., 1989; Abzug et al., 1993; Keyserling, 1997; Jankovic et al., 1999). The severity of the disease and the likelihood of death are often more pronounced in perinatally acquired cases than in nosocomially acquired cases, presumably related to the lack of maternal antibody present in the neonate (Modlin et al., 1981; Isaacs et al., 1989). Blood and cerebrospinal fluid cultures should be obtained from any patient with clinical symptoms of disease. Polymerase chain reaction analysis is helpful in making a rapid diagnosis (Nigrovic, 2001; Tebruegge and Curtis, 2009). No antiviral agents are currently available to treat enteroviral infections in newborns (Nigrovic, 2001). Although commercially available intravenous immunoglobulin (IVIG) preparations have high levels of neutralizing antibodies to common enterovirus serotypes, there is no clear evidence that administration of immunoglobulin alters the process or outcome of enteroviral infection (Dagan et al., 1983; Abzug et al., 1993; Keyserling, 1997; Tebruegge et al., 2009).

Adverse Outcomes Related to Health Care–Associated Infection

HAIs are a potentially highly modifiable contributor to a spectrum of adverse outcomes, across all gestational and postnatal ages, but especially in the most immature infants (Table 40.4). Studies from the NICHD NRN have demonstrated a striking increase in mortality in VLBW infants who experience late-onset infection (18% in infected infants vs 7% in uninfected infants), with even higher mortality rates for gram-negative or fungal sepsis (Stoll et al., 2002). Several studies have found the length of stay to increase because of sepsis: NICHD NRN data indicated that the mean length of stay increased from 60 to 79 days in VLBW infants, while the VON group found an increase in the length of stay of 4–7 days (Pessoa-Silva et al., 2001; Stoll et al., 2002; Payne et al., 2004). In the subset of infants with intestinal failure due to necrotizing enterocolitis, hospital length of stay and the duration of parenteral nutrition were greatly increased by the occurrence of infections (Cole et al., 2012). A study of VAP in pediatric intensive care units and NICU populations noted an increased duration of mechanical ventilation (by 3 days) (Foglia et al., 2007). There is also strong evidence that infections in VLBW infants are associated with an increased risk of adverse neurodevelopmental outcomes. One early study of more than 6000 ELBW infants found that infants who experienced infection had impaired head growth as well as a significantly increased risk of cerebral palsy, lower Bayley mental and psychomotor development indices, and visual deficits (Stoll et al., 2004a). A secondary analysis of the Trial of Indomethacin Prophylaxis in Preterms (944 ELBW infants) also confirmed that infection was an independent risk factor for neurodevelopmental impairment (Bassler et al., 2009). Most recently, an analysis by the NICHD NRN of trends from 2005 to 2012 noted a decrease in infections across all VLBW gestational ages in this time period, while also noting slight improvements in survival without major morbidity in infants born at 25–28 weeks' gestation; the authors suggest that at least some of this improvement

TABLE 40.4 Adverse Outcomes Associated With Bloodstream Infections in Very Low Birth Weight Infants in the Neonatal Intensive Care Unit

| Adverse Outcome | Study | Adjusted Effect |
|---------------------------------|--------------------------|--|
| Death | Stoll et al. (1996) | 2.4-fold increase (17% vs 7%) |
| | Stoll et al. (2002) | 2.6-fold increase (18% vs 7%) |
| | Makhoul et al. (2002) | 2.0-fold increase (17% vs 9%) |
| Poor neurodevelopmental outcome | De Haan et al. (2013) | OR 4.8 (1.5–15.9) (gram-negative BSI) ^a |
| | Mitha et al. (2013) | OR 2.2 (1.5–3.1) ^b |
| | Schlapbach et al. (2011) | OR 3.2 (1.2–8.5) ^b |
| | Stoll et al. (2004a) | OR 1.4 (1.3–2.2) ^a |
| Increased length of stay | Stoll et al. (1996) | 19–22-day mean increase |
| | Stoll et al. (2002) | 18.6-day mean increase |
| | Makhoul et al. (2002) | 27-day mean increase |
| | Atif (2008) | 9.2-day mean increase |
| Increased cost | Payne et al. (2004) | \$54,539 mean increase |
| | Donovan et al. (2013) | \$16,800 mean increase |

Data from all studies have been adjusted for gestational age.
^aBayley-II motor or cognitive score less than 85, blindness, deafness, or cerebral palsy.
^bCerebral palsy.
 BSI, Bloodstream infection; OR, odds ratio.
 Data from Cantey JB, Milstone AM. Bloodstream infections: epidemiology and resistance. *Clin Perinatol*. 2015;42:1–16.

may plausibly be related to the reduction in infection rates over the same period (Stoll et al., 2015).

Evidence regarding the adverse effects of excess antibiotic exposure continues to mount. Development of antibiotic resistance is a well-known concern, potentially facilitating the emergence and spread of resistant nosocomial pathogens within the NICU (Singh et al., 2002; Millar et al., 2008; Russell et al., 2012; Gibson et al., 2015). In addition, several recent investigations into the impact of gut microbial alterations in early life have suggested associations between early antibiotic exposure, gut microbial dysbiosis, and the occurrence of GI dysfunction, necrotizing enterocolitis, and sepsis, as well as long-term immune dysregulation and GI disorders (Cotten et al., 2009; Kuppala et al., 2011; Sherman et al., 2015; Vangay et al., 2015).

HAIs are also associated with significantly increased use of healthcare resources and healthcare costs (Tambyah et al., 2002; Payne et al., 2004; Kennedy et al., 2013; Zimlichman et al., 2013). One metaanalysis of the costs of HAIs in the United States estimated costs attributable to CLABSI at \$45,814 (95% CI \$30,919–\$65,245), to VAP at \$40,144 (95% CI \$36,286–\$44,220), to surgical site infections at \$20,785 (95% CI \$18,902–\$22,667), and to catheter-associated UTIs at \$896 (95% CI \$603–\$1189)

• BOX 40.2 Principles for the Prevention of Health Care–Acquired Infection in the Neonatal Intensive Care Unit

Observe recommendations for standard precautions with all patient contact.
 Observe recommendations for transmission-based precautions (gowns, gloves, masks, isolation, as indicated).
 Use good nursery design and engineering.
 Appropriate nurse-to-patient ratio
 Avoidance of overcrowding and excessive workload
 Improve hand hygiene compliance (Box 40.3).
 Minimize risk of contamination of central lines—adopt care bundles.
 Provide meticulous skin care.
 Encourage early and appropriate advancement of enteral feedings.
 Perform continuous monitoring and surveillance of health care–acquired infection rates in the neonatal intensive care unit.
 Provide education and feedback to nursery personnel.

(Zimlichman et al., 2013). A retrospective study of HAIs in NICUs calculated an incremental cost of \$16,800 attributable to bloodstream infections (Donovan et al., 2013), whereas another study in VLBW infants found a more modest cost increase but still amounting to thousands of dollars (\$1280–\$5875 per infection) (Payne et al., 2004). Attributable costs in a study of pediatric and neonatal VAP were estimated at \$30,000 (Foglia et al., 2007).

Thus decreasing HAI rates in the NICU can reduce the risk of adverse events in infants during their hospital stay, thereby decreasing the incidence of short-term and long-term adverse outcomes, length of stay, and direct healthcare costs. HAIs have therefore become a very important focus of quality improvement efforts in NICUs across the United States and the world.

Prevention of Health Care–Associated Infection

Overall Approach to Infection Control

The CDC recommends a two-tiered approach to infection control (Box 40.2). Standard precautions should be used with all patient contact regardless of the underlying diagnosis or infectious status. These precautions consist of universal precautions (designed to prevent blood and body fluid contamination) and body substance precautions (designed to prevent contamination with moist substances). Transmission-based precautions are necessary when a patient is infected with a known or suspected pathogen that is associated with a high risk of contamination via airborne or droplet transmission or contact with the skin or contaminated surfaces (Garner, 1996).

Guidelines for Hand Hygiene Practices

Despite knowledge of the importance of hand hygiene since the 1800s, as elucidated by Labarraque and Semmelweis (Boyce and Pittet, 2002), healthcare settings continue to face challenges in establishing optimal hand hygiene practices among caregivers. Good hand hygiene is the cornerstone of HAI reduction efforts (Larson, 1995; Polin et al., 2012a). Hand hygiene techniques are effective in decreasing the colonization rate of resident and transient flora and have been shown to reduce cross-contamination among patients.

• BOX 40.3 World Health Organization Recommendations for Hand Hygiene

- Wash hands with soap and water when they are visibly dirty or soiled or after use of the toilet.
- Use alcohol-based hand rub for routine activities if hands are not soiled; if alcohol-based hand rub is not obtainable, wash hands with soap and water. Brushes are not recommended (even for surgical scrubs).
- Perform hand hygiene:
 - Before and after touching the patient
 - Before handling invasive device, regardless of whether gloves are worn
 - After contact with body fluids, mucous membranes, nonintact skin, dressings
 - If moving from a contaminated body site to another body site on the same patient
 - After contact with inanimate surfaces and objects in the immediate vicinity of the patient
 - After removing sterile or nonsterile gloves
- Selection and handling of hand hygiene agents
 - Provide products with a low irritancy potential.
 - Solicit input regarding skin tolerance, feel, and fragrance of products being considered.
 - Determine known interaction between products used to clean hands, skin care products, and the types of gloves used in the institution.
 - Ensure that dispensers are accessible at the point of care.
 - Provide alternatives for individuals with adverse reactions to standard products.
 - When alcohol-based hand rub is available in the healthcare facility, use of antimicrobial soap is not recommended.
 - Soap and alcohol-based hand rub should not be used concomitantly.
- Use of gloves:
 - The use of gloves does not replace the need for hand hygiene.
 - Wear gloves when it can be reasonably anticipated that contact with blood or other potentially infectious materials, mucous membranes, or nonintact skin will occur.
 - Remove gloves after caring for a patient; do not reuse them with other patients.
 - Change or remove gloves during patient care if moving from a contaminated body site to either another body site within the same patient or the environment.
- Other aspects:
 - Do not wear artificial fingernails or extenders, keep natural nails short
- Hand hygiene promotion programs:
 - Focus specifically on factors with significant influence on behavior and not only on the type of hand hygiene product. The strategy should be multifaceted and multimodal and include education and senior executive support for implementation.
 - Educate staff about the types of patient-care activities that result in hand contamination and about the advantages and disadvantages of various methods used to clean their hands.
 - Monitor adherence to hand hygiene practices, and provide performance feedback.
 - Encourage partnerships between patients, their families, and healthcare workers.

The World Health Organization offers publicly available educational resources to encourage appropriate hand hygiene in healthcare settings and tools for implementation of hand hygiene training and auditing programs: http://www.who.int/gpsc/5may/EN_PSP_GPSC1_5May_2016/en.

Modified from Polin RA, Denson S, Brady MT, Committee on Fetus and Newborn, Committee on Infectious Diseases. Strategies for prevention of health care-associated infections in the NICU. Pediatrics. 2012;129:e1085–e1093.

Direct patient contact and respiratory tract care seem to be particularly associated with contamination (Pittet et al., 1999). Organisms such as RSV, *S. aureus*, and gram-negative bacilli are able to survive on inanimate objects (“fomites”), so holding an infant infected with one of these organisms, changing diapers, and even touching items in the infant’s environment can result in hand contamination (Goldmann, 1989; Boyce and Pittet, 2002).

Recommendations on indications and techniques for hand hygiene were published by the CDC in 2002 and updated by the World Health Organization in 2009 (Box 40.3; Storr et al., 2009). These guidelines will be effective only if every healthcare provider performs hand hygiene before and after every patient contact. Reported barriers to compliance with hand hygiene recommendations include skin irritation, poor accessibility of sinks or cleansing agents, insufficient time, heavy workload, understaffing, and lack of information. A common misconception is that use of gloves obviates the need for adequate hand hygiene. Leakage and contamination of gloves have been reported (Larson, 1995; Boyce and Pittet, 2002). Disposable single-use gloves should be removed after each patient encounter, and hands should be washed before and after their use. The World Health Organization has a number of excellent, publicly available educational resources to encourage appropriate hand hygiene in healthcare settings, including hand hygiene videos in many languages and tools for implementation of hand hygiene training and auditing programs (http://www.who.int/gpsc/5may/EN_PSP_GPSC1_5May_2016/en/).

Hand hygiene is extremely cost-effective. The additional hospital charges associated with a single HAI may almost equal the yearly

hand hygiene budget. One study estimated the cost of a hand hygiene intervention program to be approximately \$57,000 per year (Pittet et al., 2000). Assuming that 25% of the observed decrease in infections was attributable to improved hand hygiene practices, a saving of \$2100 was estimated for every infection averted.

Guidelines for Gloves and Gowns

Although gowning by healthcare workers is still a common practice in many countries, a metaanalysis of studies of gowning in newborn nurseries revealed that gowning by healthcare workers did not reduce rates of colonization, rates of infection, length of stay, or mortality in infants (Webster and Pritchard, 2003). Moreover, an observational study noted that when increasing numbers of patients were placed under contact precautions, necessitating gowning and gloving, this actually led to a decrease in compliance with precautions (Dhar et al., 2014). However, recent studies have shown that use of nonsterile gloves along with correct hand hygiene practices could reduce bloodstream infections in preterm infants compared to hand hygiene alone (Janota et al., 2014; Kaufman et al., 2014).

Recent Quality Improvement Efforts at Reduction of Health Care–Associated Infection in Neonatal Intensive Care Units

In recent years, many NICUs and quality improvement collaboratives have described their success in decreasing the rate of central

line-associated infections (Kaplan et al., 2011; Schulman et al., 2011; Wirtschafter et al., 2011; Payne et al., 2012; Fisher et al., 2013; Lee et al., 2014; Shepherd et al., 2015). Several guiding principles and common themes are present in these success stories. A key and powerful initial step is comparing institutional performance (or “benchmarking”) to improve understanding of the performance of a NICU in comparison with peer institutions and to recognize opportunities for improvement. Monitoring, surveillance, and benchmarking of the HAI rates in the nursery are critical components of any prevention program. There is power in knowing how an individual NICU compares with others, and there is empowerment in knowing that preventing HAIs is possible (Schulman et al., 2009). Next, formation of “quality improvement collaboratives” helps multiple institutions develop common sets of best practices and common definitions and attempts to minimize interhospital variation in outcomes, allowing lesser performing hospitals to learn from hospitals with lower rates of infections. Several statewide and other collaboratives have reported reductions in CLABSI rates with use of this strategy (Kaplan et al., 2011; Schulman et al., 2011; Wirtschafter et al., 2011; Payne et al., 2012; Fisher et al., 2013; Lee et al., 2014; Shepherd et al., 2015; Piazza et al., 2016). These significant successes have been accomplished with the development and implementation of care “bundles,” incorporating a group of interventions aimed at standardizing care to minimize HAIs.

Prevention of Central Line–Associated Bloodstream Infection

The care and maintenance of CVCs are key to reducing CLABSIs. On the basis of knowledge of risk factors, and quality improvement efforts, most institutions currently adopt a “care bundle” of practices surrounding central line insertion and maintenance that have been shown to be effective at reducing CLABSI rates (Box 40.4; Schulman et al., 2009; Kaplan et al., 2011; Wirtschafter et al., 2011; Payne et al., 2012; Fisher et al., 2013; Lee et al., 2014; Shepherd et al., 2015). The key elements during catheter insertion are hand hygiene, aseptic technique, skin antisepsis, and sterile dressing technique. Best practices during catheter maintenance include hand hygiene, daily review of line necessity, daily inspection of the insertion site and dressing, standardization of practices around IV tubing changes, and “scrubbing the hub” of the central line to minimize contamination (Box 40.4). Several NICUs have adopted practices targeting early removal of central lines, when infants advancing on enteral feedings demonstrate tolerance of 70%–80% of goal volume feeds. A recent study from a multicenter collaborative of Children’s Hospital NICUs used an innovative approach, combining orchestrated testing with quality improvement efforts, to pinpoint individual bundle interventions that were likely to have the greatest impact on CLABSI reduction (Piazza et al., 2016). Specific guidelines around use of umbilical catheters have also been developed, emphasizing timely removal—not later than 14 days from placement for umbilical venous catheters and ideally not later than 5 days for umbilical arterial catheters (O’Grady et al., 2011; Polin et al., 2012a).

A metaanalysis comparing skin antiseptic agents found that use of chlorhexidine gluconate for catheter site care reduced the risk of CLABSI by 50% compared with use of solutions containing povidone–iodine (Chaiyakunapruk et al., 2002). However, chlorhexidine is not approved by the Food and Drug Administration for use in infants younger than under 2 months. A randomized trial in VLBW infants showed that while chlorhexidine

• BOX 40.4 Example of Central-Line Insertion and Maintenance Bundle Elements

Insertion Bundle

- Establish a central line kit/cart to consolidate all items necessary for the procedure.
- Perform hand hygiene with a hospital-approved alcohol-based product or antiseptic-containing soap before and after palpating insertion sites or inserting the central line.
- Use maximal barrier precautions (including sterile gown, sterile gloves, surgical mask, hat, and large sterile drape).
- Disinfect skin with appropriate antiseptic (2% chlorhexidine, 70% alcohol) before catheter insertion.
- Use either sterile transparent semipermeable dressing or sterile gauze to cover the insertion site.

Maintenance Bundle

- Perform hand hygiene with a hospital-approved alcohol-based product or antiseptic-containing soap before and after accessing a catheter or changing a dressing.
- Evaluate the catheter insertion site daily for dressing integrity and signs of infection.
- If the dressing is damp/soiled/loose, change the dressing aseptically, and disinfect skin around insertion site with an appropriate antiseptic (2% chlorhexidine, 70% alcohol).
- Develop and use a standardized intravenous tubing setup and changes.
- Maintain an aseptic technique when changing intravenous tubing and when entering the catheter, including “scrub the hub.”
- Daily review of catheter necessity with prompt removal when no longer essential

Modified from Schulman J, Wirtschafter DD, Kurtin P. Neonatal intensive care unit collaboration to decrease hospital-acquired bloodstream infections: from comparative performance reports to improvement networks. Pediatr Clin North Am. 2009;56:865–892.

gluconate-containing dressings reduced colonization of central venous lines (compared with povidone–iodine), CLABSI rates were comparable with the two modes of catheter site care; of note, chlorhexidine was associated with high contact dermatitis rates in the most immature infants (Garland et al., 2001).

There are some data describing the use of prophylactic vancomycin and antibiotic lock therapy with vancomycin in neonates, suggesting a reduction in CoNS bloodstream infection; however, these studies did not demonstrate reductions in length of stay or mortality. Given the concerns for potential development of antibiotic resistance with widespread use of prophylactic antibiotics, these practices are not recommended (Craft et al., 2000; Garland et al., 2002; Taylor et al., 2015). A similar concern regarding the possibility of fluconazole-resistant fungal infections, fluconazole-related toxicity, and lack of evidence regarding mortality or long-term morbidity reduction has limited the recommendation for prophylactic fluconazole to high-risk units, despite evidence of fungal CLABSI reduction with prophylactic fluconazole from randomized trials (Clerihew et al., 2007).

Prevention of Health Care–Associated Pneumonia

Similarly to CLABSI prevention efforts, best practice bundles for VAP prevention have been adopted in many hospitals. The key elements of VAP prevention as recommended by the CDC include surveillance for VAP (which does not include routine cultures), prevention of bacterial transmission, staff education, and risk reduction in the patient (Tablan et al., 2004; Polin et al., 2012a).

Key practices that may help reduce the risk of VAP include timely removal of tracheal tubes from patients, minimizing aspiration of pathogenic bacteria by elevating the head of the bed by 30–45 degrees, and performing oral hygiene (Tablan et al., 2004). There is no clear evidence of the superiority of closed versus open suctioning systems in minimizing VAP risk (Deppe et al., 1990; Woodgate and Flenady, 2001; Tablan et al., 2004).

Other Aspects of Prevention and Management of Health Care–Acquired Infections

Skin Care

The skin of VLBW preterm infants is immature and an ineffective barrier to prevent transepidermal loss of water and invasion of bacteria. The stratum corneum has mechanical and chemical properties that decrease the risk of infection (Darmstadt and Dinulos, 2000) and matures at approximately 32 weeks' gestation. In a prematurely born neonate, the maturation process is accelerated and is usually complete by 2–4 weeks after birth (Darmstadt and Dinulos, 2000). There is no consensus on the most effective skin care practices for VLBW infants (Baker et al., 1999; Munson et al., 1999). While early studies demonstrated that topical application of emollients protected the developing epidermal layer and reduced transepidermal water loss, a metaanalysis showed an increased risk of CoNS infections with topical emollient application (Soll and Edwards, 2000).

Human Milk Feedings

Several studies have demonstrated lower risks of sepsis and necrotizing enterocolitis with early enteral feeding with human milk. The various mechanisms proposed for the beneficial effect of human milk feeding include its content of immunoprotective substances and prebiotics and probiotics that modulate the development of a protective infant gut microbiome. However, a metaanalysis of human milk studies demonstrated a lack of infection protection from human milk feedings (de Silva et al., 2004). Additional studies are required to clarify this question.

Catheter Removal Following Central Line–Associated Bloodstream Infection

A retrospective review compared outcomes in patients in whom catheters were removed at the onset of infection with those in whom catheters remained in place (Benjamin et al., 2001). Forty-six percent of infants in whom in-place catheter sterilization was attempted had complications, compared with 8% of infants whose catheters were removed. Infants with gram-negative infections were more likely to have complications if catheters remained in place (Benjamin et al., 2001). A study of infants with CoNS CLABSI found no difference in complications or mortality rate if catheter removal was delayed (Karlowicz et al., 2000); however, these patients were more likely to have persistently positive culture results when their lines were not removed with the first positive culture result (43% vs 13% for immediate catheter removal). In general, patients with CLABSI due to gram-negative or fungal pathogens should have their catheters removed as early as possible; patients with CLABSI caused by CoNS should undergo catheter removal if the culture results are persistently positive or if the patient's condition is unstable (Karlowicz et al., 2000; Benjamin et al., 2001).

Antibiotic and Adjunctive Therapies

Antibiotic choice should initially cover a broad spectrum of pathogens and should then be narrowed as soon as possible to

cover the specific bacteria identified once sensitivities are known. Antibiotic use should be discontinued if infection is not proved and is not likely. Coverage for *Pseudomonas* spp. or other resistant gram-negative organisms should be considered in patients with rapid clinical deterioration (Karlowicz et al., 2000; Stoll et al., 2002). However, empiric broad-spectrum antibiotic use should be limited as much as possible to avoid development of resistant infections (Dellit et al., 2007).

The most recent metaanalysis update on the prophylactic use of IVIG in preterm neonates included data on immunoglobulin M–enriched IVIG and found no reduction in mortality or adverse neurodevelopmental outcomes (Ohlsson and Lacy, 2015). IVIG is therefore not recommended for routine use in suspected or proven sepsis (Ohlsson and Lacy, 2015). Hemopoietic colony-stimulating factors (granulocyte and granulocyte–macrophage) are effective in raising the neutrophil count but have not consistently decreased HAI rates or mortality (Modi and Carr, 2000; Carr et al., 2003). A metaanalysis of randomized controlled trials of orally administered lactoferrin for prevention of sepsis and necrotizing enterocolitis suggests a decrease in the development of late-onset sepsis (RR 0.49, 95% CI 0.32–0.73) and all-cause mortality (RR 0.30, 95% CI 0.12–0.75) (Pammi and Abrams, 2015); additional data are needed to confirm these benefits. A recent metaanalysis of probiotic trials showed a reduction in the development of late-onset sepsis in preterm infants (RR 0.86, 95% CI 0.74–0.98), contradicting the results of large randomized controlled trials and previous metaanalyses (Garland et al., 2011; AlFaleh and Anabrees, 2014; Lau and Chamberlain, 2015; Costeloe et al., 2016; Rao et al., 2016). Conflicting results may in part be due to variability in probiotic composition between studies (AlFaleh and Anabrees, 2014; Rao et al., 2016).

Conclusion

Interventions to reduce HAI are a cornerstone of excellence in NICU care. While it is encouraging that HAIs in US NICUs have shown trends toward reduction in the last decade, CLABSI and other HAIs continue to cause significant morbidity and death in the most vulnerable NICU patients. Considerable work remains to be done in the United States and across the world to move closer to the ideal goal of zero preventable harm. Collaborative quality improvement approaches, benchmarking, and emphasizing “bundles” of good practices are key to accomplishing these goals. Clinicians must continue to focus on effective prevention strategies, including adherence to strict hand hygiene policies, minimal use of invasive devices, promotion of enteral nutrition, surveillance of infection patterns, and education of all nursery staff members.

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Complete references used in this text can be found online at www.expertconsult.com

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Fungal Infections in the Neonatal Intensive Care Unit

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KEY POINTS

- The most important risk factor for fungal infection is gestational age.
- Fungi account for approximately 12% of neonatal late-onset sepsis, with a mortality rate of approximately 32%.
- When a lumbar puncture is performed, 10%–50% of candidemic infants have associated meningitis and a significant percentage of extremely low birth weight (ELBW) infants with *Candida* meningitis have negative blood cultures.
- Any *Candida* species isolated from a blood culture should never be regarded as a contaminant.
- Rapid institution of parenteral amphotericin B deoxycholate is the therapy of choice for systemic fungal infection; if there is also meningitis, 5-flucytosine or fluconazole should be added.
- Central venous catheters should be removed within 24 hours after the identification of yeasts in the blood culture, if possible.
- The finding that prophylactic fluconazole reduces the incidence of invasive neonatal fungal infections should be interpreted with caution; nevertheless, recent guidelines recommend fluconazole prophylaxis in ELBW (<1000 g birthweight) infants in nurseries with high rates (>10%) of invasive candidiasis.

Epidemiology

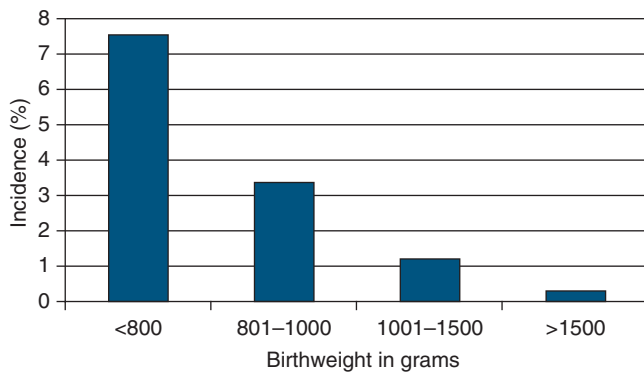
Invasive fungal infection occurs in approximately 1%–2% of all infants admitted to US neonatal intensive care units (NICU) (Stoll et al., 1996), and the incidence rises dramatically with decreasing gestational age. Among the fungi, *Candida* species (spp.) are the dominant pathogens, with infections about evenly distributed among *C. albicans* and non-*C. albicans* spp. (*C. parapsilosis*, *C. orthopsilosis*/*C. metapsilosis*, *C. glabrata*, *C. guilliermondii*, *C. krusei*, and *C. lusitanae*). *Candida* spp. are the third most common cause of late-onset sepsis in the NICU, with a fatality rate more than sevenfold greater than that of *Staphylococcus epidermidis*, the most common pathogen found in the NICU (Benjamin et al., 2000; Saiman et al., 2001). In late-onset sepsis, fungi account for approximately 12.2% of cases, with a mortality rate of 31.8% (Plano, 2010). Other fungi encountered include *Aspergillus fumigatus* and *A. flavus* and *Malassezia furfur* and *M. pachydermatis*. The yeast *Cryptococcus neoformans* and the fungi *Histoplasma capsulatum*, *Blastomyces dermatitidis*, and *Coccidioides immitis* are rarely, if ever, seen in the NICU.

The most important risk factor for fungal infection is gestational age. In a survey of 2847 infants from six different nurseries, the incidence of candidemia in infants weighing less than 800 g (7.55%) was 25 times that of the infants weighing more than 1500 g (Fig. 41.1) (Saiman et al., 2000). This latter group acquires bloodborne candidal infection in association with congenital anomalies, especially those of the gastrointestinal tract (Rabalais et al., 1996). Candidemia carries a mortality rate exceeding 25% in most studies (Chapman and Faix, 2000; Weese-Mayer et al., 1987).

Colonization with ubiquitous fungal spp. occurs in at least 25% of very low birth weight (VLBW) infants (Baley et al., 1986), and both the amount of *Candida* in the gastrointestinal tract (Pappu-Katikaneni et al., 1990) and colonization at sites such as endotracheal tubes (Rowen et al., 1994) have been correlated with increased risk of invasive disease caused by *Candida* spp. Prospective studies correlating colonization by other fungal genera (e.g., *Aspergillus*, *Malassezia*) with risk of invasive disease have not been done.

Apart from colonization and gestational age, other host factors that contribute to the susceptibility of the NICU neonate to fungal infection include a 5-minute Apgar score of less than five and an age-dependent immunocompromised state ascribable to reduced numbers of T cells, impaired phagocyte number and function, and reduced levels of complement (Zach and Hostetter, 1989; Maródi et al., 1994; Rebuck et al., 1995; Witek-Janusek et al., 2002). Concomitants of NICU care that are thought to increase the risk of fungal infections include length of stay greater than 1 week, indwelling central venous catheters (CVCs), abdominal surgery, parenteral nutrition, intralipids, H₂ blockers, endotracheal intubation, and prolonged use of broad-spectrum antimicrobials, especially third-generation cephalosporins (Saiman et al., 2000; Saiman et al., 2001; Cotten et al., 2006). Other centers have identified associations with systemic steroids and catecholamine infusions in retrospective studies and with topical petrolatum in a prospective case-control study (Botas et al., 1995; Benjamin et al., 2000; Campbell et al., 2000).

Interestingly, a number of variables appear not to associate with candidal colonization, including use of antibiotics in the mother, premature rupture of the membranes, the infant's gender, use of antimicrobial agents other than third-generation cephalosporins in the infant, surgical procedures, or frequency of intubation (Saiman et al., 2001). Although approximately 5% of NICU staff carry *C. albicans* on the hands and 19% carry *C. parapsilosis*, there is no



• **Fig. 41.1** Incidence of Candidemia Related to Birthweight in Grams. (Data from Saiman L, Ludington E, Pfaller M, et al. Risk factors for candidemia in neonatal intensive care unit patients. The National Epidemiology of Mycosis Survey study group. *Pediatr Infect Dis J.* 2000;19:319–324.)

correlation with site-specific rates of neonate colonization (Saiman et al., 2001).

This chapter will place its major emphasis on infections caused by *Candida* spp. and will also discuss infections caused by other fungi, as well as the approach to diagnosis, treatment, and management of infants with fungal infection.

Infections Caused by *Candida* Species

Congenital Candidiasis

Presenting within the first 24 hours of life in both full-term and premature neonates, congenital candidiasis, a very rare entity, manifests as a deeply erythematous skin rash in the setting of pronounced neutrophilia, with white blood cell counts often rising to 50,000 mm³ or more. *Candida* funisitis (discussed later in the chapter) can be an infrequent accompaniment. In the full-term neonate, there are usually no invasive consequences, and desquamation typically ensues within 2 to 3 days. In contrast, the condition is life-threatening in the premature neonate (Dvorak and Gavaller, 1966; Johnson et al., 1981) and is distinguished by a pustular rash, hazy infiltrates reminiscent of respiratory distress syndrome on chest radiograph, and frequently positive blood cultures. The premature neonate is thought to acquire the organism from inhalation of infected amniotic fluid.

Diagnosis in both premature and full-term neonates requires visualization of the organism on Gram stain from a bullous lesion or an opened pustule. On rare occasions, the placenta has yielded the diagnosis. Treatment for the full-term neonate requires only the full-body application of topical antifungal creams containing either nystatin or azoles such as miconazole or clotrimazole. In the premature neonate, the initiation of parenteral amphotericin B deoxycholate at a dose of 1 to 1.5 mg/kg is mandatory (this dose may be reduced to 1.0 mg/kg), but respiratory involvement typically heralds death despite antifungal therapy. Except where noted, all dosages come from *Nelson's Pediatric Antimicrobial Therapy*, 2017 edition, AAP press.

Local Infections With *Candida* Species

Diaper Dermatitis

This entity presents as an erythematous, erosive dermatitis of the perineal region, typically with pustular “satellite lesions” beyond the borders of the rash. Predisposing factors include systemic

antibiotics, glucosuria, and wet diapers. Care must be taken to differentiate this tractable condition from invasive fungal dermatitis (discussed later in the chapter). The former responds well to topical antifungal ointments.

Funisitis

Infection of the umbilical cord with *Candida* spp., while rare, is an indicator of chorioamnionitis and carries a poor prognosis, especially in the premature neonate (Qureshi et al., 1998). A minority of neonates in one study (16%) had associated congenital candidiasis. Intrauterine contraceptive devices or cervical cerclage was reported in 16% of the mothers.

Urinary Tract Infection

Isolation of *Candida* spp. from a catheterized specimen or via suprapubic bladder aspiration, as opposed to a bagged sample, is a reliable indicator of infection, although asymptomatic colonization of urinary catheters, stents, or nephrostomy tubes can be difficult to distinguish from true infection (Lundstrom and Sobel, 2001).

The presence of candiduria in the NICU neonate is associated with renal candidiasis—the latter manifested by cortical abscesses or fungal mycelia in the collecting system (“fungus balls”)—nearly half the time and may be a cause of frank obstruction (Bryant et al., 1999). Thus in contrast to older children or adults, the finding of candiduria in the NICU neonate should prompt blood cultures and renal imaging at the very least. If blood cultures prove to be positive, a full evaluation for disseminated candidiasis should be undertaken (discussed later in the chapter).

Because of the high prevalence of associated upper tract disease, imaging of the kidneys by ultrasonography should occur upon isolation of the organism from a catheterized urine specimen or from a suprapubic bladder aspiration. Approximately half of patients who eventually develop upper tract manifestations will display them on the first ultrasound (Bryant et al., 1999). Therefore follow-up imaging is recommended both to ensure the clearance of fungal mycelia, if present, and to monitor for later development of this complication. Unfortunately, no standard interval for monitoring has been proposed. However, in the infant who remains persistently funguric or candiduric, a single negative ultrasound should not be considered definitive.

Removal of a colonized urinary catheter may suffice for treatment in a patient without pyuria or systemic symptoms. Disease confined to the lower tract is best addressed with azoles (e.g., fluconazole, 4 to 6 mg/kg per day). Upper tract disease requires parenteral amphotericin B in systemic doses (1 mg/kg per day). Liposomal amphotericin B may not be an acceptable alternative. The particles in at least one liposomal preparation (Abelcet) appear to be too large to penetrate the adult kidney (Agustin et al., 1999), and this author has seen a premature newborn whose persistent candiduria failed to resolve until Abelcet was changed to amphotericin B deoxycholate.

Peritonitis

Candida peritonitis typically develops as a consequence of bowel perforation or, rarely, as a complication of peritoneal dialysis. In the former situation, multiple organisms such as gram-negative rods and enterococci may also be involved, and the neonate is at risk for sepsis with any one of them (Johnson et al., 1980). Peritonitis associated with a peritoneal dialysis catheter usually occurs as an isolated process, and the outcome is much better.

Spontaneous intestinal perforation associated with *Candida* peritonitis with or without sepsis has been described within 7–10

days of birth in neonates weighing less than 1000 g, typically in the absence of necrotizing enterocolitis (Meyer et al., 1991; Mintz and Applebaum, 1993; Adderson et al., 1998; Holland et al., 2003). Hallmarks include bluish discoloration of the abdomen and a gasless pattern on abdominal film. A substantial proportion of these neonates will have systemic candidiasis, although *S. epidermidis* can also be seen. In a small study of seven patients (Holland et al., 2003), deficiency of the muscularis propria was found in six.

Diagnosis requires visualization of the organism on a Gram stain of peritoneal fluid sterily obtained or culture of the organism from the same source. Isolation of *Candida* spp. from the peritoneal fluid should always prompt a search for bowel perforation, either by radiology or by surgical exploration, depending upon the clinical circumstances.

Treatment of *Candida* peritonitis caused by necrotizing enterocolitis or bowel perforation requires surgical evaluation, supportive therapy, and direct address of all contaminating microorganisms in the peritoneal fluid and the bloodstream. The typical regimen should address enterococci, gram-negative rods, and anaerobes together with systemic antifungal therapy, with the most likely choice being amphotericin B. *Candida* spp. isolated from peritoneal dialysate can be treated with removal of the catheter and a short course (7–10 days) of amphotericin B therapy in a dose of 0.3 to 0.5 mg/kg per day. The catheter can typically be reinserted within 24–48 hours, once the Gram stain is free of yeast cells.

Systemic Infection

Candidemia Associated With Central Venous Catheters

The association between prematurity and bloodborne candidal infections has been recognized for 25 years (Baley et al., 1984; Johnson et al., 1984). Over this same period of time, the incidence of candidemia has escalated from 25 to 123 cases per 10,000 NICU admissions (Kossoff et al., 1998; Saiman et al., 2000). The median time of onset is approximately 30 days of age (Baley et al., 1984). In a large multicenter study, colonization of the gastrointestinal tract preceded candidemia in 43% of cases (Saiman et al., 2000).

A variety of nonspecific clinical findings may be associated with this presentation of candidal disease, including respiratory decompensation, feeding intolerance, temperature instability, or mild thrombocytopenia. It is unclear whether the latter manifestation relates more to the use of heparin in intravenous (IV) and peripheral catheters or to the presence of *Candida* spp. in the bloodstream.

Any *Candida* spp. isolated from a blood culture should never be regarded as a contaminant and should prompt an immediate search for evidence of dissemination, which occurs in approximately 10% of premature newborns with candidemia (Patriquin et al., 1980; Noyola et al., 2001). A thorough evaluation includes ophthalmologic examination and ultrasonography of the heart, venous system, and abdomen. When lumbar puncture is performed, 10%–50% of candidemic neonates may have associated meningitis (Faix, 1984; Benjamin et al., 2006); in one prospective study, nearly 50% of ELBW neonates with *Candida* meningitis (13/27) had negative blood cultures (Benjamin et al., 2006).

Numerous studies have shown that CVCs should be removed within 24 hours of identification of yeasts in the blood culture (Karlowicz et al., 2000); in particular, removal of the CVCs within 3 days is associated with a significantly shorter median duration of candidemia (3 days vs 6 days) and a reduced mortality rate (0% vs 39%). In at least one study of candidemia, delayed removal of CVCs was associated with neurodevelopmental impairment at

18–22 months (Benjamin et al., 2006). Many experts recommend routine echocardiograms for patients with catheter-associated candidemia, to look for thrombi before removal of the catheter. However, even with the prompt removal of the catheter and institution of appropriate antifungal therapy, a substantial proportion of infants may exhibit prolonged candidemia lasting 1 to 3 weeks (Chapman and Faix, 2000).

Disseminated Candidiasis

Mortality rates approach 30% for this dreaded complication of candidemia. Once again, *C. albicans* is the leading pathogen. Organ involvement is most common in the vascular tree at catheter sites (15.2%), followed by the kidneys (7.7%) (Patriquin et al., 1980; Noyola et al., 2001). Eye involvement occurs in approximately 6% of infants. Thrombi within the vascular bed may be particularly difficult to eradicate with antifungal therapy; infants with right atrial thrombi may benefit from atriotomy (Foker et al., 1984). Other sites less frequently involved include the liver, spleen, and skeletal system. In infection of the bones and joints in premature newborns, *Candida* spp. are typically the second most likely pathogen, preceded by *S. aureus* (Ho et al., 1989).

Antifungal Therapy for Systemic Infection

As is true of most medications used in the NICU, dosing recommendations for antifungal therapies have not undergone rigorous testing in this patient population. With that caveat in mind, practice guidelines for this difficult clinical problem are suggested. Amphotericin B is the “gold standard” antifungal agent for treatment of systemic neonatal fungal infection. The drug binds to ergosterol in the membrane of fungi, facilitating membrane leakage. Rapid institution of parenteral amphotericin B deoxycholate in doses of 1 mg/kg per day, given by IV infusion over 2–6 hours, is the therapy of choice for systemic infection, including catheter-associated candidemia and disseminated candidiasis. No more than 24 hours should elapse before the infant is receiving a therapeutic dose of 0.7 to 1.0 mg/kg per day. Liposomal amphotericin B in a single dose of 5 mg/kg per day as an IV infusion over 2 hours (Weitkamp et al., 1998; Juster-Reicher et al., 2003) is an equivalent choice for initial therapy, but clinicians should be aware that some lipid formulations (e.g., Abelcet) have decreased renal penetration and are therefore not appropriate choices in disseminated candidiasis with renal infection.

Azoles are fungistatic agents that interfere with ergosterol synthesis by inhibiting C-14 alpha demethylase, a cytochrome P450 enzyme. Some experts recommend this class of antifungals as initial therapy for *C. albicans* infection; because of their fungistatic effects, others use them primarily to complete a course of antifungal therapy after clearance of the infecting organisms. Fluconazole, the most frequently used, requires an IV loading dose of 25 mg/kg on day 1, with subsequent IV doses of 12 mg/kg per day for the remainder of therapy. Azoles such as itraconazole and posaconazole are preferable to fluconazole for *Aspergillus* and zygomycetes. However, *Aspergillus* and zygomycetes are extremely rare in the NICU; therefore no studies have been done to recommend neonatal dosing guidelines.

Caspofungin, an echinocandin, has also been used in dosages of 25 mg/m² per day to treat invasive candidal disease in the newborn in several case reports. Caspofungin and other echinocandins such as micafungin (10 mg/kg per day) or anidulafungin (1.5 mg/kg per day) interrupt biosynthesis of β -(1,3)-D-glucan, an integral part of the fungal cell wall. However, identification of

infecting spp. has important implications for choice of therapy. Although amphotericin B formulations, the azoles, and the echinocandins are all appropriate for infections caused by *C. albicans*, non-*C. albicans* spp. such as *C. glabrata*, *C. guilliermondii*, and *C. krusei* may have decreased susceptibility to fluconazole and can be variably sensitive to the echinocandins (Odio et al., 2004; Sáez-Llorens et al., 2009; Hsieh et al., 2012). *C. lusitanae* is usually resistant to amphotericin B formulations.

Some experts recommend institution of empiric antifungal therapy in acutely thrombocytopenic neonates of less than 25 weeks' gestation, especially if there is a recent exposure to third-generation cephalosporins (Benjamin et al., 2003). Dosage adjustment for renal dysfunction is necessary only if serum creatinine increases significantly during therapy. Amphotericin B has poor cerebrospinal fluid penetration; in neonates with accompanying meningitis, fluconazole may also be added to amphotericin B. The use of 5-flucytosine in doses of approximately 25 mg/kg per dose (range 12.5–37.5 mg/kg) given orally every 6 hours in patients with normal renal function is no longer routinely recommended because of bone marrow suppression or hepatotoxicity when serum concentrations rise above 40 to 60 µg/mL.

With prompt removal of an offending CVC and no evidence of dissemination, the duration of therapy for catheter-associated candidemia is typically 10–14 days after the blood culture becomes negative (Donowitz and Hendley, 1995). Disseminated candidiasis, including *Candida* meningitis, requires at least three or more weeks of parenteral therapy; the course is typically completed when all foci have been eradicated. Most infectious disease experts will use fungicidal doses of parenteral amphotericin B or a liposomal preparation for the entire course.

Antifungal Prophylaxis

Five randomized controlled trials comparing IV fluconazole (3 mg/kg per day) with placebo or no treatment in VLBW or ELBW infants for 4 to 6 weeks (Kaufman et al., 2001; Kicklighter et al., 2001; Cabrera et al., 2002; Manzoni et al., 2007; Parikh et al., 2007) met criteria for analysis in Cochrane reviews (Clerihew et al., 2007). Kaufman et al. (2001) and Manzoni et al. (2007) reported significantly lower incidences of invasive fungal infection, while there was no difference in treated versus untreated infants in the studies of Kicklighter et al. (2001), Cabrera et al. (2002), and Parikh et al. (2007). The only study to evaluate neurologic outcomes found no difference in neurologic impairment at 16 months (Kaufman et al., 2001). Fluconazole prophylaxis did not affect a significant difference in the risk of death before discharge in any of the five studies or in a metaanalysis (Clerihew et al., 2007). No study documented clinically significant adverse effects of fluconazole or the emergence of fluconazole resistance.

One study from a single center compared nonrandomized fluconazole prophylaxis from 2002 to 2006 with an untreated, retrospective cohort from 2000 to 2001 and reported that invasive candidiasis decreased from 0.6% to 0.3%, although the proportion of invasive disease caused by non-*C. albicans* spp. increased from 26% to 41% after the introduction of fluconazole prophylaxis (Healy et al., 2008). Interestingly, fluconazole prophylaxis in this study was extended to several infants with birthweights greater than 1000 g, if risk factors (e.g., maternal human immunodeficiency virus infection, intestinal abnormalities) were present.

The finding that prophylactic fluconazole reduces the incidence of invasive fungal infection must be interpreted "with caution" (Clerihew et al., 2007; Clerihew et al., 2008) as:

1. The incidence of invasive fungal infection in the placebo groups in the Kaufman et al. (2001), Manzoni et al. (2007), and Parikh et al. (2007) studies was significantly higher (13%–16%) than in other large cohort studies of VLBW or ELBW infants in the United States (6%–7%) or the United Kingdom (1%–2%).
2. Fluconazole prophylaxis may have impaired the microbiologic isolation of some fungal spp. and led to underdiagnosis of infection in the treatment group.
3. Six years after the introduction of fluconazole prophylaxis, one study reported that non-*C. albicans* spp. with relatively reduced susceptibility to the azoles were the most common causes of invasive fungal infection (Parikh et al., 2007). This study did not detect a significant effect of fluconazole prophylaxis in reducing invasive candidal disease.

Current guidelines from the Infectious Diseases Society of America (Pappas et al., 2016) recommend IV or oral fluconazole, 3 to 6 mg/kg twice weekly for 6 weeks in neonates with birth weights less than 1000 g in nurseries with high rates (>10%) of invasive candidiasis. Oral nystatin, 100,000 units three times daily for 6 weeks, is an alternative in neonates less than 1500 g where issues with availability or resistance to fluconazole militate against its use.

Infections Ascribable to Other Fungi

Invasive Fungal Dermatitis

Invasive fungal dermatitis typically presents in the infant weighing less than 1000 g who displays macerated or bruised lesions that are contaminated with fungal spp.. In the initial report three of seven confirmed cases had *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *Trichosporon beigelii*, *Curvularia*, or *Aspergillus niger* and *fumigatus* were cultured from the remainder (Rowen et al., 1995). Among cases considered "probable," seven of eight had *C. albicans*. Systemic complications, including fungemia, meningitis, or infection of the urinary tract, occurred in four of seven confirmed cases and seven of eight probable cases. More cases than controls had postnatal steroids and prolonged hyperglycemia. Disseminated infection occurred in 69%, all ascribable to *Candida* spp.

Diagnosis requires a skin biopsy demonstrating fungal invasion beyond the stratum corneum or a positive potassium hydroxide preparation of skin scrapings; growth of the identical organism from an otherwise sterile site (blood, cerebrospinal fluid, or urine obtained via suprapubic aspiration) is confirmatory. Treatment requires systemic doses of amphotericin B in the range of 0.7 to 1.0 mg/kg per day; in those infants who do not develop systemic infection, oral therapy with fluconazole or topical antifungal creams may suffice. Oral therapy is not advisable for pathogens like *Aspergillus*, and repeated skin biopsies may be necessary to define duration of therapy.

Line Infections Caused by Lipophilic Organisms

The spp. *M. furfur* and *M. pachydermatis* are lipophilic organisms commonly carried on the skin, even in patients without tinea versicolor (Marcon and Powel, 1992). Cutaneous colonization can infect hyperalimentation fluids or parenteral lipid formulations. Infants typically present with mild but nonspecific signs: respiratory decompensation, glucose intolerance, or thrombocytopenia (Dankner et al., 1987; Stuart and Lane, 1992). Diagnosis requires isolation of the organism from blood by growth on fungal medium overlaid with olive oil, since *Malassezia* spp. will not grow in the

absence of lipids (Marcon et al., 1986). Removal of the intravascular catheter usually suffices for therapy, although some experts recommend the addition of amphotericin B in doses of 0.5 mg/kg per day for 7 days.

Miscellaneous Fungal Infections

Aspergillus Species

Although rarely seen in neonates, systemic infection with *Aspergillus* suggests severe immunocompromise, such as in DiGeorge syndrome or myeloperoxidase deficiency (Chiang et al., 2000; Marcinkowski et al., 2000). Disseminated disease has occurred in premature newborns without additional immunologic abnormalities (Rowen et al., 1992). Diagnosis requires isolation of the fungus from a normally sterile tissue site or visualization by Gomori-methenamine silver stain on biopsy of infected tissue. Of note, a commercially available enzyme-linked immunosorbent assay for diagnosis of aspergillosis on serum specimens had an 83% rate of false positives in premature newborns (Siemann et al., 1998). Treatment requires systemic amphotericin B in doses of 1 mg/kg per day. Fungistatic therapies such as the triazoles are not recommended for aspergillosis.

Trichosporon beigelii

In a cluster of five neonatal cases of infection caused by *T. beigelii*, a yeast found ubiquitously in soil, no common source was identified (Fisher et al., 1993). Two of three premature neonates infected with this organism died. Resistance to achievable concentrations of amphotericin B complicates therapy.

Suggested Readings

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42

Lung Development

ERIN PLOSA AND SUSAN H. GUTTENTAG

KEY POINTS

- Lung organogenesis is temporospatially organized into five stages, beginning at embryonic day 25 in humans.
- The mechanisms of lung organogenesis, including branching morphogenesis, stretch/mechanotransduction, alveolarization, microvascular maturation, and cellular differentiation, depend on transcriptional regulation of cell–cell and cell–extracellular matrix signaling networks.
- Alveolarization and lung growth are primarily postnatal processes that extend to early adulthood to establish a surface area that matches growing metabolic needs.
- A growing number of congenital lung malformations and acquired neonatal lung diseases can be traced to disruption of lung development.
- In addition to maturation of the surfactant system, development of lung host defense and environmental detoxification systems is critical for the transition from the fluid-filled fetal lungs to successful air breathing.

The primary function of the lung is to accomplish exchange of oxygen and carbon dioxide to accommodate the needs of aerobic cellular respiration. The oxygen consumption of the adult human ranges from 250 mL per minute at rest to 2630 mL per minute at peak exercise (Hansen et al., 1984). To accommodate these metabolic needs, a large surface area and a thin alveolocapillary membrane are required to enable efficient diffusion of oxygen, more so than carbon dioxide. Ultimately, the zone of gas exchange will attain a surface area of 50–100 m² and a volume of 2.5–3.0 L in the adult human. Therefore a primary goal of lung organogenesis is to expand the lung surface area to meet these needs. A second goal of lung organogenesis is to minimize the diffusion distance from the alveolus to the red blood cell, coordinating the development of an extensive capillary network with a thin, expansive alveolar epithelial surface. A third goal of lung development is production of a protective aqueous barrier overlying the delicate alveolar epithelium while mitigating the effects of the surface tension generated by this barrier, specifically alveolar collapse, through the production of a surface-active agent, or surfactant.

The trachea, airways, and alveoli are in constant contact with the external environment. Every inhalation brings large numbers

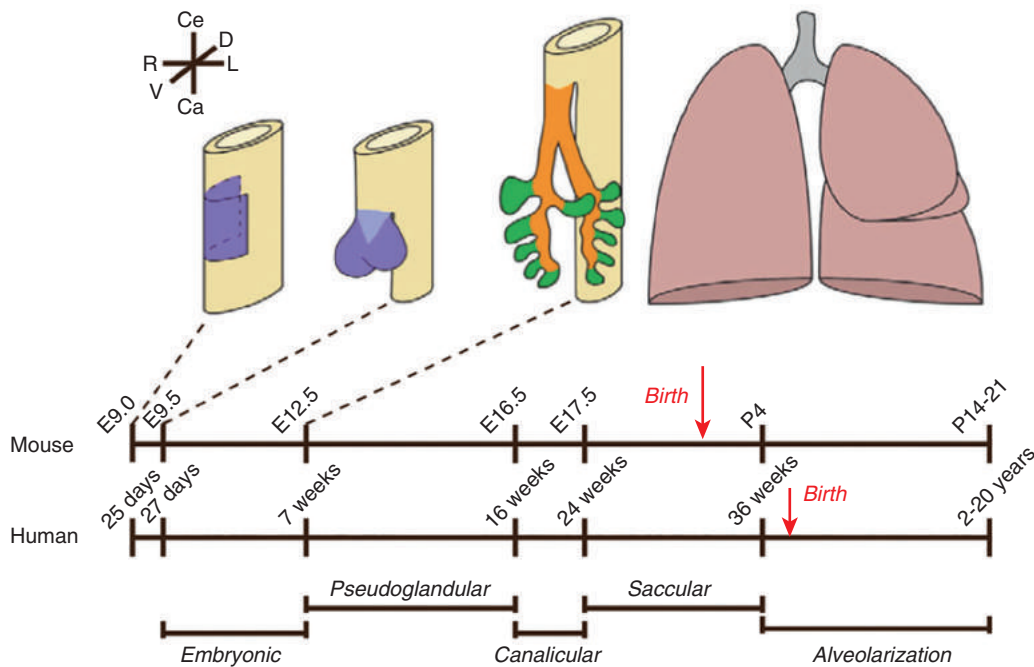
of microorganisms, and potentially toxic particles and gases, into direct contact with epithelial surfaces. Lung organogenesis must also incorporate mechanisms for clearance of microorganisms and allergens that may result in epithelial infection or injury. Similarly, the lung must defend against nonparticulate gases that are potentially harmful. Oxygen, so critical to cellular function, can be the source of harmful reactive oxygen species that require detoxification, as do inhaled pollutants. The appropriate development and maintenance of these lung functions are critical to the health and survival of newborns.

Key Events in Lung Development

Lung organogenesis begins early in human gestation (by day 25), and growth extends well into early adulthood (Kumar et al., 2005; Burri, 2006). Lung development is organized into stages (embryonic, pseudoglandular, canalicular, saccular, and alveolar), although the timing of these stages is somewhat imprecise, and considerable overlap may occur. Fig. 42.1 shows a time line of fetal and postnatal lung development in the mouse lung versus the human lung.

Development of Airways and Gas Exchange Surfaces

The initial phase of lung development, the *embryonic phase*, is marked by the formation of the lung bud and initial branching of presumptive airways. The lung bud is first recognizable as a laryngotracheal groove of the ventral foregut at 25 days of human gestation. Within a few days the groove closes such that the only remaining luminal attachment to the foregut is in the region of the developing hypopharynx and larynx. The lung bud, consisting of epithelium and surrounding mesenchyme, then begins the first of a series of dichotomous divisions that give rise to the conducting airways and five primordial lung lobes (two on the left and three on the right). Tracheoesophageal fistulae, tracheal atresia, and tracheal stenosis result from errors in separation of the laryngotracheal groove, whereas failure of partitioning of the lung bud can result in pulmonary agenesis, most typically of the right lung (Jacobs et al., 2012). Branching continues into the *pseudoglandular stage* of lung development. By 7 weeks of human gestation, the trachea, segmental bronchi, and subsegmental bronchi are evident. By the end of 16 weeks, all bronchial divisions are completed. It is important to remember that although the conducting airways



• **Fig. 42.1** Comparison of Lung Development Events in Mice and Humans. Lung endoderm specification begins at embryonic day 9 in the mouse, and the development stages in the mouse are depicted associated with key transcription factors. Comparisons are provided with the timing of equivalent events in human lung development, beginning at day 25 of gestation in the human. Ca, Caudal; Ce, cephalic; D, dorsal; L, left; R, right; V, ventral. (Modified from Herriges M, Morrisey EE. Lung development: orchestrating the generation and regeneration of a complex organ. *Development*. 2014;141:502–513.)

will certainly enlarge as the fetus and newborn grow (airway diameter and length increase twofold to threefold between birth and adulthood), large airway branching ceases after 16 weeks of human gestation.

Closure of the pleuroperitoneal folds is a critical event of the pseudoglandular phase, reaching completion by 7 weeks and resulting in separation of the thoracic cavity from the peritoneal cavity. Failure of pleuroperitoneal closure results in a diaphragmatic defect and continuity between these cavities, resulting in herniation of abdominal contents into the thorax when the midgut returns to the peritoneal cavity from the umbilical cord at 10 weeks of human gestation. The resulting congenital diaphragmatic hernia leads to pulmonary hypoplasia of the lung ipsilateral to the diaphragmatic defect as bowel and solid viscera migrate into the thorax. Pulmonary hypoplasia may also extend to the contralateral lung as the mediastinum shifts because of accumulating abdominal viscera in the thorax.

The *canalicular phase* is marked by completion of the conducting airways through the level of the terminal bronchioles and the development of the rudimentary gas exchange units that are no longer invested with cartilaginous support. The acinus is the gas exchange unit of the lung and encompasses a respiratory bronchiole and all of its associated alveolar ducts and alveoli. A terminal bronchiole with all its associated acinar structures constitutes a lobule. Branching of these distal air spaces continues on a more limited basis during the canalicular phase, finally achieving a total of 23 airway subdivisions.

Evolution of the relationships between the air spaces, capillaries, and mesenchyme takes on more significance during the *saccular phase* of lung development (24–38 weeks of human gestation), enabling an alveolocapillary membrane sufficient to participate in

gas exchange ($0.6\ \mu\text{m}$) by approximately 24 weeks of human gestation. Beyond this point the efficiency of gas exchange is determined by the available surface area. Lengthening and widening of the terminal sacs expand the gas-exchange surface area. Each saccule consists of smooth-walled air spaces with thickened interstitial spaces containing a double capillary network. These will give rise to two or three alveolar ducts, further expanding the available surface area. Expansion of these rudimentary gas exchange units continues well into the third trimester of human gestation. Thus the human lung is not fully mature structurally, even at term delivery.

Postnatal lung development can be subdivided into additional stages (Burri, 2006). True alveoli become evident as early as 36 weeks of gestation in the human fetus, initiating the *alveolar phase* of lung development. The development of primary alveoli is followed by a further expansion of the gas-exchange surface area through the formation of septae, or secondary crests (described further later). Postnatal alveolarization extends from term through 1–2 years of age. An initial phase of “*bulk alveolarization*” occurs within the first 6 months postnatally, with a more modest addition of secondary alveoli through the remainder of this period. The alveoli of the infant lung are different from adult alveoli. These immature secondary alveoli contain a double capillary bed, whereas adult alveoli are invested by a single capillary bed. *Microvascular maturation*, the next phase of postnatal lung development, occurs between the first few postnatal months of life through 3 years of age (discussed later).

There is considerable controversy regarding when the lung ceases to add alveoli. Estimates have ranged from as early as 2 years to as late as 20 years of age in humans (Narayanan et al., 2012). This is further complicated by the observation that alveolar expansion

can occur in response to pneumonectomy in adult animals (Hsia et al., 1994) and humans (Butler et al., 2012). The acquisition of alveoli after the maturation of the microvasculature has been termed *late alveolarization*. This activity has been most often demonstrated in subpleural regions of the lung and likely invokes mechanisms similar to secondary crest formation.

The addition of alveoli is not the only means for expanding the surface area of the lung. While alveolarization wanes over the first 3 years of life in the human, *growth of the lung* continues to expand the gas-exchange surface area. Between 2 years of age and adulthood, lung tissue expands with lung volume proportionately to the increase in body weight of the child. Hyperpolarized helium-3 diffusion magnetic resonance imaging, a noninvasive means of assessing alveolar volume, has been used in children as young as 4 years to demonstrate increasing lung volume during childhood and adolescence (Altes et al., 2006). The observed increase in the apparent diffusion coefficient suggests that expanding lung volume is due to not only increasing numbers of alveoli but also increasing size of alveoli. Thus owing to the combined processes of prenatal lung development, postnatal lung development, and lung growth, there is tremendous potential for expansion of the gas-exchange surface area that is developmentally programmed into the fetal lung to account for the growing needs of the infant, child, and adult for aerobic cellular respiration. The extent to which these developmental mechanisms can be harnessed after premature birth, with or without superimposed lung injury, is a topic of active investigation that relies on extrapolation of experimental data from mice.

Composition of Airways and Alveoli

As branching morphogenesis proceeds, the epithelium lining the successive generations of airways and alveoli gives rise to specialized cells that participate in gas exchange, surfactant production, mucociliary clearance, detoxification, and host defense. Differentiation proceeds in a centrifugal fashion from proximal air spaces to distal air spaces, lagging behind branching. Temporal as well as contextual signals foster the regionalization of epithelial cell types.

Proximal Airways

The airway epithelium is tall and columnar, decreasing to a more cuboidal appearance more distally (Jeffery, 1998). The endodermal epithelial lining cells of the trachea and bronchi partition into four cell types: undifferentiated columnar, ciliated, secretory/goblet, and basal cells. Ciliated cells critical to the process of mucus clearance are first apparent between 11 and 16 weeks of human gestation and become less prevalent in more distal airways. Three types of secretory cells—those with largely mucous granules, those with serous granules, and some with both types of granules—can be seen as early as 13 weeks of human gestation. The number of mucin-producing goblet cells in airways peaks at midgestation in the fetus and declines in the third trimester relative to adulthood. Finally, immature basal cells expressing epidermal keratin have been noted as early as 12 weeks of human gestation. Basal cells play a critical role in regenerating injured large airway epithelium.

Cartilaginous support of the tracheobronchial tree begins and also proceeds in a centrifugal fashion beginning in the primitive trachea at 4 weeks, reaching the main bronchi by 10 weeks, and proceeding to the most distal terminal bronchioles by approximately 25 weeks of human gestation. Cartilaginous investment of airways is complete by the second month postnatally. Submucosal glands found in the interstitium between the cartilaginous tissue and

surface epithelium play a major role in airway host defense. Submucosal gland development can be characterized by five stages: epithelial budding and invasion of the lamina propria, development of a lumen, initiation of tube branching, and repetitive dichotomous branching. The airways of infants and children contain relatively more submucous glands than those of adults. The glands are lined by mucous cells proximally and serous cells more distally, the latter constituting 60% of the total epithelial cell content of the glands. Serous cells secrete water, electrolytes, and proteins with antimicrobial, antiinflammatory, and antioxidant properties, while the mucous cells produce primarily mucins. In addition to this host defense role, submucosal glands also contain a population of basal cells that respond to injury of the airway by replenishing the airway epithelium.

Muscular investment of the airways begins as early as 6–8 weeks of gestation as smooth muscle cells are identifiable around the trachea and large airways. Fetal airway smooth muscle is innervated and able to contract during the first trimester. It contracts in response to methacholine challenge, and this is reversible with β -adrenergic agonists. Muscularization increases throughout fetal life and childhood such that there is an increased amount of smooth muscle relative to airway size when compared with adult airways. Moreover, the rapid increase in the amount of bronchial smooth muscle immediately after birth occurs regardless of the timing of delivery, term or preterm.

An additional airway cell deserves mention because of its association with a wide variety of pediatric diseases. Pulmonary neuroendocrine cells (PNECs) are found throughout the airways, often in innervated clusters known as *pulmonary neuroepithelial bodies* (NEBs) located at branch points in the bronchial tree (Gu et al., 2014; Cutz, 2015). Although they arise from foregut endoderm, the cell of origin is distinct from other epithelial components of the lung. PNECs have large numbers of dense core vesicles containing neuropeptides, including serotonin and calcitonin gene-related peptide/bombesin, and regulate bronchial tone by releasing their contents in response to stretch- and hypoxia-mediated stimuli. Recent evidence suggests that PNECs also function as airway sensors that trigger immune responses (Branchfield et al., 2016). Pathologic conditions associated with PNEC/NEB hyperplasia include bronchopulmonary dysplasia (BPD), disorders of respiratory control (congenital central hypoventilation syndrome and sudden infant death syndrome), cystic fibrosis, chronic obstructive pulmonary disease, congenital diaphragmatic hernia, and pulmonary hypertension. Neuroendocrine hyperplasia of infancy is a rare form of interstitial lung disease of infancy associated with expansion of the number of PNECs and NEBs. Although the associations are strong, it remains unclear whether PNECs/NEBs play a primary role in these diseases or a responsive secondary role.

Distal Airways

The bronchiolar epithelium differs from the more proximal airway epithelium. In addition to being more cuboidal in appearance, the epithelium contains progressively fewer ciliated cells and goblet cells, which are ultimately absent from the terminal bronchioles. Instead, the nonciliated, secretory club cell is found in increasing numbers and density down the conducting airways, such that the club cell is the most abundant cell of the terminal bronchiole (Jeffery, 1998). Club cells are first evident by 16–17 weeks of human gestation, initially exhibiting large glycogen stores that are replaced by secretory granules. Between 23 and 34 weeks of gestation there is a dramatic increase in club cell numbers in distal bronchioles.

Club cells are critical to the host defense and detoxification functions of the lung. This specialized cell produces the highest levels of cytochrome P450 and flavin monooxygenases in the lung. While critically important in detoxification, these enzymes also participate in the bioactivation of procarcinogens, placing the club cell in a precarious position as a primary target of toxic metabolites. The club cell also plays an important role in immunoregulation in the distal airways. Important host defense products of the club cell include club cell secretory protein, surfactant protein A (SP-A), surfactant protein D (SP-D), leukocyte protease inhibitor, and a trypsin-like protease. Club cells produce a precursor form of surfactant protein B (SP-B) that may contribute to host defense. The secretion of antiproteases from club cells suggests that they modulate the protease–antiprotease balance in the distal part of the lung.

Alveolar Epithelium

During the fourth through sixth month of human gestation the epithelial cells lining the acini begin to differentiate further (Khoor et al., 1994). The cuboidal epithelial cells accumulate large glycogen stores and develop small vesicles containing loose lamellae. The large glycogen pools provide a ready source of substrate required for the production of increasing amounts of surfactant phospholipids, and they decrease in size as surfactant production advances in the fetal lung. In cells destined to become type 2 cells, lamellar bodies become larger, more numerous, and more densely packed with surfactant phospholipids and proteins, whereas those cells destined to become type 1 cells, on losing their prelamellar vesicles and becoming progressively thinner, adopt a phenotype more suitable for gas exchange. Alveolar type 1 and type 2 cells are readily identified early in the sacular stage of fetal lung development. There remains considerable controversy regarding the origin of type 1 epithelial cells. These cells in culture demonstrate very slow turnover, with a doubling time estimated to be between 40 and 120 days, suggesting that functionally they are terminally differentiated *in vivo*. Furthermore, in response to epithelial denudation occurring with lung injury, type 2 cells rapidly proliferate to reestablish epithelial continuity and then lose phenotypic features such as lamellar bodies and acquire markers of type 1 cells, suggesting that rapid repopulation of type 1 cells requires a type 2 cell intermediary. More recent studies in animals have suggested that alveolar type 1 cells can be induced to exit their terminally differentiated state and proliferate (Yang et al., 2016).

There is increasing appreciation for the alveolar type 1 cell as more than just a passive membrane for gas exchange (Williams, 2003). While a large surface area and small cytoplasm-to-nucleus ratio provides a thin alveolocapillary membrane to facilitate gas exchange, it also provides a large absorptive surface in the lung. The presence of water and ion channels, some distinct from those in type 2 cells, facilitates the maintenance of a relatively dry alveolus. Type 1 cells may also regulate cell proliferation locally, signal macrophage accumulation, and modulate the functions of local peptides, proteases, and growth factors.

While most notable for its role in surfactant production (discussed later), the alveolar type 2 cell provides other important functions in the alveolus (Fehrenbach, 2001). Alveolar type 2 cells are local progenitor cells and, like type 1 cells, contain ion and water channels as well as ion pumps that contribute to the movement of water and ions across the epithelium. Type 2 cells also contain and secrete important antioxidants (superoxide dismutases 1, 2, 3 and glutathione) and molecules of innate host defense (SP-A,

SP-D, and lysozyme) to participate in detoxification and sterilization of the alveolar microenvironment.

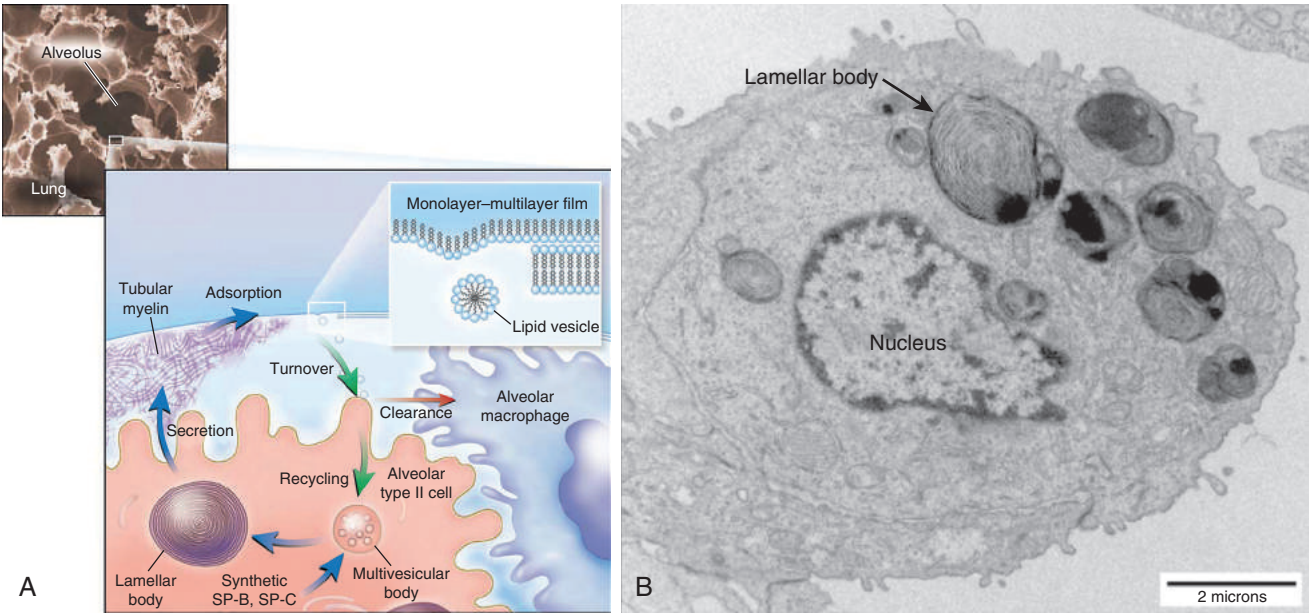
More recently, it is becoming clear that alveolar type 2 cells may play a part in exacerbating alveolar disease. The type 2 cell participates in the coagulation–fibrinolysis cascade through the production of fibrinogen, urokinase-type plasminogen activator, and tissue factor, especially under pathologic circumstances. Type 2 cells are increasingly recognized as a source of cytokine and chemokine production in the lung, as well as growth factors that can promote fibrosis. Finally, cross talk between epithelial cells, cell matrix, interstitial cells, and local inflammatory cells can foster the resolution of injury and inflammation or prolong lung remodeling after injury, with detrimental effects such as lung destruction and fibrosis. Therefore while previously heralded as the defender of the alveolus, the alveolar type 2 cell plays a much more complex role in alveolar health and disease.

Surfactant

Pulmonary surfactant is essential to alveolar health. The alveolar epithelial cells secrete a thin layer of liquid to protect the gas-exchange surface. The surface tension generated by this aqueous layer opposes alveolar inflation and promotes alveolar collapse at the end of expiration owing to the law of Laplace, whereby the collapsing pressure on the alveolus is directly proportional to the surface tension and inversely proportional to the radius of the alveolus. The film of pulmonary surfactant at the air–liquid interface lowers surface tension as the alveolar surface area decreases with exhalation, thereby preventing end-expiratory atelectasis, maintaining functional residual capacity, and lowering the force required for subsequent alveolar inflations.

Pulmonary surfactant is a complex mixture of phospholipids, neutral lipids, and proteins that is synthesized, packaged, and secreted by alveolar type 2 cells (Whitsett et al., 2015). The life cycle of surfactant is depicted in Fig. 42.2. Storage of surfactant occurs in the lamellar body, a lysosome-derived membrane-bound organelle that undergoes regulated secretion in response to a variety of stimuli, including stretch. In the alveolus, surfactant phospholipids transition through an extracellular storage form, tubular myelin. Phospholipid and protein components are recycled out of the surfactant monolayer at the air–liquid interface and taken back into the alveolar type 2 cell, where they can be repackaged into lamellar bodies. Alternatively, alveolar macrophages are able to engulf and degrade surfactant components.

The predominant surfactant phospholipid is saturated dipalmitoyl phosphatidylcholine (DPPC), with the remaining phospholipids consisting of monounsaturated phosphatidylcholine, phosphatidylglycerol, and other phospholipids (Table 42.1; Parra and Perez-Gil, 2015). DPPC is the only surface-active component of lung surfactant capable of lowering surface pressure to nearly zero. The presence of unsaturated phospholipids and other lipid components such as cholesterol enables the monolayer to remain fluid at body temperature during the respiratory cycle. Phospholipid content in the fetal lung increases with advancing gestation because of increased activity of enzymes responsible for phospholipid synthesis within alveolar type 2 cells. The expression and activity of enzymes of the choline incorporation pathway, the predominant pathway for surfactant phospholipid synthesis, are not only developmentally regulated but are also induced by hormones. The inductive hormones that have direct clinical relevance are glucocorticoids and agents that increase intracellular cyclic AMP



• **Fig. 42.2** (A) The life cycle of surfactant. (B) Electron micrograph of a type 2 alveolar epithelial cell showing the prominent lamellar bodies near the apical surface. *SP-B*, Surfactant protein B; *SP-C*, surfactant protein C. ([A] Reprinted with permission from Whitsett J, Weaver T. Hydrophobic surfactant proteins in lung function and disease. *N Engl J Med*. 2002;347:2141–2148. Copyright 2002 Massachusetts Medical Society.)

TABLE 42.1 Composition of Pulmonary Surfactant

| Component | Percentage (by Weight) |
|---------------------------------|------------------------|
| Lipid | 92 |
| Saturated phosphatidylcholine | 41 |
| Unsaturated phosphatidylcholine | 25 |
| Phosphatidylglycerol | 9 |
| Other phospholipids | 4 |
| Cholesterol | 8 |
| Neutral lipids | 5 |
| Protein | 10 |
| Surfactant proteins | 8 |

(cAMP) levels such as the β -adrenergic agonist (and tocolytic) terbutaline.

Surfactant contains a group of specific proteins with importance for surfactant function and host defense. The four surfactant proteins, SP-A, SP-B, surfactant protein C (SP-C), and SP-D, are subdivided on the basis of their physical characteristics into either hydrophobic (SP-B and SP-C) or hydrophilic (SP-A and SP-D) proteins. The hydrophobic surfactant proteins play a major role in the surface-active properties of surfactant, whereas the primary roles of the hydrophilic surfactant proteins are in host defense, immunomodulation, and surfactant clearance and metabolism.

Together the hydrophobic proteins, SP-B and SP-C, facilitate the mobilization of surfactant phospholipid from tubular myelin to the surface monolayer, promote spreading of phospholipids in the surfactant film, and assist in film stability at the end of expiration. SP-B plays a central role in alveolar health because of its critical function in surfactant homeostasis. SP-B is a secretory protein that exhibits strong association with membranes, unlike

SP-C, which contains a membrane-spanning domain and covalently attached fatty acids (palmitate) that render it integral to phospholipid membranes (Conkright et al., 2001). Both SP-B and SP-C are synthesized as large precursor proproteins that undergo extensive posttranslational processing as they pass through the secretory pathway, ultimately reaching the lamellar body. SP-B is essential for the process of lamellar body formation, and the alveolar type 2 cells of infants with an inherited deficiency of SP-B are devoid of lamellar bodies. Because the lamellar body is where SP-C proteolytic processing is completed, infants with inherited deficiency of SP-B are also deficient in mature SP-C, instead accumulating a larger, nonfunctional precursor of SP-C. Thus patients with inherited deficiency of SP-B, despite having relatively normal surfactant phospholipid profiles, make a pulmonary surfactant with very poor surface tension properties because of the combined defects in SP-B and SP-C. Conversely, because SP-C does not play either a direct or an indirect role in SP-B processing, animals with SP-C deficiency have normal SP-B, have normal lamellar bodies, and exhibit no perinatal lethality due to surfactant dysfunction.

Like the enzymes of surfactant phospholipid production, SP-B and SP-C exhibit developmental and hormonal regulation of expression (Mendelson, 2000). In human fetuses, SP-C messenger ribonucleic acid (mRNA) is detected as early as 12 weeks of gestation and SP-B mRNA by 14 weeks, yet the mature proteins are not detectable in fetal lung tissue until after 24 weeks. SP-B protein is not detectable in amniotic fluid until after 30 weeks of gestation, the amount increasing toward term (Pryhuber et al., 1991). This is due to developmental regulation of posttranslational events in the proteolytic processing of proSP-B and proSP-C (Guttenag, 2008). Consequently, infants delivered prematurely have reduced levels of both surface-active components of surfactant—phospholipid and hydrophobic surfactant proteins. The rate of type 2 cell differentiation, and secondarily surfactant production, is modulated

by the levels of endogenous corticosteroids and is accelerated by prenatal administration of glucocorticoid to women in preterm labor. The response of the surfactant system to prenatally administered glucocorticoids involves all key lipid and protein components, and occurs primarily through increased gene expression, thus representing precocious maturation mimicking the normal developmental pattern. Endogenous thyroid hormones, prostaglandins, and catecholamines also have stimulatory effects on type 2 cell maturation as well as on clearance of lung fluid at birth. Certain proinflammatory cytokines (e.g., tumor necrosis factor (TNF)- α and transforming growth factor (TGF)- β) inhibit surfactant production in experimental systems and may downregulate surfactant in conditions such as sepsis and inflammation.

Development of the Pulmonary Vasculature

The primary role of the pulmonary vasculature is to supply blood flow to the acini for gas exchange (Peng and Morrissey, 2013). During early fetal life the airways act as a template for pulmonary blood vessel development. The earliest pulmonary vessels form *de novo* in the tissue surrounding the lung bud in a process known as *vasculogenesis*. Mesodermal cells within the mesenchyme investing the developing lung tube differentiate into endothelial cells, proliferate, organize into chords, and develop a central lumen. As each new airway buds into the mesenchyme, a new plexus forms that adds to the pulmonary circulation, thereby extending the network of arteries and veins. By the fifth week of human gestation, a capillary network surrounds each bronchus, and circulation of blood between the right ventricle and the left atrium via this network is evident.

During the canalicular stage of lung development, new blood vessels form from preexisting vessels, a process known as *angiogenesis*. In contrast to *vasculogenesis*, *angiogenesis* is initiated by endothelial cell proliferation and sprouting from established vessels, resulting in extension of a vascular network into undervascularized regions. *Vasculogenesis* is the primary mode of pulmonary vascular development until the 17th week of gestation, when all preacinar airways and their accompanying vessels are present, whereas *angiogenesis* becomes the predominant mode of vascular development in the later stages of lung development. Although originally thought to be sequential processes, it is generally accepted that both occur concurrently during lung development, with *angiogenesis* dominating in the central part of the lung and *vasculogenesis* dominating in the periphery (deMello et al., 1997). Interconnections between vascular networks arising from both *angiogenesis* and *vasculogenesis* increase in the saccular phase of lung development.

In the human lung a second circulatory system, the bronchial circulation, arises from the dorsal aorta and nourishes the cellular constituents of the lung itself. The bronchial vasculature develops after the pulmonary circulation, with bronchial vessels first apparent by 8 weeks of gestation. The network of bronchial vessels is extensive, with bronchial arteries demonstrated as distal as the alveolar ducts in the adult respiratory tree. Inappropriate branching of bronchial vessels from the dorsal aorta is implicated in the formation of bronchopulmonary sequestration, a space-occupying lung malformation that can result in hypoplasia of the ipsilateral lung.

Vasculogenesis and *angiogenesis* are the primary mechanisms of vascular development throughout intrauterine life. The human lung at term contains only a small portion of the adult number of alveoli, and the air space walls are represented by a thick "primary septum" consisting of a central layer of connective tissue surrounded by two capillary beds, each of them facing one alveolar surface

(Burri, 2006). As alveolar architecture changes with the appearance of secondary septa, or secondary crests, folding of one of the two capillary layers occurs within the secondary septa. Microvascular maturation involves fusion of the juvenile double capillary network into a single capillary system present in the adult lung. Fusion is facilitated by the expansion of alveolar surface area and luminal volume, which compresses the interstitium, bringing the capillary networks in close proximity. This process is evident in the third postnatal week, during which lung volume increases by 25%, with a concomitant 27% decrease of the interstitial tissue volume. Subsequently, preferential growth of areas with a mature, fused capillary system continues.

Lastly, it is well known that lung volume increases about 23-fold between birth and young adulthood, while capillary volume expands 35-fold. It has been shown recently that this increase in capillary volume occurs by a third mechanism of vascular development: intussusceptive microvascular growth. This new concept in capillary network growth involves the formation of transluminal tissue pillars within capillaries that then expand, resulting in a net increase in capillary surface area (Burri, 2006).

Muscularization of the pulmonary arteries begins early in development (Hislop, 2005). Initially the muscular investment of the vasculature is derived from the migration of bronchial smooth muscle cells from adjacent airways. Muscularization of preacinar and resistance arteries of the pulmonary vasculature begins in the canalicular stage and continues through the remainder of gestation. This second phase of smooth muscle cells investing pulmonary vessels develops from the surrounding mesenchyme. Fibroblasts in close proximity to developing arteries alter their cellular shape and begin to express α -smooth muscle actin, a marker of their transformation into smooth muscle cells. A third phase of vascular muscularization, largely restricted to the distal part of the lung, involves the process of endothelial-mesenchymal transition, marked by endothelial cell division, separation and migration away from the endothelial layer, and expression of smooth muscle cell markers.

Muscularization of pulmonary arteries normally extends to the level of the terminal bronchiole and is minimal to absent in blood vessels surrounding respiratory bronchioles. Abnormal extension of smooth muscle along arterioles supplying acinar structures occurs in infants dying of persistent pulmonary hypertension of the newborn, as well as in infants with congenital diaphragmatic hernia and severe BPD.

Development of Pulmonary Host Defense

Every minute, the adult human lung takes in approximately 7 L of air contaminated with a variety of potential pathogens that can cause lung injury. The continuous exposure of the epithelial surface of the conducting airway to inhaled pathogens requires the presence of an efficient innate immune response system as a first-line defense against infection. The proximal and distal airway epithelia play a major role in clearing pathogens by secreting antimicrobial as well as antiinflammatory molecules into the airways and alveoli. In the proximal airways, components of mucociliary clearance appear as early as 11 weeks of human gestation, including ciliated epithelial cells, goblet cells expressing mucins, and submucosal glands described previously. The relative increase in the number of airway goblet cells over ciliated cells in prematurely born infants compared with term infants renders premature infants more prone to enhanced mucus production and obstruction caused by poor mucociliary clearance.

A number of microbial defense molecules are produced and secreted by epithelial cells into the airways (Bartlett et al., 2008). They include lysozyme, C-reactive protein, lactoferrin, collectins, β -defensins, and the cathelicidin CAP-18/LL-37 (Hiemstra, 2007). Two lung collectins originally identified as surfactant-related proteins, SP-A and SP-D, play a larger role in lung host defense than in surfactant biophysics. Although originally identified as products elaborated by epithelial cells lining airways (club cells) and alveoli (type 2 cells), they are secreted at other epithelial surfaces exposed to the external environment (Haagsman and Diemel, 2001; Kim et al., 2007; Bräuer et al., 2012; Madhukaran et al., 2015). These collectins interact with microorganisms and inflammatory cells to facilitate microorganism clearance and modulate inflammatory and apoptotic responses (Goto et al., 2014).

The basis for the interactions of the lung collectins with microbes and antigens centers on the binding of sugars by the carbohydrate recognition domains of these proteins (Wright, 2005). Interactions between collectins and gram-negative bacteria depend on the ability of SP-A and SP-D to bind lipopolysaccharide, whereas interactions with gram-positive bacteria, including group B β -hemolytic streptococcus, depend on recognition of the gram-positive outer membrane component lipoteichoic acid (Liu et al., 2012). The collectins bind a variety of fungi as well as *Pneumocystis carinii* and play an important role in inhibiting a variety of respiratory viruses, including influenza A virus and respiratory syncytial virus. Differences in the structure of the carbohydrate recognition domain provide SP-A and SP-D with altered affinities for different sugar molecules, allowing complementary functions and improving the diversity of microbial interactions.

The lung collectins also modulate the functions of a variety of immune cells, including macrophages, neutrophils, eosinophils, and lymphocytes (Atochina et al., 2004; Douda et al., 2011; Ariki et al., 2012). The collectins enhance the local production of chemotactic factors to attract macrophages and neutrophils and cytokines that activate macrophages and eosinophils, as well as attenuate lymphocyte responses by inhibiting T-cell proliferation. Other direct effects on inflammatory cells include modulating the production of reactive oxygen and nitrogen species used in killing microorganisms.

Like the hydrophobic surfactant proteins, SP-A and SP-D exhibit both developmental and hormonal regulation of expression (Mendelson, 2000). In human fetuses, SP-A mRNA is undetectable before 20 weeks of gestation, and SP-A is first detectable in amniotic fluid by 30 weeks of gestation, with the amount increasing toward term (Pryhuber et al., 1991). The SP-A gene promoter is induced by cAMP and glucocorticoids, although the response to glucocorticoids is biphasic, showing attenuation of SP-A expression at higher doses. Retinoids, insulin, and growth factors, including TGF- β and TNF- α , inhibit SP-A gene expression. Like SP-A, SP-D expression in the developing lung is quite low during the second trimester (Dulkerian et al., 1996) but is detectable in amniotic fluid in the third trimester, increasing toward term. The levels of both SP-A and SP-D increase markedly in the first few days after preterm birth and can be modulated by the local microenvironment of pathogens, toxins, and reactive oxygen species (Park et al., 2012; Zou et al., 2016).

Development of Detoxification Systems

Although oxygen is an essential component of cellular processes, concentrations beyond the physiologic limits may be hazardous to cells. The lung is particularly susceptible to reactive forms of

oxygen and free radicals since it is the organ with the highest exposure to atmospheric oxygen. The fetal lung is exposed to oxygen tensions of 20–25 mmHg in utero, and the transition to air breathing is associated with a fourfold to sevenfold increase in oxygen tension, presenting a significant oxidant stress. Oxygen free radicals arise from endogenous production through metabolic reactions or by exogenous exposure, such as air pollutants and cigarette smoke. Free radicals injure the lung through oxidation of proteins, DNA, and lipids. Therefore an antioxidant detoxification system is especially critical during the transition of the fetal lung to air breathing.

Oxygen free radicals are highly toxic substances. Superoxide is produced by the reduction of molecular oxygen through the addition of an electron, hydrogen peroxide is produced from the transfer of a single electron to superoxide, and hydroxyl radicals are produced through the interaction of hydrogen peroxide with superoxide. The free electrons of free radicals promote peroxidation of membrane lipids, alter sulfhydryl and other groups on exposed amino acids in proteins, and cause direct damage to DNA. All of these changes have been described in the lungs of animals and humans exposed to high levels of oxygen, although the dose response differs by species and with age, leading to altered epithelial integrity, interstitial and air space edema, and infiltration of inflammatory cells. Reactive oxygen species have also been implicated in enhancing production of proinflammatory mediators by lung epithelial and resident inflammatory cells, through chromatin remodeling, triggering signal transduction pathways, and activating transcription factors (Iliodromiti et al., 2013; Porzionato et al., 2015).

Antioxidants, both nonenzymatic and enzymatic, attenuate the effects of oxygen free radicals in the lungs. The major nonenzymatic antioxidants are glutathione, vitamin C (ascorbate), vitamin E (primarily α -tocopherol), β -carotene, and uric acid. Enzymatic antioxidants include superoxide dismutases (1, 2, and 3), catalase, thioredoxins, and a variety of peroxidases. Animal studies indicate that many of the antioxidant enzymes are induced before term delivery, and limited data suggest that the same is true for human fetuses (Weinberger et al., 2002). However, premature animals fail to induce antioxidant enzymes in response to oxidative lung injury (Berkelhamer and Farrow, 2014). Thus preterm infants are at significantly higher risk of oxidant lung injury owing to both increased need for oxygen in the treatment of respiratory distress syndrome and underdeveloped antioxidant defenses.

Mechanisms of Lung Development

Fetal and postnatal lung development depend on several key developmental processes: branching morphogenesis to promote branching of the lung bud into the surrounding mesenchyme, static and cyclic stretching of the lung that assist in promoting sacculization, alveolarization to enhance the expansion of the gas-exchange surface area, and vasculogenesis and angiogenesis to ensure that the developing epithelial surface area is invested with both a gas-exchange and a nourishing vascular supply.

Branching Morphogenesis

Branching morphogenesis is a fundamental mechanism of lung development. Branching is mediated by the accelerated growth of epithelial cells along the stalk of a branching airway with concomitant growth arrest at the branch tip (Affolter et al., 2009). This process requires extensive communication between epithelial cells and with the adjacent mesenchyme, as well as integration of

microenvironmental cues from the extracellular matrix (McCulley et al., 2015). Classic tissue recombination experiments in which mesenchyme from proximal airways was transplanted to distal airways (and vice versa) indicate that the mesenchyme has an important inductive role in dictating the branching pattern and cell fate of the expanding epithelium (Alescio and Dani, 1971). More recently, elegant studies using genetic mouse models indicate that three modes of branching—domain branching, planar bifurcation, and orthogonal bifurcation—are the basic mechanisms that characterize development of the complex three-dimensional respiratory tree through the pseudoglandular phase (Metzger and Krasnow, 1999; Metzger et al., 2008). These branching patterns are used repetitively during lung development and appear to be governed by a genetic clock orchestrating side branch formation, then planar bifurcation of a side branch, and planar rotation dictating the orientation of the bifurcation, thereby establishing a complex, three-dimensional structure.

Stretch and Mechanotransduction

The role of physical factors in modulating lung size is well established: normal lung growth requires adequate space in the chest cavity and appropriate tonic and cyclic distending forces. Genetic defects that compromise the thoracic skeleton and space-occupying lung masses such as congenital cystic adenomatoid malformations are associated with pulmonary hypoplasia due to the restriction of intrathoracic space. Denervation of the diaphragm to eliminate fetal breathing movements is associated with pulmonary hypoplasia, as is oligohydramnios.

Static Stretch: Fetal Lung Fluid Production

Fetal lung fluid is a product of the epithelial lining of the developing lung (Wilson et al., 2007; Helve et al., 2009), averaging 4–6 mL/kg per hour. The resistance imparted by laryngeal abduction results in fluid accumulation to a total volume of 20–30 mL/kg during gestation, which generates an end-expiratory pressure of approximately 2–4 cmH₂O. The composition of fetal lung fluid is distinct from that of both amniotic fluid and plasma, as illustrated in Table 42.2. The increased chloride content of fetal lung fluid as compared with serum is the result of active chloride secretion by the tracheal and distal pulmonary epithelium, largely because of the chloride channel CLC-2/CLCN2. A variety of growth factors, hormones, and lipid mediators influence the production of fetal lung fluid during gestation, including enhancers (prolactin, keratinocyte

growth factor, prostaglandin E₂, and prostaglandin F₂) and inhibitors (β-adrenergic agonists, vasopressin, serotonin, and glucagon).

While fetal lung fluid is an essential component of lung development, it presents a significant obstacle to the transition to air breathing on delivery. Three important events must occur to minimize the amount of fetal lung fluid and its potential impact on alveolar surface tension before the transition to air breathing: absorption, bulk removal, and maturation of pulmonary surfactant. Conversion of the pulmonary epithelium from a secretory to an absorptive surface is a critical event during the third trimester. Enhanced sodium transport across the alveolar epithelium is in part responsible for this change. Much evidence suggests that increasing expression of components of the epithelial sodium channel (ENaC) in the third trimester is a major factor in promoting sodium reabsorption from alveoli, with water passively following the movement of sodium (Helve et al., 2009; Hummler and Planès, 2010). Transgenic mouse pups that do not express the α subunit of ENaC die quickly after birth because of failure of fetal lung fluid clearance. Induction of ENaC subunits occurs at a transcriptional level in response to changes in extracellular matrix components, glucocorticoids, aldosterone, and oxygen. Drugs that increase intracellular cAMP levels (i.e., β-adrenergic agonists, phosphodiesterase inhibitors, and cAMP analogues), while not increasing the number of ENaC channels, increase the probability of a channel being open to sodium transport. In addition, glucocorticoid and thyroid hormones play an important role in priming the lung epithelium to be responsive to the actions of β-adrenergic agonists on sodium transport across lung epithelia near term. Thus the prenatal use of glucocorticoids and tocolytics may improve lung function in prematurely born infants through enhanced lung fluid reabsorption as well as by increasing surfactant production. Epithelial water channels, consisting of aquaporin proteins, are also induced during the late fetal period (Liu and Wintour, 2005). While water channels are clearly essential for fluid movement across epithelial cell membranes, their importance for fetal lung fluid clearance is less certain in the face of perinatal survival of transgenic mouse pups that do not express aquaporin 5 and/or aquaporin 1.

Conversion to an absorptive surface is not enough to minimize the amount of fetal lung fluid at the time of term delivery. The absence of uterine contractions is associated with an increased incidence of retained fetal lung fluid in infants born by cesarean delivery without the benefit of labor. On delivery of the head and neck, continued uterine contractions on the fetal thorax promote expulsion of bulk fluid from the fetal lung. However, animal studies suggest that the magnitude of the benefit of thoracic compression during labor is modest (Bland, 2001). Instead, the primary mechanism by which labor facilitates clearance of lung fluid is through hormonal effects on fluid clearance, especially through catecholamine-induced changes in the open probability of ENaC channels. The onset of air breathing, associated with increased intrathoracic negative pressure, assists in the clearance of residual fetal lung fluid into the loose interstitial tissues surrounding alveoli. Fluid is then reabsorbed via lymphatics and pulmonary blood vessels. It is generally accepted that the amount of residual liquid in the lung after transition is complete is approximately 0.37 mL/kg body weight.

Cyclic Stretch: Fetal Breathing Movements

Fetal breathing, detectable as early as 10 weeks of gestation in the human fetus, is an essential stimulus for lung growth (Kitterman, 1996). Fetal breathing occurs for 10%–20% of the time at 24–28 weeks, increasing to 30%–40% after 30 weeks of gestation.

TABLE 42.2 Composition of Human Fetal Lung Fluid Compared With Other Body Fluids

| Component | Lung Fluid | Interstitial Fluid | Plasma | Amniotic Fluid |
|---------------------|------------|--------------------|--------|----------------|
| Sodium (mEq/L) | 150 | 147 | 150 | 113 |
| Potassium (mEq/L) | 6.3 | 4.8 | 4.8 | 7.6 |
| Chloride (mEq/L) | 157 | 107 | 107 | 87 |
| Bicarbonate (mEq/L) | 3 | 25 | 24 | 19 |
| pH | 6.27 | 7.31 | 7.34 | 7.02 |
| Protein (g/dL) | 0.03 | 3.27 | 4.09 | 0.10 |

Originating from the diaphragm, fetal breathing is erratic in frequency and amplitude and changes throughout gestation. The volume of fluid moved is small and insufficient to be cleared from the trachea. Respiratory rates range from 30 to 70 breaths per minute, while periods of apnea of up to 2 hours have been recorded. Sustained periods of fetal breathing increase in duration with advancing gestation. The frequency of fetal breathing varies with sleep state (inhibited during quiet sleep) and exhibits diurnal variation, with the lowest rates recorded early in the morning. Fetal breathing is hormonally responsive, and the inhibition of fetal breathing with the onset of labor is attributed to the action of increased levels of circulating prostaglandins. Maternal medications can influence the frequency of fetal breathing movements. Central nervous system stimulants are associated with increased fetal breathing (i.e., caffeine, amphetamines), whereas depressants are associated with decreased fetal breathing (i.e., anesthetics, narcotics, nicotine, ethanol). Animal studies have clearly shown that permanent cessation of fetal breathing, regardless of the cause, is associated with impaired fetal lung growth. However, the impact on fetal lung development of short-term alterations in fetal breathing frequency and amplitude is unknown. Together, constant distending pressure from the production and retention of fetal lung fluid and episodic cyclic fetal breathing are important mechanisms for lung growth during fetal life.

Alveolarization

While branching morphogenesis is the primary developmental program that establishes the conducting airways of the lung, it is important to remember that alveolarization is the developmental program that will establish the large surface area involved in gas exchange (Galambos and Demello, 2008). This process will result in a 20-fold increase in surface area between birth (with between 0 and 50 million alveoli) and adulthood (>300 million alveoli). Primitive saccules develop low ridges (primary septa) that subdivide the saccule into an alveolar duct containing primary alveoli and outpouchings between the ridges (secondary septa/crests) that establish secondary alveoli (Fig. 42.3). Myofibroblasts and endothelial cells seed the base of developing ridges that will subdivide alveoli, while elastin localizes to the protruding edges of these developing ridges (Branchfield et al., 2016). Septation is critical for the process of microvascular maturation and also leads to the development of the pores of Kohn, allowing gaseous continuity between acini and a route for macrophages to move freely between alveoli.

The topic of alveolarization is rapidly evolving, receiving increasing attention because of observations that infants who die after severe BPD exhibit alveolar simplification with little evidence of secondary septation. It remains unclear to what extent the alveolarization potential may be permanently altered by preterm birth alone—in the absence of other injuries—and whether the developmental program can be resumed after preterm birth.

Interdependence of Alveolar and Vascular Development

Recent evidence suggests that the pulmonary capillary bed actively promotes normal alveolar development, maintains alveolar structures throughout life, and can contribute to degenerative lung disease in adulthood (Peng and Morrissey, 2013). The observation that combined abnormalities in the airways and vasculature occur in BPD supports this hypothesis. Intra-acinar arteries and

veins continue to develop after birth by angiogenesis as long as alveoli continue to increase in number and size. This may well be a reciprocal process because vascular growth around the distal air spaces suggests an inductive influence from the alveolar epithelial cells as well.

Molecular Basis for Lung Development

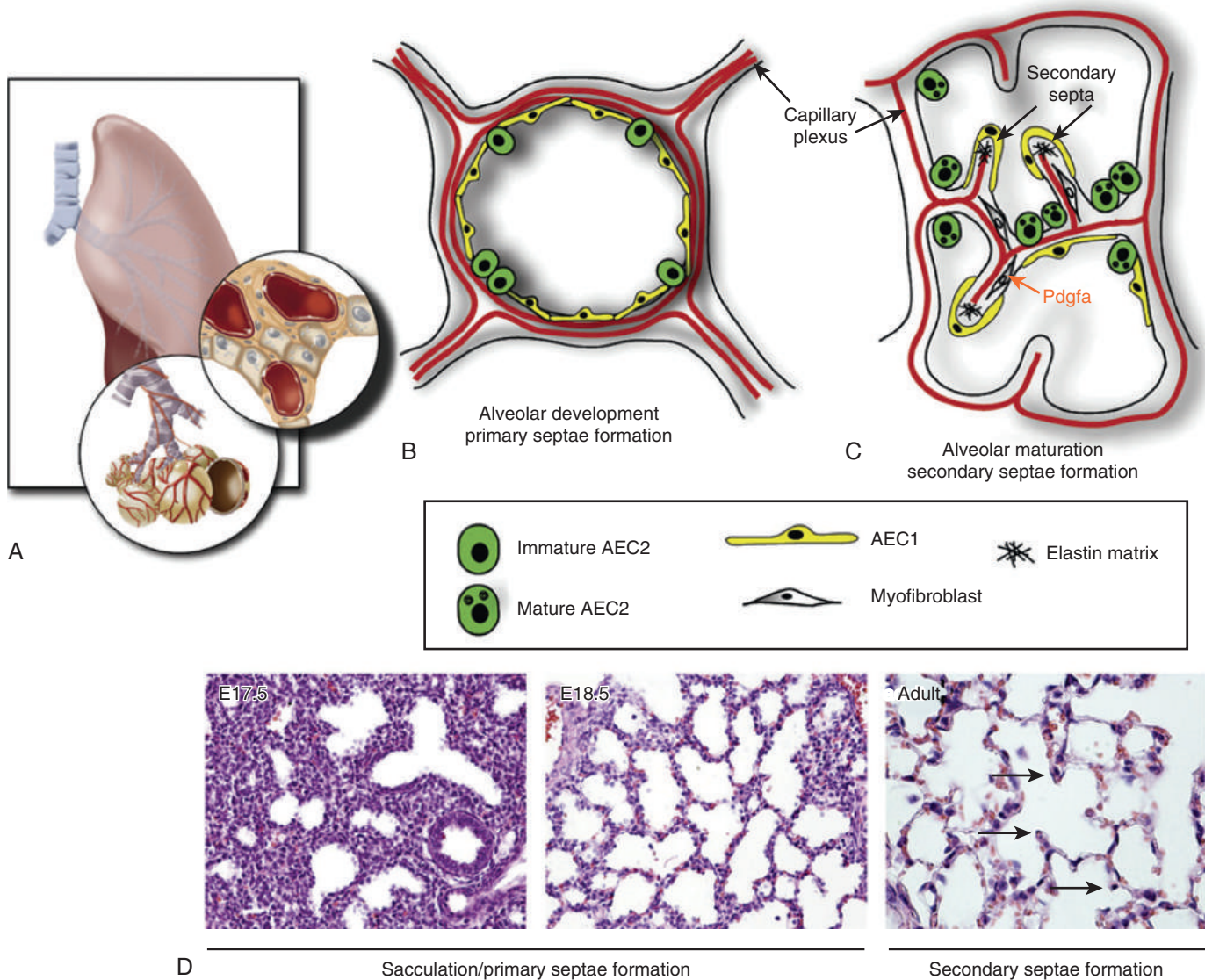
The developmental processes that contribute to lung organogenesis are under the regulation of interdependent signaling pathways mediated by secreted growth factors that are themselves under the control of large networks of transcription factors controlling gene expression. Gene regulatory networks common to other organs that depend on branching morphogenesis for organogenesis, most notably in the kidney and mammary gland, are also found in the lung. Selected regulatory networks are highlighted in the following sections. For more detailed discussion, interested readers are referred to Morrissey and Hogan (2010), Hines and Sun (2014), and Swarr and Morrissey (2015).

Growth Factors in Lung Development

The initiation of branching morphogenesis is the result of the interplay of signals between the developing lung epithelial tube and its surrounding mesenchyme. Central to this process is the family of fibroblast growth factors (FGFs) that are produced and secreted by mesenchymal cells and bind to receptors on the plasma membrane of epithelial cells, setting up a system of mesenchymal–epithelial cross talk. In particular, FGF10, secreted by mesenchymal cells, binds to its receptor, FGF receptor 2 isoform IIIb (FGFR2IIIb), on nearby epithelial cells. This signal is strongest in the epithelial cells at the tip of branching airways owing to a focused FGF10 gradient within the mesenchyme. Binding of the FGF10 ligand to the FGFR2IIIb receptor results in activation of the mitogen-activated protein kinase (MAPK) pathway within epithelial cells, setting off a cascade of downstream signaling events that regulates cell adhesion, cytoskeleton, and cell polarity, all essential elements of cell migration (Lü et al., 2005). One of the genes induced by FGF10/FGFR2IIIb signaling, *SPRY2*, inhibits the MAPK pathway, resulting in feedback inhibition of further FGF10/FGFR2IIIb signaling. Thus a signal propagated by the mesenchyme has an effect that is tightly regulated within the epithelial cell.

Proper lung development requires exquisitely precise FGF10 signaling. Animals expressing reduced levels of FGF10 develop pulmonary hypoplasia with reduced numbers of large airways (Ramasamy et al., 2007; Abler et al., 2009). Furthermore, increased FGF10 signaling during fetal lung development in mice by intrapulmonary injections of recombinant FGF10 produced cystic structures with epithelial characteristics dependent on the location of the injection: proximally with club cells, distally with alveolar type 2 cells (Gonzaga et al., 2008). Together these data provide strong evidence that FGF10 signaling has diverse responses—from initiation of branching to differentiation of epithelial cells—depending on the temporal and/or spatial context of signaling.

Like branching morphogenesis, lung vascular development is a complex and highly organized process that requires multiple vascular signaling molecules to interact in a specific temporospatial sequence. Vascular endothelial growth factor (VEGF) is a critical growth factor in angiogenesis and vasculogenesis. The expression of the VEGF ligand by epithelial cells and VEGF receptors (VEGFRs) by endothelial cells of the developing human fetal lung reinforces the interdependence of the air space and vascular



• Fig. 42.3 Alveolarization. (A) Alveolar development begins in late gestation as the endothelial plexus becomes tightly associated with the epithelium of the distal saccules. (B) Early alveoli contain several important cell types, including the flattened type 1 alveolar epithelial cells (AEC1) used in gas exchange and the cuboidal type 2 alveolar epithelial cells (AEC2) that make surfactant, which lie close to a double capillary network. (C) Maturation of the alveolar compartment is marked by the generation of secondary septa, which involves the development of alveolar crests, a process dependent on the development of myofibroblasts and the deposition of elastin. (D) Histologic sections stained with hematoxylin and eosin demonstrating the changes in distal lung morphology of the developing mouse lung, with secondary septa denoted by arrows. Pdgfa, platelet derived growth factor subunit A, found in myofibroblasts in mature alveolae. (From Morrissey EE, Hogan BL. Preparing for the first breath: genetic and cellular mechanisms in lung development. *Dev Cell*. 2010;18:8–23.)

development. The expression of VEGF mRNA and protein is localized to the epithelial cells at the distal tips of developing lung branches, and the expression levels increase with time (Hislop, 2005; Galambos and Demello, 2008). VEGF gene expression is induced in epithelial cells by the hypoxic environment of the growing fetal lung through the actions of the oxygen-sensing hypoxia-inducible factor family of transcription factors. From the single VEGF gene, five different VEGF protein isoforms are possible, although VEGFA (VEGF165) is the most studied. Each isoform has different affinities for each of the three VEGFRs (Flt-1/VEGFR1, Flk-1/kinase insert domain receptor/VEGFR2, and Flt-4/VEGFR3). Vascular endothelial cells express primarily VEGFR1 and VEGFR2,

whereas VEGFR3 is expressed on the plasma membrane of the lymphatic endothelium. VEGFRs are expressed on the plasma membrane of endothelial cells surrounding the developing airways from very early in gestation, and expression of VEGFR2 is considered the earliest marker of an endothelial progenitor cell. In vitro and in vivo experiments have shown that VEGFA induces endothelial cell proliferation and migration, both key elements of vascular sprouting, as well as tube formation through interactions with VEGFR2. VEGFR1 appears to have more importance in transforming primitive endothelial tubes into stabler vascular networks, in part by reducing endothelial proliferation through downregulation of VEGF production. The embryonic lethality of

animals with reduced VEGF expression attests to the critical importance of VEGF/VEGFR signaling to vascular development in the fetus, though not limited to the developing pulmonary vasculature.

Transcription Factors in Lung Development

The ligand–receptor interactions important for branching morphogenesis and pulmonary vascular development are in part determined by the actions of transcription factors on facilitating or reducing gene expression. Transcription factors are also critical in the differentiation of the lung epithelium from the most rudimentary lung bud out to the type 1 and type 2 alveolar epithelial cells.

The most important transcription factor in the lung is thyroid transcription factor 1 (TTF-1), a product of the *NKX2-1* gene. TTF-1 is considered a master regulator of lung development, as transgenic mice null for *NKX2-1* exhibit complete absence of lung branching (Kimura et al., 1996). *NKX2-1* plays a prominent role in establishing cell fate proximally to distally along the branching lung epithelium, with expression ultimately becoming more restricted to club cells and alveolar type 2 cells. TTF-1 is critical for the expression of genes that are unique to differentiated epithelium, such as *SCGB1A1* expression in club cells and surfactant proteins in alveolar type 2 cells. DNA binding sites are found in the promoter regions of all four surfactant genes, *SCGB1A1*, and *NKX2-1* itself, setting up a positive feedback loop for sustained TTF-1 expression. TTF-1 function is highly dependent on phosphorylation of critical amino acids, although it remains unclear which kinase is involved in this process (DeFelice et al., 2003). VEGFA expression is reduced in animals unable to phosphorylate TTF-1, providing another important link between epithelial and vascular development. TTF-1 is itself regulated by other transcription factors that bind to the promoter region of the *NKX2-1* gene, specifically hepatocyte nuclear factor 3 β and GATA-binding protein 6. Thus the ability of networks of transcription factors to bind to gene regulatory elements of DNA in a coordinated fashion during fetal lung development enables the temporospatial expression of growth factor networks that foster branching morphogenesis, the process of differentiation that ultimately gives rise to the approximately 40 cell types that constitute the human lung and the coordination of both epithelial and vascular development.

Novel Concepts in Lung Development

Stem/Progenitor Cells in the Lung

The ability of lung epithelium to replace cells damaged by normal aging or injury has become the focus of increasing attention (Herriges and Morrissey, 2014; Hogan et al., 2014). Stem cells are undifferentiated and have an unlimited capacity for self-renewal. Asymmetric divisions allow self-renewal through one daughter cell while enabling the other daughter cell to become more terminally differentiated. Progenitor cells are more committed, and although capable of self-renewal, they exhibit more restricted cell fates. Thus far, experiments combining lineage tracing with animal models of lung injury have identified several epithelial progenitor cell types having the capacity for both self-renewal and replacement of a variety of specialized lung epithelial cells. Basal cells in large airways and submucosal glands, identified by the expression of tumor protein p63/p63 and cytokeratin 5, are able to self-renew and give rise to ciliated and secretory cells. Club cells in smaller

airways, identified by the expression of club cell secretory protein, seem to be a more committed progenitor cell since they are able to self-renew but differentiate only into club cells or ciliated cells. A subset of club cells, termed *bronchoalveolar stem cells* (BASCs), located at the bronchoalveolar duct junction and resistant to certain forms of lung injury, appear to have a role in epithelial repair. BASCs are both club cell secretory protein and SP-C positive and thus have the potential to produce either club cells or alveolar type 2 cells. Also in the smaller airways, neuroendocrine cells can self-renew and give rise to club cells and ciliated cells following exposure to injury. The most limited lung epithelial progenitor cell is the alveolar type 2 cell, which divides rapidly to reestablish epithelial continuity in damaged alveoli and then transdifferentiates into alveolar type 1 cells. Less well studied are the mesenchymal progenitor cells that provide vascular and muscular components of the developing lung. Candidate cells have been identified that give rise to endothelial cells in the process of vasculogenesis and airway smooth muscle cells along the branching lung epithelial tubes.

Evidence for the existence of stem/progenitor cells in the lung is strong but limited largely to mouse models of lung development and lung repair. Therefore extrapolation to humans should be done with caution, as the murine transition between terminal bronchioles and alveoli is not equivalent to the transition in the human lung. While the capacity for self-renewal is tantalizing, it necessarily means that such cells are at risk of autonomous growth such as cancer. Controversy exists around the potential to harness these populations of cells as a means for correcting abnormalities of lung development (pulmonary hypoplasia), genetic diseases of the lung (cystic fibrosis), and abnormal repair of injured lungs (BPD).

Epithelial to Mesenchymal Transition

Fibrosis often occurs as the result of severe injury to the lung and has historically been a component of the “old BPD.” Emerging evidence suggests that expansion of fibroblast populations in fibrotic lesions is not simply the result of increased proliferation of local fibroblast populations but rather is the result of transformation of epithelial cells into mesenchymal components, a process known as *epithelial–mesenchymal transition* (EMT) (Bartis et al., 2014; Hines and Sun, 2014). EMT is an essential part of gastrulation and the development of cardiac valves. Neural crest cells are the best model system for EMT, since these epithelial cells must lose their local attachments and travel long distances before locating their final niche before differentiating. The evidence is clear that the changes in cell phenotype characteristic of EMT—from sedentary, interconnected epithelial cell expressing epithelial marker proteins to mobile, proliferative mesenchymal cell with expression fibroblast markers and secretion of collagen and other components of extracellular matrix—are under the control of local growth factors. In the lung the most prominent growth factor in EMT associated with pulmonary fibrosis is TGF β , a growth factor that is also essential for normal branching morphogenesis during early lung development.

It remains uncertain whether EMT has a similarly important role in lung development as it does in cardiac development and whether EMT alone is important for the abnormal repair response to lung injury. There is evidence that EMT makes at least a small contribution to lung development. Under the direction of *Shh* signaling, 5% of the embryonic day 14.5 lung mesenchyme in mice is derived from the mesothelium, the epithelial layer surrounding the lung (Que et al., 2008; Dixit et al., 2013).

**TABLE
42.3****Potential Impact of Premature Birth on Lung Development and Maturation**

| Event | Effect of Preterm Birth | Potential Consequences |
|--|---|--|
| Development of conducting airways | Branching: no effect; completed to the level of the respiratory bronchi by 24 weeks Tone: increased secondary to lung disease in part reflecting developmental deficiency of nitric oxide | None Increased airway resistance |
| Alveolarization | Decreased in severe bronchopulmonary dysplasia; may also be compromised by excess glucocorticoids | Reduced lung growth and lung surface area with increased alveolar size; impaired pulmonary function |
| Development of alveolocapillary membrane | Minimal effect: reaches adult diameter by 24 weeks of gestation; glucocorticoids induce precocious thinning | Gas exchange largely dependent on surface area, not alveolocapillary diameter |
| Type 1 cell differentiation | Variable depending on timing of delivery | Gas exchange largely dependent on surface area |
| Type 2 cell differentiation | Variable immaturity and deficient surfactant production depending on timing of delivery; improved with prenatal administration of glucocorticoids | Developmental deficiency of surfactant content and composition results in respiratory distress syndrome. |
| Hydrophobic surfactant proteins (SP-B, SP-C) | Variable effect depending on timing of delivery and other factors such as infection that can impair gene transcription | High alveolar surface tension; respiratory distress syndrome |
| Hydrophilic surfactant proteins (SP-A, SP-D) | Variable effect depending on timing of delivery; both proteins appear relatively late in the third trimester | Compromised host defense: impaired ability to clear microorganisms from airways and/or alveolar space; impaired ability to modulate inflammatory responses |
| Club cell differentiation | Variable effect depending on timing of delivery; these cells appear in the middle of the second trimester, but antioxidant products appear late in the third trimester | Impaired antioxidant and antimicrobial defenses; may contribute to chronic lung disease and pneumonia |
| Mucociliary clearance | Variable effect depending on timing of delivery; goblet cells decrease in number toward term | Increased mucus production may obstruct small airways |
| Development of the pulmonary capillary bed | Variable effects depending on timing of delivery in parallel to alveolar development | Variable degrees of impaired gas exchange commensurate with impaired alveologenesis and any superimposed lung injury; pulmonary hypertension |
| Pulmonary arteries | Variable effects depending on presence and severity of associated lung disease | Pulmonary hypertension associated with chronic lung disease |
| Fetal lung liquid | Fluid loss: variable effects depending on magnitude and duration of fluid loss (i.e., prolonged premature rupture of membranes) as well as timing of delivery Fluid retention: variable effects depending on timing of delivery because hormone surges near term and in labor promote reabsorption before delivery | Pulmonary hypoplasia Transient tachypnea of the newborn |
| Fetal breathing movements | Variable effects depending on timing of delivery but also depending on maternal exposure to substances that reduce fetal breathing movements | Unlikely to have effects in preterm infants unless coexisting conditions severely limit fetal breathing |
| Respiratory drive | Variable depending on timing of delivery | Apnea of prematurity |

SP-A, Surfactant protein A; SP-B, surfactant protein B; SP-C, surfactant protein C; SP-D, surfactant protein D.

EMT has the potential to reduce epithelial populations, resulting in lung destruction, while expanding mesenchymal fibroblast pools and enhancing local matrix deposition that reduces lung elasticity. Many of the models of EMT are derived from lung cancer cell lines or mouse models, limiting their applicability to humans. However, improved understanding of the processes controlling EMT may lead to novel therapies for limiting or reversing pulmonary fibrosis after lung injury. Furthermore, early events in EMT may be particularly important targets of preventive therapy in premature infants given the potential for EMT to contribute to lung destruction, a more common feature of the new BPD.

Epigenetic Regulation of Lung Development and Maturation

Regulation of normal development and the consequences of abnormal development are at the heart of understanding the implications of preterm birth and the development of potential lung protective therapies. The central dogma of DNA to RNA to protein is being challenged by increasing recognition of epigenetic mechanisms—histone modifications, modification of DNA and RNA, silencing RNA, and microRNA (miRNA)—for regulating gene expression. The evidence that miRNAs play an important role in normal fetal lung development is clear (Khoshgoo et al., 2013). MiRNAs are small, noncoding RNAs (generally 19–22 nucleotides in length) found within cells that target genes for RNA degradation or inhibition of protein synthesis. There are more than 500 recognized miRNAs, some of which are particularly enriched in lung cell populations. MiRNAs are generated from a

process that begins in the nucleus. Long primary miRNA is transcribed, processed, and exported from the nucleus, where further cytoplasmic maturation occurs before the mature miRNA is able to interact with regions of messenger RNA, usually in the 3' untranslated region.

Emerging evidence indicates that miRNAs are essential for normal lung development, since targeted deletion of *dicer*, a key enzyme in miRNA processing, results in abnormal airway development and excessive apoptosis in the lungs (Nardiello and Morty, 2016). The miR-17-92 cluster of miRNA is highly expressed in embryonic mouse lung, decreasing into adulthood as lung development progresses. Altered expression of miR-17-92 suggests a primary role for these miRNAs in maintaining E-cadherin expression in airway epithelial cells during branching morphogenesis.

A novel class of noncoding RNAs, the long noncoding RNAs (or lncRNAs), similarly regulate lung development. These large nucleotides (>200 nucleotides in length) are less stable than mRNA, have fewer posttranslational modifications, and play a role in stabilizing chromatin, thereby regulating gene transcription. The expression of several lncRNAs is spatially and temporally correlated with transcription factors critical to lung development (Herriges et al., 2014). The best characterized is *NANCI* (Nkx2.1-associated noncoding intergenic RNA), which regulates neighboring Nkx2.1 expression. Knockdown of *NANCI* in vivo results in lung sacculation and epithelial differentiation defects.

Because of their role in regulating developmental and pathologic processes, noncoding RNAs are increasingly seen as targets for therapeutic interventions. The obstacles to using miRNA or lncRNA as therapeutic agents are similar to the obstacles encountered in other gene therapies, including mode of delivery, cell and tissue specificity, and the potential for off-target effects.

Summary

Lung branching morphogenesis is coordinated with pulmonary vascular development to provide a large surface area and thin alveolocapillary membrane to accomplish adequate gas exchange in the transition to air breathing and to meet the needs of a growing infant and child. Although occurring late in fetal lung development, maturation of the surfactant system is similarly critical in the transition to air breathing and the maintenance of patency of the gas-exchange surface. Although the fetal lung developmental program requires an array of transcription factors, hormones, and growth factors promoting branching morphogenesis, lung growth is equally dependent on intact neural input to modulate fetal breathing, stability of an appropriately sized thorax, and the presence

of adequate lung and amniotic fluid. Furthermore, the maturation of the host defense and detoxification systems minimizes the effects of increased oxygen tension and exposure to potential pathogens accompanying the transition to air breathing. Premature birth impacts all of these functions, as illustrated in Table 42.3. BPD is the net result of multiple injuries to the underdeveloped lungs of premature newborns that compromise postnatal growth and development, thus impairing function. Integrated approaches to therapy that reflect the interdependency of these lung functions have the most promise for minimizing the impact of premature birth on childhood and, ultimately, adult lung function.

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43

Control of Breathing

ESTELLE B. GAUDA AND RICHARD J. MARTIN

KEY POINTS

- Apnea of prematurity is the manifestation of greater inhibitory (rather than excitatory) influences on the central respiratory network.
- The predominant influence of peripheral arterial chemoreceptors on baseline breathing in premature infants causes periodic breathing and apneas, including postsigh apnea.
- The protective upper airway reflex (laryngeal chemoreflex) prevents aspiration, but in premature infants, profound bradycardia, apnea, and oxygen desaturation can occur—in addition to laryngeal constriction.
- The excessively compliant chest wall in premature infants predisposes to low lung volumes.
- In extremely premature infants, apnea of prematurity can persist beyond 37 weeks' postconceptional age.

How the respiratory network matures is of interest not only to physiologists but also to clinicians who care for infants, newborns, and children with disorders of respiratory control. Neonatologists, in particular, are aware of the challenges of treating infants who are born prematurely. While breathing movements can be detected in the human fetus as early as 10–12 weeks' gestation, the purpose of fetal breathing is not gas exchange but instead the lung stretch that occurs during breathing is essential for lung development. The transition from fetal to neonatal life requires a rapid conversion from intermittent fetal respiratory activity not associated with gas exchange to continuous breathing on which gas exchange is dependent. Breathing in the most premature infants is akin to fetal breathing, which is episodic, punctuated by periods of disturbingly long apneic pauses interspersed with frequent periods of hyperventilation and sighs (augmented breaths). For the infant who is born prematurely, frequent apneas and periods of hypoventilation associated with oxygen desaturations and bradycardia are of significant concern. With maturation, breathing becomes more stable. Thus the premature infant provides a unique opportunity to observe how the respiratory system matures in humans.

Even though breathing is more stable in term infants than in premature infants, the respiratory system at term gestation is still undergoing significant maturation and can become unstable in response to stressors such as infection or hyperthermia. In some term infants who appear well, subtle developmental abnormalities

in the anatomy and neurochemistry of the respiratory system can lead to profound disorders of breathing. Infants who have died of sudden infant death syndrome (SIDS) and those infants with congenital central hypoventilation syndrome (CCHS) or with Rett syndrome have also provided a window of opportunity for careful epidemiologic, genetic, neurochemical, and neuroanatomic studies that have led to a more complete understanding of the genes that regulate the development of the respiratory system and how environmental factors in fetal and early neonatal life may adversely affect normal development of systems that control breathing.

The purpose of this chapter is (1) to present what is currently known about the neuroanatomy, neurocircuitry, and neurochemistry that controls breathing during development and (2) to better understand how infants breathe, why premature infants have apnea of prematurity, why term infants have apnea of infancy, and how genetic errors and environmental exposures modify mechanisms that control breathing during fetal and early neonatal life.

Animal Models of Control of Breathing

Much of our understanding of the basic mechanisms that lead to stable breathing come from studies performed in newborn and adult animals. Earlier studies in newborn pig, dog, and cat as well as the fetal and newborn sheep characterized the developmental physiology, and much has been gained from these models. However, a more detailed understanding of the neuroanatomy, neurocircuitry, and neurochemistry has been obtained with in vitro models from fetal and newborn rats and mice. Of particular relevance, the stage of respiratory development of the rat born at term is similar to that of the human born at 25–29 weeks' gestation. Two different reduced in vitro preparations from rodents have been used to better delineate components of the respiratory network in the brainstem: (1) brainstem slices that include the area that contains the “pacemaker cells mediating rhythmogenesis” and (2) isolated brainstem spinal cord preparations from fetal and newborn rodents. Because the stability and viability of these in vitro preparations are best when tissues are used from the late embryonic stage or within the first week of postnatal life, data from these in vitro preparations are relevant only to respiratory control during very early development. However, these techniques provided the opportunity to carefully characterize the different regions of the brain that are involved in respiration. We now know that the groups of neurons that control the different phases of respiration

have a genetic signature, allowing the use of genetic tools to manipulate the activity of the different regions and observe the physiologic responses in intact animals. Specifically, optogenetics and pharmacologic techniques are often combined to explore the functionality of the specific brain regions related to breathing in unanesthetized adult animals. Thus what we know about how the respiratory network is integrated and functions is based on a significant amount of information obtained from well-designed in vitro and in vivo animal experiments using immunohistochemistry, electrophysiology, fluorescent imaging, genetic manipulation, pharmacology, computational modeling (Richter and Spyer, 2001; Smith et al., 2013) and more recently optogenetics in rodents (Cui et al., 2016; Anderson et al., 2016).

Overview of Respiratory Control

Muscles of Respiration

The diaphragm is the major muscle of respiration, but other muscles of respiration are also essential for unobstructed breathing at rest and augmented breathing during exercise and stress. Thus the muscles of respiration include the pump muscles (diaphragm, intercostal muscles, and abdominal muscles) and muscles of the upper airway (alae nares, pharyngeal muscles, and laryngeal muscles). The diaphragm is innervated by the phrenic nerve, which originates in the spinal column (C3–C5). The upper airway muscles are innervated by motoneurons originating in the brainstem, specifically, the nucleus ambiguus, the dorsal motor nucleus of the vagus, and the hypoglossal nucleus (Nunez-Abades et al., 1992; Jordan, 2001). Different muscles are activated during each of the three phases of the respiratory cycle: inspiration, postinspiration, and expiration. Upper airway muscles are particularly important in modulating the rate of inspiratory and expiratory airflow. During inspiration, the diaphragm, external intercostal muscles (in infants), and posterior cricoarytenoid (laryngeal dilator) contract.

During postinspiration (also known as the *first phase of expiration*), the diaphragm and the thyroarytenoid (laryngeal constrictor) contract. Diaphragmatic and thyroarytenoid postinspiratory activity are common in newborns, both human (Eichenwald et al., 1997) and animal (England et al., 1985). Because the chest wall of newborns, particularly premature newborns, is highly compliant, diaphragmatic and laryngeal postinspiratory activity retards expiration to maintain functional residual capacity. This postinspiratory activity is often audible, known as *grunting*, and is heard in infants with low lung volume disease states, such as surfactant deficiency and atelectasis. Vocalizations, swallowing, and coughing also occur during the postinspiratory phase of respiration (Dutschmann et al., 2014).

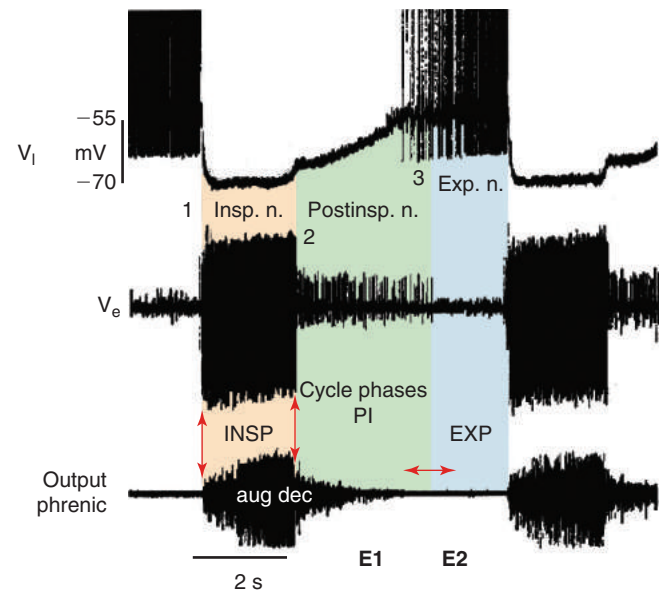
The second phase of expiration is often quiescent regarding muscle activity; the diaphragm relaxes and the lungs recoil. However, during exercise and hypercapnia the intercostal and abdominal muscles will contract during the second phase of expiration. Each phase of respiration has a corresponding excitatory region in the brainstem that initiates each phase. While the regions initiating inspiration and forced expiration (second phase of expiration) had been previously identified, the region initiating postinspiratory activity has only recently been localized and validated in rodents (Anderson et al., 2016).

It is essential that the pump muscles, particularly the diaphragm and the upper airway muscles, contract in such a way that unobstructed breathing occurs. There are several disease states in which this is not the case; upper airway obstruction during inspiration

is the hallmark of obstructive sleep apnea that can occur at any age. Premature infants may have an obstructed component of the upper airway from low upper airway tone or active closure of the glottis during apnea.

Respiratory Rhythmogenesis

The motor output of muscles of respiration is a summation of influences on, and intrinsic properties of, respiratory-related neurons in the brainstem. While abnormal breathing can result from disease of the muscle itself, it can also occur from an abnormality anywhere along the cascade, starting from the neurons and cells involved in genesis of respiration and regulation of the respiratory timing to the axons that carry the signal to the motor units. Respiration is a rhythmic motor behavior similar to locomotion that is generated by semiautonomous neural networks known as *central pattern generator*, which are located in the medulla and pons (Smith et al., 2013). Specific brainstem regions contain highly specialized and interconnected neurons that have synchronized activity that results in the three phases of the respiratory cycle (Fig. 43.1). A core group of synaptically coupled excitatory neurons in the brainstem located in the pre-Bötzinger complex (PBC) has pacemaker properties and is the putative site of genesis of inspiratory activity (Smith et al., 1991). These pacemaker neurons express the homeobox gene *DBX1* and are immunopositive for neurokinin 1 (NK1) receptor and somatostatin (Bouvier et al., 2010; Picardo et al., 2013; Wang et al., 2014). The timing of the respiratory cycle is controlled by



• **Fig. 43.1** Simultaneous triple recordings of electric activities of the main populations of respiratory neurons (1), an inspiratory neuron (large spikes, middle tracing); (2), a postinspiratory neuron (small spikes, middle tracing); (3), an expiratory neuron (top tracing) and the phrenic nerve (bottom tracing). Note the augmenting (aug) activity of the phrenic nerve during inspiration and the decrementing (dec) activity of the phrenic nerve during the postinspiratory (PI) phase of the respiratory cycle. The postinspiratory phase is the first phase of expiration (E1) and is associated with phrenic nerve activity. In the second phase of expiration (E2), the phrenic nerve is silent. (Reproduced from Richter DW, Spyer KM. Studying rhythmogenesis of breathing: comparison of in vivo and in vitro models. *Trends Neurosci.* 2001;24:464–472.)

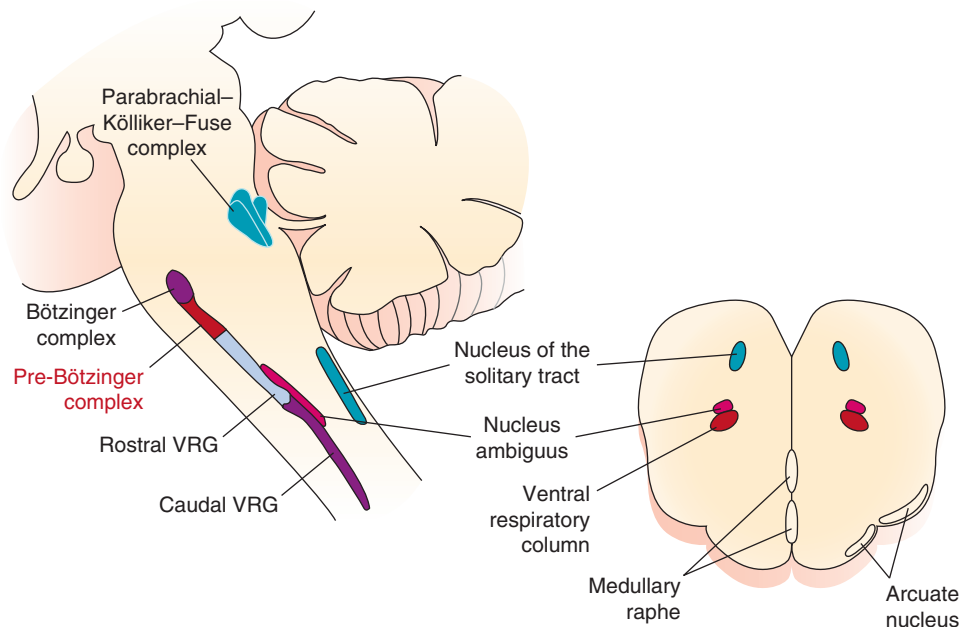
inhibitory interneurons that discharge during specific phases of the respiratory cycle (Smith et al., 1991).

Neuroanatomy of the Central Respiratory Network

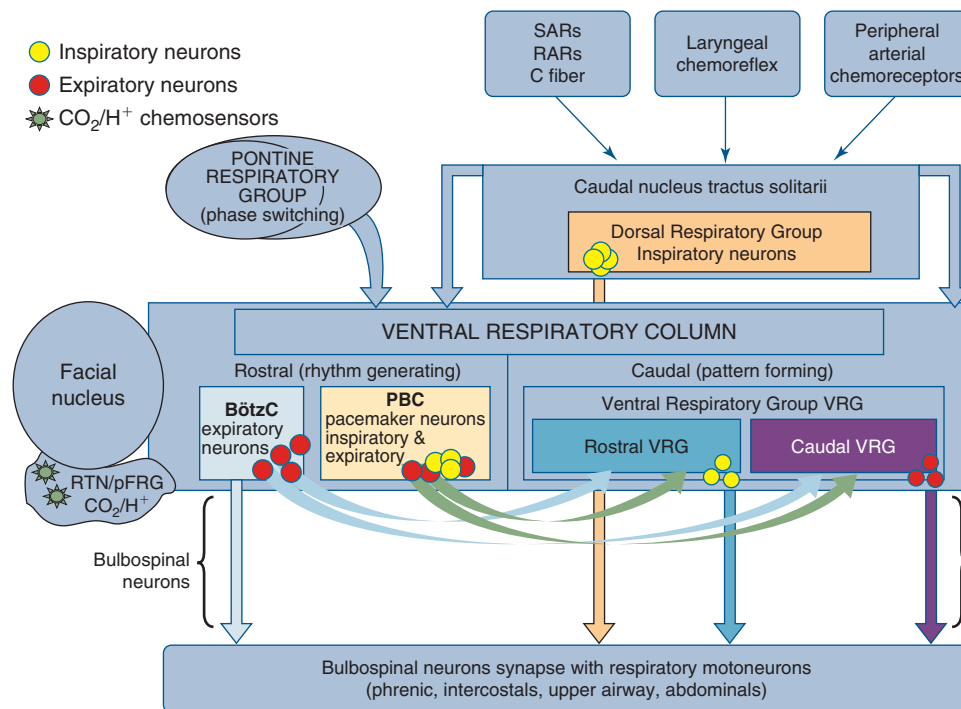
As shown in the anatomic illustration in Fig. 43.2 and the schematic in Fig. 43.3 and summarized in Table 43.1, the respiratory-related neurons are located in three main areas in the brainstem: (1) the dorsal respiratory group within the nucleus tractus solitarius (nTS); (2) the ventral respiratory column (VRC), which extends from the facial nucleus to the ventrolateral medulla at the spinal–medullary junction; and (3) the pontine respiratory group within the dorsolateral pons (Alheid and McCrimmon, 2008; Smith et al., 2013). The VRC can be subdivided into a rostral part, involved in rhythmogenesis, and a caudal part, involved in pattern formation. The VRC should not be confused with the ventral respiratory group (VRG). Bulbospinal neurons are neurons that originate in the medulla (bulbo) and synapse with motoneurons in the spinal column such as the phrenic motoneurons. The rostral VRC contains both the rostral VRG, consisting of a large proportion of bulbospinal inspiratory neurons that project directly to the phrenic and external intercostal motoneurons, and the caudal VRG, containing bulbo-spinal expiratory neurons that project to abdominal and internal intercostal motoneurons. Propriobulbar neurons are neurons originating in the brainstem that send projections to other neurons in the brainstem.

Within the rostral VRC are two areas that are essential to the formation of respiratory rhythm: the PBC and the Bötzinger complex. As outlined already, the PBC contains a core group of synaptically coupled excitatory neurons that have pacemaker properties, similar to the pacemaker cells in the atrioventricular node of the heart. These pacemaker cells are rostral to the nucleus ambiguus, have both intrinsic inspiratory and expiratory bursting properties, and are essential to maintaining respiratory rhythm (Smith et al., 1991). Progressive destruction of the PBC disrupts rhythmogenesis, leading to death in animals (Ramirez et al., 1998). Using immunocytochemistry to identify NK1 and somatostatin positive neurons and anatomical approaches, a similar area has been identified in the human brain. In individuals who had died of neurodegenerative disease with central respiratory deficits, reduced numbers of neurons were identified in the area of the putative PBC as compared with the brains of individuals with neurodegenerative disease without central respiratory deficits (Schwarzacher et al., 2011). The postinspiratory complex (PiCo) is another area, recently discovered, with autonomous rhythm-generating properties that controls postinspiratory activity (Anderson et al., 2016). The PiCo is medial to the nucleus ambiguus and caudal to the nucleus of cranial nerve VII. The Bötzinger complex contains propriobulbar expiratory neurons that provide strong inhibitory inputs to inspiratory and expiratory bulbospinal neurons in the VRC.

Another important group of neurons are those within the retrotrapezoid nucleus (RTN) located along the ventral medullary surface beneath the facial nucleus (see Fig. 43.3). These neurons



• **Fig. 43.2** The anatomic relationship between the regions in the human brainstem that constitute the respiratory network. These regions include specialized neurons in the dorsolateral pons (parabrachial and Kölliker–Fuse nuclei), nucleus of the solitary tract, and ventral respiratory column. The ventral respiratory column is organized rostrocaudally extending from the level just below the facial nucleus to the C1 level of cervical cord. The ventral respiratory column consists of the Bötzinger complex, the pre-Bötzinger complex, and the rostral and caudal ventral respiratory groups (VRGs). Vagal motor neurons of the nucleus ambiguus innervate the laryngeal muscles. The medullary raphe, arcuate nucleus, located just underneath the ventral medullary surface, contains neurons that depolarize in response to hypercapnia and hypoxia. The retrotrapezoid nucleus (not shown) is a CO_2/H^+ chemosensor and is located rostrally below the facial nucleus on the ventral medullary surface. (Modified with permission from Benarroch EE. Brainstem respiratory control: substrates of respiratory failure of multiple system atrophy. *Mov Disord.* 2007;22: 155–161.)



• **Fig. 43.3** Pontine–Medullary Brainstem Network Controlling Respiration and Afferent and Efferent Projections. The pontine respiratory group contains two nuclei that send neuronal projections to respiratory-related neurons in the ventral respiratory column (VRC). The pontine respiratory group mediates phase switching between inspiration and expiration. Within the nucleus tractus solitarius (NTS) are the inspiratory neurons of the dorsal respiratory group with projections to motoneurons in the spinal column. The NTS also receives monosynaptic inputs from vagally mediated reflexes in the lung and upper airways (including slowly adapting receptors [SARs], rapidly adapting receptors [RARs], and C-fiber receptors), laryngeal chemoreceptors, and peripheral arterial chemoreceptors. Projections from second-order neurons in the NTS then synapse with neurons in the rostral VRC and the caudal VRC. The VRC extends from the level of the facial nucleus to C1 in the cervical spinal cord. The rostral VRC is involved in respiratory rhythmogenesis and contains the expiratory neurons of the Bötzinger complex (Bötzc) and the pacemaker cells of the pre-Bötzinger complex (PBC). The Bötzc and PBC contain propriobulbar neurons that project to inspiratory neurons in the rostral ventral respiratory group (VRG) and expiratory neurons in the caudal VRG. The Bötzc also contains bulbospinal neurons that synapse with phrenic motoneurons in the spinal cord, whereas the PBC contains only propriobulbar neurons. Neurons in the VRG are responsible for shaping the respiratory pattern and receive inputs from second-order neurons in the NTS and from rhythm-generating neurons in the Bötzc and PBC. Bulbospinal neurons from the dorsal respiratory group, Bötzc, rostral VRG, and caudal VRG synapse with motoneurons that control the activity of the muscles of respiration. *pFRG*, Parafacial respiratory group; *RTN*, retrotrapezoid nucleus.

have chemosensitive properties and depolarize in response to increasing carbon dioxide (CO_2) concentration and decreasing pH and synapse with rhythm- and pattern-generating neurons in the VRC (Guyenet et al., 2008). All these neuronal groups and networks that contribute to rhythmogenesis are present in newborn animals born at term (e.g., sheep, cats, and pigs) or born prematurely (e.g., rodents) in which rhythmogenesis is well established before birth. Episodic spontaneous fetal breathing movements occur in human fetuses as early as 10 weeks' gestation (de Vries et al., 1985). In rodents, respiratory rhythmogenesis is first detected at embryonic day 15 in rats and embryonic day 17 in mice (Thoby-Brisson and Greer, 2008). The emergence of this respiratory-related activity in rats is coincident with the characteristic expression of NK1 receptors of the PBC (Thoby-Brisson and Greer, 2008).

In summary, respiratory rhythm and inspiratory–expiratory patterns emerge from dynamic interactions between (1) excitatory neuron populations in the PBC and rostral VRG, which are active during inspiration and form the inspiratory motor output, (2)

excitatory neurons in the PiCo, which are active during postinspiration, (3) inhibitory neurons in the PBC that provide inspiratory inhibition within the network, and (4) inhibitory neurons in the Bötzinger complex, which are active during expiration and provide inhibitory inputs to inspiratory and expiratory neurons within the network and to phrenic motor neurons (see Fig. 43.3). Because of the limitations in performing mechanistic experiments in humans, much of what we know about how we breathe is extrapolated from animal models, but many similarities exist between animals and humans regarding the respiratory network. Harper et al. (2014) have described the relationship between damage to specific brain regions observed on brain imaging and specific disorders of respiratory control in humans.

Neurochemical Control of Respiration

Neurotransmitters

Glutamate is the major neurotransmitter mediating excitatory synaptic input to brainstem respiratory neurons and respiratory

**TABLE
43.1****Major Anatomic Regions in the Brainstem Involved in the Pattern and Timing of Respiration**

| Neuroanatomy: Brainstem Respiratory Network | Characteristics | Comment |
|--|---|---|
| Dorsal respiratory group | <ol style="list-style-type: none"> 1. Located in the medulla 2. Receives sensory input from mechanoreceptors and peripheral chemoreceptors 3. Contains primarily inspiratory neurons 4. Synapses with nerves innervating the diaphragm and intercostal muscles | |
| Ventral respiratory group | <ol style="list-style-type: none"> 1. Located in the medulla 2. Contains inspiratory neurons in the rostral ventral respiratory group and expiratory neurons in the caudal ventral respiratory group 3. Sends inspiratory impulses to laryngeal and pharyngeal muscles, diaphragm, and external intercostal muscles 4. Sends expiratory impulses to the abdominal and internal intercostal muscles | Do not confuse the ventral respiratory group with the ventral respiratory column. |
| Pontine respiratory group | <ol style="list-style-type: none"> 1. Includes the Kölliker–Fuse and other parabrachial nuclei 2. Important in timing of inspiration: sends inhibitory signals to the dorsal respiratory group 3. Innervates laryngeal premotor neurons controlling upper airway resistance 4. Innervates spinal projecting neurons controlling phrenic motoneurons | Overstimulation causes apneustic breathing. |
| Retrotrapezoid nucleus (humans)/parafacial respiratory group (rodents) | <ol style="list-style-type: none"> 1. Located in the ventral medulla in the parafacial region 2. Parafacial respiratory group separated into ventral and lateral areas with different effects on respiration 3. Contains glutamatergic neurons expressing the transcription factor paired-like homeobox 2b 4. Express neurokinin 1 receptor 5. Activity regulated by CO₂ or pH 6. Synaptic inputs from peripheral chemoreceptors modify output of this area. 7. Enhances expiratory activity of abdominal muscles during hypercapnia 8. Thought to be insensitive to opioids | <p>Congenital central hypoventilation syndrome</p> <p>Individuals have insensitivity to hypercapnia—retrotrapezoid nucleus is affected.</p> <p>Genetic mutation: polyalanine expansion mutation in the <i>PHOX2B</i> gene</p> |
| Bötzinger complex | <ol style="list-style-type: none"> 1. Located in the medulla 2. Contains expiratory neurons 3. Inhibits inspiratory neurons 4. Involved in controlling the alteration between inspiration and expiration 5. Augments expiratory activity (second phase of expiration) | |
| Pre-Bötzinger complex | <ol style="list-style-type: none"> 1. Located in the medulla 2. Intrinsic rhythmic inspiratory excitatory drive 3. Glutamatergic neurons have widespread projections 4. Also inhibits expiratory neurons during inspiration | |
| Nucleus tractus solitarii | <ol style="list-style-type: none"> 1. Located in the dorsomedial medulla 2. Receives inputs from pulmonary mechanoreceptor, peripheral chemoreceptor, and other visceral sensory afferent inputs 3. The dorsal respiratory group (contains inspiratory neurons) is part of the nucleus tractus solitarii | |
| Brainstem raphe nuclei | <ol style="list-style-type: none"> 1. Located near the midline of the brainstem with extensive rostrocaudal extension from the caudal medulla to the pons 2. Extensive projections throughout the brain and spinal cord 3. Rich source of serotonergic neurons with extensive projections 4. Synapse with neurons that are involved in motor, somatosensory, and limbic systems | Abnormalities in serotonergic neurons in the raphe have been found in infants who died of sudden infant death syndrome. |

TABLE 43.1 Major Anatomic Regions in the Brainstem Involved in the Pattern and Timing of Respiration—cont’d

| Neuroanatomy: Brainstem Respiratory Network | Characteristics | Comment |
|---|--|--|
| Bulbospinal neurons | Neurons originating in the brainstem that synapse with motoneurons in the spinal column | For example, bulbospinal neurons synapse with phrenic motoneurons and with motoneurons for the intercostal muscles |
| Propriobulbar neurons | Neurons originating in the brainstem that send projections to other neurons in the brainstem | For example, the Bötzing complex contains propriobulbar expiratory neurons that provide strong inhibitory inputs to inspiratory and expiratory bulbospinal neurons in the ventral respiratory column |

Modified from Smith JC, Abdala AP, Borgmann A, Rybak IA, Paton JF. Brainstem respiratory networks: building blocks and microcircuits. *Trends Neurosci.* 2013;36:152–162.

premotor and motor neurons through binding to α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid kainite receptors (Bonham, 1995) and metabotropic glutamate receptors (Pierrefiche et al., 1994). γ -Aminobutyric acid (GABA) and glycine are the two major inhibitory neurotransmitters mediating inhibitory synaptic input in the respiratory network; they have a key role in pattern generation and termination of inspiratory activity (Haji et al., 2000). GABA (via GABA_A receptors) and glycine (via glycine receptors) mediate fast synaptic inhibition via activation of chloride channels (Bianchi et al., 1995). Throughout development, glutamate always functions as an excitatory neurotransmitter; however, it is not the case that GABA and glycine are always inhibitory neurotransmitters. In early development, GABA and glycine mediate *excitatory neurotransmission* in many neuronal networks, including the respiratory network (Putnam et al., 2005). GABA and glycine signaling modifies the level of chloride in the cell. Activation of the sodium (Na⁺)–potassium (K⁺)–chloride (Cl[−]) cotransporter (NKCC1) and the potassium–chloride transporter (KCC2) on the cell modulates intracellular ion concentrations. Specifically, NKCC1 brings Na⁺, K⁺, and 2Cl[−] into the cell, while activation of KCC2 moves K⁺ and Cl[−] outside the cell. During early development, the high NKCC1/KCC2 ratio causes *high* intracellular chloride concentrations in immature neurons. When GABA then binds to GABA_A receptors, there is net outward movement of Cl[−] ions, leading to membrane depolarization. With maturation, the KCC1/KCC2 ratio reverses, and there is less chloride in the cell. Now when GABA binds to GABA_A receptors, more chloride comes into the cells, leading to hyperpolarization (Rivera et al., 1999; Cellot et al., 2013). With brain injury, NKCC1 expression increases, making the GABAergic system less inhibitory. Moreover, myoclonic jerks are associated with midazolam exposure (GABA_A receptor agonist) in premature infants (Ozcan et al., 2015). GABA_B receptors, which are metabotropic G protein–coupled receptors, also have a greater role in inhibiting respiratory rhythm in adult animals as compared with newborn animals (Kerr and Ong, 1995).

Neuromodulators

The baseline excitatory and inhibitory influences mediated by glutamate and GABA–glycine respectively on major neuronal networks are further altered by many endogenously released neuromodulators that shape and fine-tune respiratory pattern and rhythm throughout development, as outlined in Table 43.2. For example, acetylcholine, substance P, cholecystokinin (CCK), and

thyrotropin-releasing hormone all exert an excitatory drive, whereas opioids, somatostatin, and prostaglandin E₂ exert an inhibitory drive on respiratory-related neurons. Dopamine, adenosine, serotonin, and norepinephrine can have excitatory and inhibitory influences depending on the specific receptors that the neuromodulator binds. Neurons within the PBC that are important in rhythmogenesis are also distinctly identified by being immunopositive for glutamate transporter, NK1, μ -opioid, and GABA_B receptors. Although these cells are primarily excitatory and release glutamate, they can be modulated by synaptic inputs that release substance P, opioids, and GABA (Doi and Ramirez, 2008). These rhythmicogenic neurons produce and are excited by brain-derived neurotrophic factor (Bouvier et al., 2008). Lastly, the presence of μ -opioid receptors and prostaglandin receptors on respiratory-related neurons contribute to the respiratory depression associated with opiate therapy for analgesia and prostaglandin E₁ therapy for congenital heart disease respectively. Similarly, apnea—a common presenting sign of illness in infants—is mediated, in part, by an increase in prostaglandin E₂ levels in the central respiratory network (Hofstetter et al., 2007).

Genetic Mutations Affecting Respiratory Control

Some neuromodulators may be more critical in supporting respiratory rhythmogenesis than others. By identifying the genetic mutations that are associated with marked abnormalities in respiratory control, we can obtain a better understanding of the key role of several neuromodulator systems. For example, serotonergic neurons in the caudal medullary raphe nuclei have extensive projections to phrenic and hypoglossal motoneurons, the nTS, the RTN, and the PBC (Pilowsky et al., 1990). The serotonergic system has a significant influence on the modulation and integration of diverse homeostatic functions, including cardiorespiratory responses and thermogenesis (Kinney et al., 2009). Individuals with Prader–Willi syndrome, who may exhibit breathing abnormalities at birth (Cohen et al., 2014), have mutations in the *necdin* gene (*NDN*) on chromosome 15 leading to abnormalities in the brainstem serotonergic system (Zanella et al., 2008a). Mice lacking the *necdin* gene also have abnormal brainstem serotonergic neurochemistry (Zanella et al., 2008b). Medullary serotonergic neurons are also CO₂ sensitive (Richerson et al., 2001). In genetically modified mice that do not develop medullary serotonergic neurons, CO₂ sensitivity is reduced by 50% (Hodges et al., 2008).

TABLE 43.2**Neurotransmitters and Neuromodulators That Mediate Respiratory Rhythm**

| Neuromodulator | Receptor Subtype | Source of the Endogenous Ligand | Excitatory or Inhibitory on Respiratory Rhythm | Comment |
|----------------|---|--|--|---|
| Glutamate | NMDA, AMPA, GluR | | Excitatory | Major excitatory neurotransmitter |
| Ach | M3 | PAG, LC, X | Excitatory | |
| NE | α 1-Adrenergic | LC | Excitatory | |
| Serotonin | 5-HT _{2A/2B} , 5-HT ₃ , 5-HT ₄ | Raphe | Excitatory | |
| Dopamine | Likely D ₁ | PVN, hypothalamus | Excitatory | |
| ATP | P2X ₂ | Ventral medulla; CO ₂ /H ⁺ -sensitive cells in the RTN | Excitatory | |
| Adenosine | P2Y ₁ | Ventral medulla | Excitatory | |
| Substance P | NK1 | nTS, NA | Excitatory | |
| CCK | CCK1 | nTS, raphe | Excitatory | |
| TRH | TRH-R (1 and 2) | Raphe | Excitatory | |
| GABA | GABA _A , GABA _B | | Inhibitory | Major inhibitory neurotransmitter (can be excitatory during fetal life) |
| Glycine | GlyR | | Inhibitory | Can be excitatory during fetal life |
| NE | α 2-Adrenergic | Pons | Inhibitory | |
| Dopamine | D ₄ | PVN, hypothalamus | | |
| Adenosine | A ₁ , A ₂ | Ubiquitous from metabolism of ATP that increases during hypoxia | Inhibitory | Contributes to respiratory depression at the baseline (A ₁), and mediates HVD |
| Opioid | μ , δ , κ | nTS, PBN, PVN, raphe | Inhibitory | Prominent inhibitory effect during early development |
| PDGF | PDGF- β | nTS | Inhibitory | Contributes to HVD |

Data from Doi A, Ramirez JM. Neuromodulation and the orchestration of the respiratory rhythm. *Respir Physiol Neurobiol.* 2008;164:96; and Simakajornboon N, Kuptanon T. Maturational changes in neuromodulation of central pathways underlying hypoxic ventilatory response. *Respir Physiol Neurobiol.* 2005;149:273.

Ach, Acetylcholine; AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate; ATP, adenosine triphosphate; CCK, cholecystokinin; GABA, γ -aminobutyric acid; GluR, glutamate receptor; GlyR, glycine receptor; HVD, hypoxic ventilatory depression; LC, locus ceruleus; NA, nucleus ambiguus; NE, norepinephrine; NMDA, N-methyl-D-aspartate; nTS, nucleus tractus solitarius; PAG, periaqueductal gray; PBN, parabrachial nucleus; PDGF, platelet-derived growth factor; PVN, paraventricular nucleus; RTN, retrotrapezoid nucleus; TRH, thyrotropin-releasing hormone; TRH-R, thyrotropin-releasing hormone receptor; X, vagal nucleus.

In some infants who have died of SIDS, neuropathologic studies have shown disruptions of the brainstem serotonergic system (Kinney et al., 2009; Paterson et al., 2006b). While specific single-gene mutations that regulate serotonin production and function have not been identified in infants who have died of SIDS, some studies have shown a higher proportion of specific polymorphisms in the 5' regulatory region of the *SLC6A4* gene, which encodes serotonin transporter, which regulates the reuptake of serotonin from the extracellular space. Specifically, infants who have died of SIDS have an increased frequency of the long allele variant and the variable number 12-tandem repeat in intron 2 polymorphisms (Weese-Mayer et al., 2007) in the promoter. The long allele occurs more frequently in African Americans; African Americans also have a 2.7-fold greater incidence of SIDS than whites. Effectively the polymorphisms result in increased activity of serotonin transporter, thereby decreasing the time that serotonin stays in the

synapse, leading to a relative serotonin deficiency causing dysregulation of the cardiorespiratory system.

Rett syndrome is an X-linked disorder with mutations in several genes, but the most common genetic defect (90%) is in the methyl CpG binding protein 2 gene (*MECP2*). Affected individuals are normal at birth and then experience progressive deterioration leading to severe motor, cognitive, and autistic behavioral systems. They also have characteristic severe respiratory disturbances with prolonged apnea and hyperventilation that can be fatal. Genetically modified mice that lack the *MECP2* gene have reduced levels of norepinephrine and serotonin in the medulla and have breathing patterns similar to those of humans with Rett syndrome (Roux et al., 2008). Pharmacologic treatment to increase brain norepinephrine and serotonin levels stabilizes breathing and prolongs the life of these mice (Roux et al., 2007). A case report demonstrating efficacy of fluoxetine and buspirone in reducing breathing

dyregulation in a patient with Rett syndrome has been published (Gokben et al., 2012). Altered GABA neurotransmission might also be causative as suggested by experiments using stem cells from a patient with Rett syndrome that demonstrated that the functional switch of GABA neurotransmission from excitation to inhibition was impaired (Tang et al., 2016).

Congenital central hypoventilation syndrome (CCHS), also known as *Ondine's curse*, is another rare autosomal dominant genetic disorder, occurring in 1 in 200,000 live births. Affected individuals characteristically have adequate ventilation during wakefulness but profound hypoventilation during sleep as well as impaired ventilatory responses to CO₂ and hypoxia during sleep and wakefulness (Berry-Kravis et al., 2006). Although the disorder most commonly presents during infancy, milder forms may present later in childhood or even during adulthood. More than 90% of individuals with CCHS have mutations in the *PHOX2B* gene (Weese-Mayer et al., 1993, 2005; Amiel et al., 2009). *PHOX2B* is a homeobox gene located on chromosome 4 that is specifically expressed in limited types of neurons involved in autonomic processes (Dauger et al., 2003). Its expression is required for the development of the carotid body, nTS, and catecholaminergic neurons. It is also expressed in chemosensitive glutamatergic neurons in the RTN that receive polysynaptic inputs from peripheral arterial chemoreceptors (Guyenet et al., 2008). Thus mutations in the *PHOX2B* gene alter the development of key structures that regulate chemical control of breathing.

The gene has a stretch of 20 alanine repeats in exon 3. Most affected individuals have the classic mutation that adds additional alanine repeats to the 20-alanine repeat, resulting in a stretch of 25–35 alanine repeats instead of 20. This mutation is classified as polyalanine repeat mutations (PARMs). The severity of the disease is correlated with the number of extra alanines. Patients with *PHOX2B*^{20/25} have 25 versus 20 alanines; they have the mildest disease, may never need 24-hour ventilatory support, and may present only after infection or exposure to agents that inhibit respiration. On the other hand, patients with 28–32 alanine repeats in the gene (*PHOX2B*^{20/28–32}) often need continuous ventilatory support. Fewer affected individuals with CCHS have nonpolyalanine repeat mutations (NPARMs) resulting in deletions in exon 3 that cause frameshift mutations. Depending on the mutation, some patients require tracheostomy and long-term ventilation and/or diaphragmatic pacing (Weese-Mayer et al., 2010). While respiratory stimulants are not effective in increasing respiratory drive, drugs that cause respiratory depression may lead to profound respiratory depression (Chen et al., 2006).

From genetically modified mouse models with mutations in the *PHOX2B* gene, we know that its expression is essential for the development of the RTN in the brainstem and catecholaminergic and cholinergic traits in the autonomic nervous system (Moreira et al., 2016). The RTN contains putative central chemoreceptors that have intrinsic pH sensitivity and release the excitatory neurotransmitter glutamate, thereby stimulating breathing during hypercapnia (Holloway et al., 2015). However, abnormalities throughout the autonomic nervous system are often seen in patients with CCHS. Specifically, patients can present with Hirschsprung disease (Haddad syndrome) and neuroblastomas (neuroblastoma–Hirschsprung disease–CCHS syndrome) (Szymońska et al., 2015). As reviewed by Moreira et al. (2016), Hirschsprung disease occurs in 80% of patients with NPARM and 10% of patients with PARM. Similarly, neural crest tumors are found in 1% of PARM patients and in 41% of NPARM patients.

Peripheral Inputs That Modulate the Central Respiratory Network

The nTS in the brainstem (see Fig. 43.3 and Table 43.1) is where sensory information from vagally mediated reflexes and chemical signals from the blood (arterial chemoreceptors) and cerebrospinal fluid (central chemoreceptors) and information from higher brain regions are integrated; neurons from the nTS synapse onto respiratory related neurons, thereby augmenting or attenuating minute ventilation.

Bronchopulmonary Reflexes

Essentially all bronchopulmonary reflexes that modify the depth and duration of inspiration and expiration are mediated through the vagus nerve. The vagus nerve has both myelinated and unmyelinated fibers. Myelinated vagal afferent fibers are activated via (1) slowly adapting stretch receptors (SARs), which are activated by volume and stretch of the lung (mediating the Breuer–Hering reflex), or (2) rapidly adapting receptors (RARs), which are activated in response to inhaled irritants (e.g., ammonia, cigarette smoke) and large inflations or deflations of the lung (Kubin et al., 2006). Activation of SARs changes the duration of inspiration and expiration, whereas activation of RARs causes sighs (i.e., augmented breaths) and cough. Unmyelinated vagal afferents, specifically C-fibers in the airway, are activated by a multitude of chemical stimuli, including CO₂ and capsaicin, in addition to lung edema and elevated temperature. Activation of C-fibers in the lung causes rapid shallow breathing and apnea. Table 43.3 lists bronchopulmonary and upper airway reflexes and their physiologic responses. How these reflexes modify breathing during development is discussed next.

Slowly Adapting Stretch Receptors: Major Modulators of Respiratory Timing

The duration of inspiratory or expiratory effort is greatly influenced by mechanoreceptors that are activated by changes in lung volume. The most well-characterized vagally mediated bronchopulmonary reflex is the pulmonary stretch reflex mediated through SARs, discovered by Josef Breuer in 1868. In adult cats, Breuer showed that expansion of the lungs reflexively inhibits inspiration and promotes expiration and that deflation of the lungs promotes inspiration and inhibits expiration (Widdicombe, 2006). In humans the contribution of the Breuer–Hering reflex to tidal breathing is determined by occlusion of the airway at the end of expiration. The following occluded inspiratory effort is prolonged, and expiratory effort is shortened. Alternatively, the occlusion can be performed at end of an inspiratory effort; then the following occluded expiratory effort is prolonged and inspiratory effort is shortened (Gauda et al., 1987). With this technique, the Breuer–Hering reflex significantly contributes to tidal breathing in infants. The reflex is strongest at birth and then decreases during the first year of life (Rabbette et al., 1994). It is reasoned that the strength of the Breuer–Hering reflex is inversely related to gestational and postnatal age because of the excessively compliant chest wall in newborns, which collapses at lung volumes below functional residual capacity. With decreasing lung volumes during expiration, the Breuer–Hering deflation reflex is activated; the expiratory time is then shorter, and the inspiratory time is longer. Several factors increase the strength of the Breuer–Hering reflex, including premature birth (Kirkpatrick et al., 1976; De Winter et al., 1995), prone sleeping position (Landolfo et al., 2008), active sleep (AS) (Hand et al., 2004), and respiratory distress syndrome (De Winter et al., 1995).

TABLE 43.3 Airway Receptors and Reflex Responses

| Receptor | Characteristics | Stimulant | Responses | Comment |
|-----------------------------------|---|--|--|--|
| Slowly adapting stretch receptors | <ol style="list-style-type: none"> 1. Mechanoreceptors 2. Mediated by fast-conducting, myelinated vagal fibers 3. Located in lung parenchyma | Lung volume and transmural pressure | <ol style="list-style-type: none"> 1. Breuer–Hering reflex 2. Termination of inspiration and prolongation of expiration 3. Bronchodilation 4. Tachycardia | Breuer–Hering reflex more active in infants than in adults |
| Rapidly adapting receptors | <ol style="list-style-type: none"> 1. Mechanoreceptors 2. Irritant receptors 3. Located throughout the airways 4. Mediated by fast-conducting, myelinated vagal fibers | <ol style="list-style-type: none"> 1. Inhaled irritants 2. Low lung volumes | <ol style="list-style-type: none"> 1. Cough 2. Mucus production 3. Augmented breaths (sighs) | Responsible for inducing sighs in premature infants—restoring functional residual capacity |
| Bronchial and pulmonary C fibers | <ol style="list-style-type: none"> 1. Located throughout the airway from the nose to alveoli 2. Stimulated by substances in the pulmonary circulation and inhaled 3. Slowly conducting, nonmyelinated vagal fibers | <ol style="list-style-type: none"> 1. Capsaicin 2. Respiratory irritants 3. Lung edema 4. Inflammatory mediators | <ol style="list-style-type: none"> 1. Rapid, shallow breathing 2. Apnea 3. Bronchoconstriction 4. Laryngoconstriction 5. Mucus secretion 6. Vasodilatation (pulmonary C fibers) 7. Bradycardia | J-receptors located in alveoli activated by lung edema |
| Laryngeal chemoreflex | <ol style="list-style-type: none"> 1. Potent airway-protective reflex from aspiration 2. Receptors in laryngeal mucosa 3. Mediated by sensory fibers in the superior laryngeal nerve | <ol style="list-style-type: none"> 1. Hyposmolarity 2. Low chloride content | <p>Response in newborns:</p> <ol style="list-style-type: none"> 1. Hypoventilation/apnea 2. Laryngoconstriction 3. Swallowing 4. Bradycardia 5. Shunting of blood flow to brain, heart, adrenals <p>Response in adults:</p> <ol style="list-style-type: none"> 1. Cough 2. Arousal 3. Swallowing | May contribute to apnea and bradycardic events associated with oral feedings in premature infants. Immature responses are exacerbated during hypoxia |

Modified from Kubin L, Alheid GF, Zuperku EJ, McCrimmon DR. Central pathways of pulmonary and lower airway vagal afferents. *J Appl Physiol* (1985). 2006;101:618–627; Thach BT. Maturation and transformation of reflexes that protect the laryngeal airway from liquid aspiration from fetal to adult life. *Am J Med*. 2001;111 Suppl 8A:69S–77S; and Widdicombe J. Reflexes from the lungs and airways: historical perspective. *J Appl Physiol* (1985). 2006;101:628–634.

Rapidly Adapting Receptors: Cough, Augmented Breaths

Lung deflation, mechanical stimulation, and chemical irritants also stimulate vagal afferents of RARs, causing augmented breaths, cough, and increased mucus production (Widdicombe, 2006). RARs are also activated at low lung volumes. Newborns, especially premature newborns, have excessive compliance of the chest wall predisposing to low lung volumes during tidal breathing. Activation of RARs and the resulting augmented breath are particularly important in restoring lung inflation in premature and term infants. The frequency of augmented breaths is inversely related to gestational age, with premature infants having the greatest frequency (Alvarez et al., 1993) when compared with term infants and adults. Moreover, augmented breaths in infants have a biphasic pattern with two large inspiratory efforts in succession, whereas in adults only one large inspiratory effort is seen. Augmented breaths in preterm and term infants are also relatively larger than those in adults; immediately after the augmented breath, preterm and term infants often hypoventilate or have apnea because partial pressure of arterial oxygen (PaO_2) rapidly increases and partial pressure of carbon dioxide (PaCO_2) rapidly decreases. These gas changes reduce excitation from peripheral arterial chemoreceptors, leading to

hypoventilation or apnea in infants. In contrast, ventilation often increases after the augmented breath in adults (Qureshi et al., 2009). Peripheral arterial chemoreceptors are also key in inducing augmented breaths (Matsumoto et al., 1997). As a result, the increased activity of RARs during lung deflation and the increased sensitivity of peripheral arterial chemoreceptors in early development contribute to the increased frequency of augmented breaths, postsigh apnea, and ventilatory instability in premature infants.

C-Fiber Receptors: Apnea, Bronchoconstriction, Rapid Shallow Breathing

Pulmonary and bronchial C-fiber receptors are unmyelinated vagal fibers located throughout the respiratory tract, extending from the nose to the lung parenchyma. Pulmonary C-fibers are accessible from the pulmonary circulation, whereas bronchial C-fibers are accessible from the bronchial circulation and have similar sensitivity to various stimuli (Coleridge and Coleridge, 1984). C-fibers are activated by a variety of substances: inflammatory mediators, capsaicin, lobeline, and phenylbiguanidine. Capsaicin and phenylbiguanidine are used experimentally to identify vagal afferents as C-fibers and characterize stimulus–response profiles. C-fiber

simulation induces central and local effects—cough, apnea, laryngospasm, and bronchoconstriction—followed by rapid shallow breathing, bradycardia, and hypotension. Juxtacapillary receptors (J receptors) are composed of C-fibers located in the alveolar walls. They are activated by lung edema and congestion and cause rapid shallow breathing. By far the most common respiratory response from C-fiber stimulation is reflex apnea characterized by prolongation of the expiratory time from excitation of postinspiratory neurons and continuous firing of central expiratory neurons (Coleridge and Coleridge, 1984).

In newborns the stimulation of pulmonary C-fibers by chemical stimulants causes bronchoconstriction and apnea (Frappell and MacFarlane, 2005). Capsaicin-induced apneic response and the sensitivity of the reflex were greatest in newborn rat pups younger than 10 postnatal days (Wang and Xu, 2006). Bronchopulmonary C-fibers are also stimulated by acidosis, adenosine, reactive oxygen species, hyperosmotic solutions, and lung edema. Furthermore, inflammatory mediators in the local environment sensitize C fibers to other stimuli (Lee and Pisarri, 2001).

Pulmonary C-fiber-mediated respiratory inhibition may cause persistent apnea beyond term gestation in infants born at the limit of viability who have lung inflammation from chronic lung disease (Eichenwald et al., 1997). Local C-fiber activation in the lung may also be the mechanism accounting for the increase in apnea observed in infants with viral infections (Pickens et al., 1989) and unlikely to be from direct viral invasion of the central nervous system (Erez et al., 2014). Prenatal exposure to nicotine/tobacco smoke is a known risk factor for SIDS. In newborn rat pups, prenatal nicotine exposure sensitizes bronchopulmonary C fibers and prolongs apnea in responses to C-fiber stimulation. As outlined already, C-fibers are also activated by inflammation (Zhao et al., 2016). The epidemiologic association between infection, prenatal tobacco smoke exposure, and SIDS could be attributed to sensitization of bronchopulmonary C-fibers from tobacco smoke exposure, confounded by acute infection leading to prolonged apnea from which the infant does not recover.

Laryngeal Reflexes

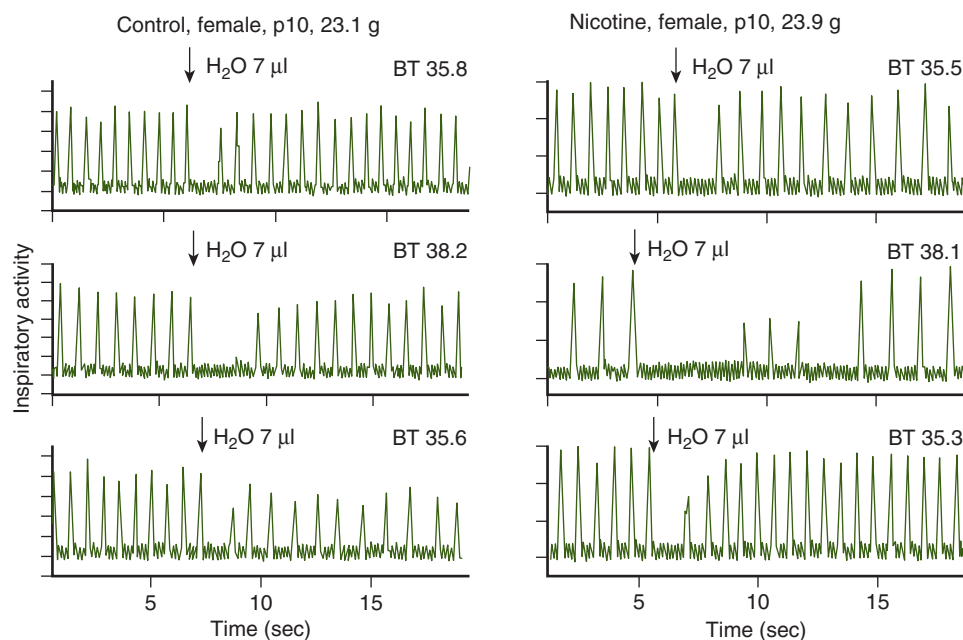
Receptors that respond to changes in upper airway pressure and chemicals are abundantly distributed throughout the laryngeal mucosa. These receptors can be slowly adapting, rapidly adapting irritant receptors, or C-fibers. Water receptors that are stimulated by hypoosmolality and low chloride content may also be involved. Stimulation of upper airway mechanoreceptors and chemoreceptors modifies the activity of upper airway muscles as well as the pattern and timing of diaphragmatic activity. The upper airway reflex that mediates significant cardiorespiratory effects that occur in newborns is the laryngeal chemoreflex (LCR). The LCR is one of the most potent defensive reflexes protecting the respiratory tract from inadvertent aspiration (Harding et al., 1978). Laryngeal chemoreceptors are stimulated by liquid in the airway, which induces coughing, swallowing, and arousal in mature models. However, the response in immature models is apnea followed by hypoventilation, laryngeal constriction, and swallowing (Thach, 2001). In addition to respiratory inhibition, bradycardia, peripheral vasoconstriction, and redistribution of blood flow also occur. The associated apnea and bradycardia can be life threatening in newborns (Sasaki et al., 1977; Boggs and Bartlett, 1982; Wetmore, 1993; Thach, 2001), and in newborns baseline hypoxemia enhances the severity of the apnea and bradycardia induced by the LCR (Wennergren et al., 1989). Afferent fibers for this reflex travel in the superior laryngeal

nerve, a branch of the vagus nerve. These afferents synapse with neurons in the nTS, which then send (1) excitatory projections to motoneurons of the recurrent laryngeal nerve in the nucleus ambiguus, causing constriction of the thyroarytenoid muscle (laryngeal constrictor), resulting in laryngospasm; (2) inhibitory projections to phrenic motoneurons in the cervical spinal cord, inhibiting diaphragmatic contraction, resulting in apnea; and (3) an excitatory pathway to cardiac vagal neurons in the nucleus ambiguus, causing bradycardia. The LCR occurs in the fetus and likely functions to prevent aspiration of amniotic fluid, which contains approximately half the chloride content of pulmonary fluid (Bland, 1990; Reix et al., 2007). In premature infants the reflex may be involved in the apnea and bradycardic responses associated with feeds and gastroesophageal reflux (GER) that reaches the larynx or nasopharynx. Whether the immature response is still present in term infants or how the maturation of the reflex is affected by premature birth has not been determined. Studies in newborn animals show that the apnea associated with the LCR is prolonged during hyperthermia in newborn rats (Xia et al., 2008) and is further accentuated if the animal was exposed prenatally to nicotine (Xia et al., 2010). This relationship is illustrated in Fig. 43.4. Because of profound inhibitory cardiorespiratory effects that are further accentuated by prenatal nicotine exposure, stimulation of the LCR may be an important reflex that is operative in some SIDS cases and infants with acute life-threatening events (Duke et al., 2001; Thach, 2001; Richardson and Adams, 2005; Gauda et al., 2007). Overheating and prenatal exposure to nicotine are both risk factors highly associated with SIDS (Kinney et al., 2009).

Chemical Control of Breathing

Central Chemosensitivity

In air-breathing animals, respiratory rhythmogenesis is primarily driven by the level of PaCO_2 in the blood and cerebrospinal fluid and, to a lesser extent, by oxygen tension. For every 1-mmHg increase in PaCO_2 , ventilation will increase by 20%–30%. Specialized chemosensitive cells in the brainstem depolarize in response to changes in CO_2/H^+ concentration; they drive breathing through synaptic inputs to respiratory-related neurons (Spyer and Gourine, 2009). Although peripheral arterial chemoreceptors in the carotid body also depolarize in response to increasing CO_2/H^+ concentration, these receptors are primarily responsible for modifying breathing in response to changes in oxygen tension (reviewed later). As a result of careful anatomic, physiologic, neurochemical, and genetic studies, the location and the development of central chemoreceptors and some of the genetic factors that drive the development of these receptors in health and disease have been determined. Several groups of neurons in the brainstem, specifically in the medullary raphe, RTN, nTS, locus ceruleus, and fastigial nucleus, are responsive to CO_2/H^+ in vitro and in vivo; therefore they are characterized as chemosensitive. Chemosensitive regions of the brain are identified by the following properties: (1) focal acidification either with acetazolamide (inhibits carbonic anhydrase) or local elevations of CO_2 concentration increase ventilation in adult animals; (2) focal ablation or disruption of the region inhibits the ventilatory response (Feldman et al., 2003). Although several regions are CO_2/H^+ sensitive, there appears to be some specificity in the contribution of each of the regions to chemical control of breathing during wakefulness and sleep (Feldman et al., 2003). The greatest density of CO_2/H^+ sensitive neurons in the brainstem is in serotonergic cells of the raphe of the caudal medulla and



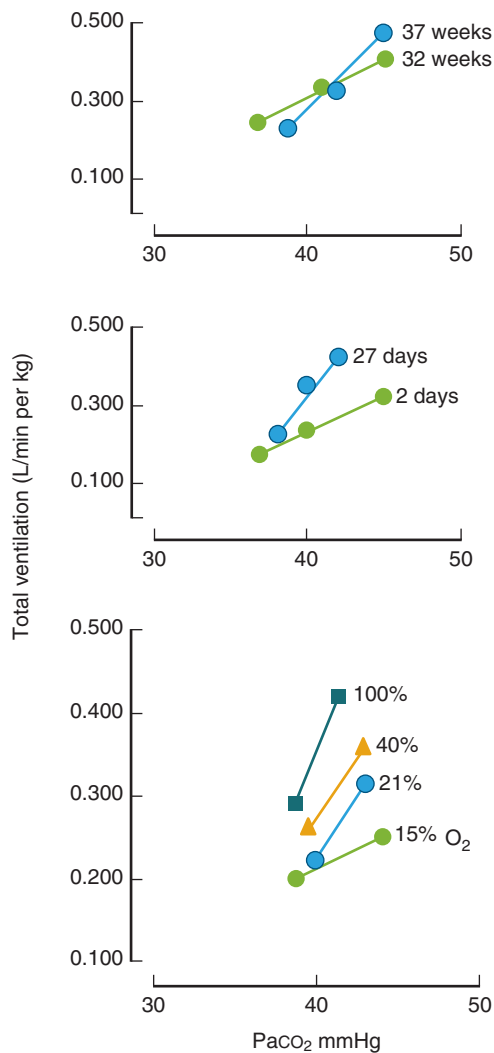
• **Fig. 43.4** Prenatal Exposure to Nicotine and Increased Body Temperature Prolongs the Laryngeal Chemoreflex in Rat Pups at Postnatal Day 10. Example of laryngeal chemoreflex from female postnatal day 10 rat pups during baseline conditions (*top panels*), during mild hyperthermia (*middle panels*), and during a final, recovery period (*bottom panels*). The data displayed on the *left* were obtained from a rat pup born to a control dam, and the data displayed on the *right* were obtained from a rat pup born to a dam infused with nicotine during pregnancy. The *downward arrows* indicate the time at which small volumes of water were injected into the larynx, and the body temperature (BT) is listed to the right of each example. Note the prolonged apnea and respiratory disruption during hyperthermia compared with normothermic conditions, and note that maternal exposure to nicotine markedly prolonged the laryngeal chemoreflex in the rat pup shown on the right. (From Xia L, Leiter JC, Bartlett Jr D. Gestational nicotine exposure exaggerates hypothermic enhancement of laryngeal chemoreflex in rat pups. *Respir Physiol Neurobiol.* 2010;171:17–21.)

glutamatergic neurons of the RTN, located just below the ventral medullary surface (see [Table 43.1](#)). The serotonergic neurons in the caudal raphe project to phrenic motoneurons, where they modulate neuronal plasticity in response to hypoxia ([Feldman et al., 2003](#)). The RTN receives polysynaptic excitatory inputs from peripheral arterial chemoreceptors ([Takakura et al., 2006](#)) and sends projections to neurons in the VRC, including the PBC ([Guyenet et al., 2008](#)). In animals older than seven postnatal days, the activity of the RTN depends on intrinsic pH sensitivity and synaptic drive. RTN neurons detect CO_2 via intrinsic proton receptors (TASK-2, GPR4), synaptic input from peripheral chemoreceptors, and signals from astrocytes ([Guyenet et al., 2015](#)). The parafacial respiratory group may be the precursor to the RTN, and these neurons have intrinsic bursting properties starting at embryonic day 19 ([Guyenet et al., 2008](#)). In humans the arcuate nucleus in the medulla (not to be confused with the arcuate nucleus in the hypothalamus) is believed to be a homologous chemosensitive region similar to that found in animals on the basis of the following findings: (1) the arcuate nucleus is located along the ventral medullary surface; (2) it contains a large population of glutamatergic neurons and a smaller population of serotonergic neurons ([Paterson et al., 2006a](#)); (3) it depolarizes in response to hypercapnia ([Gozal et al., 1994](#)); and (4) the absence of the arcuate nucleus in a human infant was associated with lack of CO_2 sensitivity during life ([Folgering et al., 1979](#)). Purinergic signaling involving the purinergic P2 receptors and adenosine triphosphate (ATP) mediating CO_2/H^+ responsiveness is unique to chemosensitive areas in the RTN

versus that of the raphe or the nTS ([Sobrinho et al., 2014](#)). For an excellent comprehensive review of central chemoreception, see [Guyenet et al. \(2013\)](#).

Maturation of CO_2/H^+ Sensitivity of Central Chemoreceptors

In fetal sheep, hypercapnia causes an increase in the depth of fetal breathing movements, with no change in respiratory rate. In humans, maternal exposure to CO_2 also increases fetal breathing ([Richie and Lakhani, 1980](#)). Ventilatory responses to CO_2 are present immediately after birth in most mammalian species, and CO_2 sensitivity increases with maturation. However, in the newborn rat, CO_2 sensitivity is robust during the first several days of postnatal life; it declines markedly in the next 2 weeks of life and then gradually increases to reach adult levels by the end of the third week ([Stunden et al., 2001](#)). Premature and term infants tested at 2 days of postnatal age have modest ventilatory responses to 2% and 4% CO_2 , although the strength of the ventilatory response is less in the more immature infants ([Frantz et al., 1976](#)). In premature and late preterm newborns, CO_2 sensitivity increases with postnatal age, reaching a mature response by 4 weeks of postnatal age ([Fig. 43.5](#); [Rigatto et al., 1975b, 1975c](#)). Similar to the response in the fetus, the increase in ventilation is predominantly due to an increase in tidal volume and not respiratory rate. If the increase in inspiratory effort against an occluded airway is used as an indicator of central respiratory drive, the increase in CO_2 sensitivity with postnatal development is due to an increase in central



• **Fig. 43.5** The relationship between ventilatory responses to carbon dioxide and gestational age (*top panel*), postnatal age (*middle panel*), and the concentration of inspired oxygen (*bottom panel*). P_{aCO_2} , Partial pressure of carbon dioxide (From Rigatto H, Brady JP, de la Torre Verduzco R. Chemoreceptor reflexes in preterm infants: II. The effect of gestational and postnatal age on the ventilatory response to inhaled carbon dioxide. *Pediatrics*. 1975;55[5]:614–620; and Rigatto H, De La Torre Verduzco R, Gates DB. Effects of O_2 on the ventilatory response to CO_2 in preterm infants. *J Appl Physiol*. 1975;39[6]:896–899.)

respiratory drive in human infants (Frantz et al., 1976). Premature infants with apnea of prematurity have reduced ventilatory responses to CO_2 compared with control infants of the same postconceptional age (Gerhardt and Bancalari, 1984; Durand et al., 1985). This finding suggests that infants with apnea of prematurity have reduced central respiratory drive to breathe when compared with infants who do not have apnea of prematurity at the same postconceptional age.

It is unknown whether maturation of synaptic inputs from chemosensitive neurons to respiratory-related neurons in the brainstem or maturation of intrinsic properties of chemosensitive neurons accounts for the increase in CO_2 sensitivity with early postnatal development. Although such studies of human infants are impossible, data from studies performed in neonatal rats show that intrinsic responses of chemosensitive neurons in the nTS and

locus ceruleus are already mature at birth. It is less clear whether there is a developmental increase in the sensitivity of chemosensitive neurons in the medullary raphe, an increase in the number of chemosensitive neurons in the RTN, or both (Putnam et al., 2005). However, within the first several weeks of postnatal development in the rat, the size of brainstem neurons changes, and both dendritic arborization in the nTS and astrocyte proliferation increase. Astrocytes contribute substantially to the pH of the extracellular milieu surrounding chemosensitive neurons (Putnam et al., 2005). It is likely that all these morphologic and neurochemical changes within and between neurons and astrocytes in the brainstem contribute to maturation of CO_2 sensitivity in the early weeks of postnatal development.

Peripheral Arterial Chemoreceptors

The peripheral arterial chemoreceptors in the carotid body, located at the bifurcation of the carotid artery, are best known for reflex control of ventilation in response to changes in arterial oxygen tension (Nurse, 2014). However, specialized cells within the carotid body also depolarize in response to changes in blood CO_2/H^+ concentration, reflexively increasing ventilation in response to acidosis and hypercapnia and decreasing ventilation in response to hypocapnia. Histologically, the carotid body chemoreceptors consist of (1) type 1 or glomus cells, similar to presynaptic neurons, which are chemosensitive and contain neurotransmitters and autoreceptors; (2) postsynaptic afferent nerve fibers from the carotid sinus nerve, which oppose glomus cells (Gonzalez et al., 1994; Nurse, 2014), contain neurotransmitters and postsynaptic receptors, and have cell bodies in the petrosal ganglion (Gonzalez et al., 1994; Nurse, 2014); (3) type 2 cells, similar to glial cells, which are not chemosensitive; (4) microganglion cells that express cholinergic traits (Gauda et al., 2004); and (5) blood vessels and sympathetic fibers innervating these vessels. In response to hypoxia or increased CO_2/H^+ concentration, type 1 cells depolarize. The major excitatory neurotransmitter in peripheral arterial chemoreceptors is ATP. ATP then binds to the rapid cation ligand-gated ion channel on postsynaptic receptors on the carotid sinus nerve. Neuromodulators, inhibitory and excitatory, further shape the response. For example, dopamine is the most abundant neuromodulator that is produced in and released from the type 1 cells when depolarized. However, dopamine then binds to D_2 receptors on postsynaptic receptors, which leads to inhibition, attenuating ventilatory responses to hypoxia and hypercapnia. Exogenous administered dopamine depresses ventilatory responses to hypoxia and hypercapnia in humans. Adenosine is the breakdown product of ATP, and adenosine A_{2a} and A_1 receptors, which are excitatory and inhibitory G protein-coupled receptors, respectively shape the response of the carotid sinus nerve (Nurse, 2014). Xanthines block both excitatory and inhibitory adenosine receptors.

The commissural nucleus of the nTS is the primary target for afferent processes from peripheral arterial chemoreceptors. Although these afferent processes contain neuromodulators, it is glutamate, binding to both *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors on second-order neurons in the nTS, that is responsible for chemical transmission of excitatory inputs from the peripheral arterial chemoreceptors (Vardhan et al., 1993). These second-order neurons then send tonic excitatory projections to (1) CO_2 -sensitive neurons in the RTN (see earlier) and (2) bulbospinal neurons in the dorsal respiratory group and the VRG that synapse with respiratory motoneurons, leading to changes in the integrated output of the muscles of respiration.

Maturation of CO₂/H⁺ Chemosensitivity of Peripheral Arterial Chemoreceptors

The sensitivity of the peripheral arterial chemoreceptors to CO₂ increases with postnatal development in newborn animals (Carroll et al., 1993); however, in human infants the contribution of peripheral arterial chemoreceptors from central chemoreceptors to ventilatory responses to changes in CO₂ is difficult to delineate. Inferences can be made from the ventilatory response that is seen within a few seconds of exposure to a particular concentration of CO₂, oxygen (O₂), or both because the response time of the peripheral arterial chemoreceptors is faster than that of the central chemoreceptors. What is inferred is that after the first 2 days after birth, peripheral arterial chemoreceptors in newborns are highly responsive to changes in PaCO₂. The postsinh apnea that occurs in infants is the manifestation of this high sensitivity of peripheral arterial chemoreceptors to PaCO₂, which abruptly falls during the sigh (Edwards et al., 2013). In premature infants the PaCO₂ apneic threshold is close to the PaCO₂ that stimulates breathing at rest (Khan et al., 2005). The level of PaCO₂ significantly modifies the sensitivity of the chemoreceptors to the arterial O₂ tension (PaO₂), and this O₂–CO₂ interaction at the carotid body increases with development (Carroll et al., 1993).

Maturation of O₂ Sensitivity of Peripheral Arterial Chemoreceptors

Peripheral arterial chemoreceptors in the carotid body are primarily responsible for changes in ventilation in response to rapid changes in oxygen tension. Changes in ventilation during the first 30 seconds of hypoxia are used to assess the maturation of hypoxic chemosensitivity of the peripheral arterial chemoreceptors, while changes in ventilation in response to hyperoxia measure the contribution of peripheral arterial chemoreceptors on baseline breathing. Hypoxic and hyperoxic sensitivity do not follow the same developmental trajectory. Although oxygen tension less than 25 mmHg stimulates the carotid body in exteriorized fetal sheep, peripheral arterial chemoreceptors are not functional at any level of hypoxia for the first several days after birth in most mammalian species (for a historical overview, see Walker, 1984), including human infants (term and preterm). The rapid increase in arterial oxygen tension during the transition from fetal to neonatal life likely contributes to the resetting of the activation of the carotid body to higher oxygen tension. After birth, hypoxic chemosensitivity gradually increases with postnatal maturation and reaches adult levels by 2–3 weeks postnatally (Rigatto et al., 1975a; Gauda et al., 2009). On the other hand, the contribution of peripheral arterial chemoreceptors to eupneic breathing determined by a drop in ventilation in response to hyperoxia gradually decreases with maturation; the drop in ventilation in response to hyperoxia is greatest in premature versus term infants and least in adults (Al-Matary et al., 2004; Nock et al., 2004). Moreover, premature infants who have a greater frequency of apnea and periodic breathing have a greater reduction in ventilation when exposed to fractional inspired oxygen of 100% (Dejours test). While activity from the peripheral arterial chemoreceptors is not essential for breathing to be established at birth, trophic factors from peripheral arterial chemoreceptors acting on central mechanisms that control breathing during early postnatal development are key in stabilizing rhythmogenesis so that breathing is maintained. The first 2 weeks of postnatal development appear to be the critical period for this trophic influence since sectioning of the carotid sinus nerve (the conduit for trophic support to the central respiratory network) results in death of several mammalian

species from respiratory failure several weeks after denervation (Gauda et al., 2013). Exposures during this critical period of development that can lead to sustained alterations in chemoreceptor function include:

- environmental exposure to the extremes of oxygen tension (chronic or intermittent hypoxia and hyperoxia) (Bavis, 2005; Gauda et al., 2009; Logan et al., 2016)
- nicotine exposure (Gauda et al., 2001; Hafstrom et al., 2005; Stéphan-Blanchard et al., 2013)
- maternal separation—in male rats (Genest et al., 2004)
- perinatal inflammation (Samarasinghe et al., 2015; Master et al., 2016)

Hypoxic Ventilatory Depression: Consequences for the Neonate

Although the peripheral arterial chemoreceptors function to increase ventilation in response to hypoxia, minute ventilation significantly declines after 2–3 minutes of hypoxic exposure. This decline is commonly referred to as *hypoxic roll off*, *hypoxic ventilatory decline*, or *hypoxic ventilatory depression*. Hypoxic ventilatory depression occurs in individuals at all ages, but it is most pronounced in the fetus and newborn (Bissonnette, 2000). Whereas the hypoxic ventilatory decline is usually still above baseline ventilation in mature models, the hypoventilatory response in newborns is usually below baseline ventilation and is often associated with apnea. This hypoxic roll off is not because of a decline in activity of peripheral arterial chemoreceptors. Mechanisms accounting for hypoxic respiratory depression are most well characterized in the fetal animals in which the central brainstem nuclei mediating this response have been attributed to the pons. Transverse section of the upper pons results in a sustained hyperventilatory response to hypoxia in fetal and newborn sheep (Gluckman and Johnston, 1987). Hypoxia activates expiratory neurons in the ventrolateral pons, and chemical blockade of this area blocks the hypoxic respiratory depression in newborn rats (Dick and Coles, 2000).

Several neuromodulators have been implicated in mediating hypoxic ventilatory decline, including norepinephrine, adenosine, GABA, serotonin, opioids, and platelet-derived growth factor, as shown in Table 43.2 (Simakajornboon and Kuptanon, 2005). All these neuromodulators contribute to the ventilatory depression in newborns, but particular attention has been paid to adenosine. Degradation of intracellular and extracellular ATP is the main source of extracellular adenosine, which then mediates its cellular effects by binding to A₁, A_{2a}, A_{2b}, and A₃ adenosine receptors. In response to hypoxia, brain adenosine levels can increase 2.3-fold in fetal sheep (Koos et al., 1994) and 100-fold in rats in response to ischemia (Winn et al., 1981). Nonspecific adenosine receptor blockers, particularly caffeine and methylxanthines, are commonly administered to premature infants to increase central respiratory drive, and aminophylline inhibits hypoxic ventilatory depression in newborns (Darnall, 1985). A₁ adenosine inhibitory receptors are found on respiratory-related neurons (Bissonnette, 2000). Specific A₁ adenosine receptor agonists depress phrenic output in a reduced brainstem spinal cord preparation, whereas A₁ adenosine receptor blockers reverse this inhibitory effect (Dong and Feldman, 1995). In fetal sheep the hypoxic respiratory depression appears to be mediated by excitatory A_{2a} receptors, because blockade of A_{2a} receptors eliminates hypoxic ventilatory roll off in conscious newborn sheep (Koos et al., 1994). Xanthines (e.g., caffeine, aminophylline) block both A₁ and A_{2a} adenosine receptors; therefore their effectiveness in stabilizing ventilation and decreasing the frequency of apnea in premature infants may be by directly

altering the ventilatory response to hypoxia as well as by nonspecifically increasing respiratory drive (see [Apnea of Prematurity](#)).

Effect of Sleep State on Breathing

Sleep state has a profound influence on breathing in the fetus and newborn, and most disorders of breathing that affect the young and old are worse during sleep. AS and quiet sleep (QS) in infants are equivalent to rapid eye movement (REM) and non-REM sleep respectively in older children and adults. Breathing during AS is mostly driven by inputs from the reticular activating system, with less influence from PaCO_2 , whereas breathing during QS is driven by chemical control. Similarly to REM sleep in adults, AS is associated with paralysis of striated muscles. Although this paralysis may be necessary to prevent the acting out of dreams, paralysis of striated muscles that are involved in breathing can be problematic for the newborn. Breathing becomes more irregular in AS and REM sleep because of inhibition of the intercostal muscles and upper airway dilating muscles. The discoordination between chest wall muscles and the diaphragm during AS causes paradoxical breathing: the chest wall moves in during inspiration, with the abdomen moving outward. The more compliant the chest wall, the greater propensity for paradoxical breathing, which is common in the most immature infants. In addition, during inspiration, intrathoracic pressure becomes more negative, and this “suction pressure” causes narrowing or collapse of the compliant upper airway, particularly pharyngeal structures, leading to upper airway obstruction. Paradoxical breathing movements seen on physical examination or detected on inductive plethysmography are often interpreted as a sign of upper airway obstruction. During QS, breathing is characterized by smooth, regular breaths of consistent frequency and depth associated with tonic and phasic activity of the muscles of respiration that are in phase with each other. The chest wall and the abdomen move outward during inspiration, whereas they move inward during expiration. AS and QS can be reliably assessed at 30–32 weeks’ gestation ([Curzi-Dascalova et al., 1993](#)). At this gestational stage, premature infants spend 80% of their sleep time in AS; the proportion decreases to 50% by term, and in adulthood, REM sleep accounts for only 20% of sleep time. Sleep state in normal infants also modifies the time to arousal and the ventilatory responses. The time to arousal on exposure to a hypoxic, somatosensory, or auditory stimulus is greater in AS than with QS in infants during the first 6 months of life ([Horne et al., 2005](#)). The arousal latency in response to a hypoxic stimulus in AS is longer in preterm infants at 2–5 weeks’ postnatal age than that of term infants at the same postnatal age ([Verbeek et al., 2008](#)). The level of oxygen desaturation at the time hypoxic arousal occurs is similar in AS and QS ([Richardson et al., 2007](#)). Respiratory pauses and periodic breathing are more common during AS in both term and premature infants, but the ventilatory response to CO_2 is greater during QS ([Cohen et al., 1991](#)). Because of the complexity of the ventilatory response to hypoxia and the frequent occurrence of arousals induced by hypoxic exposure, assessment of the effect of sleep state on the ventilatory response to hypoxia in newborns is more difficult. Other than the clear difference in arousal in response to a hypoxic stimulus between the two sleep states, differences between sleep states in other respiratory parameters are more variable ([Richardson et al., 2007](#)).

Although most disorders of breathing, such as obstructive sleep apnea, become more severe during sleep, the breathing disorder that is most significantly influenced by sleep state in the newborn is CCHS. As noted previously, CCHS is characterized by

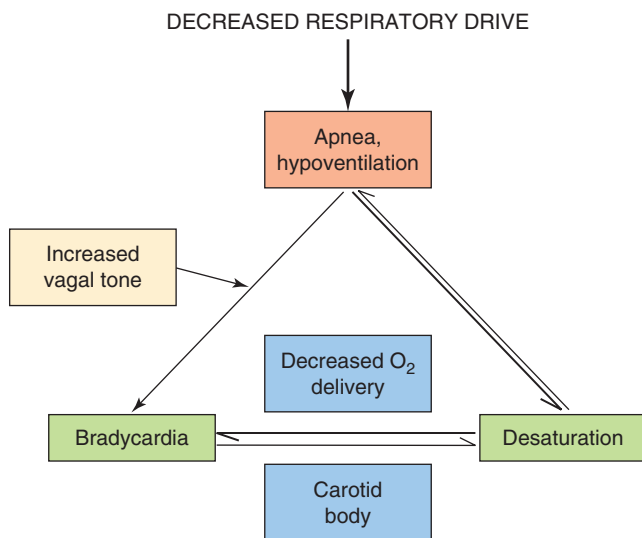
abnormalities in chemical control of breathing; therefore with maturation, as the frequency of QS increases, so does the severity of the disorder. During QS, CO_2 sensitivity is markedly impaired in affected individuals, and exposure to hypercapnia does not significantly increase minute ventilation ([Paton et al., 1989](#)). In infants who died of SIDS, their sleep state during their terminal event is unknown. However, adequate arousal mechanisms are key in preventing respiratory failure and death, and impaired arousal mechanisms are hypothesized to be causative in SIDS. Prone sleeping position increases the percentage of QS ([Horne et al., 2002](#)), and QS is associated with increased time to hypoxic arousal in human infants ([Richardson et al., 2007](#)). A hypoxic microenvironment from rebreathing with defective arousal and autoresuscitative mechanisms is hypothesized to have occurred in infants who have died of SIDS in the face-down (i.e., prone) sleeping position ([Patel et al., 2001](#)). Therefore sleep state can have a significant influence on control of breathing during health and disease, especially in the newborn.

Thus far this chapter has outlined the neurocircuitry and neurochemistry of the respiratory network along with its synaptic inputs that undergo significant maturation during the newborn period. Because these pathways are less developed in premature infants, premature infants have apnea of prematurity, which often requires active therapeutic intervention and can delay hospital discharge. The components of the respiratory network, similarly to other developing organ systems, are plastic and uniquely vulnerable to pathologic processes in premature infants. Therefore the episodes of intermittent hypoxemia and bradycardia that accompany apnea of prematurity may be a cause of acute and chronic morbidities in this high-risk population.

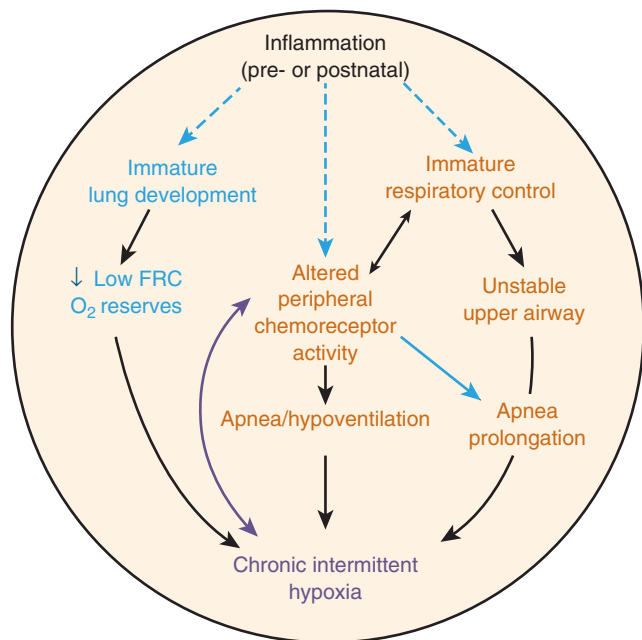
Apnea of Prematurity

Respiratory pauses are universal features in infants born prematurely and are most prominent in the lowest gestational age infants. There is no consensus as to when a respiratory pause can be defined as an apneic episode. It has been proposed that apnea be defined by its duration (e.g., longer than 15 seconds) or accompanying bradycardia and desaturations. However, even the 5-second to 10-second pauses that occur in periodic breathing can be associated with bradycardia or desaturation. It should be emphasized that periodic breathing (ventilatory cycles of 10–15 seconds with pauses of 5–10 seconds) is a normal breathing pattern that should not require therapeutic intervention. It is thought to be the result of dominant peripheral chemoreceptor activity responding to fluctuations in arterial oxygen tension. Episodic bradycardia and desaturation in preterm infants are almost invariably secondary to apnea or hypoventilation ([Fig. 43.6](#); [Martin and Abu-Shaweesh, 2005](#)). The rapidity of the fall in oxygen saturation after a respiratory pause is directly proportional to baseline oxygenation, which is related to lung volume and the severity of lung disease ([Di Fiore et al., 2013](#)). Prenatal and postnatal inflammation directly affect the developing lung and peripheral arterial chemoreceptors ([Masters et al., 2016](#)), which further increases the frequency of oxygen desaturations during short respiratory pauses ([Di Fiore et al., 2013](#); [Fig. 43.7](#)).

Apnea is classified traditionally into three categories on the basis of the absence or presence of upper airway obstruction: central, obstructive, and mixed. Central apnea is characterized by total cessation of inspiratory efforts with no evidence of obstruction. In obstructive apnea, the infant tries to breathe against an obstructed upper airway, resulting in chest wall motion without airflow through the entire apneic episode. Mixed apnea consists of obstructed



• **Fig. 43.6** The sequence of the events whereby apnea results in various combinations of desaturation and bradycardia. (From Martin RJ, Abu-Shaweesh JM. Control of breathing and neonatal apnea. *Biol Neonate*. 2005;87:288–295.)



• **Fig. 43.7** The consequences of the adverse effects of prenatal or postnatal inflammation on the developing lung and respiratory network leading to the emergence of chronic intermittent hypoxia in premature infants. *FRC*, Functional residual capacity. (From Di Fiore JM, Martin JM, Gauda EB. Apnea of prematurity – perfect storm. *Respir Physiol Neurobiol*. 2013;189:213–222.)

respiratory efforts, usually following central pauses. The site of obstruction in the upper airways is primarily in the pharynx, although it also may occur in the larynx and possibly at both sites. It is assumed that there is an initial loss of central respiratory drive, and its recovery is accompanied by a delay in activation of upper airway muscles superimposed on a closed upper airway (Gauda et al., 1987). Mixed apnea typically accounts for more than 50% of long apneic episodes, followed in decreasing frequency by central apnea and obstructive apnea. Purely obstructive spontaneous apnea

in the absence of a positional problem is probably uncommon. Because standard impedance monitoring of respiratory efforts via chest wall motion cannot recognize obstructed respiratory efforts, mixed versus obstructive apnea is frequently identified by the accompanying bradycardia or desaturation (DiFiore et al., 2016a, 2016b).

Presentation of apnea can reflect a nonspecific alteration in either the environment (e.g., thermal) or the general well-being of preterm infants. For example, neonatal sepsis can manifest itself as an increase in the frequency or severity of apnea, and the underlying cause must be treated. Studies using a rat pup model suggest that the systemically released cytokine interleukin-1 β binds to its receptor on vascular endothelial cells at the blood–brain barrier. This binding induces synthesis of prostaglandin E₂, which induces respiratory depression in the brainstem (Hofstetter et al., 2007). These studies provide insight into mechanisms whereby sepsis often manifests itself as apnea of prematurity. Anemia, presumably via decreased oxygen delivery, is also frequently implicated as a cause of apnea. Transfusion of packed red cells can provide some variable benefit for apnea of prematurity (Zagol et al., 2012). Furthermore, there may be some utility in reducing persistent intermittent desaturations in extremely low birth weight newborns younger than 7 days of age (Abu Jawdeh et al., 2014).

Therapeutic Approaches

Continuous Positive Airway Pressure

Continuous positive airway pressure (CPAP) of 4–6 cmH₂O is a relatively safe and effective therapy for apnea of prematurity. Because longer episodes of apnea frequently involve an obstructive component, CPAP appears to be effective by splinting the upper airway with positive pressure and decreasing the risk of pharyngeal or laryngeal obstruction (Miller et al., 1985). CPAP also has beneficial effect in apnea by increasing functional residual capacity, thereby improving oxygenation status. At a higher functional residual capacity, the time from cessation of breathing to desaturation and resultant bradycardia is prolonged. Heated high-flow nasal cannula therapy has been suggested as an equivalent treatment modality to allow CPAP delivery, while improving ease of care. Although this approach is used widely, its efficacy for treatment of apnea of prematurity has not been studied in depth. For example, a recent small study in 10 premature infants with a crossover design suggested that work of breathing may be greater when infants are treated with heated high-flow nasal cannula therapy (HHFNC) versus CPAP therapy (Nasef et al., 2015). Noninvasive ventilatory strategies, using a nasal mask to deliver intermittent positive pressure, avoid the need for full ventilatory support in some infants and can be effective in reducing the need for reintubation for persistent apnea (Lemyre et al., 2014). However, for severe or refractory episodes, endotracheal intubation and mechanical ventilation may be needed. Minimal ventilator settings should be used to allow for spontaneous ventilatory efforts and to minimize the risk of barotrauma.

Methylxanthines

Methylxanthines have been the mainstay of pharmacologic treatment of apnea of prematurity for several decades. Both theophylline and caffeine are used and have multiple physiologic and pharmacologic mechanisms of action. Xanthine therapy appears to increase minute ventilation, improve CO₂ sensitivity, decrease hypoxic depression of breathing, enhance diaphragmatic activity, and decrease periodic breathing. The likely major mechanism of action is through

competitive antagonism of adenosine receptors. Adenosine acts as an inhibitory neuroregulator in the central nervous system via activation of adenosine A_1 receptors (Herlenius et al., 1997). In addition, activation of adenosine A_{2a} receptors appears to excite GABAergic interneurons, and released GABA may contribute to the respiratory inhibition induced by adenosine (Mayer et al., 2006).

Methylxanthines have some well-documented short-term adverse effects. Toxic levels can produce tachycardia, cardiac dysrhythmias, feeding intolerance, and seizures (infrequently), although these effects are seen less commonly with caffeine at the usual therapeutic doses. Mild diuresis is caused by all methylxanthines. The observation that xanthine therapy causes an increase in metabolic rate and oxygen consumption of approximately 20% suggests that caloric demands can be increased with this therapy at a time when nutritional intake is already compromised (Bauer et al., 2001).

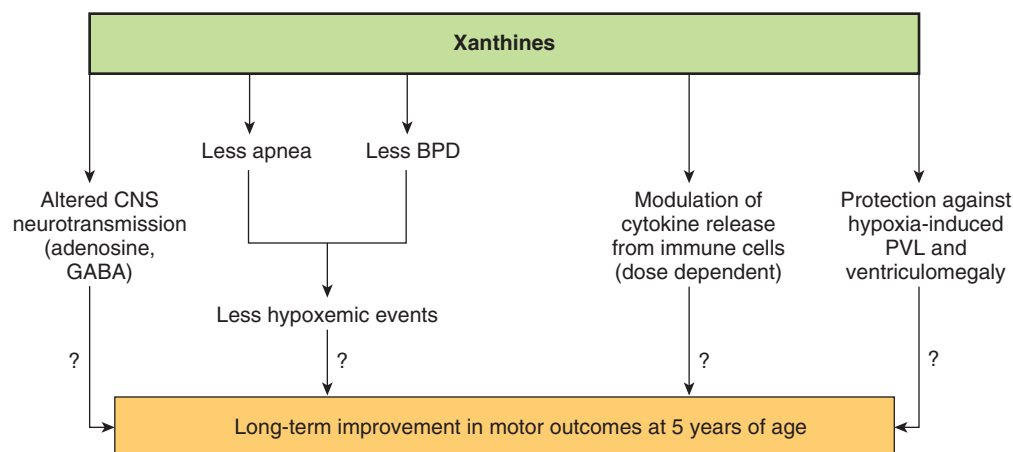
A large, international, multicenter clinical trial was designed to test short-term and long-term safety of caffeine therapy for apnea of prematurity. In the neonatal period, caffeine treatment was associated with a significant reduction in the postmenstrual ages at which both supplemental oxygen and endotracheal intubation were needed (Schmidt et al., 2007). Of even greater interest was the significant decrease in the incidence of cerebral palsy and cognitive delay in the caffeine-treated group, which was not sustained by 5 years of age (Schmidt et al., 2012) but caffeine exposure was associated with a sustained beneficial effect on developmental coordination disorders at 5 years of age (Doyle et al., 2014). This finding raises interesting questions regarding possible mechanisms underlying this beneficial effect of caffeine on neurodevelopmental outcome (Fig. 43.8). These beneficial effects include the observation in animal models that loss of the adenosine A_1 receptor gene is protective against hypoxia-induced loss of brain matter (Back et al., 2006) and a potential benefit of caffeine on immune mechanisms that mediate lung and brain injury (Chavez-Valdez et al., 2009). Recent data in rodent models also support a beneficial anti-inflammatory effect of xanthine therapy in immature lungs exposed to the proinflammatory effects of prenatal exposure to lipopolysaccharide, postnatal

hyperoxia, or both (Köroğlu et al., 2014; Nagatomo et al., 2016). The inhibitory effects of caffeine on adenosine receptor subtypes may differ depending on the xanthine concentrations; this should present a note of caution in the clinical situation when high doses of caffeine are considered. Recent data in an immature ovine model do not, however, demonstrate an adverse effect on developing white matter (Atik et al., 2014).

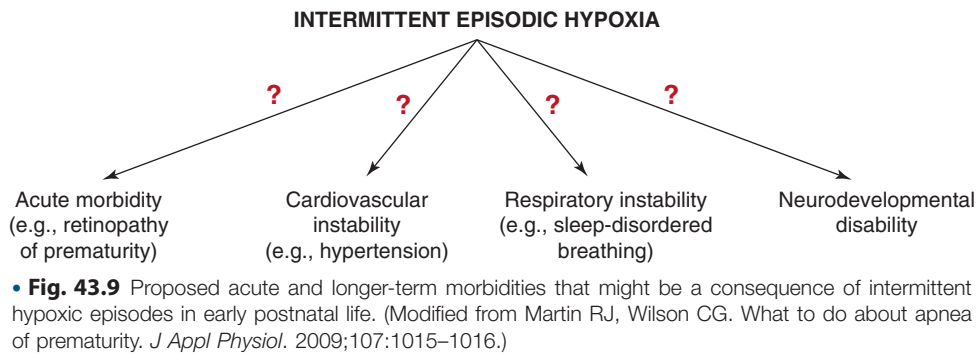
In recent years the practice of caffeine administration has expanded as a result of both increased use of noninvasive ventilation and the success of the earlier caffeine trial. Caffeine is now widely used in a prophylactic mode to prevent, rather than treat, clinically significant apnea; early onset of treatment appears beneficial (Lodha et al., 2015). Extended use beyond apparent resolution of apnea has also been proposed to decrease intermittent hypoxic episodes (Rhein et al., 2014). As already suggested, increased dosing beyond the traditional caffeine citrate loading dose of 20-mg/kg followed by a maintenance dose of 5–10 mg/kg every 24 hours should, however, proceed with caution pending further study. A recent small pilot randomized trial of high-dose caffeine therapy suggested an association between a high loading dose and cerebellar injury on magnetic resonance imaging in preterm infants (McPherson et al., 2015). Although measurement of serum caffeine levels is not widely practiced, these levels may not become subtherapeutic until 11–12 days after cessation of treatment (Doyle et al., 2016). Finally, increased respiratory drive may increase within minutes after intravenous caffeine administration as measured by diaphragmatic activity in preterm infants (Kraaijenga et al., 2015).

Gastroesophageal Reflux and Apnea of Prematurity

GER is often incriminated as a cause of neonatal apnea. Despite the frequent coexistence of apnea and GER in preterm infants, investigations of the timing of reflux in relation to apneic events indicate that they are rarely related temporally. When these events coincide, there is no evidence that GER prolongs the concurrent apnea (Di Fiore et al., 2005). Although physiologic experiments



• **Fig. 43.8** Multiple proposed mechanisms are demonstrated whereby xanthine therapy for apnea of prematurity may improve motor outcomes in former premature infants. These outcomes include functional changes in neurotransmitters in the brain, a decrease in hypoxemic episodes that accompany apnea, especially in the presence of bronchopulmonary dysplasia (BPD), a proposed protective effect of adenosine receptor inhibition on hypoxia-induced white matter injury, and the beneficial effect of adenosine receptor blockade on inflammatory cytokine-mediated lung or brain injury. CNS, Central nervous system; GABA, γ -aminobutyric acid; PVL, periventricular leukomalacia. (Modified from Abu-Shaweesh JM, Martin RJ. Neonatal apnea: what's new? *Pediatr Pulmonol*. 2008;43:937–944.)



in animal models reveal that reflux of gastric contents to the larynx induces reflex apnea, there is no clear evidence that treatment of reflux affects the frequency of apnea in most preterm infants. Therefore pharmacologic management of reflux with agents that decrease gastric acidity or enhance gastrointestinal motility generally should be reserved for preterm infants who exhibit signs of emesis or regurgitation of feedings, regardless of whether apnea is present, and infants at specific risk (e.g., exhibiting neurodevelopmental delay or following gastrointestinal surgical repair). Extreme caution should be taken when histamine H_2 receptor blockers are used to change gastric pH since use of these agents in hospitalized very low birth weight infants has been associated with increased incidence of necrotizing enterocolitis and increased mortality (Romaine et al., 2016). Therapy for such infants should begin with nonpharmacologic approaches, such as thickened feeds, because acid suppression therapy has been shown to alter the gastrointestinal microbiome and increase the risk of lower respiratory infection in infants (Gupta et al., 2013) and necrotizing enterocolitis (Terrin et al., 2012). There remain considerable differences of opinion among neonatologists, pediatric gastroenterologists, and pediatric pulmonologists regarding diagnosis and management of this problem (Golski et al., 2010).

Resolution and Consequences of Neonatal Apnea

Apnea of prematurity generally resolves by 36–40 weeks' postconceptional age; however, apnea frequently persists beyond this time in more immature infants. Available data indicate that cardiorespiratory events in such infants return to the baseline normal level at 43–44 weeks' postconceptional age (Ramanathan et al., 2001). In other words, beyond 43–44 weeks' postconceptional age, the incidence of cardiorespiratory events in preterm infants does not significantly exceed that in term infants. The persistence of cardiorespiratory events may delay hospital discharge for a subset of infants. In these infants, apnea longer than 20 seconds is rare; rather they exhibit frequent bradycardia to less than 70 to 80 beats per minute with short respiratory pauses (Di Fiore et al., 2001). The reason that some infants exhibit marked bradycardia with short pauses is unclear, but available data suggest a vagal phenomenon and benign outcome. For a few of these infants home cardiorespiratory monitoring, until 43–44 weeks' postconceptional age, is offered in the United States as an alternative to a prolonged hospital stay. The apparent lack of a relationship between persistent apnea of prematurity and SIDS has significantly decreased the practice of home monitoring, with no increase in the SIDS rate. Infants born prematurely experience multiple problems during

their time spent in the neonatal intensive care unit, and many of these conditions can contribute to poor neurodevelopmental outcomes. For example, a history of hyperbilirubinemia has been associated with persistent apnea of prematurity in preterm infants and animal models (Amin et al., 2005; Mesner et al., 2008). The problem of correlating apnea with outcome is compounded by the fact that nursing reports of apnea severity may be unreliable, and impedance monitoring techniques will fail to identify mixed and obstructive events. Despite these reservations, available data suggest a link between the number of days of assisted ventilation and the number of days of apnea after extubation with impaired neurodevelopmental outcome (Janvier et al., 2004). A relationship has also been shown between delay in resolution of apnea and bradycardia beyond 36 weeks' corrected age and a higher incidence of unfavorable neurodevelopmental outcome (Pillekamp et al., 2007). Finally, a high number of cardiorespiratory events recorded after discharge via home cardiorespiratory monitoring appear to correlate with less favorable neurodevelopmental outcome (Hunt et al., 2004).

Recent studies have focused more on the incidence and severity of desaturation events, because techniques for long-term collection of pulse oximetry data are now more advanced. Furthermore, it is likely that recurrent hypoxia is the detrimental feature of the breathing abnormalities exhibited by preterm infants. Recent data demonstrate that hypoxic episodes of at least 60 seconds' duration are associated with an unfavorable neonatal outcome in a large cohort of preterm infants (Poets et al., 2015). Fig. 43.9 summarizes proposed morbidities that might be attributable to intermittent hypoxic episodes in early life. Recurrent episodes of desaturation during early life and resultant effects on neuronal plasticity related to peripheral and central respiratory control mechanisms may serve as an important future direction for study.

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Neonatal Pulmonary Physiology

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KEY POINTS

- Gas flows in and out of the lung only in the presence of pressure gradients. Typically, gas flow ceases at the end of inspiration and the end of expiration if ventilation is uniform.
- Airway resistance varies with the fourth to fifth power of the radius of the airway, making gas flow highly sensitive to changes in airway diameter.
- Measurements of pulmonary function that rely on measurement of expiratory gas flow may not measure flow out of poorly ventilated lung units. This could cause them to underestimate total lung resistance and miss the effect of therapies such as bronchodilator therapies.
- The primary cause of hypoxemia in infants with lung disease is the continued perfusion of open but poorly ventilated lung units. Attempts to correct hypoxemia should be directed at improving oxygenation in these lung units by administration of oxygen or by improving ventilation with continuous positive airway pressure (CPAP) or mechanical ventilation.
- The major pulmonary disability of the premature infant, in the absence of respiratory distress syndrome, is a highly compliant chest wall that can allow lung volumes and PaO_2 to decrease and contribute to apnea. The treatment is to restore lung volume with use of nasal CPAP if possible.
- Pulmonary edema occurs when the rate of fluid filtration exceeds the rate of fluid removal by pulmonary lymphatics.

Lung Mechanics and Lung Volumes

The lungs possess physical, or mechanical, properties that resist inflation, such as elastic recoil, resistance, and inertance. The dynamic interaction between these properties determines the effort that must be exerted during spontaneous breathing and the resting and extreme values for the volume of gas in the lung.

Elastic Recoil

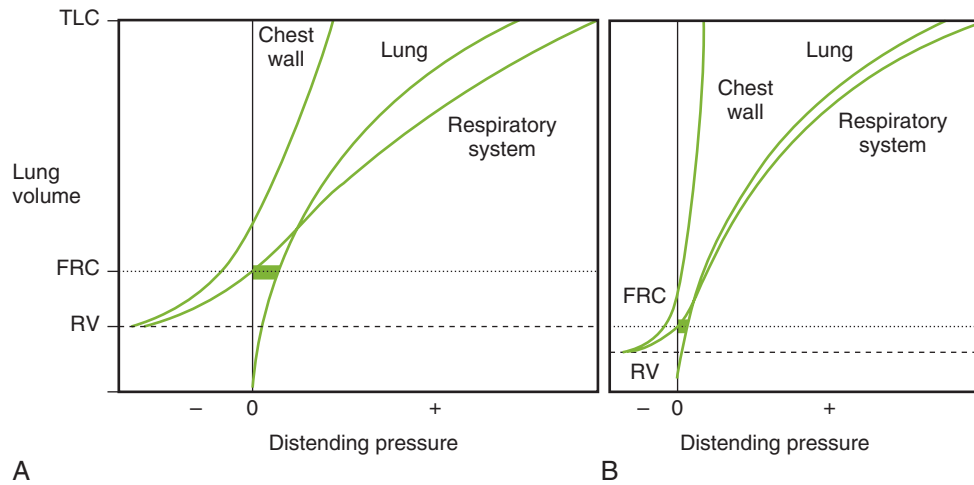
The lung contains elastic tissues that must be stretched for lung inflation to occur. Hooke's law requires that the pressure needed to inflate the lung must be proportional to the volume of inflation (Fig. 44.1). Conventionally, the volume of inflation is plotted on the y -axis, and the distending pressure is plotted on the x -axis. In this way the constant of proportionality is volume divided by pressure, or lung compliance. Throughout the range of *tidal* ventilation the relationship between pressure and volume is linear. At

higher lung volumes, as the lung reaches its elastic limit (i.e., total lung capacity), this relationship plateaus, making the pressure–volume relationship nonlinear.

The lungs and the chest wall function as a unit (the respiratory system) coupled by the interface between the parietal and visceral pleura. Therefore respiratory system compliance (CRS) can be partitioned into lung compliance (CL) and chest wall compliance (CCW), where $1/\text{CRS} = 1/\text{CL} + 1/\text{CCW}$. The tendency for the lung to collapse inward at the end of exhalation is balanced by the outward recoil of the chest wall, resulting in a negative (subatmospheric) intrapleural pressure. The functional residual capacity (FRC) is the volume of gas in the lungs when the elastic forces of the lung and chest wall reach equilibrium (Greenspan et al., 2005). Inflation of the respiratory system above FRC requires a positive distending pressure that must overcome the elastic recoil of both the lung (alveolar pressure minus intrapleural pressure) and the chest wall (intrapleural pressure minus atmospheric pressure) (Gappa et al., 2006). Deflation below FRC requires an active expiratory maneuver. Residual volume (RV) is defined as the volume of air that cannot be expired even with a forced deflation.

As depicted in Fig. 44.1, the relative compliance of the lung of the newborn is similar to that of the adult (Krieger, 1963). However, the infant's chest wall is composed primarily of cartilage, whereas in adults the chest wall is completely ossified and therefore CCW is greater in infants than in adults (see Fig. 44.1), and pleural pressure is only slightly subatmospheric. Measurements of lung and chest wall compliance suggest that the newborn should have a lower percent RV and a lower percent FRC than the adult. In fact, the percent FRC in the newborn is equal to the adult's, and the infant's percent RV is slightly greater. This seeming paradox exists because FRC and RV are measured while the infant is breathing, and predictions from the pressure–volume curves assume that there is no air movement and that there is passive relaxation of all respiratory muscles (Bryan and England, 1984).

Establishment of the FRC is vital to the role of maintaining adequate lung mechanics and gas exchange. The highly compliant newborn chest wall exerts very little outward distending pressure, and thus the lung is more prone to collapse at the end of exhalation (Hülkamp et al., 2005). Data suggest three mechanisms by which the newborn can limit expiratory flow and increase the intrapulmonary pressure to maintain a normal FRC during spontaneous breathing: (1) by increasing expiratory resistance through laryngeal adduction (glottic narrowing), (2) by maintaining inspiratory muscle activity throughout expiration, and (3) by initiating high breathing frequencies to limit the expiratory time (gas trapping) (Magnenat et al., 2004; te Pas et al., 2008).



• **Fig. 44.1** An idealized plot of volume as a function of distending pressure for the lung, chest wall, and respiratory system (lung plus chest wall) of an adult (A) and an infant (B). The curves are derived by instillation or removal of a measured volume of gas from the lung and allowing the respiratory system to come to rest against a shuttered airway. At this point only elastic forces are acting on the respiratory system, and airway pressure is equal to alveolar pressure. Intrapleural pressure can be measured with an esophageal balloon. Because airway pressure is equal to alveolar pressure, the distending pressure for the lung can be measured as *airway pressure minus intrapleural pressure*. The distending pressure for the chest wall is *intrapleural pressure minus atmospheric pressure*, and the distending pressure for the respiratory system is *airway pressure minus atmospheric pressure*. Compliance is the change in volume divided by the change in distending pressure. The shaded area is the resting intrapleural pressure at functional residual capacity (FRC). The lung volumes depicted are residual volume (RV), FRC, and total lung capacity (TLC).

Resistance

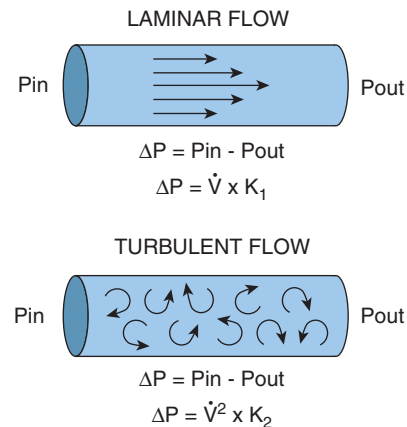
Resistance to gas flow arises because of friction between gas molecules and the walls of airways (i.e., airway resistance) and because of friction between the tissues of the lung and the chest wall (i.e., viscous tissue resistance). Airway resistance represents approximately 80% of the total resistance of the respiratory system, and tissue resistance and inertial forces account for the remaining 20% (Polgar and String, 1966). In the newborn, nasal resistance represents nearly half of the total airway resistance; in the adult, it accounts for about 65% of the airway resistance (Polgar and Kong, 1965).

Gas flows only in response to a pressure gradient (Fig. 44.2). During laminar flow the pressure difference needed to move gas through the airway is directly related to the flow rate times a constant (airway resistance). During turbulent flow, however, this pressure is directly proportional to a constant times the flow rate squared. Gas flow becomes turbulent at branch points in airways, at sites of obstruction, and at high flow rates. Turbulence occurs whenever flow increases to a point that the Reynolds number exceeds 2000. This dimensionless number is directly proportional to the volumetric flow rate and gas density, and it is inversely proportional to the radius of the tube and the gas viscosity. Obviously, turbulent flow is most likely to occur in the central airways, where volumetric flow is high, rather than in lung periphery where flow is distributed across a large number of airways. Both types of flow exist in the lung, so the net pressure drop is calculated as follows (Pedley et al., 1977):

(1)

$$\Delta P = (K_1 \times \dot{V}) + (K_2 \times \dot{V}^2).$$

It is possible to take advantage of the differences between laminar and turbulent flow to determine the site of airway obstruction in



• **Fig. 44.2** Gas flow (\dot{V}) through tubular structures occurs only in the presence of a pressure (P) gradient ($P_{in} > P_{out}$). For laminar flow, P is directly proportional to \dot{V} :

$$\Delta P = (\dot{V} \times 8 \times L \times \mu) / (\pi \times r^4).$$

In this case the constant of proportionality (K_1) is directly related to the length of the airway (L) and the viscosity of the gas (μ) and is indirectly proportional to the fourth power of the radius of the airway (r). For turbulent flow, ΔP is proportional to \dot{V}^2 . The constant of proportionality (K_2) is directly proportional to the length of the airway and the density of the gas and inversely proportional to the fifth power of the radius of the airway.

the lung. If obstruction to gas flow is in the central airways, turbulent flow is affected the most. Because turbulent gas flow is density dependent, if the patient breathes a less dense gas (such as helium mixed with oxygen), the resistance to gas flow is reduced. If the site of obstruction is peripheral, the mixture of helium and oxygen does not appreciably affect resistance.

Inflation of the lung increases the length of airways and might therefore be expected to increase airway resistance; however, lung inflation also increases airway diameter. Because airway resistance varies with the fourth to fifth power of the radius of the airway, the effects of changes in airway diameter dominate, and resistance is inversely proportional to lung volume (Rodarte and Rehder, 1986). Airway resistance is lower during inspiration than during expiration because during inspiration, pleural pressure becomes more negative, and a greater distending pressure is applied across the lung. This distending pressure increases airway diameter as well as alveolar diameter and decreases the resistance to gas flow. During expiration, pleural pressure increases and airways are compressed. Collapse of airways is opposed by their cartilaginous support and by the pressure exerted by gas in their lumina. During passive expiration these defenses are sufficient to prevent airway closure. When intrapleural pressure is high, during active expiration, airways may collapse, and gas may be trapped in the lung. This problem may be accentuated in the small preterm infant with poorly supported central airways.

Inertance

Gas and tissues in the respiratory system also resist accelerations in flow. Inertance is a property that is assumed to be negligible during quiet breathing and physiologically significant only at rapid respiratory rates.

Dynamic Interaction

Compliance, resistance, and inertance all interact during spontaneous breathing (Fig. 44.3). This interaction is described by the equation of motion for the respiratory system:

(2)

$$P(t) = (V[t] \times 1/C) + (\dot{V}[t] \times R) + (\ddot{V}[t] \times I),$$

where $P(t)$ is the driving pressure at time t , $V[t]$ is the lung volume above FRC, C is the respiratory system compliance, $\dot{V}[t]$ is the rate of gas flow, R is the resistance of the respiratory system, $\ddot{V}[t]$ is the rate of acceleration of gas in the airways, and I is the inertance of the respiratory system. If I is disregarded, the equation simplifies to

(3)

$$P(t) = (V[t] \times 1/C) + (\dot{V}[t] \times R).$$

At times of zero gas flow (end expiration and end inspiration), the equation further simplifies to

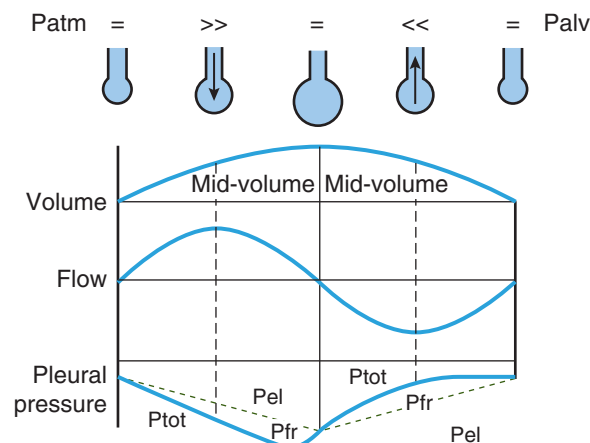
(4)

$$P(t) = (V[t] \times 1/C) \text{ and } C = V(t)/P(t).$$

This series of equations and Fig. 44.3 demonstrate that at points of no gas flow (end of expiration and end of inspiration), only elastic forces are operating on the lung. During inflation or deflation of the lung, however, both elastic and resistive forces are important.

Work of Breathing

The work of breathing (WOB) is a reflection of the amount of energy required to overcome the elastic and resistive elements of the respiratory system and move gas into and out of the lung



• **Fig. 44.3** Gas flows from the atmosphere into the lung only if atmospheric pressure (P_{atm}) is greater than alveolar pressure (P_{alv}). At the end of exhalation, when P_{atm} equals P_{alv} , there is no gas movement in or out of the lung. During a spontaneous inspiration, the diaphragm contracts, the chest wall expands, and the volume in the intrathoracic space increases. As a result, pleural pressure (P_{pl}) decreases relative to P_{atm} , and a gradient is created between P_{pl} and P_{alv} , distending the lung, increasing alveolar volume, and decreasing P_{alv} . A gradient is also created between P_{atm} and P_{alv} , and gas flows from the atmosphere into the alveolar space. The rate of gas flow increases rapidly, reaches a maximum (peak flow), then decreases as the alveolus fills with gas and P_{alv} approaches P_{atm} . At peak inspiration, P_{alv} equals P_{atm} , and lung volume is at its maximum, as is P_{pl} . The curved solid line connecting the end of expiration to the end of inspiration is the total driving pressure for inspiration (P_{tot}). The dotted line represents the pressure needed to overcome elastic forces alone (P_{el}). The difference between the two lines is the pressure dissipated overcoming flow resistive forces (P_{fr}). During exhalation, this cycle is reversed.

during spontaneous breathing. WOB is defined as the cumulative product of distending pressure and the given volume displaced during inhalation or exhalation (Fig. 44.4):

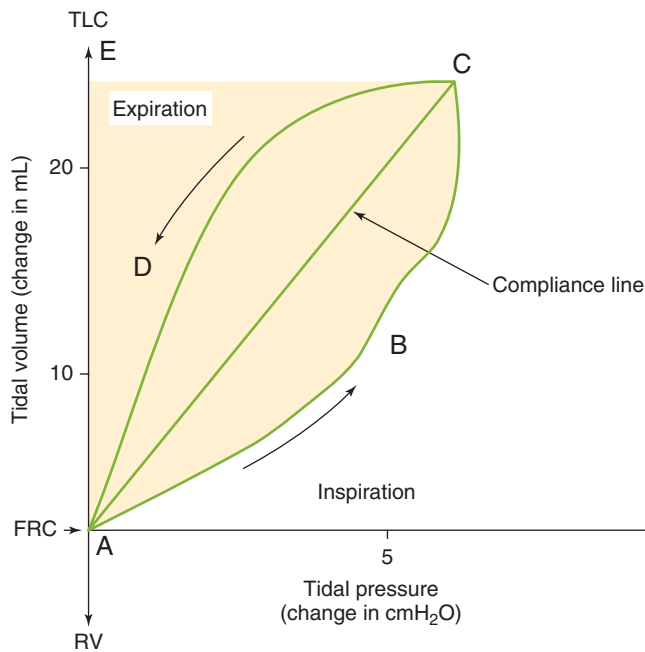
$$WOB = \int P dV,$$

where P is pleural pressure (in time) above resting pleural pressure and V is the volume (in time) relative to the resting thoracic volume (see Fig. 44.4).

The WOB required to ventilate the lungs of normal newborns is approximately 10% of that required in adults (McIlroy and Tomlinson, 1955). However, infants have been shown to have a higher oxygen cost and lower mechanical efficiency associated with the WOB than adults (Thibeault et al., 1966). In healthy infants, most of the WOB is done by the diaphragm during inhalation. Approximately one-third of the total inspiratory WOB is related to overcoming the resistance to gas flow in the airways (Mortala et al., 1982). Exhalation is usually passive because of the potential energy stored in the lung and the chest wall at the end of inhalation but may become active as expiratory resistance increases or lung volumes decrease below FRC.

Measurements of Respiratory System Mechanics

Respiratory system mechanics are objective measurements used to determine the severity of lung disease, changes in pathophysiology,



• **Fig. 44.4** Pressure–volume loop showing the compliance line (AC, joining points of no flow); work done in overcoming elastic resistance (ACEA), which incorporates the frictional resistance encountered during expiration (ACDA); work done in overcoming frictional resistance during inspiration (ABCA); and total work done during the respiratory cycle (ABCEA), or the entire shaded area. FRC, Functional residual capacity; RV, residual volume; TLC, total lung capacity. (From Wood BR. Physiologic principles. In Goldsmith JP, Karotkin EH, eds. *Assisted Ventilation of the Neonate*. 4th ed. Philadelphia: WB Saunders; 2003:15–30.)

response to therapeutic interventions, and progression of lung growth and development. Table 44.1 shows mechanics values for well and sick newborn infants. Lung mechanics in infants are typically measured during spontaneous breathing or during assisted ventilation or during short periods of apnea induced by taking advantage of the Hering–Breuer reflex.

There are a number of methods to calculate resistance and compliance of the respiratory system and their individual components. This section will deal with some of the more commonly used methods (Mammel and Donn, 2015; Peterson-Carmichael et al., 2016).

Resistance of the total respiratory system can be calculated from measurements of driving pressure and the rate of gas flow and can be measured only when the lung is moving. The choice of driving pressure determines the site of the measurement. Most commonly the driving pressure is airway pressure minus atmospheric pressure (resistance of the respiratory system) or airway pressure minus intrapleural pressure (total lung resistance). Gas flow rates are calculated from measurements obtained from devices placed in the infant's airway via a face mask, nasal prongs, or an endotracheal tube. If there are no leaks, all of the gas flow in and out of the infant's lungs will travel through a pneumotachometer, which calculates flow as the pressure drop across a fixed resistance or a hot wire anemometer, which calculates flow by measuring the transfer of heat from a heated wire in the circuit just proximal to the infant.

More recently, investigators have placed elastic bands embedded with coils arranged in a sinusoidal pattern around the chest and

TABLE 44.1 Lung Volumes and Mechanics of Well and Sick Neonates

| Measurements | Units | Normal | RDS | BPD |
|--------------|----------------------------|--------|---------|---------|
| Tidal volume | mL/kg | 5–7 | 4–6 | 4–7 |
| FRC | mL/kg | 25–30 | 20–33 | 20–30 |
| Compliance | mL/cmH ₂ O | 1–2 | 0.3–0.6 | 0.2–0.8 |
| Resistance | cmH ₂ O/L per s | 25–50 | 60–160 | 30–170 |

BPD, Bronchopulmonary dysplasia; FRC, functional residual capacity; RDS, respiratory distress syndrome.

Data from Cook CD, Sutherland JM, Segal S, et al. Studies of respiratory physiology in the newborn infant: III. Measurements of mechanics of respiration. *J Clin Invest*. 1957;36:440–448; Polgar G, String ST. The viscous resistance of the lung tissues in newborn infants. *J Pediatr*. 1966;69:787–792; Reynolds RN, Etsten BE. Mechanics of respiration in apneic anesthetized infants. *Anesthesiology*. 1966;27:13–19; Polgar G, Promadhat V. Pulmonary function testing in children: techniques and standards. Philadelphia, PA: WB Saunders; 1971:273; Gerhardt T, Bancalari E. Chestwall compliance in full term and premature infants. *Acta Paediatr Scand*. 1980; 69:359–364; McCann EM, Goldman SL, Brady JP. Pulmonary function in the sick newborn infant. *Pediatr Res*. 1987; 21:313–325; and Choukroun ML, Tayara N, Fayon M, Demarquez JL. Early respiratory system mechanics and the prediction of chronic lung disease in ventilated preterm neonates requiring surfactant treatment. *Biol Neonate*. 2003;83:30–35.

abdomen. As an infant breathes, changes in the rib cage and abdominal cross-sectional areas defined by the bands can be transduced into electric signals (respiratory inductance plethysmography [RIP]). The change in cross-sectional area can be used to estimate the rate of gas flow and if calibrated can be used in the calculation of lung mechanics. In addition, with calibration the resting values can be used to measure or estimate chest and abdominal volumes. RIP can also be used to measure the synchrony between the chest and the abdomen as the infant breathes, with asynchronous breathing implying increased respiratory effort and in some infants impending respiratory failure (Allen et al., 1990; Reiterer et al., 2015).

Traditionally, resistance of the total respiratory system was calculated from measurements of distending pressure, volume, and flow (see Fig. 44.3). For this calculation, points of equal volume are chosen during inspiration and expiration. The gas flow and the distending pressure are measured at each point. The pressure needed to overcome elastic forces should be the same for inspiration and expiration, and therefore the pressures should cancel out. Total resistance, consequently, is equal to distending pressure at the inspiratory point minus distending pressure at the expiratory point divided by the sum of the respective inspiratory and expiratory point gas flows. Subsequently, investigators simplified these calculations by measuring distending pressure, gas flow, and volume (see Fig. 44.3), then fitting these measurements to the equation of motion (Eq. 3) using multiple linear regression techniques and solving for the coefficients $1/C$ and R (Bhutani et al., 1988). Calculations of pulmonary function by modern ventilators use much the same process.

Calculation of lung compliance, however, requires measurements of distending pressure and volume (see Figs. 44.1 and 44.3) obtained when the lung is at rest. The choice of distending pressure determines the site of the pressure measurement, and gas volume is obtained by integration of the measurements of flow. As mentioned previously, the mechanical behavior of the respiratory system (chest wall and

lung) can be decoupled effectively by measurement of pleural pressure. Because it is not feasible to measure pleural pressure, esophageal pressure measured in the distal third of the esophagus, with use of an air-filled catheter attached to a pressure transducer, can be used to estimate pleural pressure. Thus transpulmonary pressure is estimated as the difference between airway pressure and esophageal pressure and is useful in measuring lung and chest wall mechanics.

Static compliance is the compliance measured when the infant is completely passive and can be estimated with use of an inspiratory hold at the end of inhalation during assisted ventilation (McCann et al., 1987) by instillation of a known volume of gas into the lung, followed by measurement of airway pressure at equilibrium in the absence of airflow and respiratory muscle activity (see Figs. 44.1 and 44.3) or by the use of a weighted spirometer (Tepper et al., 1984).

Calculations of compliance are affected by lung size. For example, if a 5-cmH₂O distending pressure results in a 25-mL increase in lung volume in a newborn, the calculated lung compliance is 5 mL/cmH₂O. In an adult, the same 5-cmH₂O distending pressure increases the lung volume by roughly 500 mL, and the calculated compliance is 100 mL/cmH₂O. Although the calculated lung compliances are different, the forces needed to perform tidal ventilation are similar (i.e., lung function is normal in both circumstances). This example points out that if lung compliances are to be compared, they must be corrected for size. One usually does this by dividing compliance by resting lung volume to get *specific compliance*. For the newborn, resting lung volume is roughly 100 mL, so specific compliance is 0.05 mL/cmH₂O per milliliter lung volume. For the adult, resting lung volume is nearly 2000 mL, so specific compliance is 0.05 mL/cmH₂O per milliliter lung volume—identical to that of the newborn.

Lung compliance changes with volume history, meaning that it decreases with fixed tidal volumes and increases after deep breaths that recruit air spaces that may have been poorly ventilated or collapsed. The periodic sigh in spontaneous breathing is typically associated with an increase in lung compliance and in oxygenation (Frappell and MacFarlane, 2005).

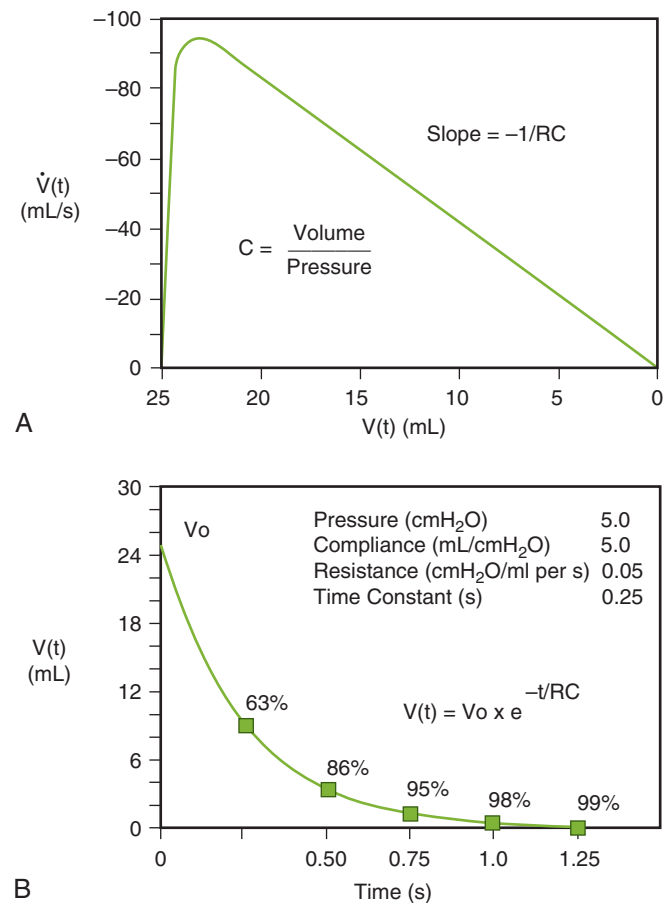
Because of the difficulties associated with trying to achieve a steady state to measure static compliance, many investigators calculate a “dynamic compliance” and take advantage of the fact that gas flow in and out of the lung is transiently equal to zero at the end of inspiration and the end of expiration (see Fig. 44.3). Measuring lung volume and distending pressures at these two points of rest allows calculation of compliance by division of the change in volume by the concomitant change in distending pressure.

In the normal infant it is generally assumed that *dynamic* compliance is equal to *static* compliance. However, many respiratory disorders result in nonhomogeneous increases in small airway resistance in the lung (Table 44.1). As a result, in infants who are tachypneic or who have elevated airway resistance, the *dynamic* compliance may underestimate the *static* compliance of the lung (Katier et al., 2006).

Discussion of a partial solution to the equation of motion for the respiratory system can shed some light on this discrepancy. The behavior of the respiratory system during passive exhalation is a special situation for which a solution can be obtained relatively easily with use of the occlusion technique (McIlroy et al., 1963; Lesouef et al., 1984; Katier et al., 2005). Before a passive exhalation maneuver, the infant is given a positive pressure breath, and the airway is occluded—invoking the Hering–Breuer reflex and a brief

apnea. Airway pressure is measured, and the occlusion is released. Expired gas flow is measured with a pneumotachometer and integrated to volume; flow is then plotted as a function of volume (Fig. 44.5A). During a passive exhalation, no external forces are acting on the respiratory system (airway pressure or $P(t) = 0$), so the equation of motion simplifies to a first-order differential equation:

$$(V[t] \times 1/C) + (\dot{V}[t] \times R) = 0.$$



• **Fig. 44.5** (A) Flow of gas out of the lung versus volume of gas remaining in the lung, $V(t)$, for a passive exhalation. Flow of gas out of the lung is negative by convention. After an initial sharp increase, flow decreases linearly as the lung empties. Static compliance of the respiratory system is obtained by division of the exhaled volume by the airway pressure at the beginning of the passive exhalation. Resistance is calculated from the slope of the flow–volume plot ($-1/RC$) and the compliance. This technique has the advantage of not requiring measurements of pleural pressure and being relatively unaffected by chest wall distortion. (B) $V(t)$ as a function of time for a passive exhalation. The graph is an exponential with the equation

$$V(t) = V_0 \times e^{-t/RC},$$

where V_0 is the starting volume, and e is the base of the natural logarithm (roughly 2.72). For this example the time constant of the respiratory system (Trs) is roughly 0.25 second. Calculations show that when exhalation persists for a time equal to the time constant ($t = 0.25$ second = $1 \times Trs$), 63% of the gas in the lung is exhaled. For $t = 2Trs$, 86% of the gas is exhaled; for $t = 3Trs$, 95% of the gas is exhaled; for $t = 4Trs$, 98% of the gas is exhaled; and for $t = 5Trs$, 99% of the gas is exhaled. If expiration is interrupted before $t = 3Trs$, gas is trapped in the lung.

Rearrangement yields the linear equation

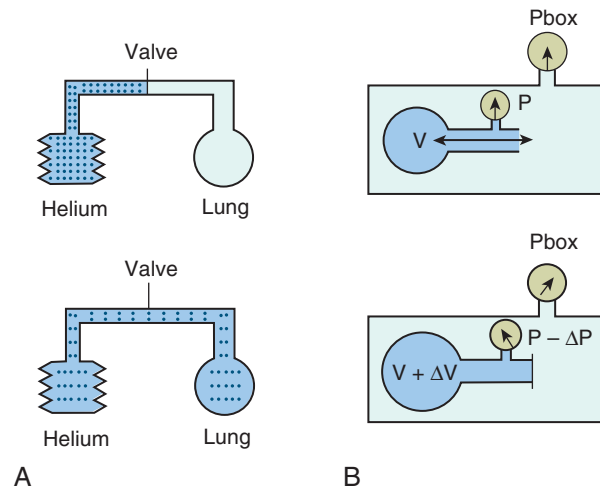
$$(5) \quad \dot{V}(t) = -\left(\frac{1}{RC}\right) \times (V[t]),$$

where the slope is $-1/RC$, which can be determined by a linear regression of $\dot{V}(t)$ versus $V(t)$. This equation states that during passive exhalation, flow plotted against volume is a straight line with slope $-1/RC$. Since we can calculate volume from the flow-volume curve, we can use the pressure measured during the inspiratory hold to calculate compliance and the slope of the line to calculate resistance. The quantity RC has the units of time, is termed the *respiratory system time constant* (Trs), and defines the rate at which the lung deflates during a passive exhalation (see Fig. 44.5B). Time constants affect the rate of lung inflation in much the same manner.

Several investigators have noted that with obstructive lung disease the expiratory flow-volume plot may not be linear but curved (concave), and the fit to a straight line is poor (Jarriel et al., 1993; Numa et al., 2001). These studies found that the plots fit a biexponential function much better—with one exponential describing the behavior of well-ventilated lung units and the other describing the behavior of poorly ventilated lung units. The studies were designed to measure the effects of bronchodilators on airway resistance in infants with obstructive lung disease. The effect of bronchodilators on airway resistance in well-ventilated lung units was small (Numa et al., 2001) in one study and absent in the other (Jarriel et al., 1993). Both studies found that administration of bronchodilators resulted in a substantial reduction in airway resistance in poorly ventilated lung units. In both studies the time constants of well-ventilated lung units were relatively normal and much smaller than those of the poorly ventilated lung units (2.5 to 7 times smaller). These data imply that the well-ventilated lung units would have almost completely emptied before the poorly ventilated units reached even one time constant. Any measurements of mechanics that depend on the initial rate of gas flow only (from direct measurements of flow or from estimates obtained by RIP would miss most of the flow from poorly ventilated lung units, underestimate resistance for the lung as a whole, and miss the important effect of the bronchodilators and other interventions on the poorly ventilated parts of the lung.

This compartmentalization of lung units also impacts lung inflation. If lung compliance remains relatively uniform, the product of resistance and compliance (Trs) will vary throughout the lung. During lung inflation, units with normal resistance have the lowest Trs and fill rapidly; units with high resistance have a greater Trs and fill more slowly. At rapid respiratory rates when the duration of inspiration is short, only those lung units with a small Trs are ventilated. In effect, the ventilated lung becomes smaller. As discussed earlier, as the lung becomes smaller, its measured compliance decreases. Therefore in infants with ventilation inhomogeneities, dynamic lung compliance decreases as respiratory rate increases. This decrease in lung compliance with increasing respiratory rate is termed the *frequency dependence of compliance*, and it is suggestive of inhomogeneous small airway obstruction.

FRC is measured by inert gas dilution techniques (helium dilution) or inert gas displacement (nitrogen washout) (Fig. 44.6). Both of these techniques measure gas that communicates with the airways. The total volume of gas in the thorax at the end of expiration (thoracic gas volume) can be measured with a body plethysmograph and application of Boyle's law. This technique measures



• **Fig. 44.6** (A) Measurement of functional residual capacity (FRC) by helium dilution. At the end of exhalation, the infant breathes from a bag containing a known volume (V_{bag}) and concentration of helium (He_1) in oxygen. The gas in the infant's lungs dilutes the helium oxygen mixture to a new concentration (He_2): $FRC = bag\ volume \times (He_1 - He_2)/He_1$. (B) Measurement of thoracic gas volume (TGV) using a plethysmograph. The infant breathes spontaneously in a sealed body plethysmograph (Pbox). At the end of exhalation, the airway is closed with a shutter. As the infant attempts to inspire against the shutter, the volume of the thorax increases, and airway pressure decreases. The increase in volume of the thorax can be measured from the change in the pressure inside of the Pbox. By Boyle's law, $P \times TGV = (P - \Delta P) \times (TGV + \Delta V)$, where P is atmospheric pressure, $(P - \Delta P)$ is airway pressure during occlusion, and $(TGV + \Delta V)$ is thoracic volume during occlusion. Therefore $TGV = (P - \Delta P) \times \Delta V / \Delta P$. Because ΔP is small compared with P , this can be simplified to $TGV = P \times \Delta V / \Delta P$.

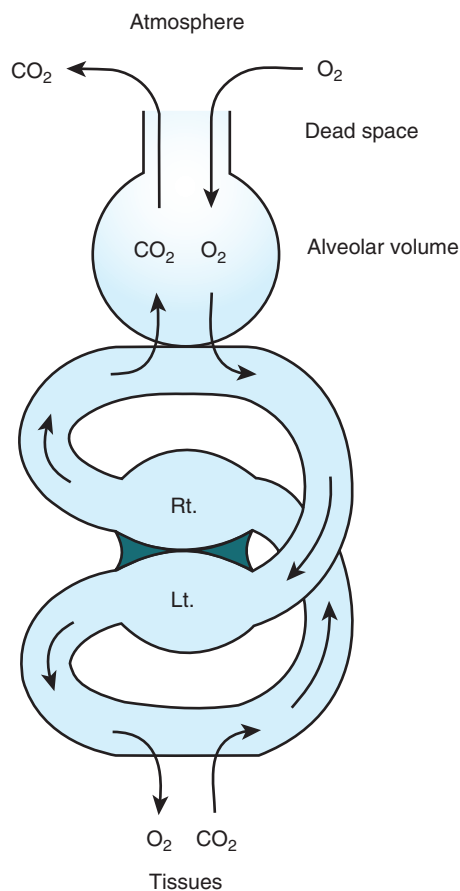
all gas in the thorax—even trapped gas that is not in contact with the airways. Obviously, FRC measured by inert gas dilution is less than thoracic gas volume if significant volumes of trapped gas are present.

Alveolar Ventilation

The tissues of the body continuously consume oxygen (O_2) and produce carbon dioxide (CO_2) (Fig. 44.7). The primary function of the circulation is to pick up O_2 from the lungs and deliver it to the tissues and to pick up CO_2 from the tissues and deliver it to the lungs. The exchange of O_2 and CO_2 with the blood occurs within the alveolar volume of the lungs. The alveolar volume acts as a “large sink” from which O_2 is continuously extracted by the blood and to which CO_2 is continuously added. This mechanism for acquiring O_2 from the atmosphere and excreting CO_2 into the atmosphere is the alveolar ventilation (Slonim and Hamilton, 1987).

The alveolar volume of the lung includes all lung units capable of exchanging gas with mixed venous blood: respiratory bronchioles, alveolar ducts, and alveoli. Because the conducting airways do not participate in gas exchange, they constitute the anatomic dead space (V_{DS}). At the end of exhalation, the FRC is the sum of the volume of gas in the alveolar volume and in the anatomic V_{DS} . During normal breathing the amount of gas entering and leaving the lung with each breath is the tidal volume (V_T):

$$Minute\ Ventilation\ (\dot{V}) = V_T \times Respiratory\ Rate\ (RR).$$



• **Fig. 44.7** Coupling of alveolar ventilation with tissue oxygen consumption. *Lt.*, Left side of the heart; *Rt.*, right side of the heart.

Part of each V_T is wasted ventilation because it moves gas in and out of the V_{DS} . Therefore alveolar ventilation (\dot{V}_A) can be expressed as

$$(6) \quad \dot{V}_A = (V_T - V_{DS}) \times (RR).$$

Alveolar ventilation is an intermittent process, whereas gas exchange between the alveolar space and the blood occurs continuously. Because arterial O₂ and CO₂ tensions (PaO₂ and PaCO₂) are roughly equal to the O₂ and CO₂ tensions within the alveolar space, these fluctuations in breathing could result in intermittent hypoxemia and hypercarbia. Fortunately the lung has a large buffer—the FRC. The FRC is four to five times as large as the V_T ; therefore only a fraction of the total gas in the lung is exchanged during normal breathing. This large buffer continues to supply O₂ to the blood during expiration and acts as a sump to accept CO₂ from the blood, so alveolar O₂ and CO₂ tensions (PAO₂ and PACO₂) change little throughout the ventilatory cycle.

Alveolar ventilation is linked tightly to metabolism. When alveolar ventilation is uncoupled from the body's metabolic rate, hypoventilation or hyperventilation results. During hypoventilation, less O₂ is added to the alveolar space than is removed by the blood, and less CO₂ is removed from the alveolar space than is added by the blood. As a result, PAO₂ decreases and PACO₂ increases. The net result of hypoventilation is hypoxemia and hypercapnia.

Administration of supplemental O₂ increases the quantity of O₂ in each breath delivered to the alveolar space, and it may prevent arterial hypoxemia. For example, suppose a 1-kg male infant has a V_T of 6 mL, an anatomic V_{DS} of 2 mL, and a respiratory rate of 40 breaths per minute. His alveolar ventilation is 160 mL per minute $[(6 \text{ mL} - 2 \text{ mL}) \times 40/\text{minute}]$. If he breathes room air (21% O₂), he delivers 33.6 mL of O₂ to the alveolar space every minute $(160 \text{ mL/minute} \times 0.21)$. If he maintains the same V_T but breathes only 20 times per minute, his alveolar ventilation decreases to 80 mL per minute, only 16.8 mL of O₂ $(80 \text{ mL/minute} \times 0.21)$ is delivered to the alveolar space each minute, and his PAO₂ and PaO₂ decrease. If he is allowed to breathe 50% O₂, O₂ delivery to the alveolar space increases to 40 mL per minute $(80 \text{ mL/minute} \times 0.50)$, and his PAO₂ and PaO₂ both increase. Because O₂ administration has no effect on the accumulation of CO₂, it does not prevent hypercapnia.

Hyperventilation delivers more O₂ to the alveolar space than can be removed by the blood and removes more CO₂ than can be added by the blood. As a result, PAO₂ increases and PACO₂ decreases.

Measurements of alveolar ventilation and anatomic V_{DS} in the infant rely on the relationship between CO₂ production \dot{V}_{CO_2} , \dot{V}_A , and P_ACO₂. The mathematical expression of this relationship states (Cook et al., 1955)

$$(7) \quad F_A \text{CO}_2 = \dot{V}_{CO_2} / \dot{V}_A.$$

$F_A \text{CO}_2$ is the fraction of CO₂ in total alveolar gas, or

$$(8) \quad F_A \text{CO}_2 = P_A \text{CO}_2 / (P_B - 47).$$

P_B is the barometric pressure, and 47 mmHg is the vapor pressure of water at body temperature.

Therefore

$$(9) \quad \dot{V}_A = [\dot{V}_{CO_2} \times (P_B - 47)] / P_A \text{CO}_2.$$

If minute ventilation (\dot{V}) is measured, dead space ventilation (\dot{V}_D) is calculated as

$$(10) \quad \dot{V}_D = \dot{V} - \dot{V}_A,$$

and V_{DS} is calculated by multiplication by the respiratory rate.

This method measures the anatomic V_{DS} in the lung. As seen in the next section, portions of some gas-exchanging units in the lung can also function as V_{DS} ; therefore the total V_{DS} , or the physiologic V_{DS} , may be greater than the anatomic V_{DS} . Physiologic V_{DS} is calculated by substitution of PaCO₂ for PACO₂ in Eq. (9). When PaCO₂ = PACO₂, all the dead space is anatomic dead space, and the gas-exchanging units are all functioning normally. As physiologic V_{DS} increases, however, PaCO₂ increases relative to PACO₂. Therefore the difference between PaCO₂ and PACO₂ (aA.DCO₂) is a measure of the efficiency of gas exchange in the lung.

For clinical purposes, \dot{V}_{CO_2} in Eq. (9) is assumed to be a constant so that \dot{V}_A is proportional to $1/\text{PACO}_2$. Thus increased PACO₂ means that alveolar ventilation has decreased; decreased PACO₂ means that alveolar ventilation has increased.

TABLE 44.2**Indices of Ventilation–Perfusion Imbalance in the Normal Newborn Breathing Room Air**

| | Aa.D _O ₂ (mmHg) | $\dot{Q}_t - \dot{Q}_{va}$ | aA.DN ₂ (mmHg) | $\dot{Q}_t - \dot{Q}_o$ | $\dot{Q}_t - \dot{Q}_s$ | aA.DCO ₂ (mmHg) |
|---------|---------------------------------------|----------------------------|---------------------------|-------------------------|-------------------------|----------------------------|
| Newborn | 25 | 0.25 | 10 | 0.10 | 0.15 | 1 |
| Adult | 10 | 0.07 | 7 | 0.05 | 0.02 | 1 |

BPD, Bronchopulmonary dysplasia; FRC, functional residual capacity; RDS, respiratory distress syndrome.

Adapted from Nelson NM. Respiration and circulation after birth. In Smith CA, Nelson NM, eds. *The Physiology of the Newborn Infant*. Springfield: Charles C Thomas; 1976.

Ventilation–Perfusion Relationships

Ideally, ventilation and perfusion of the lung are evenly matched, $\dot{V}/\dot{Q} = 1$, both in the lung as a whole and in each individual air space. The air spaces receive O₂ from the inspired gas and CO₂ from the blood. O₂ is transported into the blood, while CO₂ is transported to the atmosphere. Even though $\dot{V}/\dot{Q} = 1$, CO₂ and O₂ are exchanged in the lung at the same ratio at which they are exchanged in the tissues: a little less CO₂ is transported out than O₂ is transported in, and the respiratory exchange ratio *R* equals 0.8. If there is no diffusion defect, the gas composition of the air spaces and the blood comes into equilibrium. Nitrogen (N₂) makes up the balance of dry gas. The sum of partial pressures of all gases in the air spaces must equal atmospheric pressure or they will collapse. The ideal alveolar gas composition is PAO₂ = 100 mmHg, PACO₂ = 40 mmHg, PAN₂ = 573 mmHg, and PAH₂O = 47 mmHg at an atmospheric pressure of 760 mmHg. The ideal arterial blood composition is the same. Therefore differences between alveolar and arterial gas composition under ideal circumstances are all zero. In the following equations, inspired gas is denoted “I” (inspired oxygen tension is written as P_{IO}₂, the fraction of oxygen in inspired gas is F_{IO}₂, the inspired nitrogen tension is P_{IN}₂, and the fraction of nitrogen in inspired gas is F_{IN}₂. The values for PACO₂, the inspired oxygen tension (P_{IO}₂), and the fraction of oxygen in inspired gas (F_{IO}₂) can be used to calculate the ideal alveolar gas composition with use of the alveolar gas equation (Farhi, 1966):

(11)

$$PAO_2 = P_{IO_2} - PACO_2 \times [F_{IO_2} + (1 - F_{IO_2})/R],$$

where

(12)

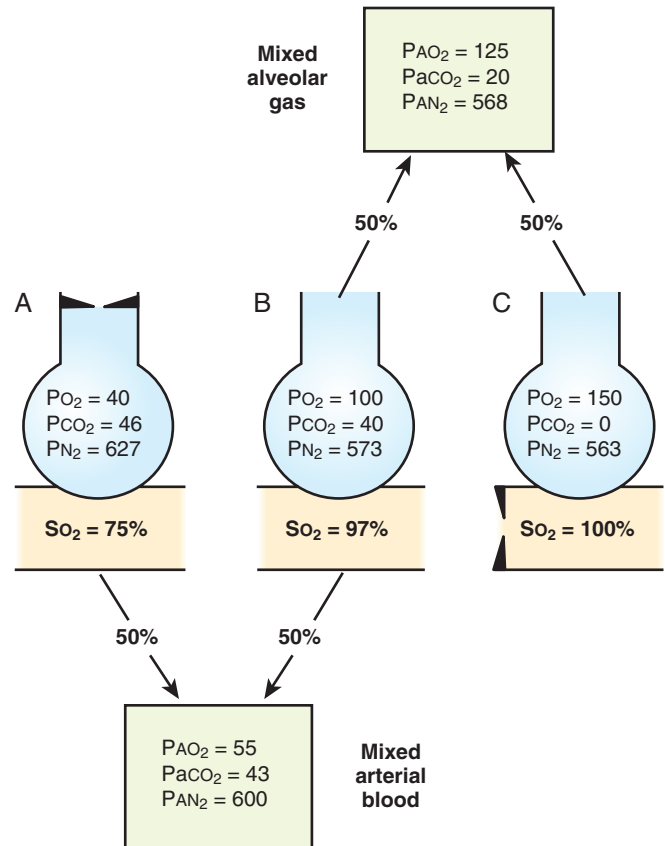
$$P_{IO_2} = F_{IO_2} \times (P_B - PH_2O)$$

and

(13)

$$PAN_2 = F_{IN_2} \times [PACO_2 \times (1 - R)/R + (P_B - PH_2O)].$$

Under normal circumstances, and certainly in the presence of lung disease, this ideal situation is not the case; some air spaces receive more ventilation than perfusion, and others receive more perfusion than ventilation (Table 44.2). A reduction of ventilation may occur because of atelectasis, alveolar fluid, or airway narrowing. Reduced ventilation in one part of the lung may cause increased ventilation elsewhere. A reduction of perfusion may occur if air spaces are collapsed or overdistended or because of gravitational effects, and increased perfusion may occur in congenital heart disease. As with ventilation, reduced perfusion in one part of the lung may cause



• **Fig. 44.8** Three-compartment model of the lung with $\dot{V}/\dot{Q} = 0$ (A), $\dot{V}/\dot{Q} = 1$ (B), and $\dot{V}/\dot{Q} = \text{infinity}$ (C). The inspired gas is room air, and (B) is the ideal compartment. The sum of alveolar gas partial pressures is always 713 mmHg. So₂ is oxygen saturation in capillary blood. PaO₂ is read from the oxygen dissociation curve for a saturation of 86%. By calculated differences, Aa.DO₂ is 70 mmHg, aA.DCO₂ is 23 mmHg, and aA.DN₂ is 32 mmHg.

increased perfusion in other regions. If an air space is relatively overventilated (high \dot{V}/\dot{Q}), its gas composition trends toward that of inspired gas, which in the case of room air is PO₂ = 150 mmHg and PCO₂ = 0 mmHg. If an air space is relatively underventilated (low \dot{V}/\dot{Q}), its gas composition trends toward that of mixed venous blood, which is PvO₂ = 40 mmHg and PVCO₂ = 46 mmHg. What counts is the $\dot{V}/\dot{Q} = 1$ ratio, not absolute values of \dot{V} or \dot{Q} (West, 1986).

To understand \dot{V}/\dot{Q} imbalance, it is common to view the lung as a three-compartment model (Fig. 44.8): $\dot{V}/\dot{Q} = 0$ (see Fig. 44.8A); $\dot{V}/\dot{Q} = 1$ (see Fig. 44.8B); and $\dot{V}/\dot{Q} = \text{infinity}$ (see Fig. 44.8C). The O₂ saturation of blood in each compartment depends on PO₂

and the O_2 dissociation curve. For illustrative purposes, in a badly diseased lung, 50% of ventilation goes to $\dot{V}/\dot{Q} = 1$, and 50% goes to $\dot{V}/\dot{Q} = \text{infinity}$, whereas 50% of perfusion goes to $\dot{V}/\dot{Q} = 1$, and 50% goes to $\dot{V}/\dot{Q} = 0$. Perfusion of $\dot{V}/\dot{Q} = 0$ causes venous admixture, whereas ventilation of $\dot{V}/\dot{Q} = \text{infinity}$ causes alveolar V_{DS} . The mixed alveolar gas composition is easily calculated as the mean. For mixed arterial blood, PO_2 must be read from the O_2 dissociation curve, but because the CO_2 dissociation curve is fairly linear, the values for CO_2 are easily calculated as the mean. The abnormalities in the distribution of \dot{V} and \dot{Q} have created $Aa.DO_2 = 70$ mmHg, $aA.DCO_2 = 23$ mmHg, and $aA.DN_2 = 32$ mmHg (see Fig. 44.8; Markello et al., 1972; Corbet et al., 1974). $Aa.DO_2$ is greater than the sum of $aA.DCO_2$ and $aA.DN_2$ because the O_2 dissociation curve is not linear. Of course, the situation in most lungs is not as extreme as the one illustrated. From this illustration, however, it can be seen that:

1. Open low \dot{V}/\dot{Q} units produce increased $Aa.DO_2$, significant hypoxemia, and increased $aA.DN_2$, but because they are poorly ventilated and have $PACO_2$ close to the ideal value, they do not change $aA.DCO_2$ significantly.
2. High \dot{V}/\dot{Q} units produce increased $Aa.DO_2$ without hypoxemia and increased $aA.DCO_2$, but because they are poorly perfused and have PAN_2 close to the ideal value, they do not change $aA.DN_2$ significantly.

For the calculation of $Aa.DO_2$ and $aA.DN_2$, it is customary to calculate the ideal alveolar gas composition for O_2 and N_2 from the alveolar gas equations and use these values with those measured for arterial PO_2 and PN_2 . This emphasizes that part of the $Aa.DO_2$ and $aA.DN_2$ is responsible for hypoxemia. For $aA.DCO_2$, both an arterial and mixed alveolar sample are required.

In the newborn a third compartment in the model is important. A significant part of the venous return may be shunted from right to left at the foramen ovale, ductus arteriosus, pulmonary arteriovenous vessels, or lung mesenchyme without airway development, thus adding mixed venous to mixed arterial blood. This substantially increases $Aa.DO_2$ but has little effect on $aA.DCO_2$ and no effect on $aA.DN_2$. The last of these is because there is no significant exchange of N_2 in the body, so venous and arterial PN_2 are the same. The effect on $aA.DCO_2$ is small because venous PCO_2 is only slightly higher than arterial PCO_2 . From this analysis it can be seen that hypoxemia is produced by a true right-to-left shunt and open low \dot{V}/\dot{Q} units. Diffusional problems are not thought to be important in the newborn. Hypoxemia may be modeled as a venous admixture, the part of mixed venous blood, expressed as a fraction of cardiac output, that when added to blood equilibrated with an ideal lung would produce the measured arterial oxygen saturation. It is calculated as follows:

$$(14) \quad \frac{\dot{Q}_{va}}{\dot{Q}_t} = \frac{C_cO_2 - C_aO_2}{C_cO_2 - C_vO_2},$$

where \dot{Q}_{va}/\dot{Q}_t refers to a venous admixture, CO_2 refers to oxygen content, subscript c refers to a pulmonary capillary, subscript a refers to arterial blood, and subscript v refers to mixed venous blood. For practical application, C_vO_2 is calculated from a constant $av.O_2$ difference, which does introduce an error.

If an infant breathes 100% O_2 for 15 minutes, most N_2 is washed out of the lung, and PO_2 in open low \dot{V}/\dot{Q} units becomes so high that associated blood is 100% saturated with O_2 . The remaining venous admixture is attributed to true right-to-left shunt (\dot{Q}_s/\dot{Q}_t). If the total venous admixture \dot{Q}_{va}/\dot{Q}_t is measured while

the infant is breathing room air, then true shunt (\dot{Q}_s/\dot{Q}_t) measured while breathing 100% O_2 , the venous admixture caused by open low \dot{V}/\dot{Q} units (\dot{Q}_o/\dot{Q}_t) can be calculated as the difference.

Heart–Lung Interaction

Effects of the Lung on the Heart

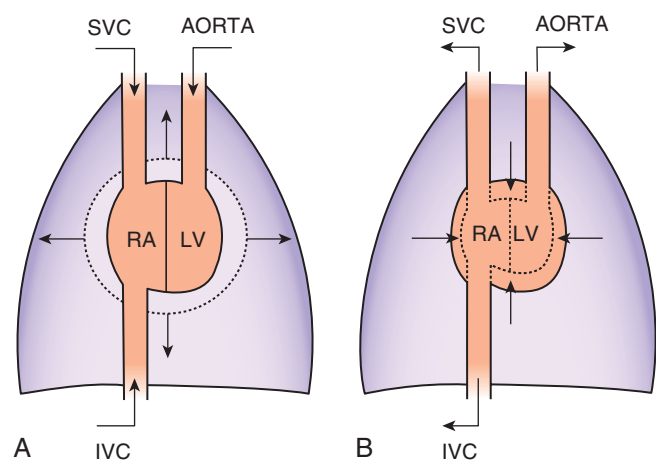
There exists considerable potential for the lung to affect the heart. Because they share the thoracic cavity, changes in intrathoracic pressure accompanying lung inflation are transmitted directly to the heart. In addition, all of the blood leaving the right ventricle must traverse the pulmonary vascular bed, so changes in pulmonary vascular resistance may greatly affect right ventricular function.

Effects of Changes in Intrathoracic Pressure on the Heart

Negative Intrathoracic Pressure

During spontaneous inspiratory efforts, the chest wall and diaphragm move outward, intrathoracic volume increases, and intrathoracic pressure decreases (Fig. 44.9A). The heart also resides within the thoracic cavity and is subject to the same negative intrathoracic pressure during inspiration. With a decrease in intrathoracic pressure, the heart increases in volume, and the pressure within its chambers decreases relative to atmospheric pressure. Analogously to the lung, when the pressure within the heart decreases, blood is literally sucked back into the heart from systemic veins and arteries. On the right side of the heart, the phenomenon serves to increase the flow of blood from systemic veins into the right atrium, increasing right ventricular preload and ventricular output. On the left side of the heart, ventricular ejection is impaired. During systole the left ventricle must overcome not only the load imposed by the systemic vascular resistance but also the additional load imposed by the negative intrathoracic pressure (McGregor, 1979).

In infants with normal lungs, spontaneous respiratory efforts result in relatively small swings in pleural pressure (2 to 3 mmHg) that have little effect on the pressure within the heart. With airway



• **Fig. 44.9** (A) Negative intrathoracic pressure increases the volume of the heart and decreases the pressure within the chambers. This facilitates return of blood from the superior vena cava (SVC) and inferior vena cava (IVC) to the right atrium (RA) and impedes ejection of blood from the left ventricle (LV) into the extrathoracic aorta. (B) Positive intrathoracic pressure decreases the volume of the heart and increases pressure within its chambers. This impedes blood return to the RA and augments ejection of blood from the LV.

obstruction or parenchymal lung disease, however, swings in pleural pressure can be much greater (5 to 20 mmHg), and systemic arterial pressure may fluctuate by as much as 5 to 20 mmHg depending on where in the respiratory cycle ventricular systole occurs. In older children with asthma or some other form of airway obstruction, these fluctuations in blood pressure constitute *pulsus paradoxus* and are indicative of severe airway obstruction.

Positive Intrathoracic Pressure

During positive pressure ventilation, the lung inflates and pushes the chest wall and diaphragm outward (see Fig. 44.9B). This outward push generates a pressure in the thoracic space that is greater than atmospheric pressure. The magnitude of the increase (relative transmission of airway pressure to the pleural space) is determined by the volume of lung inflation (which, in turn, is determined by the airway pressure and lung compliance) and by the compliance of the chest wall and diaphragm. If the lung is compliant and the chest wall rigid, little airway pressure is lost inflating the lung, but considerable pressure is generated in the thoracic cavity as the lung attempts to push the rigid chest wall outward. In this instance, intrathoracic pressure (intrapleural pressure) is much greater than atmospheric pressure and is nearly equal to airway pressure. If the lung is poorly compliant and the chest wall is highly compliant, most of the airway pressure is dissipated in an attempt to inflate the lungs, and little is transmitted to the thoracic cavity.

The effects of positive intrathoracic pressure on the heart are opposite those of negative intrathoracic pressure. The heart is compressed by the lungs and chest wall, and blood is squeezed out of the heart and the thoracic cavity. Return of blood from systemic veins is impaired, and right ventricular preload and output decrease. If the increase in intrathoracic pressure coincides with ventricular systole, the effect is to augment left ventricular ejection and reduce the load on the left ventricle.

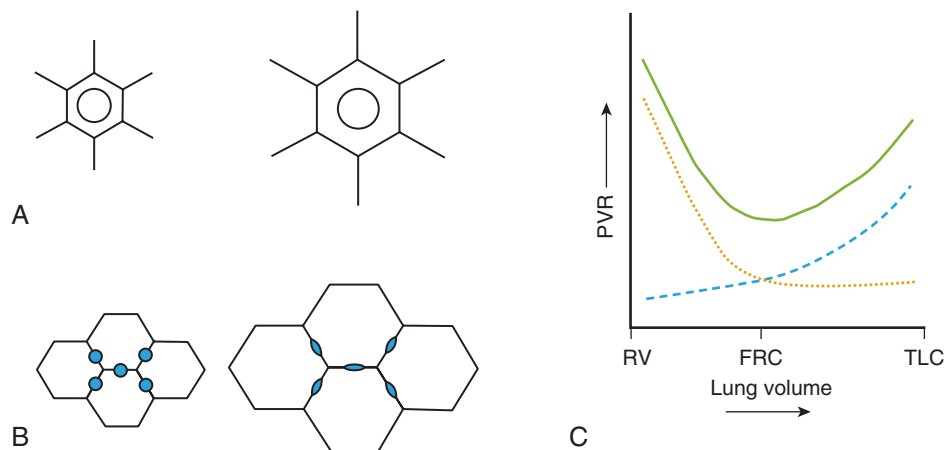
In the infant undergoing positive pressure ventilation, the degree to which lung inflation compromises venous return is related to the relative compliances of the lung and chest wall. If the infant's lung is poorly compliant and the chest wall is compliant, as in hyaline membrane disease, there is little effect of lung inflation on venous return. If the infant's lung is normally compliant but tight abdominal distention prevents descent of the diaphragm, intrathoracic pressure increases dramatically during positive pressure

ventilation, and venous return and cardiac output can be impaired. This mechanism may help explain the circulatory instability of infants after gastroschisis repair. A similar situation may arise in the preterm infant with pulmonary interstitial emphysema and massive lung overinflation. In such an infant, the heart is tightly compressed between the hyperinflated lungs and the other structures of the mediastinum and the diaphragm. Venous return may be severely limited and venous pressures so increased that massive peripheral edema often accompanies the reduction in cardiac output.

Although the effects of increased pleural pressure on the right atrium are detrimental, the effects on the left ventricle may be extremely beneficial (Niemann et al., 1980). During cardiopulmonary resuscitation the chest wall is compressed against the lung, and intrathoracic pressure increases. Because the left ventricle is in the thorax, left ventricular pressure increases as well. A gradient is created, favoring flow of blood out of the ventricle and thorax and into the extrathoracic systemic circulation. Between chest compressions, elastic recoil causes the chest wall to pull away from the lung and heart, decreasing pleural pressure, favoring return of venous blood, and priming the heart for the next chest compression. A similar phenomenon may result in augmentation of systemic pressure when ventilator breaths coincide with ventricular systole.

Effect of Lung Inflation on Pulmonary Vascular Resistance

The pulmonary interstitium comprises three different interconnected connective tissue compartments, each containing a different element of the pulmonary circulation (Fishman, 1986). The first—the perivascular cuffs—consists of a sheath of fibers that contain the preacinar pulmonary arteries, lymphatics, and bronchi. The second consists of the intersegmental and interlobular septa and contains pulmonary veins and additional lymphatics. The third connects these two within the alveolar septa and contains most of the pulmonary capillaries. The first and second compartments represent the extra-alveolar interstitium, whereas the third represents the alveolar interstitium. The perivascular cuffs are surrounded by alveoli and expand during lung inflation (Fig. 44.10A). As a result, pressure within each cuff decreases, distending extra-alveolar blood vessels and decreasing their resistance to blood flow. The alveolar interstitium lies between adjacent alveoli and contains most of the gas-exchanging vessels in the lung. These vessels are exposed to



• **Fig. 44.10** (A) Effects of lung inflation on extra-alveolar vessels. (B) Effects of lung inflation on alveolar vessels. (C) Effect of lung volume on pulmonary vascular resistance (PVR; solid line). Inflation is from residual volume (RV) to functional residual capacity (FRC) to total lung capacity (TLC). The dashed line represents alveolar vessels; the dotted line represents extra-alveolar vessels.

alveolar pressure on both sides and during lung inflation (see Fig. 44.10B) are compressed so that their resistance to blood flow increases.

Therefore during lung inflation (see Fig. 44.10C), the resistance in extra-alveolar vessels decreases, whereas resistance in alveolar vessels increases. As a result, the overall pulmonary vascular resistance decreases initially, with lung inflation reaching its nadir at FRC, and then increases with further inflation.

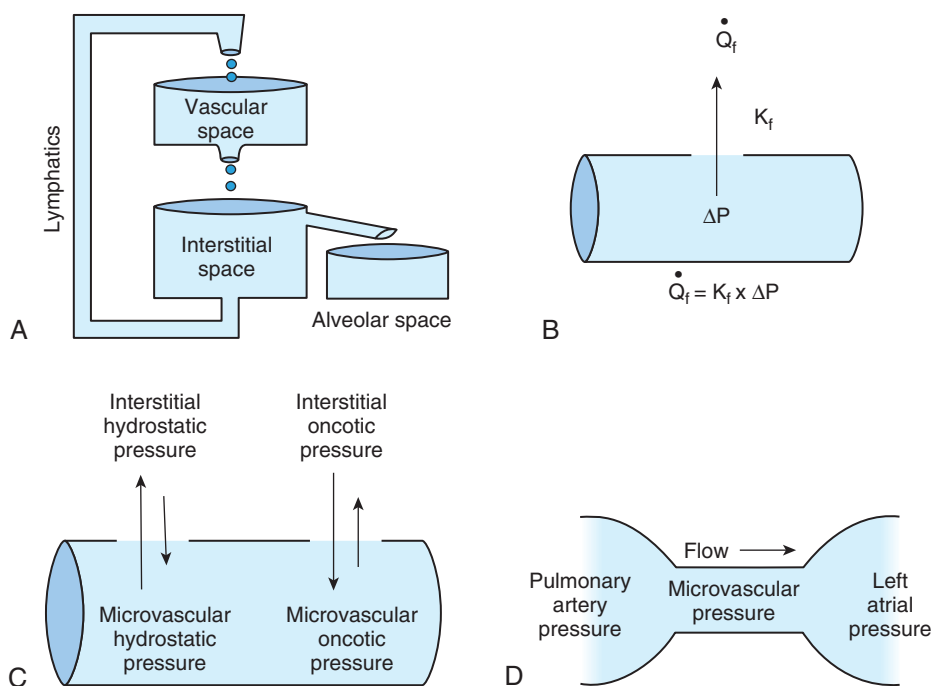
If transition from intrauterine to extrauterine life is to be successful, after birth all of the right ventricular output must traverse the pulmonary vascular bed. To some extent this adaptation is facilitated by a reduction in pulmonary vascular resistance that occurs with inflation of the lungs (see Fig. 44.10C) to a stable FRC. Inflation of the lung beyond FRC increases pulmonary vascular resistance. If care is not taken during positive pressure

ventilation, it is possible to inflate the lung to the point that alveolar vessels close and blood flow through the lung is impaired. When this occurs, either cardiac output decreases or the blood bypasses the lung via the foramen ovale or ductus arteriosus. Clinically, this is manifest as circulatory insufficiency from impaired right ventricular output or hypoxemia from right-to-left shunting of blood or both.

Effects of the Heart on the Lung

Pulmonary Edema

Pulmonary edema is the abnormal accumulation of water and solute in the interstitial and alveolar spaces of the lung (Staub, 1974; Bland et al., 1985). In the lung, fluid is filtered from capillaries in the alveolar septa into the alveolar interstitium (Fig. 44.11A)



• **Fig. 44.11** (A) In the lung, fluid is continuously filtered out of vessels in the microcirculation into the interstitium and then returned to the intravascular compartment by the lymphatics. Only when the rate of filtration exceeds the rate of lymphatic removal can fluid accumulate in the interstitium. Spillover of fluid into the alveolar space occurs only when the interstitial space fills or when the alveolar membrane is damaged. It has recently been shown that the alveolar epithelium is capable of pumping fluid from the alveolar space back into the interstitium as protection against alveolar flooding or to aid in the resolution of alveolar edema. (B) Fluid flows out of vessels at a flow rate (\dot{Q}_f) that is equal to the driving pressure for fluid flow (ΔP) times the filtration coefficient (K_f): $\dot{Q}_f = K_f \times \Delta P$. K_f can be thought of as the relative permeability of the vascular bed to fluid flux. K_f in the normal lung is a small number, so despite a driving pressure of roughly 5 mmHg, the net rate of fluid filtration is approximately 1 to 2 mL/kg per hour. (C) The driving pressure for fluid flow out of the microvascular bed represents a balance of two sets of pressures. Within the blood vessel, hydrostatic pressure tends to push fluid out of the vessel into the interstitium. This pressure is partially opposed by a smaller hydrostatic pressure within the interstitium pushing fluid back into the blood vessel. Within the blood vessel, there also exists a discrete oncotic pressure that results predominantly from intravascular albumin that tends to draw fluid from the interstitium back into the blood vessel. This pressure is partially opposed by an interstitial oncotic pressure tending to draw fluid from the blood vessel into the interstitium. (D) The intravascular hydrostatic pressure must be less than pulmonary artery pressure (P_{pa}) for blood to flow into the microvascular bed and greater than left atrial pressure (P_{la}) for blood to flow out. Intravascular pressure within the microvascular bed is given roughly by $P_{mv} = [0.4 \times (P_{pa} - P_{la}) + P_{la}]$. The interstitial hydrostatic pressure is roughly equal to alveolar pressure. The intravascular oncotic pressure can be calculated from the plasma albumin concentration. The interstitial oncotic pressure is roughly two-thirds of the intravascular oncotic pressure. The balance of these pressures favors filtration out of the vessel (in the normal lamb, this pressure is roughly 5 mmHg).

• BOX 44.1 Increased Intravascular Hydrostatic Pressure

Increased Left Atrial Pressure

Intravascular volume overload
Overzealous fluid administration
Overtransfusion
Renal insufficiency
Heart failure
Left-sided obstructive lesions
Left-to-right shunts
Myocardopathies

Increased Pulmonary Blood Flow

Normal pulmonary vascular bed
Patent ductus arteriosus
Increased cardiac output
Reduced pulmonary vascular bed
Bronchopulmonary dysplasia
Pulmonary hypoplasia

and then siphoned into the lower-pressure extra-alveolar interstitium. The extra-alveolar interstitium contains the pulmonary lymphatics, and under normal conditions, they remove fluid from the lung so that there is no net accumulation in the interstitium. Pulmonary edema results only when the rate of fluid filtration exceeds the rate of lymphatic removal. There are only three mechanisms by which this can occur (see Fig. 44.11B): (1) the driving pressure for fluid filtration (filtration pressure) increases, (2) the permeability of the vascular bed (hence the filtration coefficient K_f) increases, or (3) lymphatic drainage decreases.

Increased Driving Pressure

Filtration pressure can be increased by increased intravascular hydrostatic pressure, decreased interstitial hydrostatic pressure, decreased intravascular oncotic pressure, or increased interstitial oncotic pressure (see Fig. 44.11C–D). By far the most common cause of increased filtration pressure is increased intravascular hydrostatic pressure (Box 44.1). In the newborn, intravascular hydrostatic pressure increases with increased left atrial pressure from volume overload or a number of congenital and acquired heart defects. In the preterm and term newborn, evidence suggests that alterations in pulmonary blood flow that are independent of any change in left atrial pressure may also influence fluid filtration in the lung. Preterm infants with patent ductus arteriosus and left-to-right shunts exhibit signs of respiratory insufficiency before they develop any evidence of heart failure, and experiments performed in newborn lambs show that fluid filtration in the lung can be increased by increase of pulmonary blood flow without increase of left atrial pressure (Feltz and Hansen, 1986). In the newborn with a reduced pulmonary vascular bed, either from lung injury or from hypoplasia, cardiac output appropriate for body size may represent a relative overperfusion to the lung and can result in increased fluid filtration. This phenomenon has been invoked to explain the lung edema that often complicates the course of the infant with bronchopulmonary dysplasia.

The exact cause of pulmonary edema that accompanies severe hypoxia or asphyxia in the newborn is still a controversial issue. Data suggest that it is the result of increased filtration pressure and not the result of any alteration in permeability. Heart failure accounts for some of the increased filtration pressure following

severe asphyxia. In addition, there may be some element of pulmonary venous constriction. Finally, there is evidence that hypoxia and acidosis may redistribute pulmonary blood flow to a smaller portion of the lung and result in relative overperfusion and edema, similar to that seen with anatomic loss of vascular bed (Hansen et al., 1984).

Several investigators have suggested that upper airway obstruction may cause pulmonary edema by decreasing interstitial hydrostatic pressure relative to intravascular hydrostatic pressure. Other data suggest, however, that with airway obstruction, vascular pressures decrease with intrapleural pressure in such a way that filtration pressure remains unchanged (Hansen et al., 1985).

Hypoproteinemia in infants results in a decrease in intravascular oncotic pressure. Its effects on filtration pressure, however, are blunted by the simultaneous decrease in protein concentration in the interstitial space of the lung. As a result, edema is unlikely to occur unless hydrostatic pressure also increases (Hazinski et al., 1986).

Increased Permeability

Another possible mechanism for increased fluid filtration in the lung is a change in the permeability of the microvascular membrane to protein—high-permeability pulmonary edema. In this form of edema the sieving properties of the microvascular endothelium are altered such that K_f increases and patients may develop pulmonary edema despite relatively normal vascular pressures (Albertine, 1985). Furthermore, even small changes in vascular pressures can result in a dramatic worsening of pulmonary status. High-permeability pulmonary edema usually implies either direct or indirect injury to the capillary endothelium of the lung. Direct injuries result from local effects of an inhaled toxin such as oxygen, severe alveolar overdistention (Carlton et al., 1990), or a massive increase in pulmonary capillary pressure. The latter is proposed as the mechanism for hemorrhagic pulmonary edema following surfactant administration in the presence of a patent ductus arteriosus. However, the benefits of surfactant administration vastly outweigh the risk of pulmonary hemorrhage (Raju et al., 1993; Pappin et al., 1994). Indirect injuries imply that the initial insult occurs elsewhere in the body and that the lung injury occurs secondarily. Sepsis is a classic example of indirect lung injury where neutrophils activated by bacterial toxins attack endothelial cells in the lung and increase permeability to water and protein (Brigham et al., 1974). Massive trauma to the central nervous system can also injure the pulmonary capillary bed by inducing massive arterial and venous constriction (Malik, 1985). In general, indirect injuries usually involve bloodborne mediators, such as leukocytes, leukotrienes, histamine, or bradykinin. In some instances the injury to the capillary membrane may be so severe that red blood cells may leak into the alveolar space (*pulmonary hemorrhage*). It is important to note that Cole et al. (1973) found that the hemorrhagic fluid has a very low hematocrit suggestive of severe pulmonary edema rather than any disorders of the coagulation system and that treatment should be focused on lowering vascular pressures.

Decreased Lymphatic Drainage

In the normal lung the rate of lung lymph flow is equal to the net rate of fluid filtration, and as long as lymphatic function can keep up with the rate of fluid filtration, water does not accumulate in the lung. Although lymphatics can actively pump fluid against a pressure gradient, this ability is limited, and lung lymph flow varies inversely with the outflow pressure (pressure in the superior vena cava). Several groups of investigators have demonstrated that,

in the presence of an increased rate of transvascular fluid filtration, the rate of fluid accumulation in the lung is substantially greater if systemic venous pressure is increased (Drake et al., 1985). Data suggest that the ability of the lymphatics to pump against an outflow pressure is impaired in the fetus and newborn. In fetal lambs, lymph flow ceases at an outflow pressure of roughly 15 mmHg (Johnson et al., 1996). This explains why pulmonary edema often complicates the course of infants with bronchopulmonary dysplasia and cor pulmonale and explains the particular problem of edema with pleural effusions complicating the postoperative course of patients following cavopulmonary shunts. Subsequent investigations showed that the ability of the lymphatics to pump can be affected by other mediators, with pumping increased by α -adrenergics and certain leukotrienes and impaired by nitric oxide, β -adrenergics (von der Weid, 2001), and products of hemolysis (Elias et al., 1990).

Congenital pulmonary lymphangiectasis is a rare form of pulmonary lymphatic dysfunction that can be characterized into two groups: (1) cases associated with congenital heart disease and (2) cases not associated with congenital heart disease. The cardiac anomalies may include hypoplastic left-sided heart syndrome, total anomalous pulmonary venous drainage, and pulmonary stenosis, including Noonan syndrome (France and Brown, 1971). The group that does not include associated cardiac anomalies may be of early or late onset and has a wide spectrum of severity. In some individuals the lesion is asymptomatic, whereas in others it can lead to severe respiratory failure, usually in the first few hours after birth but sometimes during the first few weeks or months of life. Most infants with this condition die early in the neonatal period. Pulmonary lymphangiectasis has been reported twice as often in males and has been seen in families (Scott Emaukpor et al., 1981).

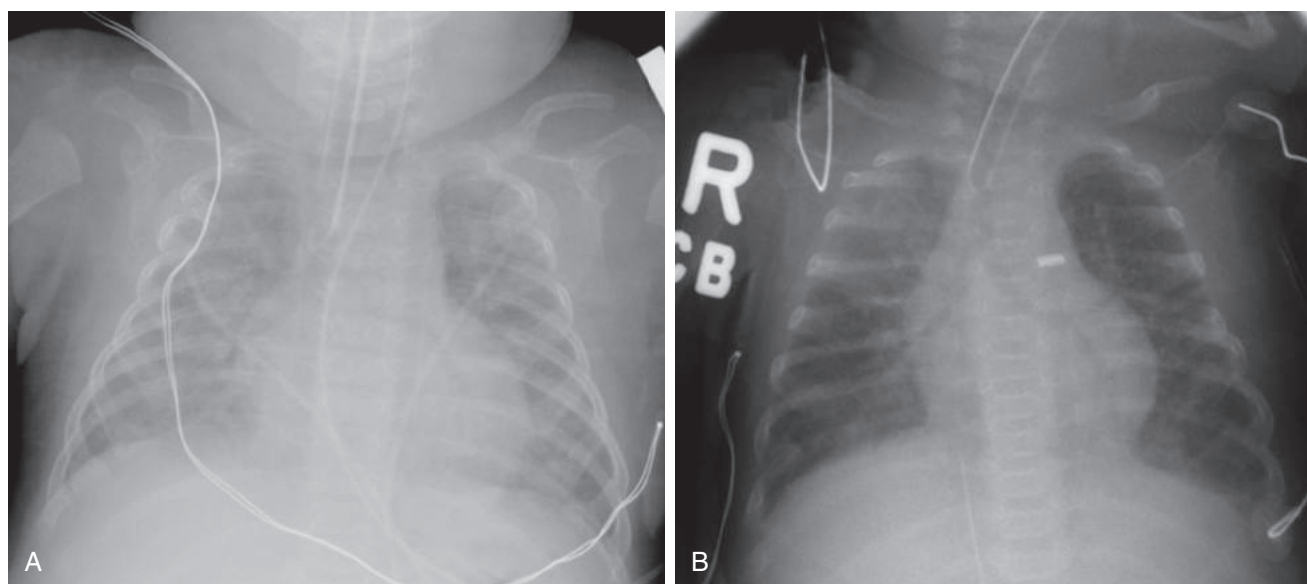
Alveolar Space

The same factors that affect fluid balance across the lung are also present within the alveolar space. The absence of protein in the alveolar space means that the oncotic pressure gradient dramatically favors fluid flow from the alveolus into the pulmonary interstitium.

In addition, fluid filtered into the alveolar interstitium ordinarily moves rapidly along pressure gradients into the extra-alveolar interstitium, where it is removed by the lymphatics. The extra-alveolar interstitium has a large storage capacity, and fluid does not begin to spill over into the alveoli and airways until total lung water is increased by more than 50%. Even then, because K_f for the alveolus is very low, alveolar flooding probably implies damage to the alveolar membrane. This damage may result from many of the same factors affecting vascular permeability, such as oxygen toxicity, sepsis, or alveolar overdistension. There is a growing body of evidence that the alveolar epithelium is capable of pumping fluid from the alveolar space back into the interstitium, and this represents another defense mechanism against alveolar flooding (see Fig. 44.11A). This clearance of fluid from the alveolar space may be accelerated by β -adrenergic agents (Frank et al., 2000) and by dopamine (Saldias et al., 1999). Whether these agents will have any clinical efficacy remains to be determined.

Symptoms of Pulmonary Edema

As discussed previously, fluid filtered into the alveolar interstitium ordinarily moves rapidly along pressure gradients into the extra-alveolar interstitium, where it is removed by the lymphatics. A delay in this process at birth can result in clinical transient respiratory distress. The first signs and symptoms of pulmonary edema are related to the presence of extra fluid in the interstitial cuffs of tissue that surround airways. As fluid builds up in these cuffs, airways are compressed, and infants develop signs of obstructive lung disease. The chest may appear hyperinflated, and auscultation reveals rales, rhonchi, and a prolonged expiration. Early in the course, chest radiographs reveal lung overinflation and an accumulation of fluid in the extra-alveolar interstitium—linear densities of fluid that extend from the hilum to the periphery of the lung (the so-called sunburst appearance) and fluid in the fissures. With more severe edema, fluffy densities appear throughout the lung as alveoli fill with fluid. Heart size may be increased in infants with edema from increased intravascular pressure. Initially, infants present with increased PaCO_2 secondary to impaired ventilation. Later PaO_2



• **Fig. 44.12** (A) Preterm infant with a large patent ductus arteriosus and pulmonary edema. (B) The same infant 24 hours after the ductus arteriosus had been closed by surgical ligation.

decreases secondary to ventilation–perfusion mismatching and alveolar flooding. In adults a ratio of protein concentration in tracheal aspirate to that in plasma greater than 0.5 may help to differentiate permeability pulmonary edema from high-pressure pulmonary edema (Fein et al., 1979).

Treatment of Pulmonary Edema

Treatment of pulmonary edema is directed at relieving hypoxemia and lowering vascular pressures. Hypoxemia should be treated with the administration of oxygen and, if necessary, positive pressure ventilation. Positive end-expiratory pressure frequently increases oxygenation in individuals with pulmonary edema by improving ventilation–perfusion matching within the lung. Available evidence suggests that positive pressure ventilation does not reduce the rate of transvascular fluid filtration in the lung (Woolverton et al., 1978). Optimal treatment of pulmonary edema requires correction of the underlying cause. In infants with patent ductus arteriosus (Fig. 44.12) or other heart disease amenable to surgery, this is often easily accomplished. In cases of permeability edema or edema from nonsurgical heart defects, correction of the underlying cause may not be possible. In these instances the only remaining option is to lower vascular pressures (even in permeability edema, lowering vascular pressures lowers the rate of fluid filtration and may also improve lymphatic function). This can be accomplished by the lowering of circulating blood volume by use of diuretics and fluid restriction, by improvement of myocardial function with the use of digitalis or other inotropic agents, or in severe cases by use of a systemic vasodilator to reduce afterload and lower vascular pressures directly.

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45

Neonatal Respiratory Therapy

EDUARDO BANCALARI, NELSON CLAURE, AND DEEPAK JAIN

KEY POINTS

- In addition to measurement of blood gases and oxygen saturation, determination of adequacy of ventilation in mechanically ventilated neonates consists of clinical assessment of chest expansion, breathing frequency, and breath sounds and may be complemented by the monitoring of tidal volume and minute ventilation with use of flow sensors.
- Strategies to avoid the harmful effects of hyperoxemia in preterm infants may have detrimental effects because of unintended hypoxemia.
- While the use of a high-flow nasal cannula in premature infants has increased because of ease of use, less risk of nares injury, and perceived improvement in patient comfort, there is little evidence of improved outcomes when compared with use of nasal continuous positive airway pressure.
- The decision to initiate invasive mechanical ventilation is usually based on gestational age, the severity of respiratory failure, the underlying disease process, the availability of noninvasive respiratory support alternatives, and the experience of the clinical team, the rate of complications, and patient outcomes.
- Metaanalyses comparing different volume-targeted ventilation strategies with pressure-limited ventilation favor the use of volume-targeted ventilation because of decreased duration of ventilation and decreased incidence of bronchopulmonary dysplasia, pneumothorax, and severe neurologic injury.
- Serious complications of mechanical ventilation include pulmonary gas leaks, airway damage, ventilator-associated pneumonia, and neurologic sequelae.
- Neonatal respiratory monitoring could be significantly improved by advances in noninvasive monitoring of arterial and venous blood gases and monitoring of brain and organ oxygenation, perfusion, and hemodynamics.

Respiratory Monitoring

One of the most important aspects of newborn intensive care is continuous monitoring of cardiorespiratory function, ventilation, and oxygenation. This can be accomplished by bedside monitoring devices and laboratory analysis. The information provided by these monitoring techniques is an essential tool in the diagnosis and treatment of respiratory problems in the newborn.

Breathing Frequency, Apnea, and Heart Rate Monitoring

Transthoracic impedance is the standard method used to monitor neonatal respiration. This technique is based on changes in the

thorax's electrical impedance caused by changes in gas volume during respiration measured by surface electrodes. Transthoracic impedance is mainly used to monitor breathing frequency and to detect apnea (Olsson and Victorin, 1970). Breathing is detected when the change in impedance exceeds a set threshold. A low threshold decreases sensitivity for apnea, and small disturbances such as cardiogenic oscillations can be falsely considered as breathing. Conversely, a high threshold may lead to false apnea alarms during shallow breathing. Monitors can automatically adjust this threshold or the impedance amplitude or use sophisticated methods for detecting breathing or apnea. Transthoracic impedance is not reliable in assessing absolute tidal volume (V_T), but it can be used to assess relative changes.

This technique is more effective in detecting apneas of central origin than obstructive apnea because the latter can produce internal displacement of gas volumes that still change impedance. More specific techniques are used for diagnosis and classification of apnea, including detection of gas exhalation by thermistors and CO_2 monitors and respiratory inductance plethysmography, which measures expansion of the chest and abdomen. Transthoracic impedance monitors can also measure heart rate and are used to detect bradycardia and tachycardia. More sophisticated devices or built-in algorithms are used to detect cardiac rhythm anomalies.

Ventilation Monitoring

The basic clinical determination of the adequacy of ventilation in the mechanically ventilated neonate consists of assessment of chest expansion, breathing frequency, and auscultation. This can be complemented by the monitoring of and minute ventilation with flow sensors available in most neonatal ventilators. This allows the monitoring of spontaneous breathing, assessment of respiratory system mechanics, and detection of excessive or insufficient V_T , hypoventilation, and gas trapping (Becker and Donn, 2007). These flow sensors are also used to synchronize positive pressure ventilation with the infant's spontaneous inspiration (discussed in detail later).

Flow sensors are classified as mainstream when they are connected between the endotracheal tube (ETT) and the ventilator circuit, also known as *proximal flow sensors*, or built into the ventilator. In small infants, mainstream flow sensors have better accuracy than those built into the ventilator because V_T and flow are only a fraction of the gas volumes compressed in the circuit and the circulating bias flow (Cannon et al., 2000; Chow et al., 2002). Although mainstream flow sensors are usually small and typically have a dead space volume of less than 1 mL, they can induce rebreathing of exhaled gases and affect CO_2 elimination in small preterm infants (Figueras et al., 1997; Claure et al., 2003).

Ventilation monitoring based on flow measurement has become the standard in modern neonatal ventilators, and monitoring of the adequacy of V_T has been shown useful in reducing the incidence of pneumothorax in preterm infants (Rosen et al., 1993; Vellanki et al., 2012). More recently V_T monitoring has also been shown to be useful in assessing ventilation during high-frequency ventilation (HFV) (Sturtz et al., 2008).

Blood Gas Monitoring

Blood Gas Sampling

Determination of arterial blood gas status is key to the management of respiratory failure and lung disease in the neonate. Measurements of arterial oxygen tension (PaO_2) and arterial carbon dioxide tension (PaCO_2) are considered the reference standard by which the efficacy and adequacy of ventilation and oxygenation are assessed.

To perform repeated arterial blood sampling, placement of invasive catheters is required. In neonates, umbilical artery catheters (UACs) are commonly used during the acute phases of respiratory failure. Samples obtained from a UAC are the most reliable when proper sample handling and laboratory procedures are followed. However, there are important issues that must be considered before their placement and during their use to ensure that the risk-to-benefit ratio remains favorable. UACs have risks during placement and use, including damage and perforation of the vessel, formation of thrombi and emboli, vasospasm, and infection. UAC lines with the tip above the celiac plexus are associated with fewer complications than those with the tip below the renal or mesenteric artery (Barrington, 2000).

Alternatively, percutaneous lines in the radial, ulnar, or dorsalis pedis artery can be used instead of a UAC. When an invasive line is not available, arterial blood can be obtained by puncture of peripheral arteries. The results should be interpreted carefully because the procedure frequently disturbs the infant and can alter blood gases. An alternative is to obtain blood samples from the peripheral capillary bed of the medial or lateral plantar surface. These are obtained after warming of the area, which produces hyperemia or “arterialization.” These samples provide an approximate estimate of arterial blood gases, and the results should also be interpreted cautiously (Courtney et al., 1990). This procedure can also disturb the infant and alter the basal status.

Errors in blood gas measurement are often technique related. Contamination by air bubbles or venous blood can alter the results. Contamination by fluids can reduce PO_2 and PCO_2 while also affecting pH. Because blood cell metabolism continues, reduction of the time from sampling to analysis and cold temperature storage and transport attenuate its effects.

During conditions such as transport or when the turnaround time is important, bedside portable blood analysis devices have proven to be most effective (Murthy et al., 1997). Another alternative measurement method is that of indwelling electrodes inserted through a UAC that provide continuous blood gas information, but they are not used in clinical practice (Rais-Bahrami et al., 2002). This is likely because of the need for an invasive catheter, which, in general, is avoided to reduce the risk of thrombus formation and bloodstream infections.

Transcutaneous Blood Gas Monitoring

Measurements of transcutaneous O_2 tension (TcPO_2) and transcutaneous CO_2 tension (TcPCO_2) provide a continuous noninvasive estimate of the composition of arterial blood gases. Local hyperperfusion of the skin induced by heating creates a skin–electrode unit

under the transcutaneous sensor, and electrochemical measurements in an electrolyte solution within this unit determine the partial pressure of O_2 and CO_2 .

Because skin metabolism continues, local hyperemia is needed to maintain skin perfusion sufficiently high so that the measured TcPO_2 is not affected by the skin's O_2 consumption. For this reason, the accuracy of TcPO_2 measurement depends on electrode temperature, with better accuracy at or above 43°C (Huch et al., 1976). Under adequate conditions, TcPO_2 correlates with PaO_2 (Peabody et al., 1978). However, conditions such as arterial hypotension and acidosis often result in underestimation because of insufficient skin perfusion (Versmold et al., 1979).

During TcPCO_2 measurement, CO_2 molecules diffusing from the capillary bed change the pH of the electrolyte solution in the skin–electrode unit. This changes the electric potential, which is then translated into TcPCO_2 . Metabolism in the skin produces CO_2 , and if the local perfusion is not adequate, the measured values can exceed those of PaCO_2 . TcPCO_2 measurements can also be affected by conditions affecting peripheral perfusion (Peabody and Emery, 1985) and tend to overestimate PaCO_2 in hypercapnia as local perfusion decreases (Martin et al., 1988).

Some reports have demonstrated improved TcPCO_2 measurement accuracy in preterm infants of less than 29 weeks' gestation but inconsistent precision because of large between-patient variability (Aliwalas et al., 2005; Bernet-Buettiker et al., 2005; Tingay et al., 2005). TcPCO_2 measurements have also been shown to be tightly correlated to capillary PCO_2 . Although capillary blood gases may not be the optimal reference, their use is often the only available method for long-term monitoring because indwelling lines are available only during the acute phase of respiratory failure. In preterm infants, TcPCO_2 may reduce the need for blood sampling and the number of painful punctures, but the main benefit is the ability of continuous monitoring. For this reason, TcPCO_2 measurement is commonly used as an adjunct to standard blood gas sampling to provide information on trends and respiratory stability. This is particularly useful in the management of invasive ventilatory support, where close tracking of the effects of ventilator changes is required.

The thin epidermal skin layer of the neonate has a relatively low metabolism. Nonetheless, some transcutaneous monitors include a metabolic correction factor. Measurements of TcPO_2 and TcPCO_2 require a period of stabilization after sensor application until skin perfusion increases. A tight seal around the skin–electrode unit is required for accuracy. Similarly to blood gas sampling, an air bubble between the skin and the electrode can lower TcPCO_2 and shift TcPO_2 toward the ambient partial O_2 pressure.

The transcutaneous electrode is usually applied on the thorax or the thigh. The need for high electrode temperature combined with the premature infant's skin sensitivity requires frequent change of the application site to avoid thermal injury. Transcutaneous measurements have an intrinsic delay of a few seconds with respect to changes occurring in the arterial blood.

End-Tidal Carbon Dioxide Monitoring

Monitoring of the partial pressure of CO_2 in end-tidal gases (PetCO_2) is based on the assumption that gases measured at the airway opening at the end of exhalation represent alveolar gases and that their levels match the arterial levels. PetCO_2 is measured by infrared sensors placed mainstream or by side-stream gas sampling. PetCO_2 measurements are dependent on V_T size because the exhaled gas has to carry alveolar gas. The accuracy of PetCO_2 measurement is affected by lung disease, with an increased arterial to alveolar CO_2

gradient resulting in underestimation of PaCO_2 (Sivan et al., 1992). For this reason, PetCO_2 is more often used in term or near-term neonates and pediatric patients who require CO_2 monitoring for reasons other than lung disease. Measurements of PetCO_2 in mechanically ventilated infants can be affected by gas leaks around the ETT. A way to circumvent this limitation is to sample exhaled gas from the distal end of a multilumen ETT (Kugelman et al., 2008), which has been shown to improve accuracy. This technique has been shown to be useful in improving the maintenance of PaCO_2 within the intended range in preterm infants (Kugelman et al., 2016).

Arterial Oxygen Saturation Measured by Pulse Oximetry

Estimation of the oxygen saturation in arterial blood (SaO_2) by pulse oximetry (SpO_2) is based on the differences in the rates of light absorption between oxygenated hemoglobin (Hb) and deoxygenated or reduced Hb in the red and infrared regions of the light spectrum. Deoxygenated Hb absorbs more red light and less infrared light than oxygenated Hb. As SaO_2 increases, the ratio of the absorption of red light to that of infrared light decreases. It is assumed that in the circulation, changes in this ratio can be produced only by pulsating arterial blood.

The amount of light absorbed by pulsatile blood is only a small fraction of the light absorbed by tissue and venous blood. Thus changes in pulse amplitude or patient movement that disrupt the optical pathway from the transmitter to the receiver side of the probe, or that produce venous blood fluctuations, can affect SpO_2 accuracy, although newer techniques have reduced the effect of the latter (Hay et al., 2002). The accuracy of SpO_2 is also affected by conditions such as low perfusion or by inappropriate placement, such as excessive tightening of the probe (Bucher et al., 1994).

In a prospective evaluation of the accuracy of SpO_2 in neonates, SpO_2 data were shown to be well correlated with measured SaO_2 (Hay et al., 1989). However, more recent retrospective studies that examined paired pulse oximetry and blood gas data documented in medical records suggested SpO_2 can overestimate arterial oxygen levels (Quine et al., 2009; Rosychuk et al., 2012). The accuracy and reliability of the current SpO_2 technology and the factors that affect them should be further examined in well-controlled prospective studies.

The use of pulse oximetry to avoid hyperoxemia and hypoxemia during oxygen supplementation must be done in the context of the sigmoid-shaped relationship between PaO_2 and SpO_2 . High SpO_2 levels are almost always induced by an excessive fraction of inspired oxygen (FIO_2), and although SpO_2 levels in the upper range can be associated with a wide range of PaO_2 (Brockway and Hay, 1998), data indicate that an SpO_2 threshold of 93% or 95% can be used to avoid most PaO_2 values greater than 80 mmHg (Hay et al., 1989; Poets et al., 1993; Bohnhorst et al., 2002; Castillo et al., 2008). On the other hand, most SpO_2 values below 80% are associated with PaO_2 below 40 mmHg. In this situation a moderate increase in supplemental oxygen is usually enough to correct the hypoxemia without inducing hyperoxemia.

Data indicate reliable detection of hypoxemia spells by pulse oximetry (Bohnhorst et al., 2000; Hay et al., 2002). Nonetheless, some hypoxemia episodes detected by pulse oximetry are deemed artifactual by the caregiver because of their temporal association with infant movement. However, these hypoxemia episodes may still need to be attended to in view of data showing increased infant activity leading to changes in heart rate, lung volume, and ventilation that trigger hypoxemia episodes (Bolivar et al., 1995; Dimaguila et al., 1997) and that hypoxemia episodes are more

frequent during periods when the infants are awake than when they are in active or quiet sleep (Lehtonen et al., 2002).

Oxygen Therapy

Principles

Neonates with acute respiratory failure or with some degree of respiratory distress experience abnormalities of gas exchange that almost invariably result in hypoxemia. Depending on the severity and duration of hypoxemia and the metabolic demands for oxygen, this can lead to reduced oxygen availability and tissue hypoxia. Hypoxemia in the neonate can result from reduced alveolar oxygen content, low ventilation-perfusion ratio, reduced diffusion capacity, and extrapulmonary right-to-left shunting.

The most common form of respiratory therapy for the neonate with hypoxemia consists of oxygen supplementation. A higher FIO_2 increases the alveolar O_2 tension (PAO_2) in both well-ventilated and partially ventilated areas of the lung. The resulting increase in the alveolar-arterial O_2 gradient in part compensates for the conditions producing hypoxemia mentioned earlier. The proportion of neonates requiring supplemental O_2 increases with lower gestational age because premature birth is associated with many of the factors that contribute to hypoxemia.

The primary goal of oxygen therapy is to maintain adequate oxygen availability to the tissues, especially to the central nervous system and the heart, and to improve an incomplete hemodynamic adaptation to extrauterine life evident by persistently elevated pulmonary vascular resistance and patency of the ductus arteriosus. These goals, however, need to be attained while minimizing the side effects of oxygen toxicity on the eye, brain, and other organs that are common in the premature infant.

Normal SaO_2 for room air-breathing term or healthy preterm infants is reportedly greater than 93%, with PaO_2 levels above 70 mmHg (Fenner et al., 1975; Richard et al., 1993; O'Brien et al., 2000). Maintenance of such oxygenation levels in premature infants with lung disease and immaturity would almost invariably require high inspired O_2 levels. Earlier strategies to "normalize" oxygenation with the use of high FIO_2 in this population resulted in high rates of retinopathy of prematurity (ROP) and blindness (Campbell, 1951; Crosse and Evans, 1952; Cross, 1973). Conversely, strict curtailment of supplemental O_2 regardless of the oxygenation level was associated with increased rates of neurologic damage and death (Patz et al., 1952; Avery, 1960; Bolton and Cross, 1974).

The preterm infant is at risk of O_2 -induced injury because of an immature antioxidant system that is unable to balance the oxidative effects of O_2 radicals. In the past, severe neonatal lung injury was only partly attributed to exposure to high FIO_2 . However, animal experiments have shown that lung damage was caused by high alveolar O_2 independent of PaO_2 (Northway et al., 1967; Miller et al., 1970; Taghizadeh and Reynolds, 1976). In preterm infants, hyperoxia has been linked to neurologic damage and impairment (Ahdab-Barmada et al., 1980; Collins et al., 2001; Haynes et al., 2003). For this reason, when supplemental O_2 is administered to hypoxemic neonates, oxygenation is continuously monitored to avoid hyperoxemia.

Methods of Administration

In neonates, supplemental O_2 is usually administered by means of a head box, mask, nasal cannula (NC), nasal continuous positive airway pressure (N-CPAP), or a mechanical ventilator. In

mechanically ventilated infants or in infants receiving N-CPAP, supplemental O₂ is administered by the mixture of air and O₂ in the ventilator or continuous positive airway pressure (CPAP) device. In all four methods, verification that the desired FIO₂ is delivered by means of an O₂ analyzer is recommended.

A head box, the least invasive of these methods, is generally used for infants who only need supplemental O₂. Depending on the size of the infant, a minimum flow is required to flush exhaled gases and minimize entrainment of ambient air. Gas warming and humidification are recommended to avoid drying of the airways and secretions as well as to avoid heat losses.

NCs deliver a constant flow of the air–O₂ mixture to the nostrils. The effective FIO₂ is determined by the delivered flow and the infant's inspiratory flow. With increasing flows the actual inspired O₂ concentration approaches that of the mixture delivered by the cannula, whereas higher inspiratory flows, in larger infants or during periods of increased demands, reduce the actual inspired O₂ by entraining more room air (Walsh et al., 2005). The actual FIO₂ during oral breathing has not been determined. Cannula flows greater than 1 L/min can produce positive pressure at the nose at levels similar to or above typical CPAP levels (Locke et al., 1993), which can become very high if the prongs fit tightly in the nostrils. Gas conditioning is recommended to avoid drying of the nose and mucosal damage (Kopelman and Holbert, 2003). NCs have gained popularity because they are flexible and facilitate access to and mobility of the infant.

Treatment Strategies

The effects of the introduction of continuous monitoring of oxygenation in the care of preterm infants in relation to ROP have been inconsistent (Bancalari et al., 1987; Grylack, 1987; Yamamoto et al., 1987). Data showing that ROP severity was associated with the duration of hyperoxemia emphasized the importance of oxygen monitoring and avoidance of hyperoxemia (Flynn et al., 1987). However, infants with severe ROP were also found to spend considerable periods in hypoxemia.

Continuous monitoring of arterial oxygen saturation by pulse oximetry (SpO₂) has become standard practice in neonatal intensive care. The use of SpO₂ has been extended to the delivery room for titration of FIO₂ during resuscitation (Escrig et al., 2008; Dawson et al., 2009). Although the optimal range of SpO₂ in premature infants has not been fully defined, existing data suggest deleterious effects of hyperoxemia, with higher rates of ROP and worse respiratory course in neonatal centers that have tolerant policies toward high SpO₂ levels (Tin et al., 2001; Anderson et al., 2004). Observational data indicate that more restrictive policies toward high SpO₂ can reduce the rates of ROP (Chow et al., 2003; Wright et al., 2006).

Tolerance of high SpO₂ levels in convalescent infants beyond the neonatal period has been proposed to improve growth and neurologic outcome, as well as to stop the progression of threshold ROP. However, clinical trials to test this showed minimal benefits that were outweighed by deterioration in lung function likely caused by the additional O₂ required to maintain higher SpO₂ (STOP-ROP Multicenter Study Group, 2000; Askie et al., 2003).

Implementation of policies to curb hyperoxemia should also consider the potential deleterious effects of insufficient oxygenation. Hypoxemia can increase patency of the ductus arteriosus (Skinner et al., 1999; Noori et al., 2009) as well as increase the resistance of the pulmonary vasculature and airways, particularly in infants with established lung disease (Cassin et al., 1964; Halliday et al.,

1980; Abman et al., 1985; Teague et al., 1988; Tay-Uyboco et al., 1989). Premature infants often experience hypoxemia in the form of spells. These spells increase in frequency with postnatal age and are more common in infants with chronic lung disease (Bolivar et al., 1995; Dimaguila et al., 1997; Garg et al., 1988; Di Fiore et al., 2010). Although most episodes are mild, some can be severe and prolonged. Frequent hypoxemia episodes and particularly those of long duration have been linked to increased incidence of severe ROP (Di Fiore et al., 2010). More recently, a post hoc analysis of the Canadian Oxygen Trial revealed an association between prolonged hypoxemia episodes and neurodevelopmental impairment (NDI) (Poets et al., 2015).

The goal of oxygen supplementation is to maintain adequate oxygenation while minimizing hyperoxemia and oxygen exposure. To achieve this, FIO₂ is adjusted to keep SpO₂ within a target range and avoid exposure to extreme high and low levels. However this is only partially achieved during routine care. A multicenter study showed that preterm infants receiving supplemental oxygen spent only half of the time with SpO₂ within the target range, more than one-third of the time with SpO₂ above the target range, and almost one-fifth of the time with SpO₂ below the target range (Hagadorn et al., 2006; Lupton et al., 2006; Lim et al., 2014). This is due to their oxygenation instability and frequent stimulation related to routine neonatal care.

In preterm infants the stability of oxygenation can be influenced by the target range of SpO₂. Relatively small decreases in the target range can produce significant increases in the proportion of time spent with SpO₂ below 80% or 85% (McEvoy et al., 1993; Lupton et al., 2006). This is in agreement with data from the Surfactant, Positive Pressure, and Oxygenation Randomized Trial (SUPPORT), where the frequency of hypoxemia spells was higher among infants assigned to the 85% to 89% target range compared with the 91% to 95% target range (Di Fiore et al., 2012). These hypoxemia episodes often require a transient increase in FIO₂, but a delayed response can prolong the hypoxemia, whereas a delayed reduction of FIO₂ after hypoxemia ends can induce hyperoxemia. Caregivers often tolerate or maintain high SpO₂ levels with the purpose of reducing the frequency of the spells or attenuating their severity (Claure et al., 2009, 2011; Van Zanten et al., 2014). When FIO₂ is increased in response to a hypoxemia spell, SpO₂ should be continuously observed, and FIO₂ should be reduced as the spell resolves. In clinical practice this is not always the case, and many infants experience hyperoxemia because FIO₂ is not quickly returned to the basal level when the hypoxemia ends (Van Zanten et al., 2014). This exposure to high saturations can further increase the risk of ROP (McColm et al., 2004). Neonatal center policies should clearly define the response of the caregiver to these events to minimize both excessive and inadequate oxygenation.

In recent years clinicians targeted progressively lower SpO₂ in preterm infants to reduce the complications associated with hyperoxemia and oxygen exposure. This was mainly driven by observational data that showed that policies targeting low SpO₂ ranges were associated with better outcomes (Chow et al., 2003; Wright et al., 2006). However, it is unknown how closely such ranges were actually maintained. Multiple clinical trials have been conducted to determine if targeting SpO₂ in the lower portion (85%–89%) versus the upper portion (91%–95%) of the commonly accepted range of 85% to 95% would reduce ROP and improve neurologic outcome. SUPPORT and the Benefits of Oxygen Saturation Targeting II (BOOST II) trials showed that targeting the 85% to 89% range reduced the rate of severe ROP compared with targeting the 91% to 95% range (SUPPORT Study Group

of the Eunice Kennedy Shriver NICHD Neonatal Research Network 2010a; Stenson et al., 2011). Unexpectedly, these trials showed higher mortality among infants assigned to the lower SpO₂ range. In contrast, in the Canadian Oxygen Trial, mortality and severe ROP did not differ significantly between the two SpO₂ target ranges (Schmidt et al., 2013). In these trials the actual SpO₂ levels achieved by manual titration of FiO₂ did not exactly match the assigned targets. The exposure to very high SpO₂ was greater with the higher target range, and there was a greater exposure to very low SpO₂ with the lower range.

These data suggest that strategies to avoid the harmful effects of hyperoxemia in preterm infants may have detrimental effects due to the unintended hypoxemia. This underlines the importance of striking a balance between avoidance of oxygen toxicity and possible hypoxemia to avoid long-term complications in this vulnerable population.

Automated systems to adjust the inspired oxygen to maintain SpO₂ within a target range have been developed for use in neonates. Short-term studies showed consistent improvement in the maintenance of SpO₂ within the target range as well as reductions in hyperoxemia, severe hypoxemia episodes, oxygen exposure, and staff workload with these automated systems compared with manual adjustments by the clinical staff or by a caregiver fully dedicated to this task (Claure et al., 2001; Urschitz et al., 2004; Claure et al., 2009; Morozoff et al., 2009; Claure et al., 2011; Hallenberger et al., 2014; Zapata et al., 2014; Claure et al., 2015; Lal et al., 2015; van Kaam et al., 2015; Waitz et al., 2015). Large trials are needed to determine the effects of extended use of automatic FiO₂ control on short-term and long-term neonatal outcomes.

Policies of oxygenation monitoring should clearly identify both the intended range and the alarm limits of SpO₂, and sufficient staff must be allocated to achieve this task. Setting SpO₂ alarm limits near or at the limits of the prescribed target range increases the proportion of time premature infants spent within the target (Hagadorn et al., 2006). Maintenance of SpO₂ within the target range can be affected by staff limitations during routine clinical care (Sink et al., 2011; Lim et al., 2014). Staff compliance with SpO₂ alarms plays an important role in keeping the target, but frequently the high SpO₂ alarm level is increased to reduce the number of alarms (Clucas et al., 2007). Insufficient staff education and communication can also influence the maintenance of SpO₂ within the intended range. A survey showed that only one-third of the caregivers were aware of and could identify their center's oxygenation policies (Nghiem et al., 2008), while a concerted effort involving protocol development and staff training showed clear improvements in SpO₂ targeting (Ford et al., 2006). These efforts can include the use of SpO₂ histograms to monitor compliance with the target range (Bizzarro et al., 2014). All these initiatives must, however, consider the potential for reduced staff attentiveness to SpO₂ alarms and alarm fatigue, which may be particularly important when SpO₂ target or alarm limits are changed (Ketko et al., 2015).

Policies for SpO₂ targeting at the time preterm infants reach near-term postmenstrual age (e.g., 36 weeks' postmenstrual age) can differ significantly between centers, and this can influence the classification of bronchopulmonary dysplasia (BPD) (Ellsburly et al., 2002). This is because BPD classification is commonly based on the need for supplemental oxygen. Centers with policies that target higher SpO₂ have greater proportions of infants receiving supplemental O₂, and many of those infants need not receive supplemental O₂ if lower SpO₂ levels are tolerated and if their actual needs are

monitored closely (Walsh et al., 2004). This is also the case at the time of discharge and during home oxygen therapy.

Noninvasive Respiratory Support

Because of the severe complications associated with invasive mechanical ventilation, there has been a persistent search for less invasive alternatives to support infants with respiratory failure. This is especially relevant in the small preterm infant, who is more susceptible to acute complications and chronic pulmonary sequelae. The strategies that have shown themselves to be most clinically effective are use of N-CPAP, nasal intermittent positive pressure ventilation (IPPV), and, more recently, a high-flow NC (HFNC). These strategies can be used to support infants with respiratory failure in lieu of invasive mechanical ventilation.

Nasal Continuous Positive Airway Pressure

Use of CPAP was introduced in 1971 by George Gregory to stabilize lung volume in preterm infants with respiratory distress syndrome (RDS) (Gregory et al., 1971). Initially, the pressure was delivered through a mask or head box and later was applied through nasal prongs. With the introduction of surfactant therapy, more infants were intubated and treated with mechanical ventilation, and the use of N-CPAP declined for several years; in the past decade, however, it has been reintroduced as a safe alternative to mechanical ventilation.

The physiologic effects of N-CPAP include prevention of alveolar collapse and stabilization of the functional residual capacity (FRC), thereby reducing pulmonary shunts and resulting in better oxygenation. N-CPAP also stabilizes large and small airways and can decrease the work of breathing and obstructive apnea episodes. By preventing alveolar closure and reopening with each breath, N-CPAP can preserve surfactant function.

N-CPAP is used primarily in infants with RDS who because of surfactant deficiency have decreased FRC. N-CPAP is effective during the transition period immediately after birth while reabsorption of fetal lung fluid and establishment of FRC are occurring. It is also used for reducing frequency and severity of apneic episodes and in stabilizing respiratory function after extubation (Locke et al., 1991) and in infants with airway obstruction. The application of continuous distending pressure during use of N-CPAP can be beneficial in infants with increased pulmonary blood flow and pulmonary edema secondary to heart lesions with left-to-right shunting such as patent ductus arteriosus.

As mentioned earlier, the major indication for N-CPAP use in preterm infants is RDS. In recent years most centers have used N-CPAP soon after birth in infants who have sufficient respiratory drive. The exceptions are infants who are depressed at birth or those who have severe respiratory failure and are intubated to administer surfactant. The success rate with use of CPAP soon after birth depends on the maturity of the infant and the severity of the respiratory failure, but more than 50% of preterm infants who are not depressed at birth can be managed successfully with N-CPAP, avoiding the use of invasive ventilation (Kamper and Ringsted, 1990; Dani et al., 2004; Ammari et al., 2005; Reininger et al., 2005). The sooner N-CPAP therapy is started, the better the results are in infants with RDS (Hegyi and Hiatt, 1981; Gittermann et al., 1997; Jonsson et al., 1997; Verder et al., 1999). There is some evidence that early use of N-CPAP instead of invasive ventilation could lead to a small reduction in the incidence of BPD (Morley et al., 2008; SUPPORT Study Group of the Eunice

Kennedy Shriver NICHD Neonatal Research Network, 2010b; Schmolzer et al., 2013).

The other frequent indication for use of N-CPAP is to stabilize respiratory function after the infant has been weaned off mechanical ventilation and extubation. Although it is not clear whether the use of N-CPAP in this situation reduces the need for reintubation, it clearly prevents a deterioration of respiratory function (Davis and Henderson-Smart, 1999; Peake et al., 2005).

The levels of N-CPAP that are used in clinical practice range from 3 to 8 cmH₂O, with evidence of the higher end of this range being more effective in preventing extubation failure in preterm infants who still need supplemental oxygen at the time of extubation (Buzzella et al., 2014). These findings indicate beneficial effects of the higher distending pressure and improved stability of lung volume in infants with residual lung disease.

In practice, the level of N-CPAP used is determined by the severity of the lung disease, reflected by the inspired oxygen concentration and the degree of lung expansion in the chest radiograph. The more severe the disease is, the higher the level of N-CPAP that is used. As the respiratory condition improves and the oxygen requirement decreases, the level of N-CPAP is reduced to 3 to 4 cmH₂O before the prongs are removed.

Devices for Application of Nasal Continuous Positive Airway Pressure

N-CPAP has been applied with use of a variety of systems and devices. Most of the evidence suggests that the best results are obtained with double short nasal prongs (De Paoli et al., 2002). The devices used to generate the pressure can be a water column such as the one used for “bubble CPAP” or an adjustable valve at the end of a continuous-flow system or a variable-flow system. Although there are data suggesting that some of these systems are superior to others, in clinical practice the stability and permeability of the nasal interface are probably the most important factors determining the success or failure of this form of respiratory support. Recently, NCs have been used as a method for generating N-CPAP. The limitation of this method is that it is difficult to control and measure the actual pressures and inspired oxygen concentrations that are delivered to the infant because both depend on the gas flow and the leaks around the cannulas and through the mouth.

Complications

The complications of N-CPAP can be related to the pressure that is applied or to the interface with the nose. The application of excessive pressure can cause overdistension and alveolar rupture with pulmonary interstitial emphysema (PIE) and pneumothorax. It can also reduce venous return, increase pulmonary vascular resistance, and reduce cardiac output. Overdistension of the lung can also reduce compliance and induce hypoventilation.

When the nasal prongs are too large or are applied with too much pressure over the nasal septum, they can produce erosions or pressure necrosis that sometimes requires the interruption of the N-CPAP. Avoiding these complications and keeping the nasal prongs in place is a task that requires considerable time and skill. During application of N-CPAP there is a risk of gas being pushed into the stomach. A nasogastric catheter is often used to avoid excessive accumulation of gas and gastric distention.

Noninvasive Ventilation

IPPV via nasal devices was among the original forms of support used in preterm infants in respiratory failure (Llewellyn et al.,

1970). Recently, it has been reintroduced in neonatal care for indications including respiratory distress and apnea and to facilitate weaning of neonates off invasive mechanical ventilation. Noninvasive ventilation (NIV) consists in the application of intermittent positive pressure at the nose or upper airway. The resurgence of the use of NIV in preterm infants is largely because N-CPAP that is effective in improving gas exchange by stabilizing lung volume and the airways is not fully effective in maintaining ventilation in infants with weak inspiratory effort and inconsistent respiratory drive.

In infants with apnea, the cycling positive pressure at the upper airway may produce an intermittent stimulus that prevents or attenuates the duration of breathing pauses of central origin. In infants with central apnea, resumption of breathing following ventilator cycles has been described, and the reduction of apnea in comparison with use of N-CPAP is more striking among infants with more frequent apnea spells (Ryan et al., 1989; Lin et al., 1998; Bisceglia et al., 2007).

NIV can improve gas exchange and ventilation compared with N-CPAP in infants with respiratory insufficiency during the first few hours after birth as well as during their weaning off mechanical ventilation (Moretti et al., 1999; Bisceglia et al., 2007). In contrast, infants with mild respiratory distress who are stable while receiving N-CPAP and are able to maintain adequate gas exchange reduced their spontaneous breathing effort only when receiving NIV (Aghai et al., 2006; Ali et al., 2007; Chang et al., 2011). In the preterm infant the chest wall is excessively compliant, and the breathing effort is in part dissipated by an inward motion of the chest. Hence the reduced breathing effort with NIV can also be explained by a reduction in chest distortion (Kiciman et al., 1998; Ali et al., 2007).

In spite of the application of cycles of relatively small positive pressure, bilevel positive airway pressure (Bi-PAP) can increase CO₂ elimination and oxygenation (Migliori et al., 2005) compared with N-CPAP. Noninvasive HFV has been used and was shown to be useful as a rescue for avoidance of intubation in a group of infants in whom use of N-CPAP failed (van der Hoeven et al., 1998; Fischer et al., 2015).

In addition to the effects on apnea, ventilation, and breathing effort, it is possible that the increase in mean airway pressure during NIV can better maintain lung volume than can N-CPAP alone. It is also possible that CO₂ removal is increased during NIV by clearance of exhaled gases from the upper airway.

The use of NIV to achieve a reduction in the need for intubation or the duration of invasive mechanical ventilation is aimed at reducing the associated risks of lung injury. This is obviously more relevant in the smaller and more immature infants in whom use of N-CPAP more frequently fails. In infants with RDS, nasally delivered intermittent mandatory ventilation (IMV) has been shown to reduce the need for invasive ventilation early in life (Kugelman et al., 2007; Meneses et al., 2011, 2012) and facilitate early extubation after surfactant administration (Bhandari et al., 2007). In some of these trials, NIV reduced incidence of BPD appreciably, but this was not a consistent finding. The possible long-term beneficial effects of nasal ventilation during the initial respiratory failure still need to be confirmed.

During weaning of infants off mechanical ventilation, adequate maintenance of lung volume is often achieved by use of N-CPAP. However, this fails in many infants because of insufficient ventilation resulting from central apnea, a weak respiratory pump, or poor lung mechanics due to the underlying lung disease. NIV has consistently been shown to be an effective way to reduce extubation failure (Friedlich et al., 1999; Barrington et al., 2001; Khalaf et al., 2001; Moretti et al., 2008), mainly by reducing apnea and

improving gas exchange. Smaller infants and those with poor lung function at extubation were more likely to benefit from nasal ventilation than larger infants or infants with better lung function (Khalaf et al., 2001).

The devices used for neonatal noninvasive positive pressure ventilation consist of conventional time-cycled pressure-limited ventilators that use the same circuits and gas-conditioning devices used for invasive ventilation, with the main difference being that in lieu of the ETT, positive pressure is applied through the same interfaces used for application of N-CPAP.

Synchronization of Noninvasive Ventilation

Noninvasive positive pressure can be delivered in the IMV mode, where the positive pressure cycles are delivered at fixed intervals. In some ventilators, ventilator cycles can be synchronized to the neonate's inspiration to provide nasal synchronized IMV (SIMV). Synchronized NIV can also be provided in the assist/control (A/C) and pressure support modes to assist every spontaneous inspiration.

The most frequently reported method for synchronization in NIV is the Graseby pressure capsule placed on the abdomen, but this system is no longer available in the United States (Friedlich et al., 1999; Barrington et al., 2001; Khalaf et al., 2001; Bhandari et al., 2007). Another method of synchronization is by detection of spontaneous inspiratory flow of the patient by use of mainstream flow sensors. However, large and variable gas leaks around the interface and the mouth may result in the risk of autocycling of the ventilator. Although this leak can be compensated by automatic adjustment of the flow trigger threshold in the ventilator, the efficacy of this mechanism in neonates has not been fully evaluated. One of the newer methods for synchronization is neurally adjusted ventilatory assist (NAVA), which uses the electrical activity of the diaphragm during inspiration to determine the timing and magnitude of the delivered pressure. Although this method has the advantage of not being affected by leaks and of achieving an early response to spontaneous inspiratory effort (Beck et al., 2009; Lee et al., 2015), further studies are needed to evaluate the impact of noninvasive use of NAVA in preterm infants.

Devices that alternate between two levels of positive airway pressure (Bi-PAP) are also available. In these devices the increase in pressure is achieved by an increase in circulating flow instead of a valve. For this reason, with Bi-PAP devices the increase in pressure may not be as fast or large as with conventional ventilators. Some Bi-PAP devices are capable of synchronizing the increase in airway pressure with the infant's inspiration using the Graseby capsule, but these devices are not available in all countries.

Ventilator cycles delivered at fixed intervals can fall toward the end of spontaneous inspiration or during exhalation and disturb the infant's breathing pattern, whereas delivery of the ventilator cycle when the upper airway is patent may increase transmission of the pressure and reduce the risk of gas being pushed into the esophagus. Nonsynchronized and synchronized modes of nasal ventilation have both been shown to be more effective than N-CPAP, but data are lacking on the superiority of synchronization in terms of efficacy or safety. Data on the most effective mode, frequency, and duration of the cycle and, most importantly, peak pressures during NIV are also lacking. Until more data are available a conservative approach with relatively low ventilator settings and in ranges near those used for intubated infants recovering from RDS or near extubation is recommended. This is particularly important in the setting of peak pressures, because V_T monitoring is not available during NIV.

Potential Drawbacks

The risk of gastrointestinal distention may be higher during NIV than during the use of N-CPAP because of the positive pressure cycles. However, clinical trials have not confirmed this.

There may also be some risk of pneumothorax and PIE during NIV as compared with the use of N-CPAP. Although there are no data on these side effects, caution should be exercised, and high peak pressures or ventilator rates should be avoided.

The risks of nasal damage and obstruction often observed during use of N-CPAP are also present in NIV. Proper application and maintenance of the nasal interface and avoidance of excessive force on the nasal septum are important to avoid these complications.

High-Flow Nasal Cannula

Traditionally, NC devices were used to deliver supplemental oxygen, but more recently they have been used to deliver continuous distending pressure as well (Sreenan et al., 2001). The pressure generated by the nasal cannula is dependent on the flow rate and the fit of the nasal cannula to the nares. As opposed to a regular NC, an HFNC uses heated and humidified gas at flow rates ranging from 2 to 8 L/min. In addition to continuous distending pressure, dead space washout from the HFNC may contribute to the increased gas exchange.

Use of an HFNC in preterm infants has increased significantly over the last decade with little evidence of improved outcomes when compared to CPAP (Shetty et al., 2016). The increased usage is largely due to ease of use, less risk of injury to nares, and increased patient comfort with use of an HFNC.

The most studied clinical application of an HFNC has been as respiratory support after extubation in preterm infants. In comparison with CPAP, trials have shown higher extubation failure rates and less nasal damage with HFNC (Collins et al., 2013; Manley et al., 2013; Wilkinson et al., 2016).

The effect of an HFNC has been evaluated as a primary form of respiratory support in preterm infants of 28 weeks' gestational age or more. Compared with use of N-CPAP, use of an HFNC resulted in similar failure rates or need for intubation (Yoder et al., 2013), but a more recent larger multicenter trial showed higher rates of failure and intubation with use of an HFNC (Roberts et al., 2016).

Drawbacks

One of the main concerns with use of an HFNC is lack of control and variability in distending pressure, leading to risk of excessive airway pressure and air leaks. The correct NC size and close monitoring of the position of the NC are both crucial to avoid these complications.

Invasive Mechanical Ventilation

Mechanical ventilation has been one of the main therapies contributing to the progress in neonatal critical care. This is especially relevant in the more immature infants who, besides lung immaturity, have a weak respiratory pump and poor central respiratory drive, making the need for mechanical ventilation very common.

Indications

The decision to initiate invasive mechanical ventilation in the newborn is very important because of the serious complications

associated with this mode of therapy and because in smaller infants it is often difficult to wean them off respiratory support. There is considerable variation between different centers in the criteria used to initiate mechanical ventilation. Most often, this decision is based on the gestational age of the infant, the severity of the respiratory failure, and the disease process that is underlying the respiratory failure. This is also done considering the alternatives available to support the infant's respiratory function. The experience of the team and the outcomes of infants exposed to mechanical ventilation in each institution should also be an important consideration. In units with vast experience and good outcomes, invasive mechanical ventilation may be used more liberally, whereas in units with limited experience and high rates of complications, other alternatives should be considered before they embark on invasive ventilation.

The decision to initiate mechanical ventilation is usually based on the clinical condition of the infant and the evaluation of arterial blood gases. In the preterm infant, mechanical ventilation is frequently started because of recurrent episodes of apnea and hypoxemia that require intervention for the infant to recover from them. In more immature infants, ventilation is often begun in the delivery room because of severe respiratory depression and bradycardia not responsive to stimulation. The other indication for mechanical ventilation is when PaCO_2 levels rise rapidly, indicating alveolar hypoventilation. Although there are no specific PaCO_2 levels, most clinicians initiate mechanical ventilation when PaCO_2 rises acutely above 55 to 65 mmHg and the pH decreases below 7.25 to 7.20.

The introduction of IPPV may result in a transient improvement in gas exchange, but prolonged invasive ventilation frequently results in further deterioration in pulmonary function because of the negative effects of high inspired oxygen concentrations, ventilator-associated infections, and overdistension of the lung. The effectiveness of mechanical ventilation is primarily due to the support of the infant's failing respiratory pump and the reduction in the work of breathing. One exception to this is the infant with RDS, in whom the positive airway pressure produces recruitment of distal air spaces, with improvement in ventilation-perfusion matching and gas exchange.

In other instances, mechanical ventilation is started because of hypoxemia not responsive to N-CPAP. Although there are no set levels of PaO_2 or FIO_2 to start ventilation in preterm infants with RDS, the initiation of mechanical ventilation is frequently associated with the decision to administer exogenous surfactant. The indications for surfactant differ between institutions, but there is good evidence that early administration of surfactant results in better outcomes, and therefore in an infant with RDS, surfactant is usually given when the inspired oxygen concentration required to maintain acceptable O_2 saturation levels increases above 30% to 40%. If the infant has hypercapnia or clinical signs of significant distress and impending failure, ventilation may be started earlier. In some centers, surfactant is given as prophylaxis to infants below a certain gestational age, usually 28 weeks. After the infant becomes more stable, he or she is extubated to N-CPAP.

In term infants, the indication for mechanical ventilation is usually more conservative because these infants are better able to cope with increased work of breathing. The indication also differs depending on the underlying cause of the respiratory failure. For example, in an infant with respiratory failure due to a congenital diaphragmatic hernia, ventilation is usually started immediately after birth, whereas in an infant with a congenital pneumonia or meconium aspiration, a more expectant approach can be taken,

and ventilation may not be started until there is evidence of rising PaCO_2 and hypoxemia requiring inspired oxygen concentrations up to 40% to 60%. A careful and continuous assessment of the infant is critical in deciding whether invasive ventilation should be started or not.

Inspired Gas Conditioning

During normal breathing, inspired gas is heated and humidified in the nasal passages and upper airways. By the time it reaches the distal airways, it is fully saturated with water vapor at core body temperature (i.e., 100% relative humidity at 37°C for an absolute humidity of 44 mg H_2O per liter of gas). The isothermal saturation point region in the respiratory tract, where the temperature of inspired gas equilibrates with core body temperature and the inspired gas is fully saturated, is near the main bronchi. As inspiratory flow increases, this point moves distally into the airways.

The nose and airways function as heat and moisture exchangers. There, a portion of heat and water added to the inspired gas is recovered during exhalation, with the net loss depending on the temperature and relative humidity of the gas. This requires continuous replenishment of water to the aqueous mucosal layer by the airway epithelium and loss of heat. In contrast to ambient air at 22°C and 50% saturated (absolute humidity of 9.7 mg H_2O per liter of gas) medical gases are typically colder ($\leq 15^\circ\text{C}$) and dry ($< 2\%$ relative humidity) and therefore require more heat energy and humidity. Hence all forms of respiratory support where medical gases are used require conditioning of the inspired gas. This is a key component of mechanical ventilation because the ETT bypasses the nose and upper airway.

Inadequate conditioning of the inspired gas can increase water and heat loss (Fonkalsrud et al., 1980). Exposure to dry and cold inspired gas can also produce inflammation of the airway epithelium and increase the risk of airway damage (Marfatia et al., 1975; Todd et al., 1991). Insufficient humidification can also affect the mucociliary transport system, reducing clearance of secretions, pathogens, and foreign particles as well as increase the risk of airway blockage by mucus plugs (Fonkalsrud et al., 1975). These effects are likely to be more striking in small infants, infants with impaired thermoregulation, and those who are fluid and energy limited. In small preterm infants, a few minutes of mechanical ventilation with inadequately conditioned gases can increase airway resistance and reduce lung compliance (Greenspan et al., 1991). Inadequate gas conditioning has also been associated with increased risk of air leaks and augmented need for O_2 (Tarnow-Mordi et al., 1989).

The standard method for conditioning of the inspired gases consists of a heater/humidifier device and heated breathing circuits. Dry and cold medical gases are heated in the heater/humidifier chamber to 37°C to increase the water-carrying capacity to 44 mg per liter of gas at 100% relative humidity. The gas travels through the ventilator circuit, where it is heated to 39°C to prevent condensation. The gas temperature decreases as the gas travels through the ETT, and gas is delivered at approximately 37°C and near 100% saturated at the distal end.

Many conditions can affect gas conditioning. Air temperature in an incubator or radiant warmer above the 39°C setpoint can inadvertently reduce heating at the ventilator circuit and result in condensation. To avoid this, the temperature probe can be insulated or placed outside the incubator or radiant warmer. If gas heating at the ventilator circuit is not adequate, low ambient temperatures and low circulating gas flows can also produce condensation. In general, the presence of water condensate in the ventilator circuit

and minimal consumption of the humidifier water indicate inadequate conditioning of the gas. Water condensation can occur in the ETT and connector when the incubator temperature is low and can result in water droplets being pushed into the airway.

The humidity of the inspired gas can also be increased by water nebulization. In contrast to the size of the water vapor molecules ($0.0001\ \mu\text{m}$), the size of aerosolized water particles ranges between 0.5 and $5\ \mu\text{m}$, which is large enough to potentially transport viruses or bacteria. Thus water nebulization is not an efficient or safe method, particularly for prolonged use.

Gas conditioning is also recommended during use of N-CPAP or nasal ventilation and oxygen head box or NC use because the nasal passages and airways may not achieve adequate conditioning of the cold and dry medical gases. These gases are typically heated to 32°C , but heating at or above room temperature may be sufficient. On the other hand, passing the gas through a water bath may not produce a sufficient gain in humidity because of the low water-carrying capacity of cold gas. Thick and dry nasal mucus and airway secretions are observed when gas conditioning is insufficient. Lack of conditioning may also affect body temperature and increase water losses.

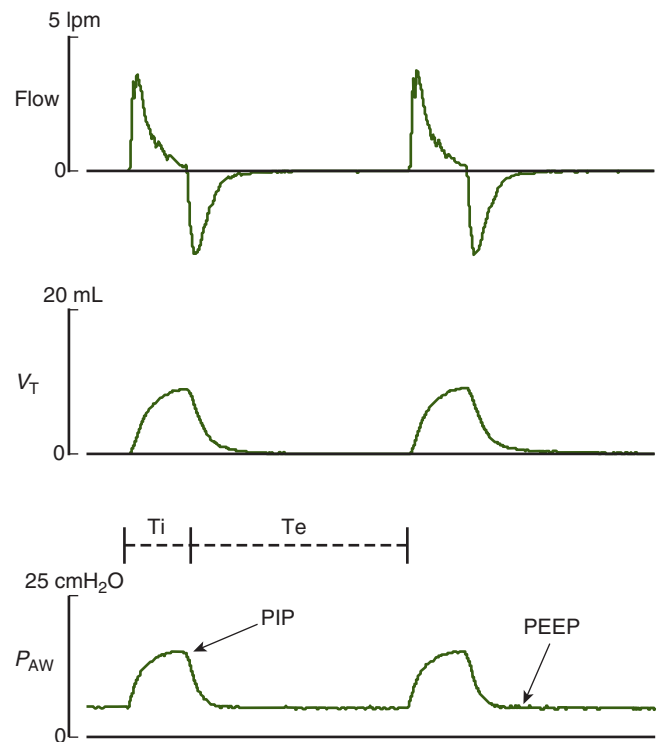
Conventional Positive Pressure Ventilation

Principles

IPPV can be described in its basic form as the cycled application of two different levels of positive pressure at the infant's airway. The positive end-expiratory pressure (PEEP) provides a continuous distending pressure to the lung and is aimed at maintaining lung volume. This is important because the ETT bypasses the upper airway mechanism that normally prevents lung volume loss by active closure of the glottis. The positive pressure applied at the airway opening is intermittently increased to a predetermined peak inspiratory pressure (PIP) during a set inspiratory time (T_i). The rise in airway pressure produces a gradient with respect to the alveolar pressure that drives a V_T of gas into the lung. This form of ventilation is known as *time-cycled pressure-limited ventilation*.

Neonatal ventilators use a constant flow of conditioned gas, also known as *bias flow*, through the breathing circuit to produce positive pressure. A controlled obstruction by a valve at the expiratory port of the ventilator produces PEEP and the intermittent increase to PIP at intervals determined by the set expiratory time (T_e) as shown in Fig. 45.1.

The circulating gas flow determines the profile of the increase in airway pressure by modulating the rise to the set PIP. The airway pressure rise is faster, and PIP is reached earlier at higher circulating flow rates. A fraction of this bias flow is driven into the infant's airways during tidal inflation. The infant's inspiratory flow, which indicates how fast the lung is being inflated, is in part determined by the profile of the airway pressure. The rapid rise in airway pressure produces a rapid increase in the infant's inspiratory flow to a high peak inspiratory flow that decays as the lung is inflated. In this pattern of rapid lung inflation, most of the V_T is delivered early in the inspiratory phase, as shown in Fig. 45.2. In contrast, a slowly rising airway pressure produces a slower inflation and a smaller peak inspiratory flow. A low bias flow may not be sufficient to reach the set PIP within a fixed T_i and in consequence produces a smaller V_T , also shown in Fig. 45.2. In older neonatal ventilators, the bias flow is constant during both the inspiratory phase and the expiratory phase, whereas newer ventilators self-adjust the flow necessary to produce a desired profile. In some ventilators the caregiver can set a maximal flow to be delivered by



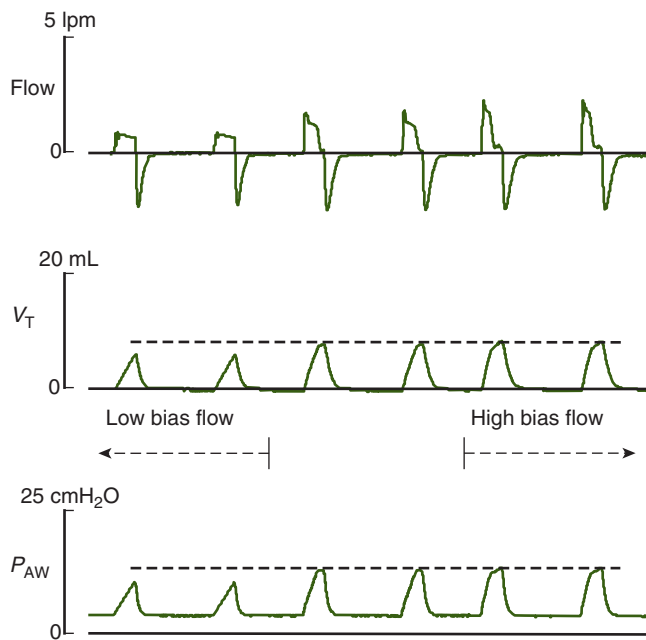
• **Fig. 45.1** Time-Cycled Pressure-Limited Ventilation. Recordings of flow, tidal volume (V_T), and airway pressure (P_{AW}) showing that ventilator cycles, occurring at intervals determined by the expiratory time (T_e), increase the airway pressure from the positive end-expiratory pressure (PEEP) to the peak inspiratory pressure (PIP) during the set inspiratory time (T_i). The increase in pressure with respect to the alveolar pressure drives gas into the lung to achieve V_T . The inspiratory flow, which determines the rate of lung inflation, peaks initially and subsequently declines to zero as the lung is inflated. *lpm*, Liters per minute.

the ventilator to the circuit during the cycle, which also modifies the airway pressure profile.

The bias flow should be sufficient to sustain the PEEP when the infant's spontaneous respiratory flow demands increase. Otherwise, it may create an inspiratory load that the infant has to overcome with each breath. This is noted as a decline in PEEP during spontaneous inspiration that can even become negative. The bias flow is also responsible for removal of exhaled gases, and an insufficient bias flow may cause rebreathing. On the other hand, a very high bias flow produces an almost square inspiratory airway pressure waveform that increases the velocity of lung inflation. This may have undesirable consequences because the lung will be expanded much faster than during a normal physiologic inflation. In general, circulating bias flow rates for ventilated preterm infants are set between 5 and 8 L/min, with higher circulating flow rate settings necessary to accommodate gas leaks around the tube or the inspiratory demands of larger neonates.

The neonate's respiratory system mechanical properties (i.e., compliance and airway resistance) determine its time constant as their product. The respiratory time constant, which is a measure of the time to achieve equilibrium between the applied pressure and the alveolar pressure, differs with different lung diseases and their severity.

The compliance of the respiratory system (CRS) is a measure of the recoil pressure that opposes expansion and is determined by the compliance of lung and the compliance of the chest wall.

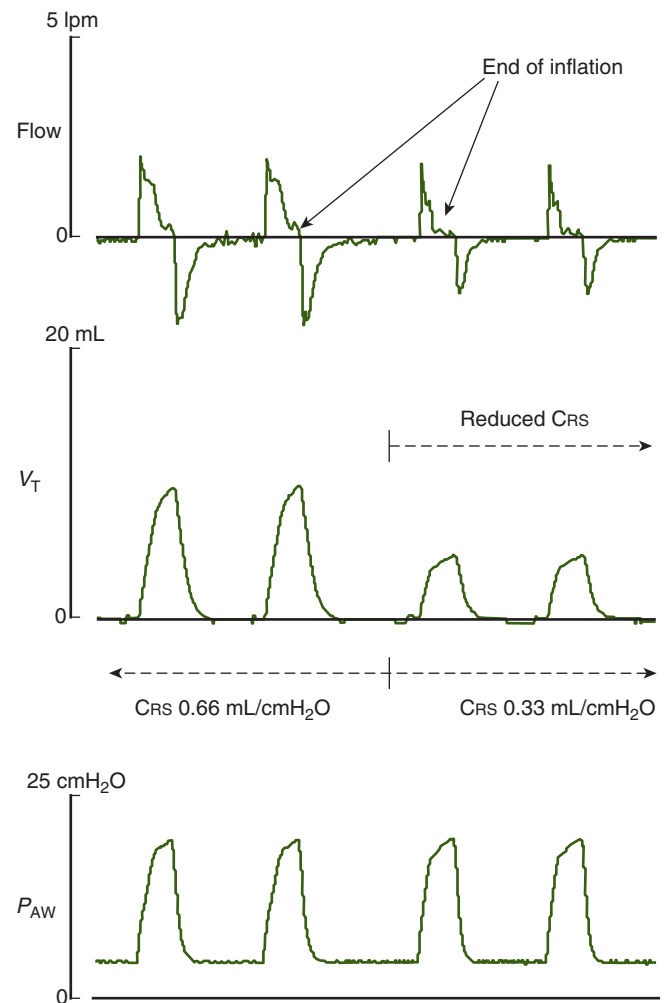


• **Fig. 45.2** Effect of Circulating Bias Flow. Recordings of flow, tidal volume (V_T), and airway pressure (P_{AW}) showing how increasing bias flow rates in the ventilator circuit change the profile of the airway pressure, with a more rapid rise toward the peak pressure. This produces a higher peak flow, which indicates faster lung inflation. An insufficient bias flow rate does not permit the generation of the desired peak pressure with each ventilator cycle and results in a smaller V_T . *lpm*, Liters per minute.

In the neonate, and most particularly in the preterm infant, CRS is mainly determined by the compliance of the lung because the chest wall is highly distensible. Lung diseases characterized by lung restriction, such as RDS, where the increased lung recoil is indicated by a low CRS and a short time constant, are quite prevalent in preterm infants. In addition, they have a respiratory pump that is often too weak to generate the pressure required to achieve an adequate V_T , and thus they require mechanical ventilation.

During positive pressure ventilation, a decreased CRS is characterized by a smaller V_T for a given transpulmonary pressure and a relatively brief duration of inflation. As shown in Fig. 45.3, the shorter time constant is illustrated by a shorter duration of inflation with an earlier return of the inspiratory flow to zero. This marks the point when the airway and alveolar pressures are at equilibrium. At this point, the PIP equals the lung's recoil pressure, with CRS as the ratio between the V_T and the applied pressure. When CRS decreases, prolonging the T_i is ineffective, and a higher PIP is required to maintain a constant V_T .

The resistance of the airways opposes the flow of gas, which dissipates part of the driving pressure during lung inflation. In diseases characterized by increased airway resistance, this attenuates the infant's inspiratory flow and results in a slower inflation rate. The longer time constant indicates the increased time required for alveolar pressure to rise and equilibrate with the applied pressure. When airway resistance increases, the set T_i may not be long enough to achieve a desired V_T , as shown in Fig. 45.4. Although setting a longer T_i may be reasonable, this should be done cautiously because the increased airway resistance also limits the expiratory flow and prolongs the time required to achieve complete exhalation. A long T_i combined with a T_e that does not allow complete exhalation will result in gas trapping.



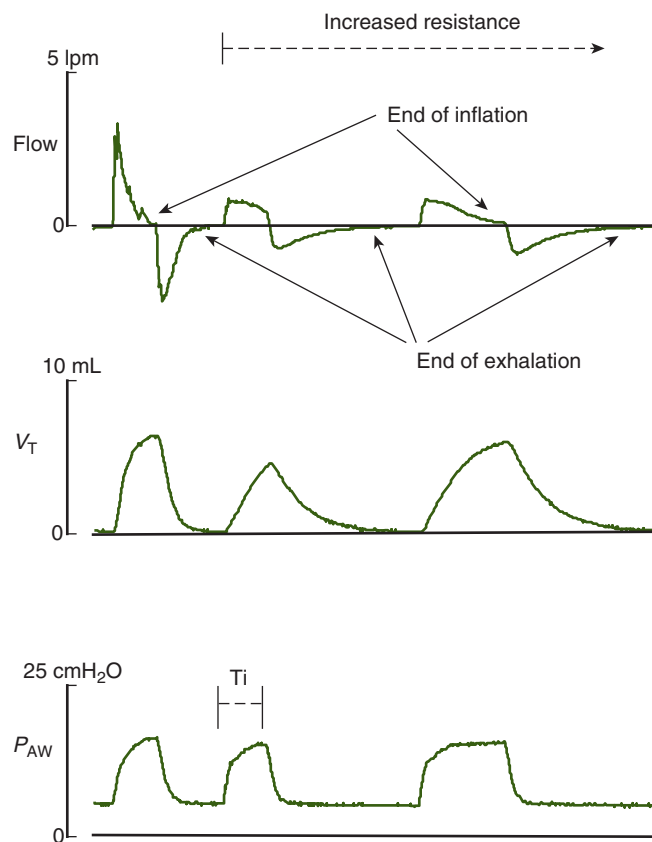
• **Fig. 45.3** Effect of Lung Compliance. Recordings of flow, tidal volume (V_T), and airway pressure (P_{AW}) showing the effects of a decrease in lung compliance. A reduction in lung compliance results in a proportional decrease in V_T for the same driving airway pressure. The effect is also noted by a shorter time constant, indicated by an earlier return of the inspiratory flow to zero, marking the end of lung inflation. *CRS*, Compliance of the respiratory system; *lpm*, liters per minute.

Modes of Conventional Positive Pressure Ventilation

The modes of conventional neonatal ventilation are generally classified according to the parameter controlled by the ventilator in each cycle as well as by the timing and duration of the cycle. Ventilator cycles can be pressure controlled or volume controlled depending on whether the ventilator targets a set peak pressure or volume respectively. Ventilator cycles can be delivered at fixed intervals regardless of the timing with respect to the infant's spontaneous breathing, or they can be delivered in synchrony with the spontaneous inspiration. The duration of the inspiratory phase of each ventilator cycle can be constant, or it can adapt to the time required to complete lung inflation, deliver the set volume, or reach the end of spontaneous inspiration.

Intermittent Mandatory Ventilation

The time-cycled pressure-limited ventilation mode described earlier and illustrated in Fig. 45.1 has been most commonly known as *intermittent mandatory ventilation* (IMV). This mode can be classified as pressure controlled because ventilator cycles deliver the PIP set

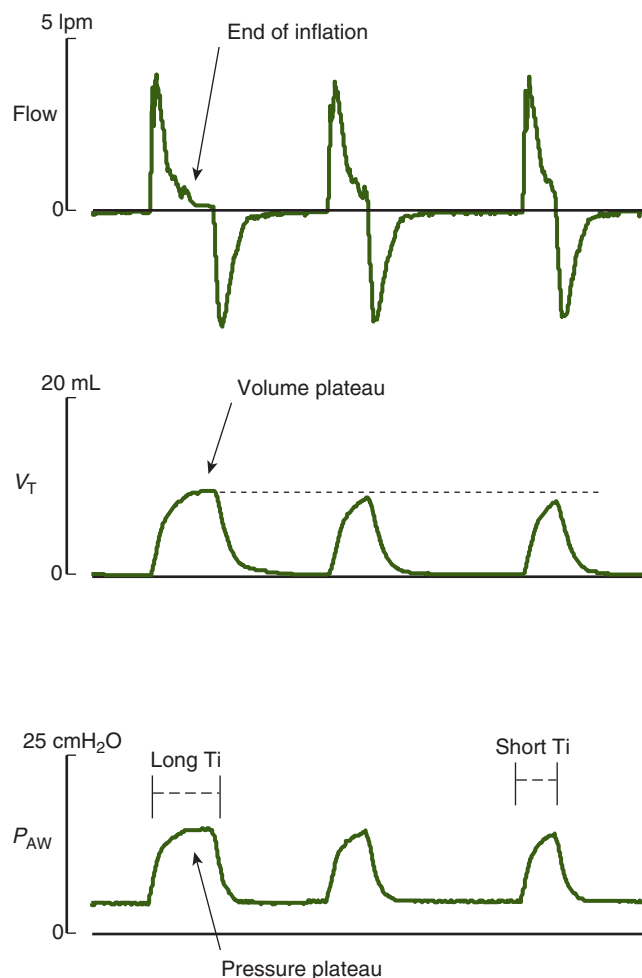


• **Fig. 45.4** Effect of Airway Resistance. Recordings of flow, tidal volume (V_T), and airway pressure (P_{AW}) showing the effects of an increase in airway resistance. The increase in resistance results in a decrease in inspiratory flow that does not permit delivery of V_T in the set inspiratory time (T_i). The prolonged time constant is noted by the increased time required to achieve full inflation and to complete exhalation. *lpm*, Liters per minute.

by the caregiver. These cycles are of constant inspiratory duration (i.e., T_i) and are delivered at fixed intervals (i.e., T_e is determined by the set ventilator frequency). For many years IMV was one of the most commonly used modes of neonatal ventilation during the acute phase as well as the more chronic phases of neonatal respiratory disease.

In IMV the increase in airway pressure in each ventilator cycle produces a given lung inflation during the set T_i . If the T_i is too short, complete lung inflation may not be achieved, whereas a long T_i that maintains a pressure plateau does not achieve a larger V_T and instead produces an inspiratory hold while the lung is kept inflated as illustrated in Fig. 45.5. In infants with respiratory failure, the T_i is usually set in the range of 0.25 to 0.4 seconds. Caution should be exercised when one is setting ventilator cycles of longer T_i or high ventilator frequencies that could result in gas trapping due to an insufficient T_e to allow full exhalation as shown in Fig. 45.6. This is particularly important when the infant's time constant is long because of a high airway resistance or when the ventilator frequency results in a T_e of less than 0.5 seconds.

During IMV, total minute ventilation results from the ventilation produced by the ventilator and the contribution of the infant's spontaneous breathing effort. During clinical use, PIP is usually adjusted to maintain the V_T between 3 and 6 mL/kg of body weight, which is considered adequate for infants with lung disease. The ventilator frequency is adjusted depending on the infant's ability to contribute to minute ventilation and maintain



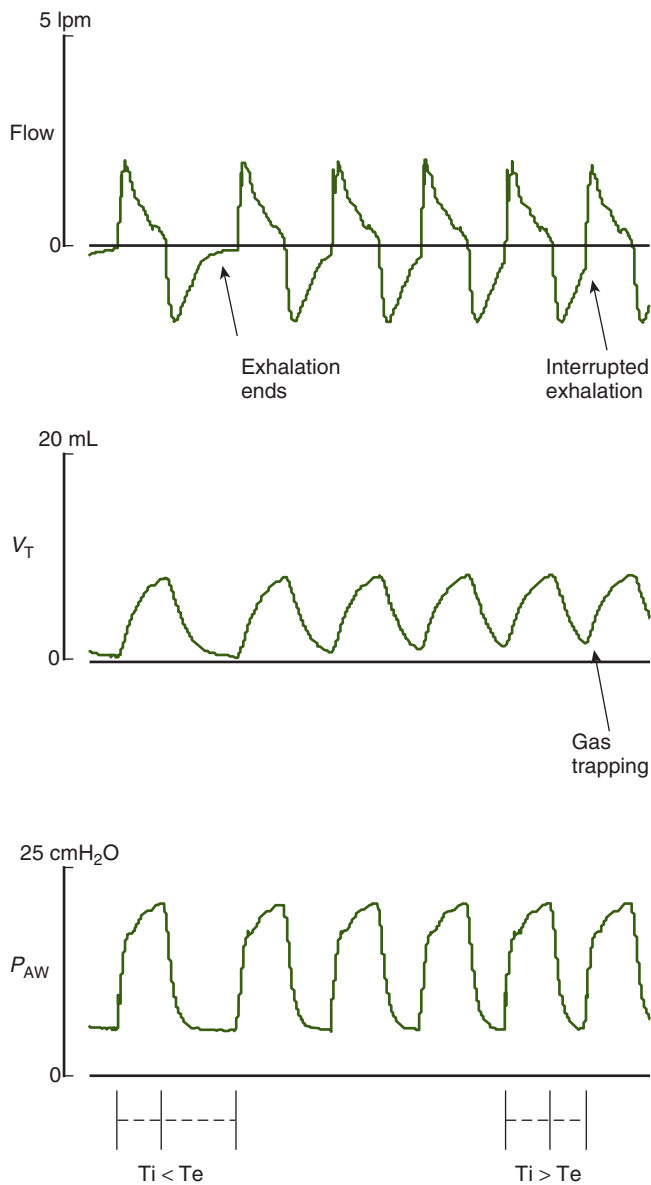
• **Fig. 45.5** Inspiratory Duration. Recordings of flow, tidal volume (V_T), and airway pressure (P_{AW}) illustrating the effects of inspiratory time (T_i). A T_i that prolongs the inspiratory phase beyond the time needed to achieve inflation does not increase the V_T and only keeps the lung expanded. A T_i of insufficient duration does not permit achievement of full inflation, as indicated by an inspiratory flow that does not return to zero and leads to a decrease in V_T . *lpm*, Liters per minute.

sufficient gas exchange. PaCO_2 levels between 40 and 50 mmHg are considered adequate in infants with acute respiratory failure, but higher levels are often accepted in infants with long-term ventilator dependency.

Although the management of IMV is relatively simple, there are some important considerations. With IMV, cycles are often delivered out of synchrony with the infant's inspiration (Greenough et al., 1983a, 1983b). Asynchronous ventilator cycles delivered toward the end of the infant's inspiration can prolong or increase lung inflation. IMV cycles delivered during the infant's exhalation can prolong its duration and in some cases elicit active exhalation against the positive pressure.

Synchronized Intermittent Mandatory Ventilation

SIMV is for the most part similar to IMV except that ventilator cycles are delivered in synchrony with the onset of spontaneous inspiration. In SIMV, ventilator management is similar to that in IMV during the different phases of respiratory failure. SIMV was rapidly accepted after its introduction in the 1980s and has become one of the most common modes of neonatal ventilation. During



• **Fig. 45.6 Gas Trapping.** Recordings of flow, tidal volume (V_T), and airway pressure (P_{AW}) illustrating the potential for gas trapping at high ventilator rates. As the ventilator rate increases, the expiratory time (T_e) becomes insufficient to complete exhalation where every exhalation is interrupted by a new ventilator cycle. This produces a volume of gas trapped in the airways and alveoli and a reduction in V_T . *lpm*, Liters per minute; *Ti*, inspiratory time.

SIMV, spontaneous breathing is continuously monitored by the ventilator, and the first spontaneous inspiration during a time window of constant duration triggers a ventilator cycle. These time windows are opened consecutively, and their duration is similar to the interval between cycles in IMV. If no spontaneous inspiration is detected by the time the window elapses, a backup ventilator cycle is delivered. In this manner the number of ventilator cycles the infant receives every minute is the same as in IMV, but the interval is not constant.

Different techniques have been incorporated into neonatal ventilators to achieve synchronization, including the Graseby abdominal pressure capsule, esophageal balloons, transthoracic impedance, airway pressure changes, phrenic electrical activity,

and flow sensors. The last are used by most neonatal ventilators for synchronization and V_T monitoring.

SIMV produces a greater and more consistent V_T in comparison with IMV because of the increased transpulmonary pressure in ventilator cycles that are in synchrony with spontaneous inspiration (Bernstein et al., 1994; Hummler et al., 1996). Thus lower PIP and slightly shorter T_i are often sufficient because the infant's inspiratory effort contributes to the generation of the V_T . As lung disease subsides, the infant's lung mechanics improve, spontaneous breathing effort becomes more consistent, and PIP is gradually adjusted to maintain a V_T between 3 and 6 mL/kg. During acute respiratory failure, a ventilator SIMV frequency ranging between 40 and 60 breaths per minute is usually required to maintain P_{aCO_2} between 40 and 50 mmHg. At this frequency, SIMV is likely to assist almost all spontaneous breaths. During weaning, the SIMV frequency is gradually reduced as spontaneous unassisted breathing can better maintain acceptable P_{aCO_2} levels. In infants with chronic lung disease, higher P_{aCO_2} levels are often tolerated for reduction of the ventilator settings as long as respiratory acidosis is not observed. The relative advantages of SIMV in comparison with IMV include faster weaning and a shorter ventilator dependency, with improved respiratory outcome in the smaller infants (Bernstein et al., 1996; Chen et al., 1997).

As the SIMV frequency is reduced, the spontaneous breathing effort must be able to sustain most of the ventilation, thus requiring a greater breathing effort. It has been suggested that the work of breathing in unassisted spontaneous breaths between SIMV cycles is counterproductive and can potentially lead to diaphragmatic fatigue. Conversely, it has also been suggested that exposure to fewer ventilator cycles may reduce the risk of lung injury and improve diaphragmatic fitness toward an eventual extubation. Failure to trigger breaths leads the ventilator to cycle at the backup IMV rate. Delayed cycling prolongs the duration of inspiration and can disrupt the breathing pattern (Beck et al., 2004), whereas autocycling produces asynchrony between the ventilator and the infant.

Assist/Control Ventilation

In A/C ventilation, every spontaneous inspiration is assisted by a synchronous ventilator cycle. If the ventilator does not detect spontaneous breaths because of either apnea or very shallow breathing, it provides an IMV rate with the same PIP as the assisted breaths. A/C ventilation can also be used during the acute and weaning phases of respiratory failure.

A/C ventilation is primarily managed by adjustment of PIP to maintain the V_T within an adequate range. As the respiratory failure resolves with improving lung mechanics and stronger spontaneous breathing effort, PIP is gradually reduced as long as the V_T remains within an acceptable range. In the more immature infants, the respiratory drive is not consistent, and such infants have frequent apnea. Thus the "controlled" or backup IMV rate becomes more relevant in the maintenance of ventilation during apnea.

In infants with respiratory failure, the T_i required to achieve lung inflation usually ranges between 0.25 and 0.4 seconds. A longer T_i is not recommended because of the potential for gas trapping with ventilator cycles delivered at the infant's often high breathing frequency. A long T_i can also disrupt spontaneous breathing (Upton et al., 1990; Dimitriou et al., 1998; Beck et al., 2004). Most modern ventilators provide flow cycling where the T_i terminates at the end of the spontaneous inspiration or at the end of lung inflation. The advantages of A/C ventilation include

a reduction in spontaneous breathing effort relative to IMV or SIMV because in contrast to these modes, A/C ventilation assists every inspiration (Bernstein et al., 1994; Kapasi et al., 2001). It has been suggested that assisting every spontaneous inspiration in A/C mode ventilation prevents diaphragmatic fatigue and can reduce the duration of weaning and ventilator dependency compared with IMV (Baumer, 2000; Beresford et al., 2000; Chan and Greenough, 1993; Donn et al., 1994). However, A/C ventilation has not been shown to be more effective than SIMV (Chan and Greenough, 1994; Dimitriou et al., 1995).

In A/C ventilation, PIP should be adjusted to avoid a large V_T that can produce hyperventilation, whereas the backup rate should be just sufficient to prevent hypoventilation during apnea. Auto-cycling is a problem in A/C mode ventilation because neonatal ventilators do not have a limit on the cycling frequency and therefore can produce hyperventilation and gas trapping.

Pressure Support Ventilation

Similarly to A/C mode ventilation, in pressure support ventilation (PSV) the ventilator provides a positive pressure breath with every spontaneous inspiratory effort. The start of the support pressure is in synchrony with the onset of the spontaneous inspiration, and termination of the support pressure occurs toward the end of spontaneous inspiration or when lung inflation is completed (i.e., flow cycling). The use of PSV is aimed at unloading the respiratory pump with support pressure that helps overcome the elastic and resistive loads imposed by the preterm infant's underlying lung disease. PSV can be used as a stand-alone mode with an IMV backup rate for apnea or in some ventilators as an adjunct to SIMV to assist the spontaneous breaths, whereas the SIMV cycles with a higher peak pressure are used to maintain lung volume.

Although PSV can be used during both the acute phase and the weaning phase of respiratory failure, it is most commonly used during weaning. A consistent respiratory drive is required for the use of PSV as a stand-alone mode. For this reason, a backup IMV rate is recommended particularly in preterm infants to prevent hypoventilation. During PSV, gas leaks around the ETT can produce autocycling and extend the duration of the cycle. To avoid the risk of hyperventilation or gas trapping, the trigger and termination sensitivity levels must be adjusted, and limits on the cycle duration must be set.

When PSV is used as an adjunct to SIMV, it can reduce the spontaneous breathing effort compared with SIMV alone even with support pressures set at a fraction of the peak pressures of the SIMV cycles (Osorio et al., 2005; Gupta et al., 2009; Patel et al., 2009). More importantly, by reducing the need for high SIMV rates, PSV can facilitate weaning in comparison with SIMV alone (Reyes et al., 2006). In that study, PSV levels set at 30% to 50% of the peak pressure of the SIMV cycles were sufficient to maintain acceptable PaCO_2 levels with significantly lower SIMV rates, and infants supported with SIMV plus PSV were weaned faster off respiratory support and supplemental oxygen.

Volume-Targeted Ventilation

In volume-targeted ventilation, automatic adjustments to the peak positive pressure or the duration of the ventilator cycle are done to maintain a target V_T . Volume-targeted ventilation modes have been proposed as a means to reduce ventilator-associated lung injury caused by ventilation with excessive or insufficient V_T s during conventional pressure-controlled ventilation. A number of volume-targeted modes are available in neonatal ventilators. These differ in the timing of the adjustment or in the duration of the mechanical

cycle (i.e., whether it occurs as the cycle is delivered to the infant or in the subsequent cycle). These modes also differ in the volume parameter that is controlled—whether it is the V_T received by the infant or the volume delivered by the ventilator to the circuit and whether this is measured during the inspiratory phase or during exhalation.

Metaanalyses comparing different volume-targeted ventilation strategies with pressure-limited ventilation showed decreased duration of ventilation and decreased incidence of BPD, pneumothorax, severe intraventricular hemorrhage (IVH), and periventricular leukomalacia with use of volume-targeted ventilation (Wheeler et al., 2011; Peng et al., 2014).

Volume-Controlled Ventilation. In volume-controlled (VC) ventilation the ventilator delivers a set volume of gas into the ventilator circuit in each cycle. VC ventilation cycles are delivered in the IMV, SIMV, or A/C ventilation modes described earlier. The time required to deliver the set volume depends on the ventilator flow rate, which can be constant during the cycle or variable, with an initial peak followed by a gradual decline. The flow continues until the set volume is delivered. The cycle ends before the set volume is delivered if its duration exceeds the set T_i or the airway pressure exceeds the set PIP. In small infants, most of the volume delivered by the ventilator is compressed in the circuit, and the actual V_T delivered to the infant is only a fraction. Some ventilators use algorithms to correct the measured volume by the compressed volume to estimate the true V_T .

VC ventilation has been proposed as a strategy to facilitate weaning and reduce the complications of positive pressure ventilation. Clinical trials showed that compared with pressure-limited ventilation in the A/C mode, VC ventilation reduced the weaning time and the duration of mechanical ventilation in infants weighing at least 1200 g at birth (Sinha et al., 1997). Weaning was also faster with VC ventilation in infants of birth weight less than 1000 g, but the total duration of mechanical ventilation did not change, and respiratory outcome did not differ significantly (Singh et al., 2006).

Pressure-Regulated Volume-Controlled Ventilation. In pressure-regulated VC (PRVC) ventilation, the peak pressure of pressure-controlled ventilator cycles is adjusted from one cycle to the next to maintain a target volume. The targeted volume can be that delivered by the ventilator or the actual estimated or measured V_T . During PRVC ventilation, gas leaks around the ETT can produce overestimation of volume during the inspiratory phase and consequently lead to inappropriate reductions in PIP.

Compared with conventional IMV, PRVC ventilation in A/C mode was effective in reducing the duration of mechanical ventilation and the incidence of IVH in infants with RDS of birth weight less than 1000 g (Piotrowski et al., 1997). These advantages can be attributed, in addition to volume targeting, to synchronized delivery PRVC ventilation cycles. These advantages were not evident when PRVC ventilation was compared with SIMV (D'Angio et al., 2005).

Volume Guarantee Ventilation. Volume guarantee (VG) ventilation is one of the more commonly used volume-targeted modes of ventilation in neonates. In VG ventilation, the peak pressure of each ventilator cycle is adjusted to maintain a target V_T based on the basis of exhaled V_T measured in previous cycles. Measurement of exhaled V_T is aimed at circumventing the effects of gas leaks around the ETT during the inspiratory phase. VG ventilation can be used in combination with the A/C, PSV, SIMV or IMV modes of ventilation. Practical details on how to use VG ventilation are discussed in Klingenberg et al. (2011).

VG ventilation was proposed as a potential alternative to avoid both extremes of V_T and to achieve a consistent reduction of peak pressure. The stability of V_T and gas exchange in infants with RDS can be enhanced by VG ventilation when combined with A/C mode ventilation or PSV as noted by fewer breaths with too small or large V_T and less hypocapnia (Abubakar and Keszler, 2001; Herrera et al., 2002; Keszler and Abubakar, 2004; Cheema et al., 2007). In infants with RDS or who had received surfactant, VG ventilation achieved the proper reduction in PIP, but this was dependent on the setting of a lower target V_T than the V_T attained with the pressure-controlled modes (Abubakar and Keszler, 2001; Cheema and Ahluwalia, 2001; Herrera et al., 2002; Olsen et al., 2002; Abd El-Moneim et al., 2005; Nafday et al., 2005). However, the smaller target V_T was in some cases not sufficient, resulting in increased PCO_2 and spontaneous breathing effort (Herrera et al., 2002). Although there is some evidence that use of VG ventilation may improve short-term respiratory outcomes, such as the duration of mechanical ventilation, its effect on long-term outcomes, such as BPD and NDI, has not been fully evaluated (Duman et al., 2012; Stefanescu et al., 2015).

VG ventilation has also been proposed as a means to attenuate hypoxemia spells triggered by hypoventilation during periods of agitation and decreased compliance in preterm infants. Studies evaluating the use of VG ventilation to reduce hypoxemia have shown mixed results. While use of VG ventilation in controlled settings with target V_T higher than that during SIMV reduced the duration of the hypoxemia spells (Polimeni et al., 2006), the effect was less marked in a study where the target V_T during VG ventilation was matched to that during SIMV (Jain et al., 2016).

Proportional Assist Ventilation

In proportional assist ventilation (PAV) the ventilator pressure is increased in proportion to the measured volume or flow, or both, generated by the infant's inspiratory effort. This achieves a perceived reduction of the elastic and resistive loads imposed by lung disease that commonly prevent the infant from producing an adequate V_T . The proportionality factors by which the positive pressure increases in relation to volume or flow are the elastic (volume proportional) and resistive (flow proportional) gains. The elastic and resistive gain factors must be individualized to each infant's lung compliance and airway resistance and the infant's ability to cope with the loads. Elastic gain factors that exceed the lung elastance (inverse of compliance) can lead to a runaway increase in pressure, whereas excessive resistive gain factors can produce oscillations in airway pressure. For this reason, PAV devices provide peak pressure and volume limits. Because PAV amplifies only the spontaneous breathing effort, a backup IMV rate is needed to prevent hypoventilation during apnea.

By compensating for the loads induced by lung disease, PAV reduced the breathing effort in infants recovering from RDS (Musante et al., 2001). Compared with conventional modes that deliver a constant pressure during inspiration, PAV produced similar ventilation with lower ventilator and transpulmonary pressures (Schulze et al., 1999, 2007) as well as improved oxygenation (Bhat et al., 2015; Shetty et al., 2016).

Neurally Adjusted Ventilatory Assist

NAVA is a mode where the ventilator pressure is adjusted in proportion to the electrical activity of the diaphragm measured by esophageal electrodes. NAVA was developed to enhance the coupling of the infant's inspiration and the ventilator response.

Short-term studies in premature infants showed that NAVA can increase synchrony between the infant's spontaneous breaths and the ventilator and that it can maintain similar or better ventilation and gas exchange with less breathing effort and lower ventilator pressures compared with conventional pressure-controlled ventilation (Beck et al., 2009; Lee et al., 2012; Stein et al., 2013; Longhini, 2015). Of note, these effects are relative to the NAVA gain level used as well as the settings during conventional ventilation. Further studies are needed to determine the impact of NAVA in the preterm infant population.

Management of the ventilatory support during NAVA differs substantially from that with conventional modalities. In NAVA the support level is adjusted by the setting of the NAVA gain (Stein et al., 2014). However, data are lacking on the effects of different NAVA proportionality factors between diaphragmatic electrical activity and ventilator pressure within the same infant or between infants, because electrical activity of the diaphragm cannot be normalized. The respiratory drive in premature infants is inconsistent, and they frequently have apneic episodes. For this reason a backup mechanical support is needed to prevent hypoventilation during these episodes.

Targeted Minute Ventilation

Targeted minute ventilation (TMV) is a mode where the ventilator rate is adjusted to maintain minute ventilation at a target level. If minute ventilation exceeds or decreases below the target level, the ventilator rate is reduced or increased respectively. If spontaneous breathing can maintain a normal minute ventilation, the ventilator rate is reduced. In preterm infants recovering from RDS, TMV reduced the ventilator rate to half the original rate without impairing gas exchange compared with SIMV. Although these infants were able to sustain their ventilation for long periods, at times they required increased ventilator rates (Claure et al., 1997).

Mandatory minute ventilation (MMV) is a form of TMV where the ventilator rate is transiently turned to zero when spontaneous breathing maintains ventilation. If minute ventilation falls below the target level, a constant ventilator rate of VC ventilation cycles is provided. In near-term infants ventilated for reasons other than lung disease, MMV reduced the rate and reduced the mean airway pressure compared with SIMV (Guthrie et al., 2005).

Adaptive backup ventilation is a form of backup support. In this mode a ventilator rate is provided during apnea as well as during hypoxemia detected by SpO_2 . In preterm infants recovering from RDS, this hybrid backup mode compared with a backup ventilator rate for apnea alone reduced the incidence and duration of hypoxemia spells (Herber-Jonat et al., 2006).

High-Frequency Ventilation

Because of the association between pulmonary overdistension, lung injury, and the development of BPD, the possibility of achieving gas exchange with very small V_T s has been of great interest to neonatologists for many years. This led to the development of instruments that can generate changes in airway pressure at rates in excess of 10 Hz (600 per minute) with the aim of producing ventilation with very small V_T s, usually much smaller than the dead space.

Gas Transport During High-Frequency Ventilation

During conventional ventilation, gas exchange occurs by introduction of fresh gas into the distal air spaces with each inspiration. In contrast, during HFV, the volume of fresh gas delivered by each

cycle is very small and does not reach the most distal portions of the lung. Therefore different mechanisms must explain alveolar ventilation and gas exchange. These include bulk flow into the more proximal portions of the lung, enhanced mixing of gas within the conducting airways, and out-of-phase movement between different regions of the lung that have different time constants. There is also enhanced diffusion of gas in large and medium-sized airways due to asymmetric velocity profiles during inspiration and expiration. Finally, there is molecular diffusion in the more distal air spaces that moves the different gas molecules from areas of higher concentration to areas of lower concentration. These mechanisms have been mostly explored in adult lung models, and therefore it is not clear how well they apply to the immature or sick neonatal lung.

Devices for High-Frequency Ventilation

Several types of high-frequency ventilators have been used in the neonate, including jet ventilators, oscillators, and flow interrupters.

Jet ventilators generate a high-velocity gas flow that is injected through a small-diameter tube that opens into the airway connector. Expiration is passive, and the cycling rate is determined by an electrically operated valve that opens and closes the jet at a pre-determined rate and timing. The high velocity of the gas injected into the airway produces a Venturi effect that pulls additional gas from the ventilator circuit. This is known as *gas entrainment*. V_T is determined primarily by the driving pressure of the gas, the T_i , and the resistance of the injection port. Because expiration is passive during high-frequency jet ventilation (HFJV), there is a risk of gas trapping and lung overdistension (Bancalari et al., 1987). This risk is higher when the time constant of the respiratory system is increased by airway obstruction and when large V_T s are delivered. Because of this, jet ventilators are used at lower rates (4 to 10 Hz). Jet ventilators are used in combination with conventional ventilators that provide PEEP and may also provide conventional positive pressure cycles.

High-frequency oscillatory ventilators use a piston or a membrane driven by an electromagnetic force and connected to the ventilator circuit. The mean airway pressure is determined by the gas flow through the circuit and a variable resistance, whereas the V_T is generated by the size of the excursion of the piston or membrane. With these devices, the expiratory phase is active because during expiration, airway pressure falls below the baseline, which reduces the risk of gas trapping.

Flow interrupters are a hybrid between jet and oscillatory ventilators. They produce airway pressure changes by interrupting the gas flow at very high rates using a standard ventilator circuit rather than an injection cannula. They are relatively simple and are usually offered as an additional mode on some conventional neonatal ventilators. They are often used on transport ventilators. However, they may not have enough power to generate sufficient pressure amplitude to effectively ventilate large infants or infants with very stiff lungs.

Ventilator Settings During High-Frequency Ventilation

The ventilator settings during HFV are simpler to adjust than with conventional ventilation. The mean airway pressure determines the lung volume and is adjusted to optimize the ventilation-perfusion ratio and oxygenation. This is done considering the severity and type of lung disease. Most infants with RDS are managed with mean airway pressures between 8 and 15 cmH₂O. Higher levels may be necessary in some cases with severe lung disease and poorly compliant lungs.

The V_T is determined by the pressure amplitude, and this, in combination with the frequency, determines the CO₂ elimination. In contrast to conventional ventilation, during HFV the V_T is reduced as the frequency increases because the shorter times for inspiration and expiration prevent the equilibration of pressures between the ventilator circuit and the distal portions of the lung. Therefore during HFV a reduction in rate may result in larger V_T s, more CO₂ elimination, and lower PaCO₂ levels.

During high-frequency oscillation or with flow interrupters, the frequencies used range from 8 to 15 Hz, whereas during jet ventilation lower frequencies between 4 and 10 Hz are commonly used.

The pressure transmission to the distal airways is greatly influenced by the resistance of the tube and the airway, so only a small fraction of the delta pressure generated by the ventilator is transmitted to the terminal air spaces. For this reason, the delta pressure during HFV represents more a relative value used to adjust the ventilator than the real pressure change in the distal airways.

Clinical Use of High-Frequency Ventilation

The indications for HFV differ widely among different centers. Although some use HFV routinely as a primary mode of support, most centers use it as rescue when conventional ventilation has failed. This includes use in preterm infants who require increasing ventilator settings to maintain CO₂ elimination and oxygenation within acceptable limits and those with evidence of pulmonary interstitial emphysema. In larger infants, HFV is also indicated in situations where conventional ventilation is not sufficient to maintain acceptable CO₂ elimination or oxygenation and especially in infants with persistent pulmonary hypertension secondary to hypoplastic lungs due to congenital diaphragmatic hernia or oligohydramnios or to severe lung disease due to meconium aspiration or pneumonia.

High-Frequency Ventilation in Respiratory Distress Syndrome

The clinical results with the use of HFV in infants with RDS have been inconsistent. Whereas some studies have shown better outcomes, such as increased survival with no BPD (Courtney et al., 2002; Sun et al., 2014), others have not shown differences (Johnson et al., 2002). Some studies have suggested a higher risk of air leaks (Thome et al., 1999), and others have suggested a higher incidence of intracranial hemorrhage with HFV (HIFI Study Group, 1989; Moriette et al., 2001).

Many of the earlier studies were performed before exogenous surfactant was available, and therefore the results are not entirely applicable to the present situation, where most preterm infants are exposed prenatally to steroids and, when indicated, receive exogenous surfactant. A metaanalysis of the most recent trials suggests a possible advantage for HFV in the outcomes “BPD at 36 weeks” or “death or BPD” at 36 weeks’ corrected age. However, when the results were analyzed with use of adjustments for heterogeneity between trials, the beneficial effect disappeared (Thome et al., 2005). The results of this metaanalysis revealed a significant increase in the risk of air leaks and a trend for higher risk of IVH grades III and IV with HFV. When the results of HFV were compared with those of conventional ventilation, including only studies where HFV was used with high-volume strategies and conventional ventilation was used with an optimized low positive pressure and V_T strategy, there were no significant differences in any of the outcomes (Thome et al., 2005; Bollen et al., 2007). Because of the inconsistency of the results and the lack of solid evidence for benefits of HFV over conventional ventilation in

infants with RDS, the selection of one modality over the other is mostly based on individual preference (Cools et al., 2015). This is also true for the use of HFJV because of the limited evidence of a positive impact on mortality, BPD, or other neonatal morbidities (Rojas-Reyes et al., 2015).

High-Frequency Ventilation in Persistent Pulmonary Hypertension of the Newborn

The clinical evidence with the use of HFV in infants with persistent pulmonary hypertension is not solid and comes from a few, relatively small trials. Some of these studies suggested a decreased need for extracorporeal membrane oxygenation in infants with persistent pulmonary hypertension treated with HFV compared with those treated with IPPV (Clark et al., 1994). However, other studies have not shown a clear advantage of HFV over conventional ventilation (Kinsella et al., 1997; Rojas et al., 2005). HFV may offer some advantage over conventional ventilation in infants with hypoplastic lungs secondary to congenital diaphragmatic hernia or other prenatal conditions (Desfrere et al., 2000).

Other Indications for High-Frequency Ventilation

HFV has also been used in infants with bronchopleural fistulas in an attempt to reduce the amount of gas leak into the pleural space (Gonzalez et al., 1987; Walsh and Carlo, 1989). It can also be used during bronchoscopy or during airway surgery because in contrast to conventional ventilation, it can produce adequate gas exchange with a partially open airway and large gas leaks (Nutman et al., 1989).

Side Effects of High-Frequency Ventilation

Because during HFV the V_T s are extremely small, when HFV is used in unstable lungs, there is a possibility of progressive loss of lung volume and atelectasis. For this reason it is necessary to use mean airway pressures that are usually higher than those used during conventional IPPV to maintain lung recruitment. There is evidence that during HFV the use of an open lung strategy using recruiting maneuvers and high mean airway pressures produces better outcomes than use of lower pressures (Thome et al., 2005). As a result of these higher pressures, it is possible that HFV may negatively influence cardiovascular function (Trindade et al., 1985; Truog and Standaert, 1985; Weiner et al., 1987; Osborn and Evans, 2003), and this may explain the higher incidence of pneumothorax reported in some studies with HFV (Thome et al., 2005).

Because of the effectiveness of HFV in enhancing alveolar ventilation, it is easy to drive PaCO_2 to very low values within a very short period. For this reason it is important to monitor PaCO_2 values closely, especially when HFV is initiated or when settings are changed. Because of the very short inspiratory and expiratory times, it is extremely important to maintain the airway as patent as possible, ensuring a correct position of the ETT. Any obstruction will produce a decrease in pressure transmission and in V_T and can lead to gas trapping. This is even more important with jet ventilation because it can deliver larger V_T s. During jet ventilation, it is also critical to ensure proper humidification of the inspired gas to prevent airway damage that can be produced by the high velocity of gas injected into the airway.

Weaning of Infants off Mechanical Ventilation

Weaning of infants off IPPV may be difficult in very immature infants who have poor and inconsistent respiratory drive, a weak respiratory pump, and immature and frequently damaged lungs.

This combination of factors explains why they frequently become ventilator dependent for long periods.

For many years, infants who were ventilated received most of their minute ventilation from the ventilator and were not allowed to have effective spontaneous ventilation. In recent years this has changed, and ventilators are used as an assist support, preserving the patient's respiratory drive and effort. This has been possible by the introduction of synchronized patient-triggered ventilation (PTV) and has resulted in better outcomes and shorter times for mechanical ventilation.

Because of the high rate of complications associated with prolonged mechanical ventilation, weaning should start as soon as mechanical ventilation is started and respiratory function is stabilized. The order in which the different ventilator parameters are decreased is determined by the relative risk of complications associated with each of them. With the possibility of measuring V_T , it has become much simpler to define the appropriate PIP that is required to generate an adequate V_T of 4 to 6 mL/kg body weight. As lung compliance increases, PIP can be reduced to keep the V_T within this range.

PEEP is usually kept between 4 and 8 cmH₂O depending on the type of underlying lung disease and the level of oxygenation and FiO_2 requirement. PEEP is decreased gradually as the oxygenation increases until a level of 4 to 5 cmH₂O is reached, and this level is maintained until extubation. The inspired oxygen concentration is adjusted according to the level of arterial oxygen tension or O_2 saturation measured by pulse oximetry. The ideal ranges of oxygenation have not been defined, but most clinicians accept saturations between 88% and 95% in preterm infants and up to 98% in term infants. In infants with evidence of pulmonary hypertension, higher levels are targeted to prevent pulmonary vasoconstriction.

If V_T measurement is not available, the reduction in PIP is based on the observation of chest movement, the degree of aeration on chest radiograph, and PaCO_2 levels. The ventilator rate is adjusted depending on the type of ventilation strategy being used. When the infant is ventilated with synchronized modes such as A/C mode or pressure support mode, the infant determines the rate of the mechanical breaths, so the set rate is only the backup that the ventilator will provide when the infant's own rate falls below that level. Therefore, this rate is relevant only when the infant becomes apneic or hypoventilates. When the infant is controlled, the rate in the ventilator is not determined by the infant, and the adjustment of the mechanical rate is based on the PaCO_2 level.

During weaning, it is advisable to make gradual changes and adjust one parameter at a time to evaluate the response of the infant to each change. With the availability of continuous oxygen and CO_2 monitoring, it is not always necessary to wait for the results of arterial gas measurement to change the ventilator settings, and the weaning can proceed faster.

Synchronized Patient-Triggered Ventilation for Weaning

The use of patient-triggered synchronized ventilation has become common practice in neonatal units. Most randomized trials comparing PTV with nonsynchronized ventilation have shown a reduction in the duration of mechanical ventilation in infants treated with synchronized modes (Greenough, 2001). This may be because during PTV, the infant retains more control of ventilation, and the effectiveness of the mechanical and spontaneous breaths is enhanced by the summation of the ventilator positive pressure and the negative pressure generated by the respiratory muscles.

A/C and SIMV are the most common modes of synchronized ventilation in the neonate. It has been suggested that assisting each spontaneous inspiration in A/C mode ventilation may avoid respiratory muscle fatigue and facilitate weaning. However, the duration of weaning has not been consistently shorter with A/C mode ventilation than with SIMV (Chan and Greenough, 1994; Beresford et al., 2000). On the other hand, a randomized trial comparing the use of PSV as an adjunct to SIMV to assist every spontaneous inspiration revealed a faster weaning and shorter duration of ventilation compared with SIMV alone in preterm infants (Reyes et al., 2006).

Volume Monitoring and Volume-Targeted Ventilation During Weaning

The continuous monitoring of V_T allows rapid reduction of PIP as the mechanical conditions of the lung improve. This can be achieved by a manual decrease of PIP as the V_T increases or automatically by use of volume-targeted ventilation where weaning is achieved automatically independent of the clinician, who only sets the V_T targeted by the ventilator. Evidence from randomized trials using volume-targeting strategies suggests that faster weaning off mechanical ventilation can be achieved, although the results have not been entirely consistent (Sinha et al., 1997; Singh et al., 2006).

Weaning of Infants off High-Frequency Ventilation

Infants ventilated with HFV are frequently switched to conventional ventilation before extubation. However, infants can be extubated directly from HFV following similar steps to those used to wean infants off conventional ventilation. The reduction in mean airway pressure is done following lung expansion by use of chest radiographs, while FIO_2 is adjusted to maintain the desired oxygenation levels.

The pressure amplitude is gradually reduced in response to the levels of PaCO_2 . If the mean airway pressure is reduced to low levels before the infant has spontaneous respiratory effort, this can lead to hypoventilation, loss of lung volume, and atelectasis. For this reason, it is advisable to lower the delta pressure and allow the PaCO_2 to rise and stimulate spontaneous respiration before the mean airway pressure is lowered below 8 or 10 cmH_2O .

Permissive Hypercapnia

Tolerance of higher carbon dioxide levels may reduce the need for support and reduce the duration of ventilation. However, the results of clinical trials have been inconsistent. Whereas initial trials suggested faster weaning of infants off mechanical ventilation in the group with higher CO_2 levels (Mariani et al., 1999; Carlo et al., 2002), more recent studies showed no benefit with regard to the duration of ventilation or BPD and a trend toward an increase in mortality and central nervous system impairment in infants with higher PaCO_2 (Thome et al., 2006, 2015). These results shed doubt on the benefits of maintaining high CO_2 levels in premature infants during the acute stages of their clinical course and also demonstrate that safe ranges of CO_2 levels have yet to be determined. However, in infants with chronic lung disease, it may be necessary to tolerate high CO_2 levels so as to wean them off mechanical ventilation.

Dead Space Reduction

The large anatomic dead space in preterm infants in addition to the instrumental dead space can also delay their weaning off mechanical ventilation (Figueras et al., 1997).

Continuous tracheal gas insufflations through small capillaries to the distal end of the ETT to produce a continuous washout can reduce arterial CO_2 and shorten the weaning process (Dassieu et al., 2000). This method is limited by the need for special ventilators and an ETT. Continuous washout of the flow sensor by a controlled gas leak can also increase CO_2 elimination and facilitate weaning (Claire et al., 2003; Estay et al., 2010).

Automated and Computer-Assisted Weaning

In the TMV mode described earlier, the ventilator rate is automatically reduced during periods of consistent spontaneous breathing where minute ventilation is maintained at or above the target level. In a study of preterm infants recovering from RDS, this experimental mode reduced the ventilator SIMV rate set by the clinical team by half while SpO_2 and TcPCO_2 remained unchanged (Claire et al., 1997). A similar reduction in rate was observed in near-term infants without lung disease when supported by MMV, a mode where the ventilator rate is transiently turned to zero when spontaneous minute ventilation exceeds a set level or delivers a set rate of volume-controlled breaths when minute ventilation decreases below this level (Guthrie et al., 2005).

Preterm infants often need supplemental O_2 , which increases their risk of eye and lung injury, particularly when exposure to oxygen is prolonged. In these infants, hyperoxemia is induced by an excessive FIO_2 , and therefore it is modifiable by appropriate weaning. Automated weaning of infants off supplemental oxygen was achieved by systems developed to adjust FIO_2 in a continuous manner. Systems of automated FIO_2 control have been shown to be as effective as or more effective than routine or dedicated manual control in maintaining oxygenation within a desired range, with most of the improvement due to significant reductions in hyperoxemia (Claire et al., 2009, 2011).

Ventilator management involves adjustments of several parameters that affect the infant's ventilation and gas exchange. Computerized algorithms for ventilator management have been proposed to achieve efficient and consistent weaning. In infants with RDS, ventilator management assisted by one of these algorithms led to improvements in gas exchange and avoided unnecessary increases in ventilator settings (Carlo et al., 1986). During routine care, hypoxemia and hypercapnia were more diligently corrected than hyperoxia and hypocapnia, whereas computer-assisted management was similarly effective in correcting both extremes. The potential beneficial effects of computer-assisted weaning on long-term outcome have not been explored.

Extubation

It is difficult to decide the best time for extubation in ventilated infants. It is obvious that many infants remain intubated for longer than necessary. This is evidenced by the fact that many infants who are accidentally extubated tolerate it well without needing further respiratory support. Various tools have been evaluated to predict successful extubation. These include measurement of lung mechanics, minute ventilation, inspiratory effort strength and ability to cope with mechanical loads, and stability of the respiratory pattern during periods when the ventilator cycling is stopped (Kamlin et al., 2006). Some of the tools have been shown to predict with some accuracy successful extubation, but these tests have not been widely accepted in neonatal clinical practice.

The decision to extubate an infant is usually based on the level of inspired oxygen and ventilator support that the infant is requiring to maintain acceptable arterial blood gas levels. In general terms, if an

infant needs less than 30% to 40% oxygen, a ventilator rate less than 15 per minute, and a PIP below 15 cmH₂O and maintains acceptable blood gas levels, most clinicians attempt extubation. The lower the gestational age, the more likely it is that the infant will not tolerate extubation and will require reintubation. In most cases this failure is because of poor respiratory effort or severe apneic episodes.

Nasal Continuous Positive Airway Pressure and Noninvasive Ventilation for Extubation

After extubation, the infant is exposed to a number of mechanical impediments that explain the frequent need for reintubation in the smaller preterm infants. These include upper airway damage and retained secretions leading to obstruction and atelectasis, loss of lung volume due to poor respiratory effort, and a highly compliant chest wall. For these reasons the use of CPAP applied through the nose can significantly reduce the deterioration that occurs frequently after extubation. Surprisingly, despite this improvement in respiratory function, the need for reintubation has not been consistently shown to be reduced by the use of N-CPAP after extubation (Davis and Henderson-Smart, 2003).

In contrast with the use of N-CPAP, the use of nasal ventilation after extubation has been shown to significantly reduce extubation failure (Davis et al., 2001; De Paoli et al., 2003). Although these studies have included small numbers of infants, the effects have been consistent. This is a promising therapeutic alternative that needs further evaluation and the development of suitable equipment to provide synchronized noninvasive support.

Use of an HFNC for respiratory support after extubation has been explored recently in randomized controlled trials, with most of the trials showing trends toward higher rates of extubation failure with use of an HFNC compared with use of CPAP (Manley, 2013; Yoder, 2013). It is important to note that many of these trials enrolled infants of higher gestational age and with lower risk of extubation failure. It is likely that infants with significant lung disease at the time of extubation will also benefit from use of CPAP or nasal ventilation.

Respiratory Stimulants for Extubation

Respiratory stimulants such as aminophylline and caffeine have been shown to be effective in increasing respiratory center activity in preterm infants and in decreasing the incidence of severe apneic episodes. These drugs have also been shown to facilitate successful weaning of infants off mechanical ventilation and to decrease the need for reintubation. For this reason most preterm infants receive a loading dose of caffeine or aminophylline before extubation, and they are maintained with these stimulants at least during the first few days after extubation while they are also maintained with CPAP therapy or nasal ventilation (Henderson-Smart and Davis, 2003).

Acute Complications of Respiratory Support

Pulmonary Gas Leaks

One of the most serious complications of mechanical ventilation is pulmonary gas leaks. These include pulmonary interstitial emphysema, pneumothorax, pneumomediastinum, pneumopericardium, pneumoperitoneum, and intravascular gas.

Pulmonary Interstitial Emphysema

PIE is the result of rupture of air spaces from overdistension. Although it may be seen in infants breathing spontaneously, it is more common in preterm infants undergoing mechanical ventilation



• **Fig. 45.7** Pulmonary Interstitial Emphysema. A grossly hyperinflated lung with coarse radiolucencies extending from the pleura to the hilum. These radiolucencies represent gas bubbles in the perivascular and peribronchial interstitial cuffs.

and in infants with evidence of pulmonary infection. Once alveolar rupture occurs, gas is forced from the air spaces into the connective tissue sheaths surrounding airways and vessels and into the interlobular septa containing pulmonary veins. The air follows a track along these sheaths to the hilum of the lung, producing the characteristic radiographic appearance of PIE (Fig. 45.7). The PIE occupies space within the lung parenchyma, decreasing lung compliance. Gas trapped within the interstitial cuffs compresses airways and increases airway resistance. In addition, gas in the interstitial space impairs lymphatic drainage, increasing the amount of interstitial fluid. This can lead to a significant deterioration in gas exchange, with an increase in PaCO₂ and a decrease in PaO₂.

The main cause of PIE is air space overdistension and rupture. Therefore minimization of pulmonary overdistension should reduce the risk of PIE. Shorter T_{is} also decrease the incidence of PIE by reducing overdistension and avoiding an inspiratory hold (Heicher et al., 1981; OCTAVE Study Group, 1991).

PIE is a serious complication of mechanical ventilation. Infants with PIE have a significantly increased risk of developing chronic lung disease as well as higher mortality rates (Gaylord et al., 1985; Powers and Clemens, 1993).

Because the gas leak can behave like a check valve, gas trapping occurs, resulting in further alveolar overdistension and rupture. Therefore the first step in treatment must be to interrupt this cycle by putting to rest the more severely involved areas in the lung. If PIE is unilateral, this can be accomplished by the positioning of the infant with the involved side down (Swingle et al., 1984). Mechanical ventilation using short T_{is} (0.1 to 0.2 seconds), low inflation pressures, and small V_Ts can reduce the gas leak (Meadow and Cheromcha, 1985). Unfortunately, it is difficult to maintain oxygenation and ventilation while low volumes are being used. A multicenter controlled trial found that HFJV allowed the use of lower peak and mean airway pressures in infants with PIE than did rapid-rate conventional ventilation and led to more rapid relief of PIE (Keszler et al., 1991).

Pneumothorax

Pneumothorax can occur spontaneously in healthy infants because of the very high transpulmonary pressures produced at birth. However, the incidence is much higher in the presence of underlying lung disease and in infants exposed to mechanical ventilation. Pneumothorax develops in 5% to 10% of spontaneously breathing infants with hyaline membrane disease. Although positive pressure ventilation increases the risk dramatically, treatment with surfactant has markedly lowered this risk.

Pneumomediastinum occurs when gas tracks through the perivascular and peribronchial cuffs to the hilum and then enters into the mediastinum. From there, gas can enter into the pleural space, producing a pneumothorax. If the leak is large, it produces a tension pneumothorax that collapses the lung and results in severe hypoxia and hypercapnia. In addition, by increasing intrathoracic pressure and compressing mediastinal structures, venous return to the heart may be impeded, resulting in circulatory compromise.

Because of the severe consequences of a tension pneumothorax, it is important to diagnose it as early as possible. In the spontaneously breathing infant, pneumothorax usually produces tachypnea, grunting, pallor, and cyanosis. The cardiac sounds may be shifted away from the side of the pneumothorax, and the affected hemithorax and abdomen may appear to be bulging. In the infant receiving positive pressure ventilation, the signs are frequently more dramatic, with sudden onset of hypoxemia and cardiovascular collapse (Ogata et al., 1976). Transillumination of the chest is positive over the affected side, and the pneumothorax is confirmed by chest radiograph that shows gas in the pleural space and partial collapse of the lung with a shift of the mediastinum to the opposite side (Fig. 45.8).

A small pneumothorax in a spontaneously breathing infant may be observed closely until spontaneous resolution occurs. Infants with larger symptomatic pneumothoraces and infants receiving positive pressure ventilation require thoracostomy and pleural tube

placement. The tube may be inserted at the midaxillary line and directed anteriorly or may be placed in the second intercostal space in the midclavicular line and directed toward the diaphragm so that the tip lies between the lung and the anterior chest wall. When placing the tube, the operator must avoid puncturing the lung, especially when a trocar, rather than a curved hemostat, is used to direct the tube. It also is possible to drain pneumothoraces with pigtail catheters that are placed percutaneously into the pleural space. This technique produces less trauma but may not be effective when large gas leaks are present. The thoracostomy tube is connected to a water seal with 10- to 20-cmH₂O negative pressure and is left in place until draining ceases. Application of the negative pressure should be discontinued, and the tube should be left under the water seal for 12 to 24 hours before removal to ensure that the gas leak has stopped.

Pneumopericardium

Pneumopericardium results from direct tracking of interstitial gas along the great vessels into the pericardial sac. Gas under tension in the pericardium can impair atrial and ventricular filling, decrease stroke volume, and ultimately decrease cardiac output and systemic blood pressure. Infants have increasing hypoxemia, decreased heart sounds, and decreased systemic blood pressure and pulse pressure. The chest radiograph is diagnostic, showing gas surrounding the cardiac silhouette (Fig. 45.9). Needle aspiration alleviates the acute symptoms, but because the recurrence rate is high, continuous tube drainage is necessary in most cases.

Pneumoperitoneum

Pneumoperitoneum results from dissection of air from the mediastinum along the sheaths of the aorta and vena cava into the peritoneal cavity. Infants with this condition experience sudden abdominal distention and have a typical abdominal radiograph. Occasionally, the pneumoperitoneum may be large enough to cause respiratory embarrassment by compromising descent of the diaphragm and may require drainage. This cause of peritoneal free gas must be distinguished from a primary gastrointestinal perforation, which is much more common.



• **Fig. 45.8** Tension Pneumothorax. The lung on the involved side is collapsed, and the mediastinum is shifted to the opposite side, with bulging of the pleura into the intercostal spaces.



• **Fig. 45.9** Pneumopericardium. A thin rim of pericardium is visible and clearly separated from the heart by gas within the pericardial sac.

Intravascular Gas

It has been suggested that the intravascular gas is pumped under high pressure through the pulmonary lymphatics into the systemic venous circulation (Booth et al., 1995). The intravascular gas results in immediate cardiovascular collapse and is often diagnosed when gas is seen in vessels or in the heart chambers when a chest radiograph is taken to determine the cause of cardiovascular collapse. Intravascular gas is usually a fatal complication.

Airway Complications

Prolonged endotracheal intubation can produce airway damage and subglottic stenosis. The risk is increased by a too snugly fitting ETT, prolonged duration of intubation, and traumatic intubation. Some infants may require tracheostomy and even surgery to repair the stenosis. Inadequate humidification of the inspired gas can produce necrotizing tracheobronchitis, a necrotic inflammatory process involving the trachea and main bronchi. Infants have acute respiratory deterioration due to airway obstruction, hyperexpansion on chest radiograph, and poor chest movement. Emergency bronchoscopy may be necessary to relieve airway obstruction.

Lobar or segmental atelectasis frequently occurs during prolonged ventilation and after extubation from mechanical ventilation. This can be secondary to airway injury due to trauma produced by suction catheters, by inadequate conditioning of the inspired gas, and by airway obstruction due to retained secretions.

Ventilator-Associated Pneumonia

Ventilator-associated pneumonia is a very common complication of mechanical ventilation at any age, and it is likely to occur even more frequently in the immune-compromised premature infant. Unfortunately, it is not commonly diagnosed in this population because of the lack of specific criteria and the difficulty of differentiating pneumonia from other acute and chronic pulmonary diseases observed in infants requiring prolonged mechanical ventilation. Despite these limitations, it should be suspected whenever there is deterioration in lung function with radiographic changes suggestive of pneumonia. Change in the amount and quality of the secretions obtained from the airway and colonization with pathogens is another indication of possible pulmonary infection that may require antibiotic therapy.

Some evidence indicates that a large proportion of ventilated premature infants have aspiration of gastric contents into their airways. This is more common in fed infants and those receiving methylxanthines (Farhath et al., 2006). The consequences of this are not clear, but this could further contribute to the risk of pulmonary infection and chronic lung damage.

Ventilator- and Oxygen-Associated Neurologic Sequelae

Prolonged need for mechanical ventilation and oxygen supplementation has been associated with increased risk of NDI in premature infants (Collins et al., 2001; Walsh et al., 2005). Although these associations may be in part determined by the fact that infants with worse respiratory course are also those with worse neurologic outcome, there is a considerable amount of data suggesting that NDI may be in part mediated by excessive hyperoxia and hypocapnia or fluctuating arterial levels of oxygen and carbon dioxide (Fabres et al., 2007; Di Fiore et al., 2010; Pappas et al., 2011; Poets et al.,

2015). Periventricular white matter injury is among the causes of NDI in premature infants. Postmortem studies showed elevated levels of markers of oxidative stress and cellular damage in infants with periventricular leukomalacia (Haynes et al., 2003). The white matter vulnerability to oxidant damage in the preterm infant has been attributed to selective lipid peroxidation and death of oligodendrocyte progenitors (Back et al., 2005). As indicated by animal experiments, the interaction of these conditions with genetic and molecular mechanisms could lead to significant brain injury (Albertine et al., 2012).

Future Directions

Although neonatal respiratory support has advanced considerably in the last few decades, there are still numerous areas that need improvement.

Respiratory monitoring could be significantly enhanced by advances in noninvasive monitoring of blood gases, both arterial and venous, as well as monitoring of brain and organ oxygenation and perfusion and hemodynamics. A promising area is that of development of noninvasive methods to assess lung volume and perfusion that until this point have been limited to complex techniques that have been primarily used in research studies or invasive clinical procedures.

With increased awareness of the importance of adequate maintenance of oxygenation and ventilation, development of more reliable and smarter alarms could reduce alarm fatigue and increase staff attentiveness.

Noninvasive modes of support could be advanced by more patient-friendly yet effective interfaces as well as by the more general availability of simple techniques to provide synchronized ventilation to the extremely preterm infant.

Significant advances have already occurred in regard to automation of ventilatory support and oxygen supplementation. Still, there is a need to fully assess the impact and safety of these new devices on important neonatal outcomes. The availability of these automated systems may facilitate the determination of optimal oxygenation and ventilation levels for infants of different gestational and postnatal ages as well as for the different respiratory conditions.

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46

Respiratory Disorders in the Preterm Infant

J. CRAIG JACKSON

KEY POINTS

- In extremely preterm newborns with respiratory distress, respiratory distress syndrome (RDS) is by far the most common diagnosis.
- The development of exogenous surfactant for treatment of RDS is one of the most important advances of newborn medicine.
- Despite widespread consensus on the antenatal use of steroids, controversies remain, including the type of steroid, the dose, frequency and timing, and the route of administration.
- Application of nasal continuous positive airway pressure (CPAP) started at or soon after preterm birth may be considered an alternative to routine endotracheal intubation with early surfactant administration.
- Severe hypercarbia immediately after birth suggests one of the following diagnoses: pulmonary hypoplasia, tension pneumothorax, congenital diaphragmatic hernia, or severe airway obstruction.
- When preterm prolonged rupture of membranes occurs at 15 weeks, the incidence of pulmonary hypoplasia is 80%; when it occurs at 19 weeks, the incidence is 50%, and when it occurs after 26 weeks, the incidence is nearly zero.
- Pulmonary hemorrhage occurs in 3%–5% of preterm infants needing respiratory support, usually at 1 to 3 days of age.

This chapter on respiratory distress in the preterm infant will focus primarily on RDS, also known as *hyaline membrane disease*. However, it also includes information about two less frequent causes: pneumonia/sepsis and pulmonary hypoplasia. Other causes of respiratory distress that are more commonly seen in term infants are briefly mentioned since they are discussed in greater depth in Chapter 47. Table 46.1 lists the most common causes of respiratory distress in newborns and demonstrates the relative frequency of each diagnosis. This table provides a useful differential diagnosis for respiratory distress in preterm neonates, but polycythemia and hypoglycemia should also be considered. More than half of extremely low birth weight newborns will have some type of respiratory distress (Fig. 46.1). In that population, RDS is by far the most common diagnosis (51%), followed by transient tachypnea of the newborn (TTNB; 4%) and pneumonia/sepsis (2%). In higher birth weight preterm newborns, the incidence of any type of respiratory distress is much lower, but the proportion with TTNB increases with higher

birth weight. Compared with the incidence of three diagnoses featured in Fig. 46.1, the incidence of other causes of respiratory distress (Table 46.1) is very low in preterm infants, but the causes should be considered in those newborns with an atypical clinical course.

Respiratory Distress Syndrome

Risk Factors

The main risk factor for RDS, by far, is prematurity (see Fig. 46.1). Other factors that increase the risk of RDS include perinatal asphyxia, maternal diabetes, absence of labor, absence of prenatal steroid administration to the mother, male sex, and white race. The central feature of RDS is surfactant deficiency due to lung immaturity, commonly a result of premature birth or delayed lung maturation associated with maternal diabetes or male sex. Surfactant dysfunction can also be caused by genetic abnormalities of surfactant-associated proteins, perinatal asphyxia, pulmonary infection, or excessive fetal lung liquid due to delivery without labor.

Pathophysiology of Respiratory Distress Syndrome

Because alveoli with insufficient (or dysfunctional) surfactant are unstable and tend to collapse, patients with RDS develop generalized atelectasis, ventilation–perfusion mismatching, and subsequent hypoxemia and respiratory acidosis.

During breathing (either spontaneous or assisted), shear stresses in the alveoli and terminal bronchioles occur because of the repetitive reopening of collapsed alveoli and the overdistention of open alveoli (Nilsson et al., 1978). These forces can quickly damage the fragile lung architecture, leading to leakage of proteinaceous debris into the airways (i.e., hyaline membranes). This debris (Fig. 46.2) may impair the function of what little surfactant is present, leading to a downward spiral that may end in respiratory failure and death if not interrupted.

If supportive therapy is successful, the repair phase usually begins during the second day after birth with the appearance of macrophages and polymorphonuclear cells (Jackson et al., 1987). Debris is phagocytosed, and the damaged epithelium is regenerated.

Edema fluid in the interstitium is mobilized into lymphatics, leading to the diuretic phase of RDS characterized by high urine output.

With uncomplicated RDS, the patient's condition improves by the end of the first week after birth. However, infants with a birth weight less than 1250 g and larger newborns needing high concentrations of oxygen and positive pressure ventilation

for severe RDS may develop inflammation and inappropriate repair of the growing lung, leading to emphysema and fibrosis (see Chapter 48).

Purpose of Surfactant

Surface tension is generated from molecular attractive forces within a liquid that oppose spreading; this is the reason that water “beads up” on a clean surface. If you try to inflate a bubble under water with a straw (Fig. 46.3A), the spreading of the water's surface and enlargement of its surface area will be opposed by its surface tension (T). According to Laplace's law, the pressure (P) required to inflate the bubble is proportional to T divided by the radius of the bubble (r). If you simultaneously try to inflate two interconnected bubbles, the smaller one will deflate into the larger one, because of its smaller radius (Fig. 46.3B).

Surfactants are surface-active materials that lower surface tension; a detergent is a type of surfactant that causes water to spread out on a surface rather than bead up. The surface tension of clean water resists the creation of bubbles, but the addition of detergent allows bubbles to form. Because the surfactant properties of detergents do not persist, all of the small bubbles in the foam eventually collapse into the large ones, and finally even the large ones collapse.

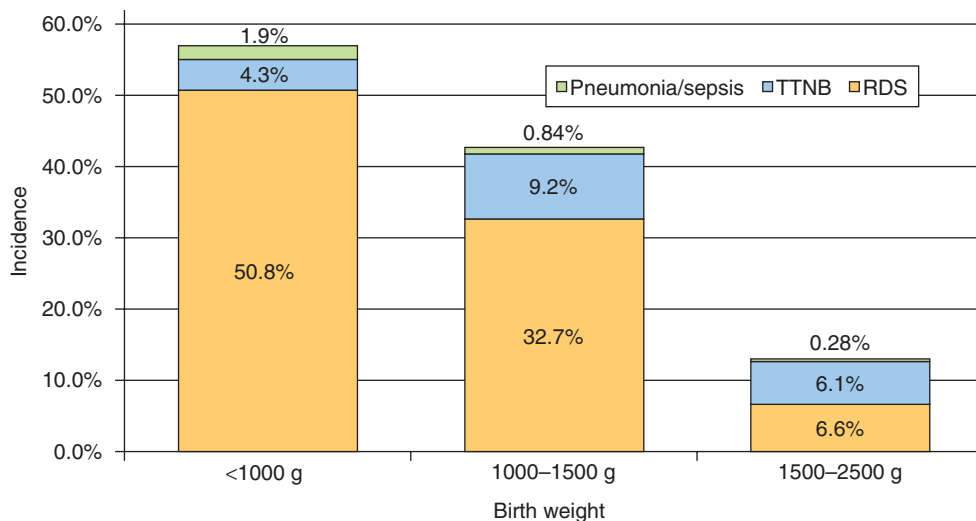
Lung surfactant has the miraculous property of reducing surface tension as the size of the bubble decreases. If the surface tension remained high, the pressure required to keep smaller alveoli open as their radius decreased would be greater than the pressure keeping open larger alveoli, so the small alveoli would collapse into larger ones, as shown in Fig. 46.3B. However, if the bubbles are lined with high-quality surfactant, the surface tension falls quickly as the radius gets smaller because the surfactant molecules become crowded during deflation (Fig. 46.4). When the radius is very small, the surface tension falls almost to zero, and the pressure required to keep the smaller bubble open is negligible, and thus it does not collapse.

During inflation, as the radius of each alveolus increases, surface tension increases even faster. This means that the pressure in the larger alveoli will be higher than that in smaller ones; in the lung

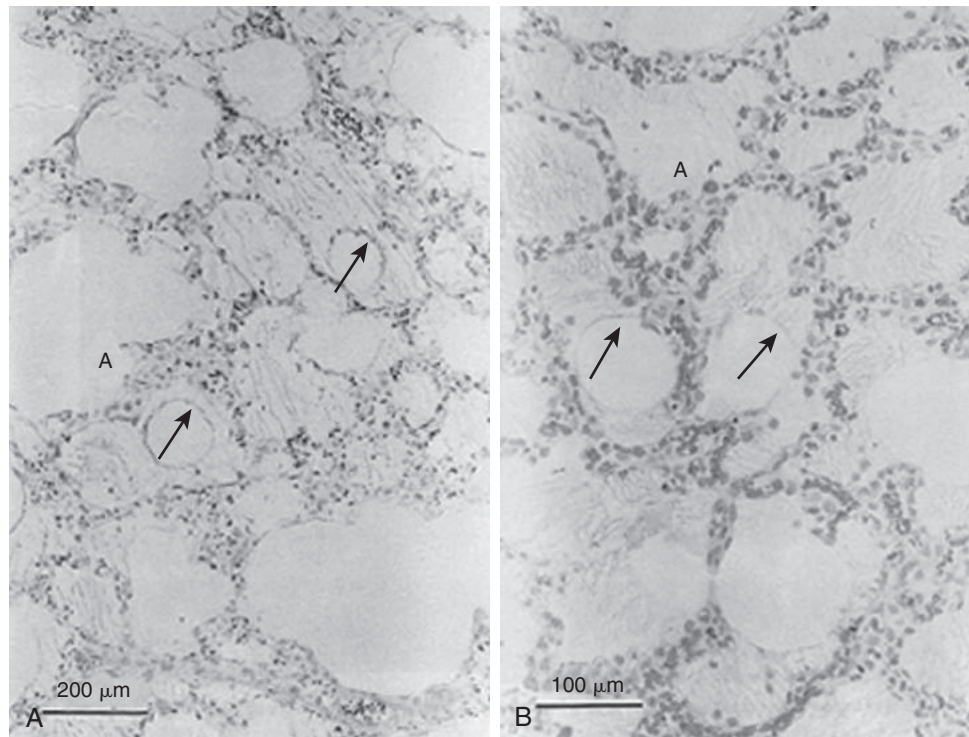
TABLE 46.1 Causes of Respiratory Distress

| Cause | Frequency % |
|--|-------------|
| Respiratory distress syndrome | 46 |
| Transient tachypnea of newborn | 37 |
| Pneumonia/sepsis | 5 |
| Meconium aspiration syndrome | 2 |
| Congenital cardiac malformation | 2 |
| Chromosomal disorder/multiple congenital anomalies | 1.4 |
| Spontaneous pneumothorax | 1.2 |
| Perinatal asphyxia | 1.1 |
| Pulmonary hemorrhage | 1.0 |
| Persistent pulmonary hypertension | 0.8 |
| Diaphragmatic hernia | 0.8 |
| Apnea of prematurity | 0.6 |
| Pulmonary hypoplasia | 0.3 |
| Pulmonary dysplasia | 0.2 |
| Hydrothorax | 0.2 |
| Postsurgical diaphragmatic palsy | 0.2 |

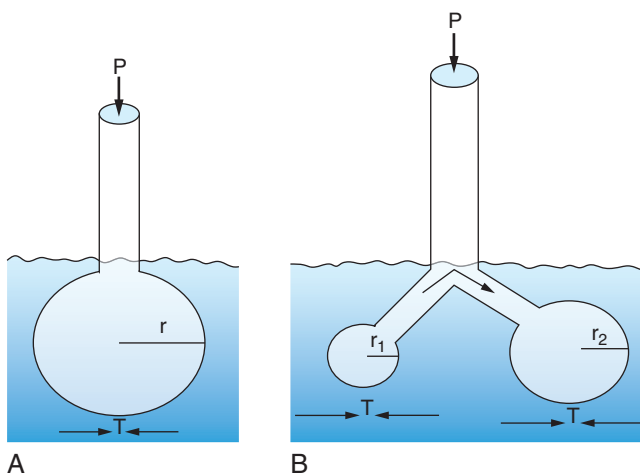
Data from Rubaltelli FF, Dani C, Reali MF, et al. Acute neonatal respiratory distress in Italy: a one-year prospective study. *Acta Paediatr.* 1998;87:1261–1268.



• **Fig. 46.1** Incidence of Respiratory Problems in Preterm Newborns. RDS, Respiratory distress syndrome; TTNB, transient tachypnea of the newborn. (Data from Rubaltelli FF, Dani C, Reali MF, et al. Acute neonatal respiratory distress in Italy: a one-year prospective study. *Acta Paediatr.* 1998;87:1261–1268.)



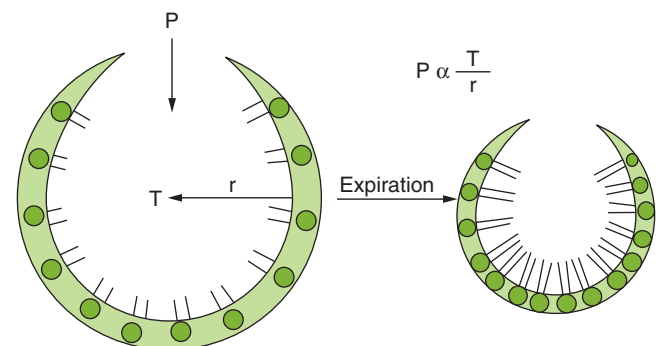
• **Fig. 46.2** Photomicrographs of lung tissue from an experimental animal with respiratory distress syndrome, flash frozen during inflation. Note the liquid–air interface (arrows). The alveolar debris forms hyaline membranes. (A) Low power. (B) Higher power. (Reproduced with permission of the American Thoracic Society. Copyright © 2017 American Thoracic Society. From Jackson JC, MacKenzie AP, Chi EY, et al: Mechanisms for reduced total lung capacity at birth during hyaline membrane disease in premature newborn monkeys, *Am Rev Respir Dis* 142:413–419, 1990. The *American Review of Respiratory Disease* is an official journal of the American Thoracic Society.)



• **Fig. 46.3** Effect of Surface Forces Generated by Inflation of Bubbles Under Water. (A) A single bubble of radius r resists inflation and thus requires pressure P to overcome the surface tension T . (B) If the surface tension is the same in two bubbles of unequal size, the smaller one will collapse into the larger one, because of the Laplace relationship, $P = T/r$ (small r requires higher pressure for the bubble to stay inflated).

where alveoli are interconnected, this pressure difference will cause flow *to* the smaller alveoli, thus keeping all the alveoli about the same size.

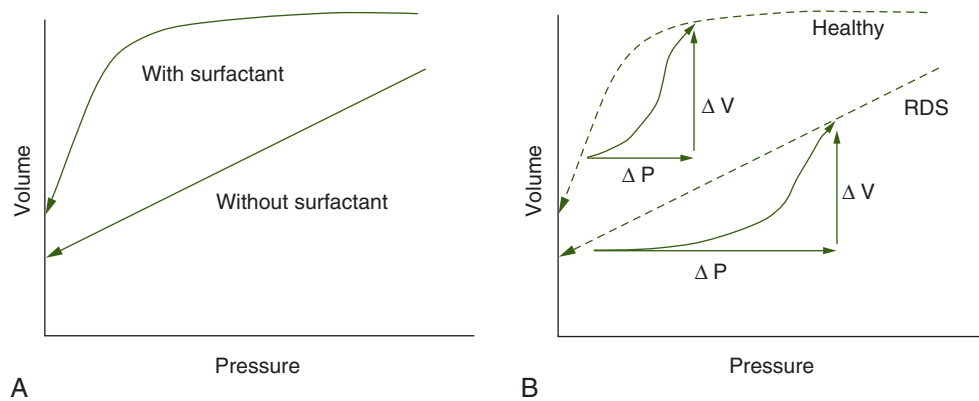
The cumulative result of the alveoli remaining open during deflation is a nonlinear pressure–volume relationship. The lung



• **Fig. 46.4** Surfactant Molecules on Surface Crowding During Deflation. (From Jackson JC. Respiratory distress syndrome. In: Osborn LM, DeWitt TG, First LR, Zenil JA, eds. *Pediatrics*. Philadelphia, PA: Mosby; 2005: 1402–1404.)

with sufficient surfactant retains gas during expiration, compared with rapid and almost complete loss of gas in the surfactant-deficient lung (Fig. 46.5A). During inflation, more pressure is required to achieve a similar tidal volume (see Fig. 46.5B), because of poor compliance ($\Delta V/\Delta P$) from having to reopen collapsed alveoli. For instance, to achieve a tidal volume of 5 mL/kg, an infant with RDS may require a pressure increase of 25 cmH₂O; dividing the volume change, ΔV , by the pressure change, ΔP , we calculate that the compliance is only 0.25 mL/kg per centimeter of water, which is about one-third of normal.

In the absence of adequate amounts of functional surfactant in the newborn lung, there is widespread alveolar collapse with



• **Fig. 46.5** Effect of Surface Forces on Pressure-Volume Relationships. During deflation (A) the lungs with surfactant retain gas even at very low pressures, because of falling surface tension as the alveoli get smaller. The alveoli without surfactant collapse as they get smaller. During inflation of respiratory distress syndrome (RDS) lungs (B), the starting lung volume (functional residual capacity) is lower, and much more pressure is required during inflation, compared with healthy lungs.

overdistention of open alveoli (Fig. 46.6). Because reopening collapsed alveoli requires high pressure, the spontaneously breathing newborn with surfactant deficiency must generate highly negative intrathoracic pressure. Clinically, this is manifested by retractions of the chest wall and use of accessory muscles during inspiration. Newborns may also attempt to prevent alveolar collapse by grunting. This partial closure of the glottis during expiration helps maintain an end-expiratory pressure that may keep some unstable alveoli open.

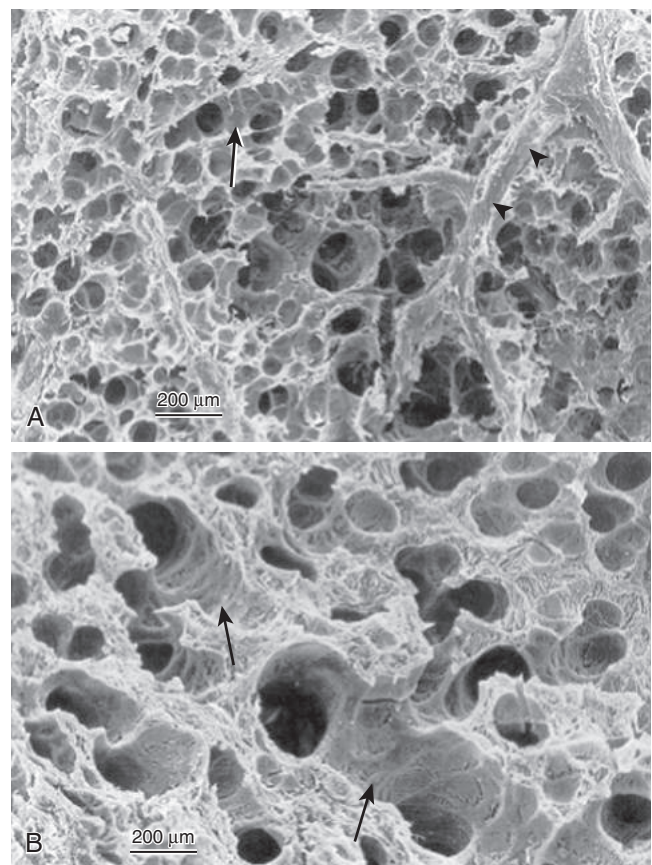
A consequence of widespread alveolar collapse is intrapulmonary shunting of blood past nonaerated lung tissue, without the opportunity for blood in pulmonary capillaries to pick up oxygen from, or deliver carbon dioxide to, the alveoli. In addition, lungs that are poorly inflated have partially collapsed intrapulmonary vessels, leading to pulmonary hypertension. The elevated pulmonary artery pressures lead to right-to-left shunting of unoxygenated blood across the patent ductus arteriosus to the descending aorta (see Chapter 52).

Origin and Composition of Surfactant

Pulmonary surfactant is composed of approximately 90% lipids and 10% proteins. The main phospholipid in surfactant is dipalmitoylphosphatidylcholine (DPPC), also known as *lecithin*. It is surface active because of its hydrophilic head and hydrophobic tails (Fig. 46.7). However, DPPC by itself does not adsorb efficiently at the air-liquid interface and is in the form of a gel at body temperature. The presence of some unsaturated phospholipids and cholesterol helps to make it more fluid (Mingarro et al., 2008).

The other main ingredients of surfactant are shown in Fig. 46.8. One of them, phosphatidylglycerol, is sometimes used as a marker of lung maturation; it interacts with the hydrophobic surfactant proteins to increase biophysical activity. Even minor components of pulmonary surfactant play important roles; for instance, free fatty acids increase the stability of the interfacial film, especially after repeated compression. The composition of surfactant is complex because it has evolved to balance the need for low viscosity for optimal spreading and redistribution along the smallest airways with the need for a stable and low surface tension.

Surfactant phospholipids are assembled in the type II pneumocytes of the lung epithelium into lamellar bodies in the form



• **Fig. 46.6** Histology of Respiratory Distress Syndrome. Scanning electron micrograph of lung frozen during inflation with air in a healthy premature monkey (A) compared with one with respiratory distress syndrome (B). Lungs affected by respiratory distress syndrome have collapsed alveoli full of liquid and proteinaceous debris, with overdistended terminal airways. (Reproduced with permission of the American Thoracic Society. Copyright © 2017 American Thoracic Society. From Jackson JC, Truog WE, Standaert TA, et al: Effect of high-frequency ventilation on the development of alveolar edema in premature monkeys at risk for hyaline membrane disease, *Am Rev Respir Dis* 143:865–871, 1991. The *American Review of Respiratory Disease* is an official journal of the American Thoracic Society.)

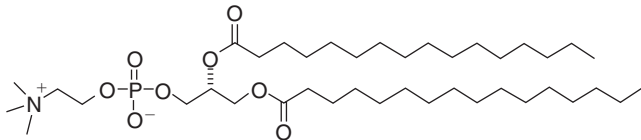
of bilayer membranes (Fig. 46.9). Surfactant-associated protein B (SP-B) and surfactant-associated protein C (SP-C) are essential for the transition to a monolayer at the air–liquid interface. The molecular structure of the hydrophobic SP-B is complex, and it interacts with the phospholipid monolayer as shown in Fig. 46.10. Its absence is associated with fatal neonatal respiratory failure. Surfactant-associated protein A and surfactant-associated protein D are hydrophilic and have roles in immune defense. Surfactant-associated protein A is also involved in reuptake and reuse of secreted surfactant (see Chapter 42).

Clinical Signs of Respiratory Distress Syndrome

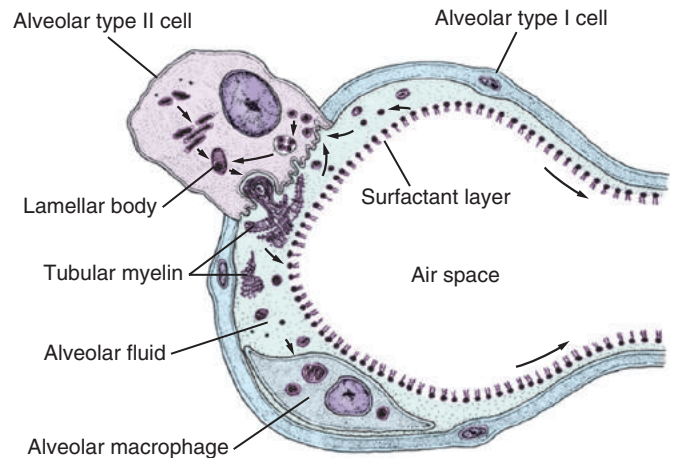
The cardinal clinical signs of RDS are tachypnea, grunting, and increased work of breathing (Box 46.1). The respiratory rate is elevated in an attempt to increase the exchange of oxygen and carbon dioxide, but with exhaustion, the rate may decline or even become zero. The newborn may grunt to create positive pressure in the lungs in an attempt to prevent collapse of air sacs. Signs of increased work of breathing include nasal flaring and retraction of the chest wall from vigorous use of the intercostal and subcostal muscles and use of accessory muscles in the neck. Because the rib cage in premature infants is so flexible, the sternum may deeply retract during inspiration, with abdominal distention, leading to a “see-saw” type of respiration. Cyanosis results from inadequate

• BOX 46.1 Clinical Signs of Respiratory Distress Syndrome

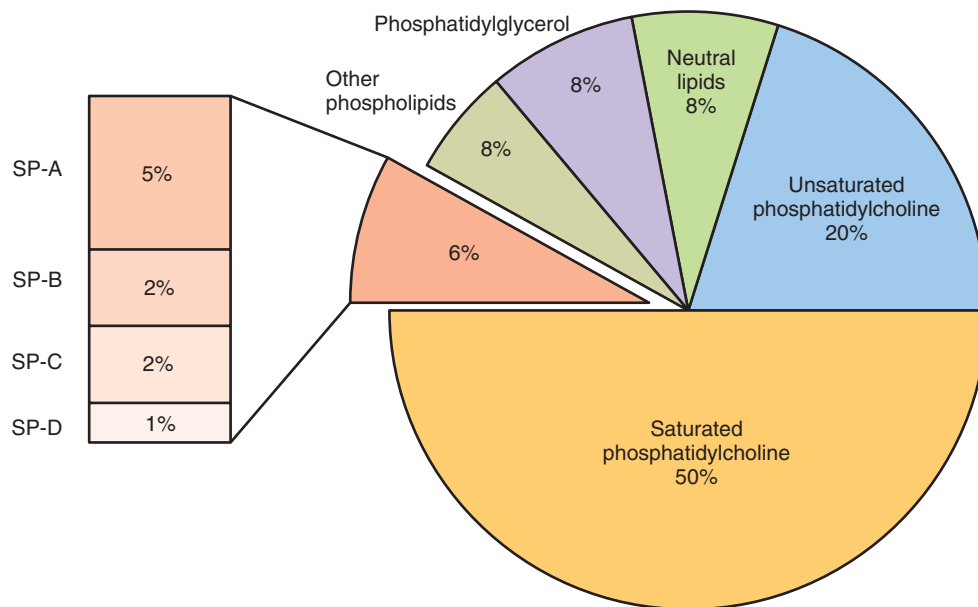
Tachypnea
Grunting
Increased work of breathing
Nasal flaring
Retraction of respiratory muscles (intercostal, subcostal, sternal)
Cyanosis
Pallor
Lethargy
Disinterest in feeding
Apnea



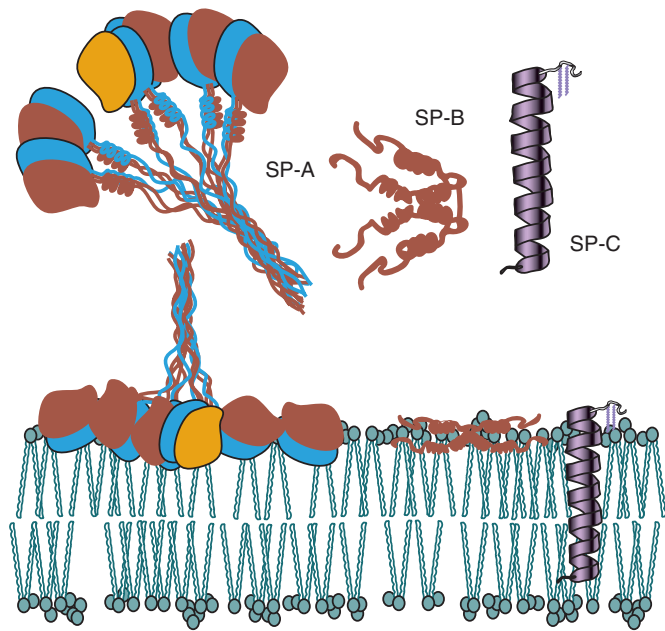
• Fig. 46.7 The main ingredient of lung surfactant, dipalmitoylphosphatidylcholine.



• Fig. 46.9 Assembly of Surfactant. (Modified from Hawgood S, Clements JA. Pulmonary surfactant and its apoproteins. *J Clin Invest*. 1990;86:1–6.)



• Fig. 46.8 Composition of Surfactant. SP-A, Surfactant-associated protein A; SP-B, surfactant-associated protein B; SP-C, surfactant-associated protein C; SP-D, surfactant-associated protein D. (From Jobe AH, Ikegami M. Biology of surfactant. *Clin Perinatol*. 2001;28:655–669.)



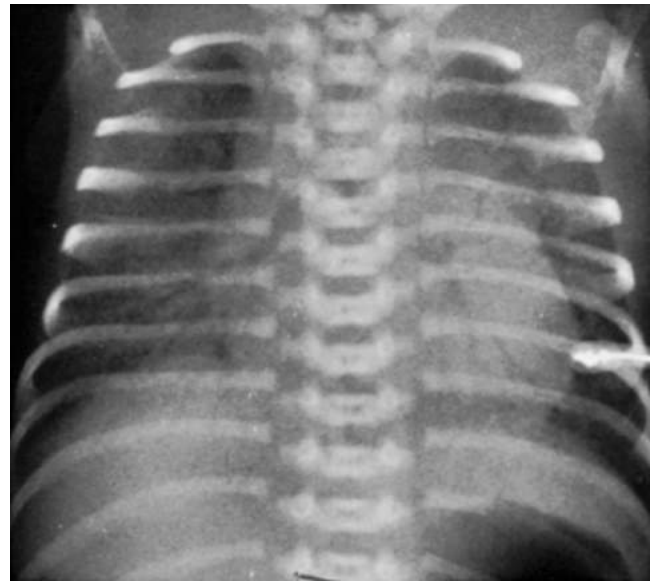
• **Fig. 46.10** Structure and interactions of surfactant and proteins. SP-A, Surfactant-associated protein A; SP-B, surfactant-associated protein B; SP-C, surfactant-associated protein C. (From Pérez-Gil J. Molecular interactions in pulmonary surfactant films. *Biol Neonate*. 2002;81:6–15.)

oxygenation, and pallor results from acidosis due to poor elimination of carbon dioxide. The combination of increased work of breathing, cyanosis, and acidosis causes lethargy and disinterest in feeding and eventually apnea. Rather than progressing through these signs during the first hours of life, newborns with intrapartum asphyxia or extreme prematurity may have apnea immediately after birth.

On auscultation, breath sounds may be distant or shallow from the fast inspiratory rate and low tidal volume, and fine inspiratory rales may be heard because of the reopening of moist, collapsed small airways. The onset of symptoms is always within hours after birth and, in severe cases, may occur with the first few breaths after delivery. In general, the respiratory distress from RDS tends to get worse in the first 1 to 3 days after birth and then usually abates gradually in a few days (although the natural course may be interrupted by exogenous surfactant therapy or application of CPAP).

Differential Diagnosis

Clinical improvement during the first 12 hours after birth suggests TTNB, whereas onset of respiratory distress after the first 24 hours suggests pneumonia and sepsis. Tachypnea without increased work of breathing suggests cyanotic heart disease; the diagnosis of obstructed pulmonary veins from total anomalous venous return is occasionally confused with RDS. Hypoventilation without increased work of breathing suggests a central nervous system problem such as intracranial hemorrhage or asphyxia. Asymmetric breath sounds may be due to pneumothorax (which is a complication of RDS), congenital diaphragmatic hernia, or unilateral pleural effusion. Meconium staining of amniotic fluid suggests the possibility of meconium aspiration syndrome, but this is rare in premature infants—green-stained amniotic fluid in this population is more likely to be due to bile refluxed into the esophagus because of intestinal obstruction rather than meconium.



• **Fig. 46.11** Radiograph of a patient with respiratory distress syndrome. Note low lung volumes and a reticulogranular pattern. (From Welty S, Hansen TN, Corbet A. Respiratory distress in the preterm infant. In: Taeusch HW, Ballard RA, Gleason CA, eds. *Avery's Diseases of the Newborn*. 8th ed. Philadelphia, PA: Elsevier; 2005:687–703.)

Laboratory Features of Respiratory Distress Syndrome

Initially the arterial blood gases will show hypoxemia, and the oxygen saturation monitor will show desaturation unless supplemental oxygen is provided. PaCO_2 may be normal because of tachypnea but is almost always elevated. Later, as the infant tires, PaCO_2 will rise further and cause respiratory acidosis. With imminent respiratory failure, there may be progressive metabolic acidosis due to inadequate oxygen delivery to tissues from poor peripheral perfusion.

Differential Diagnosis

Extremely elevated PaCO_2 within minutes of birth suggests pulmonary hypoplasia, tension pneumothorax, congenital diaphragmatic hernia, or obstruction of the airways due to debris or an anatomic cause. The tachypneic, cyanotic newborn with low PaCO_2 may have TTNB or cyanotic congenital heart disease. A positive blood culture result suggests pneumonia and sepsis. Low blood glucose level (<40 mg/dL) suggests symptomatic hypoglycemia, and high hematocrit ($>65\%$) suggests symptomatic polycythemia (Uslu et al., 2011).

Radiographic Features of Respiratory Distress Syndrome

The classic radiographic findings of RDS include a reticulogranular (i.e., ground-glass) pattern and air bronchograms (Fig. 46.11). The lungs are diffusely and homogeneously dense because of widespread collapse of alveoli. The appearance is reticular (i.e., netlike) because the small airways are open (black) and surrounded by interstitial and alveolar fluid (white); in severe cases the lungs may appear completely white on the film. Air bronchograms are commonly seen because the large airways beyond the second or third generation are more visible than usual as a result of radiodensity

• BOX 46.2 Initial Diagnostic Evaluation for Possible Respiratory Distress Syndrome

Arterial or capillary blood gas
Blood glucose
Complete blood count
Blood culture
Anteroposterior and lateral chest radiograph
Echocardiogram (if clinically indicated)

from engorged peribronchial lymphatics and fluid-filled or collapsed alveoli. Another cardinal feature is low lung volume (e.g., the diaphragms are at the eighth rib level or higher) due to widespread alveolar collapse and low functional residual capacity.

Differential Diagnosis

Normal or high lung volumes, especially with prominent interstitial fluid pattern, suggest TTNB. In this case there are coarse white lines (engorged lymphatics and interstitial water) radiating from the hilum rather than the crisp black lines (air bronchograms) of RDS. Other causes of a coarse (rather than diffuse) fluid pattern include pneumonia with sepsis and obstructed pulmonary venous drainage due to total anomalous pulmonary venous return. An abnormal cardiac silhouette or size should suggest congenital heart disease, and asymmetry of the lungs suggests pneumothorax, congenital diaphragmatic hernia, or congenital pulmonary airway malformation. Very low lung volumes, especially when accompanied by pneumothorax, may indicate pulmonary hypoplasia.

Initial Diagnostic Evaluation for Possible Respiratory Distress Syndrome

The initial diagnostic evaluation (Box 46.2) is shaped by the differential diagnoses listed in Table 46.1 and by the urgency to intervene quickly for serious but infrequent conditions such as bacterial pneumonia and sepsis, hypoglycemia, and polycythemia. It is often helpful to obtain both anteroposterior and lateral radiographs for the initial evaluation. If congenital heart disease is suspected on clinical grounds, an echocardiogram is indicated.

Treatment of Patients With Respiratory Distress Syndrome

The treatment of patients with RDS is outlined in Box 46.3. All patients with RDS need the basics of warmth, hydration, and nutrition appropriate for the degree of prematurity, as described in other chapters. Because pulmonary edema contributes to surfactant dysfunction in RDS, it is important to avoid excessive intravenous fluid administration.

Newborns with significant tachypnea (e.g., more than 60 breaths per minute) or increased work of breathing (moderate or severe) often do not have the energy required for oral feeding, and there is some risk of aspiration if nipple feeding is attempted. Initially, administration of intravenous fluids and nothing by mouth status may be appropriate, with consideration of small gavage tube gastric feedings if the baby is otherwise stable. Parenteral nutrition may be indicated because of the increased caloric expenditures associated with work of breathing. Administration of antibiotics should be considered unless the risk of pneumonia and sepsis is negligible

• BOX 46.3 Treatment of Established Respiratory Distress Syndrome

Warmth by radiant warmer or incubator
Hydration at approximately 60–80 mL/kg per day
Nutrition
Initially D5W or D10W (with protein, if possible)
Nothing by mouth if respiratory rate more than 60 breaths per minute or if moderate/severe work of breathing
Gavage feeds if stable
Consider parenteral nutrition if enteral feeds are delayed
Antibiotics if at risk of pneumonia and sepsis
Supplemental oxygen
Oxygen saturation monitoring, with appropriate target for infants at risk of retinopathy of prematurity
Exogenous surfactant
CPAP or mechanical ventilation, as needed

CPAP, Continuous positive airway pressure; D5W, dextrose 5% in water; D10W, dextrose 10% in water.

(e.g., premature delivery for maternal indications with no risk factors for chorioamnionitis).

Because exogenous surfactant and CPAP are used for both prevention and treatment of RDS, they are described in the Prevention and Treatment of Respiratory Distress Syndrome section below. See Chapter 45 regarding the risks and benefits of different modes of respiratory therapy.

Respiratory Complications of Respiratory Distress Syndrome

Air leak complications occur in patients with RDS because of the asymmetry of alveolar inflation and the sheer stresses in terminal bronchioles, leading to dissection of air into the interstitium (causing pulmonary interstitial emphysema) and through the visceral pleura (causing pneumothorax). The former can be seen in up to 50% of patients with RDS, and the latter can be seen in 5%–10%, even in those treated with exogenous surfactant.

Pulmonary hemorrhage occurs in about 3% of preterm infants needing respiratory support (Scholl and Yanowitz, 2015). For more information, see Pulmonary Hemorrhage at the end of this chapter.

Bronchopulmonary dysplasia (BPD), also known as *chronic lung disease*, may be exacerbated by abnormal lung repair following lung injury from RDS in the setting of interrupted normal lung development compounded by lung inflammation (see Chapter 48).

Prevention and Treatment of Respiratory Distress Syndrome

Prenatal administration of steroids was shown in 1972 to be effective in reducing the risk of RDS (Liggins and Howie, 1972). A consensus panel convened by the National Institutes of Health concluded that “antenatal corticosteroid therapy is indicated for women at risk of premature delivery with few exceptions and will result in a substantial decrease in neonatal morbidity and mortality, as well as substantial savings in health costs” (NIH Consensus Development Panel on the Effect of Corticosteroids for Fetal Maturation on Perinatal Outcomes, 1995). It recommended that the treatment be used from 24 to 34 weeks’ gestation without limitation by sex or race and whether or not surfactant therapy was available. Although the beneficial effects were found to be greatest if treatment was begun more than 24 hours before delivery, there was also a

benefit when treatment was given for less than 24 hours. Recently, it has been shown to be beneficial to late preterm infants delivered before 37 weeks' gestation (Gyamfi-Bannerman et al., 2016).

A review of 21 published trials (Roberts and Dalziel, 2006) concluded that prenatal administration of corticosteroids did not increase a mother's risk of death, chorioamnionitis, or puerperal sepsis. Treatment was associated with a 31% reduction in neonatal death, 34% reduction in RDS, 46% reduction in cerebroventricular hemorrhage, and 54% reduction in necrotizing enterocolitis (all significant at $P \leq .05$). In a metaanalysis, Brownfoot et al. (2013) reviewed 12 trials and found that prenatal administration of dexamethasone appears to decrease the incidence of intraventricular hemorrhage without reducing the incidence of RDS or perinatal death. Although there is now widespread consensus on the antenatal use of steroids, some issues remain controversial, including the type of corticosteroid to use; the dose, frequency, and timing of use; and the route of administration.

Because the effectiveness appears to wane if steroids are given more than 1 week before premature delivery, several trials have been conducted to determine whether one or more repeated doses at weekly intervals are beneficial. Although a metaanalysis (Crowther and Harding, 2007) found modest reductions in the occurrence and severity of RDS, there were concerning findings of lower birth weight and smaller head circumference, indicating that there is insufficient evidence to recommend repeated doses (Bonanno and Wapner, 2009). An international randomized clinical trial did not find differences in the risk of death or disability at 5 years of age when comparing multiple course of steroids administered prenatally with a single course (Asztalos et al., 2013).

Prevention of Asphyxia

Prevention of asphyxia may decrease the incidence and severity of RDS, because asphyxia leads to hypoxemia and acidosis, which reduce surfactant synthesis. Leakage of fluids from capillaries into alveoli may also impair surfactant function. Premature infants are at much higher risk of needing intervention at birth, and thus preparation and staffing for full neonatal resuscitation are necessary. Maternal transfer to a center experienced in management of premature infants is associated with improved neonatal outcome.

Continuous Positive Airway Pressure

Gregory et al. (1971) first introduced CPAP therapy for newborns with RDS, primarily by an endotracheal tube. Since then a variety of devices have been developed, including short nasal prongs that do not add much to the work of breathing (De Paoli et al., 2002). For more information on the principles and use of CPAP therapy, see Chapter 45. Avery et al. (1987) reported that centers that used early nasal CPAP therapy for RDS had a lower incidence of BPD, and now CPAP is well established as the most common respiratory therapy for prevention and treatment of respiratory distress in premature infants.

The goals of CPAP therapy in preterm infants at risk of respiratory failure are to prevent end-expiratory alveolar collapse, reduce the work of breathing, and better match ventilation to perfusion. If it is started in the delivery room, it may help the newborn establish functional residual capacity, in addition to stabilizing the chest wall and reducing airway resistance. Furthermore, adequate expansion of the lungs at birth increases pulmonary blood flow. The prophylactic use of CPAP in the delivery room might make intubation for exogenous surfactant unnecessary (De Klerk and De Klerk, 2001), and avoidance of intubation and mechanical ventilation may lower the risk of BPD.

The COIN trial randomized 25 to 28 weeks' gestation newborns who were breathing spontaneously at 5 minutes of age to receive either CPAP or prophylactic intubation with early extubation to CPAP if possible. The investigators found that 46% of the CPAP group eventually required intubation anyway during the first 5 days, and the rate of pneumothorax was 9%, compared with only 3% in those intubated routinely ($P < .001$) (Morley et al., 2008). However, a metaanalysis (Schmölzer et al., 2013) concluded that one additional infant could survive to 36 weeks without BPD for every 25 babies treated with nasal CPAP therapy in the delivery room rather than being intubated.

Some centers report use of humidified high-flow nasal cannulas in infants with RDS (Lampland et al., 2009) to achieve the benefits of positive airway pressure with fewer of the perceived disadvantages of CPAP (challenges in keeping the nasal prongs in the nares, more difficult handling of the patient, and greater risk of pressure necrosis of the nasal septum). However, the airway pressure generated from this therapy, although proven to produce a clinical effect, is variable, unpredictable, and unregulated, and the commercially available systems are not approved by the US Food and Drug Administration for this indication. It should be used only by practitioners aware of the balance of risks and benefits and those prepared to recognize and treat pneumothorax and respiratory failure. A recent review outlines the main advantages and disadvantages of the many types of CPAP delivery devices, as well as implications for developing countries (Gupta et al., 2015).

Exogenous Surfactant

Historical Summary

The development of exogenous surfactant for treatment of RDS is one of the most important advances in the history of newborn medicine. This history is well told elsewhere (e.g., Halliday, 2008). The key milestones include von Neergaard's discovery in 1929 that surface tension contributes to lung recoil, Gruenwald's demonstration in 1947 that lungs of stillborn infants have high surface tension, Prattle's speculation in 1955 that absence of surfactant active material contributes to RDS, Clement's description in 1957 of surfactant dysfunction in experimental animals, and Avery and Mead's demonstration in 1959 that RDS in human infants is due to surfactant deficiency. There was an increase in research interest and funding for RDS treatment after President Kennedy's son, born at 34.5 weeks' gestation, died of RDS in 1963. In 1972, Enhorning and Robertson used natural surfactant to delay the progression of RDS in preterm rabbits, and in 1980 Fujiwara et al. demonstrated the first successful use of exogenous surfactant in human infants. By 1990, exogenous surfactant was widely used throughout the developed world, and many large clinical trials have been conducted since then to refine and improve surfactant treatment and prevention of RDS. A metaanalysis of 13 randomized controlled trials suggests that animal-derived exogenous surfactant compared with standard therapy without surfactant reduces the risk of pneumothorax by 58%, the risk of pulmonary interstitial emphysema by 55%, the risk of death by 32%, and the risk of the combined outcome of BPD or death by 17% (Seger and Soll, 2009). However, the "standard therapy" did not include use of CPAP started immediately after birth (see later comments).

Types of Surfactants Available for Clinical Use

A list of currently available surfactant preparations and sources can be found in Table 46.2. The first generation of commercially available artificial surfactants (e.g., colfosceril [Exosurf]) was composed mainly of DPPC and did not have SP-B or SP-C.

TABLE 46.2 Commercially Available Surfactant Preparations and Their Sources

| Brand Name | Generic Name | Constituents | Amount per Dose |
|---|-------------------|---------------------------|------------------------------|
| Protein-Containing Animal Surfactants | | | |
| Curosurf | Poractant alfa | Porcine lung tissue | 2.5 mL/kg (redose 1.5 mL/kg) |
| Infasurf | Calactant CLSE | Bovine (calf) lung lavage | 3 mL/kg |
| Survanta | Beractant | Bovine lung tissue | 4 mL/kg |
| Peptide-Containing Synthetic Surfactants | | | |
| Surfaxin | Lucinactant | DPPC, POPG, PA, KL4 | 5.8 mL/kg |

CLSE, calf lung surfactant extract; DPPC, dipalmitoylphosphatidylcholine; PA, palmitic acid; POPG, palmitoyloleoylphosphatidylglycerol.
Modified from Walsh BK, Daigle B, DiBlasi RM, Restrepo RD. AARC clinical practice guideline. Surfactant replacement therapy: 2013. *Respir Care*. 2013;58:367–375.

However, a metaanalysis of 11 randomized controlled trials showed that natural surfactants were faster acting than artificial surfactants, with lower incidence of pneumothorax and death (Soll and Blanco, 2001). Work continued on development of synthetic surfactants to lower the cost and because of theoretical concerns about immunologic or infectious complications from animal-derived surfactant. These newer products have synthetic peptides or proteins, such as KL4 in lucinactant, which mimics the actions of natural SP-B and SP-C.

Surfactant Selection

The important issue for clinical providers and managers is which surfactant to stock in their pharmacy because of the expense of the preparations and the need to standardize dosing guidelines. A recent metaanalysis of clinical trials (Ardell et al., 2015) indicated that use of animal-derived surfactants leads to greater early improvement in ventilator support, fewer pneumothoraces, and fewer deaths when compared with use of artificial surfactants but may be associated with greater risk of necrotizing enterocolitis and grade 1 and grade 2 intraventricular hemorrhage. The most commonly used animal-derived surfactants that are commercially available include poractant alfa (from minced porcine lung; Curosurf) and beractant (from minced bovine lung; Survanta). A recent metaanalysis of clinical trials comparing these two drugs for prevention of RDS or treatment of RDS in premature infants at risk of or having RDS suggested that treatment with poractant alfa is associated with lower risk of death, BPD, and clinically significant patent ductus arteriosus and reduced need for repeated dosing (Singh et al., 2015). Poractant alfa also has the advantage of being more concentrated than beractant, and thus the lower volume of tracheal administration can be an advantage when larger and older newborns are dosed.

Timing of Surfactant Administration

In theory, surfactant would ideally be given with the first breath. This concept was evaluated by Kattwinkel et al. (2004) by delivering the head of the infant, suctioning the nasopharynx, and then instilling calfactant into the airway before delivery of the shoulders. CPAP therapy was initiated immediately after delivery, but the trachea was not routinely intubated. The treatment appeared feasible and safe, but the sample size (23) was too small to prove that the approach was beneficial compared with surfactant instillation via

an endotracheal tube during the first few minutes after delivery. Some have proposed administration of surfactant into the pharynx before the first breath in extremely preterm infants at high risk of RDS, but no clinical trials have been published yet (Abdel-Latif and Osborn, 2011). Preliminary clinical trials suggest that surfactant can be successfully aerosolized or nebulized (Pillow and Minocchieri, 2012), but as yet no benefits have been demonstrated in preterm infants with respiratory distress (More et al., 2014). However, work in animal models of preterm infants with RDS is encouraging (Walther et al., 2014).

Prophylactic surfactant generally refers to the administration of doses within the first 15 minutes after birth. To avoid giving an unnecessary, expensive medication and subjecting a newborn to the risks of tracheal intubation, prophylactic endotracheal surfactant should ideally be given only to patients who would have eventually developed RDS and met the treatment criteria for surfactant anyway. Several studies have compared “early” surfactant administration in infants intubated within 2 hours of delivery for respiratory distress with “rescue” surfactant administration in infants with established RDS, usually at several hours of age. Those receiving early surfactant therapy had decreased risk of pneumothorax and pulmonary interstitial emphysema and decreased risk of neonatal death and BPD (Bahadue and Soll, 2012). The benefits of early, prophylactic surfactant in older clinical trials of very preterm infants appear to have disappeared in the modern era of greater antenatal use of steroids and routine use of CPAP in the delivery room (Rojas-Reyes et al., 2012).

Combining and Comparing Surfactant Therapy With the Use of Continuous Positive Airway Pressure

Because of advances in the use of CPAP since the first clinical trials of surfactant, immediate extubation to CPAP after surfactant dosing (Verder et al., 1999) was proposed to limit complications from the endotracheal tube (including excessive tidal volumes and airway inflammation that may lead to BPD). This approach is now commonly referred to as the intubation, surfactant, and extubation (InSurE) technique. However, currently available studies are underpowered to ascertain whether this method is superior to use of nasal CPAP alone (Isayama et al., 2015).

Because of continuing concerns that even the brief period of endotracheal intubation for the InSurE method is risky and possibly associated with BPD, there have been several clinical trials comparing the InSurE method with administration of surfactant through a small endotracheal catheter while CPAP is being received, sometimes referred to as *less invasive surfactant application* (Kribs et al., 2015) or *minimally invasive surfactant therapy* (Aguar et al., 2014). Although the findings of small studies are encouraging, a recent metaanalysis did not find a difference in mortality or incidence of BPD compared with use of CPAP alone (Ali et al., 2016). The studies were underpowered to detect anything other than very large differences.

A recent policy statement from the American Academy of Pediatrics states that current evidence indicates that, for preterm infants, CPAP started at or soon after birth may be considered as an alternative to routine intubation with prophylactic or early surfactant administration (Carlo et al., 2014). The policy is based in part on the outcome of a large study known as the Surfactant Positive Pressure and Pulse Oximetry Randomized Trial (SUPPORT), which was designed to compare nasal CPAP started immediately after birth with prophylactic surfactant given within 60 minutes after birth in newborns at 24 to 27 weeks' gestation (Finer et al., 2010). There was no difference in the rate of death or BPD in the

TABLE 46.3 Delivery Room Respiratory Management of Preterm Infants

| Situation | Treatment |
|--|--|
| Not breathing at birth | Provide PPV with T-piece until breathing spontaneously. |
| ≤28 weeks' gestation <i>or</i> moderate-to-severe retractions at any gestational age | Initiate nasal CPAP at 5 cmH ₂ O as soon as possible, and titrate up to 8 cmH ₂ O as needed to reduce work of breathing and FiO ₂ . |
| Heart rate persistently <100 beats per minute <i>or</i> hemodynamic instability despite PPV or oxygen requirement >70% with CPAP <i>or</i> persistent apnea for >5 min | Intubate if necessary for resuscitation and then consider exogenous surfactant if requiring >30% oxygen and chest radiograph suggestive of RDS. |
| Improving FiO ₂ and reduced work of breathing | Extubate to nasal CPAP. |

CPAP, Continuous positive airway pressure; FiO₂, fraction of inspired oxygen; PPV, positive pressure ventilation; RDS, respiratory distress syndrome.

two groups. However, for babies born at 24 to 25 weeks' gestation, those in the CPAP group had lower mortality (20% vs. 29%, $P = .01$), and thus use of CPAP may be superior to the prophylactic surfactant strategy, despite the observation that two-thirds of the CPAP group eventually received surfactant anyway. At a corrected age of 18 to 22 months, there was less respiratory morbidity in those treated with early CPAP therapy rather than intubation/surfactant (Stevens et al., 2014).

Given the rapid evolution in published evidence contrasting and combining CPAP therapy and surfactant therapy, it is difficult to give firm recommendations on their use in preventing and treating respiratory distress in premature infants. However, most studies suggest that for either therapy to work most effectively in improving survival and reducing morbidity, they should be started as soon as possible. Furthermore, the duration of endotracheal intubation should be kept to a minimum, and endotracheal intubation should be avoided if possible. General strategies for management in the delivery room are offered in Table 46.3.

Methods of Surfactant Dosing

Animal studies suggest that surfactant is better distributed when administered as a bolus rather than by slow infusion in several minutes (Fernandez-Ruano et al., 1998). The package inserts of many surfactant preparations recommend moving the infant into multiple positions for better distribution of surfactant, but there are no clinical trials supporting one particular method. Therefore surfactant should be given as quickly as tolerated, with alveolar recruitment techniques, and with the least disruptive infant positioning (Nouraeian et al., 2014).

Number of Surfactant Doses and Dosing Intervals

A metaanalysis of two clinical trials that compared one dose versus multiple doses of animal-derived surfactant suggests a 49% reduction in the incidence of pneumothorax and a trend toward a 37% reduction in mortality when multiple doses are used (Soll and Ozek, 2009). There are no data to suggest that dosing should continue once the patient's ventilator and oxygen requirements are at minimal levels, or beyond four doses. The interval between

doses is usually at least 6 hours, and most research protocols discontinue dosing after 48 hours. Late treatment with up to five doses of surfactant in ventilated premature infants receiving inhaled nitric oxide was well tolerated but did not improve survival without BPD (Ballard et al., 2016).

Clinical Care After Dosing

Because natural surfactants may work quickly, the clinician must be prepared after dosing to immediately lower FiO₂ while carefully monitoring the pulse oximeter. The tidal volume, as measured by the ventilator and/or by careful observation of chest wall movement, may gradually increase, resulting in a need to lower inspiratory pressures to avoid air leak syndrome, lung injury, and possibly pulmonary hemorrhage. Blood gases and oxygen saturation should be monitored transcutaneously and/or by intermittent blood sampling. Positive end-expiratory pressure should be maintained but may be reduced if the starting levels were high, given that functional residual capacity increases shortly after surfactant administration.

A poor response to exogenous surfactant may occur because the patient does not have surfactant deficiency but rather has lung hypoplasia, pneumonia, or congenital heart disease. Other causes for a lack of response may be poor distribution of the surfactant, such as administration down the right stem bronchus due to malposition of the endotracheal tube, plugging of the tube, or malposition of the tube in the esophagus. A less likely reason is an inadequate dose of surfactant.

The rapid improvement in lung compliance after exogenous surfactant therapy may lead to excessive pulmonary blood flow from left-to-right shunting from a patent ductus arteriosus (Raju and Langerberg, 1993). It is uncertain whether early and aggressive intervention to close the ductus with medication or surgery will reduce the risk of pulmonary hemorrhage.

Pulmonary Hypoplasia

Definition and Incidence

One or both lungs of newborns with pulmonary hypoplasia are smaller than normal, including reduced numbers of lung cells, airways, blood vessels, and alveoli. Pulmonary hypoplasia is the cause of respiratory distress at birth in only 0.3% of newborns with respiratory symptoms (Rubaltelli et al., 1998), but it is commonly fatal, especially in preterm infants. The incidence is about 1 per 1000 live births, and 90% of cases are associated with congenital anomalies or pregnancy complications (De Paepe et al., 2005). There is a continuum of severity of pulmonary hypoplasia—from negligible to severe—and therefore all but the most severe cases are difficult to diagnose. This is especially true when one is attempting to diagnose pulmonary hypoplasia prenatally by imaging studies but is also true during clinical assessment of the newborn and even after postmortem examination of the lungs.

When one is considering the causes of pulmonary hypoplasia, it is useful to categorize them into associated conditions, each with representative diagnoses, as in Table 46.4. This chapter on respiratory distress in preterm infants focuses on the problem of pulmonary hypoplasia associated with oligohydramnios from preterm premature rupture of membranes because this cause of pulmonary hypoplasia occurs almost exclusively in preterm infants. When preterm premature rupture of membranes occurs at 15 weeks' gestation, the incidence of pulmonary hypoplasia is 80%, and when it occurs at 19 weeks, the incidence is 50%, whereas

TABLE 46.4 Categories of Conditions Associated With Pulmonary Hypoplasia

| Category | Representative Diagnoses |
|--|---|
| Restriction of thoracic space | Diaphragmatic hernia or eventration Intrathoracic mass Congenital cystic adenomatoid malformation Bronchogenic cyst Extralobar sequestration Thoracic neuroblastoma Pleural effusions Chylothorax Hydrothorax |
| Oligohydramnios | Renal Bilateral renal agenesis or dysplasia Bladder outlet obstruction (posterior urethral valves) Nonrenal Prolonged preterm rupture of membranes |
| Skeletal anomalies | Chondroectodermal dysplasia Osteogenesis imperfecta Thanatophoric dwarfism |
| Hydrops fetalis | Rhesus isoimmunization |
| Neuromuscular and central nervous system anomalies | Fetal akinesia Anencephaly Arnold–Chiari malformation |
| Cardiac anomalies | Hypoplastic right or left side of the heart Pulmonary stenosis Ebstein anomaly |
| Abdominal wall defects | Omphalocele Gastroschisis |
| Syndromes | Trisomy 13, 18, 21 Larsen syndrome Cerebrocostomandibular Jarcho–Levin syndrome Roberts syndrome Lethal multiple pterygium |

Modified from Langston C. Pulmonary Disorders in the Neonate, Infant, and Child. In: Churg AM, Myers JL, Tazelaar H, Wright J, eds. *Thurbeck's Pathology of the Lung*. 3rd ed. New York, NY: Thieme; 2005.

after 26 weeks the incidence is near zero (Rothschild et al., 1990). Other conditions associated with pulmonary hypoplasia are covered in other chapters.

Pathology

The easiest method of defining pulmonary hypoplasia during postmortem examination is to calculate the ratio of lung weight to body weight. At 28 weeks' gestation, a ratio of 0.015 is at the fifth percentile, whereas at 35 weeks' gestation, the fifth percentile is at a ratio of 0.012. The lung weight to body weight ratio may be artificially elevated if the lungs are wetter than usual from edema, hemorrhage, inflammation, or lymphangiectasia, and this may lead to the false conclusion that the patient does not have pulmonary hypoplasia. Conversely, the ratio may be artificially low if the body is heavier than usual because of renal cystic disease, hydrops, ascites, tumors, hydrocephaly, and so forth. Therefore a better method for postmortem diagnosis of pulmonary hypoplasia

is to measure the lung volume by inflating the lung at physiologic pressure and then measuring the displacement of fluid when the lung is immersed. The average lung volume is 33 mL/kg of body weight from 16 to 31 weeks' gestation, but it falls to 23 mL/kg at term (De Paepe et al., 2014). This method facilitates comparison of postmortem estimates with in utero estimates of lung volume made during prenatal imaging. However, low lung volume does not necessarily correlate with deficiency of lung structure and function. A postmortem technique more physiologically relevant than lung volume is the radial alveolar count, which is proportional to alveolar surface complexity (De Paepe et al., 2005) and thus gas exchange surface area; however, this procedure is complex and time consuming.

Impairment of lung development before 16 weeks causes reduced airway branching, reduced cartilage development, reduced acinar complexity and maturation, delayed vascularization, and delayed thinning of the air–blood barrier (see Chapter 42). Impairment after 16 weeks typically causes reduced acinar complexity and maturation. These outcomes are predictable, given the time in gestation when these structures are developing (Fig. 46.12A).

Because the growth of lung blood vessels parallels the development of the airways, pulmonary hypoplasia causes decreased total size of the pulmonary vascular bed, decreased number of vessels per unit of lung tissue, and increased amount of pulmonary artery smooth muscle. This last phenomenon accounts for persistent pulmonary hypertension after birth.

Clinical Signs

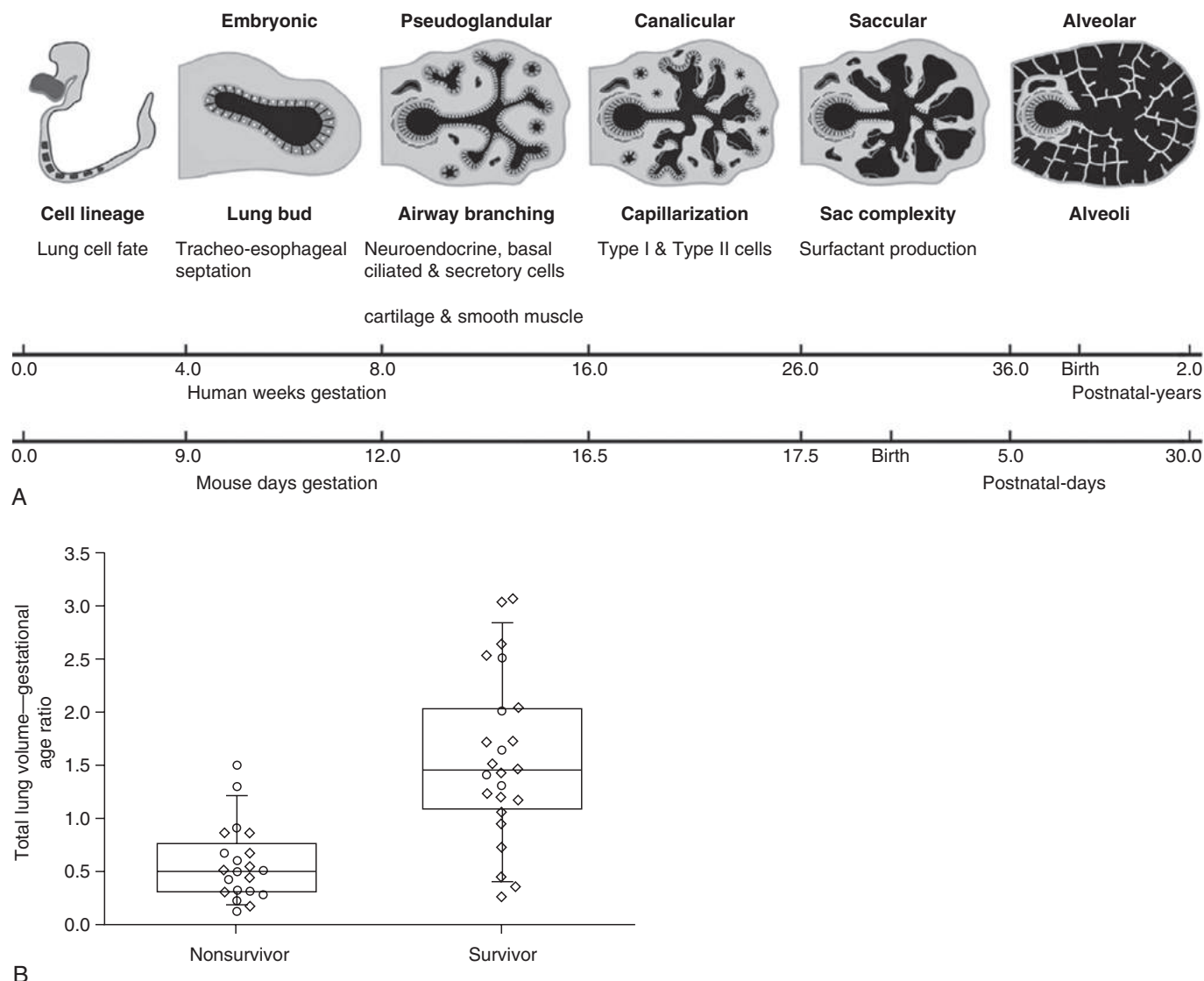
The preterm newborn with pulmonary hypoplasia often has immediate signs of respiratory distress and cyanosis indistinguishable from those in the newborn with severe RDS (see [Clinical Signs of Respiratory Distress Syndrome](#) earlier in this chapter). However, respiratory failure from severe pulmonary hypoplasia often becomes apparent within minutes of birth, whereas respiratory failure from RDS usually progresses over the first few hours after birth. The thorax may appear small or bell shaped, and, if oligohydramnios was severe, there may be flattening of the face and deformation (such as contractures of the extremities). Hypercarbia may be severe on the earliest blood gas measurement, despite aggressive mechanical ventilation. The hypoxemia from surfactant deficiency and lung immaturity may be compounded by right-to-left shunting of deoxygenated blood due to pulmonary hypertension, leading to severe desaturation. If there is also a tension pneumothorax, there may be asymmetry of breath sounds or malposition of heart sounds, as well as decreased cardiac output from impaired venous return to the thorax.

Radiographic Signs

Because lung immaturity and surfactant deficiency accompany pulmonary hypoplasia, particularly in preterm infants, the lungs may be radiographically dense with air bronchograms, as with RDS, but the lung volumes may be smaller and the diaphragms higher than in RDS. With severe pulmonary hypoplasia, the thorax may appear bell shaped, and early pneumothorax is common.

Treatment

With careful management, the survival rate of infants born at less than 32 weeks' gestation after rupture of membranes before 24 weeks' gestation has been reported as greater than 75%



• **Fig. 46.12** (A) Stages of lung development. (B) Measurement of fetal lung volume by magnetic resonance imaging and outcomes after delivery. ([A] From Kimura J, Deutsch GH. Key mechanisms of early lung development. *Pediatr Dev Pathol.* 2007;10:335–347. [B] From Zaretsky M, Ramus R, McIntire D, Magee K, Twickler DM. MRI calculation of lung volumes to predict outcome in fetuses with genitourinary abnormalities. *Am J Radiol.* 2005;185:1328–1334.)

(Soylu et al., 2010) and 90% (Brumbaugh et al., 2014). In addition to all of the treatments described earlier for the preterm infant with RDS, the ventilator strategy must be tailored to smaller lungs that may have relatively normal compliance, high pulmonary vascular resistance, and sometimes cardiac dysfunction. In the initial hours after birth, the goal should be to avoid excessive intrathoracic pressure from lung overdistention, which may lead to catastrophic pneumothorax and which may also further aggravate pulmonary hypertension and right-sided heart afterload. The neonatal team should be prepared for urgent needle decompression of the chest and insertion of a chest tube. Permissive hypercapnia is appropriate, and high-frequency ventilation may be necessary for adequate ventilation and reduction of very high arterial PCO_2 levels.

Exogenous surfactant should be considered early because RDS may complicate the management of pulmonary hypoplasia in the preterm infant. To avoid adding hypoxic pulmonary vasoconstriction to the already high pulmonary vascular resistance, the predudal

arterial oxygen saturation should be kept at 92% or higher. Although acidosis may compound hypoxic pulmonary vasoconstriction, hypercapnia is usually well tolerated and will allow a less aggressive ventilator strategy. Routine paralysis in preterm infants is usually not needed and leads to third spacing of fluid; usually narcotics will suffice to manage discomfort. Spontaneous ventilation may be beneficial, and some infants with pulmonary hypoplasia have been managed with use of nasal CPAP (Welzing et al., 2011).

Because increasing ventilator support for persistent hypoxemia is usually counterproductive, efforts should instead be directed toward treatment of pulmonary hypertension, even though the component that is reversible may be small if pulmonary hypoplasia is severe. There are several small studies demonstrating the beneficial effects of inhaled nitric oxide in infants after preterm prolonged rupture of membranes (de Waal and Kluckow, 2015), and the effects on shunt can be followed with serial echocardiography. Other medications that have been used in term infants with

pulmonary hypertension, such as sildenafil and milrinone, may be helpful. Because systemic hypotension causes worse right-to-left shunting, pressors such as dopamine may be helpful, acknowledging that these medications may increase both systemic and pulmonary vascular pressures. For more detail, see the review by [de Waal \(2015\)](#), which includes a good description of cardiovascular support agents, mechanisms of action, and physiologic targets. Before the start of administration of agents such as inhaled nitric oxide that are intended to increase pulmonary blood flow, severe left ventricular systolic dysfunction with increased left atrial pressure should first be corrected to avoid pulmonary edema.

Extracorporeal membrane oxygenation may be appropriate for late-gestation preterm infants if the pulmonary hypoplasia, surfactant deficiency, and pulmonary hypertension are expected to abate within a few days. However, it is very difficult to select appropriate candidates with pulmonary hypoplasia for extracorporeal membrane oxygenation, and it is often unsuccessful with severe disease or if other major life-threatening malformations are present.

Prenatal Diagnosis

An accurate prenatal test for pulmonary hypoplasia is important because it may affect the obstetric management. It would be particularly helpful to be able to discriminate lethal from nonlethal pulmonary hypoplasia, especially early in gestation, when termination of the pregnancy may be an option. However, quantifying the degree of fetal pulmonary hypoplasia is challenging even for the pathologist who has access to the tissue. A systematic review and metaanalysis of imaging parameters for prediction of fatal pulmonary hypoplasia found all to be poor to mediocre ([van Teeffelen et al., 2012](#)).

Nonsurvivors with pulmonary hypoplasia due to fetal urinary anomalies were noted to have lower in utero lung volumes as determined by fetal magnetic resonance imaging (MRI), adjusted for gestational age ([Zaretsky et al., 2005](#)), but there was no clear separation between the survivors and nonsurvivors (see [Fig. 46.12B](#)). Furthermore, there was considerable overlap of the confidence intervals before 26 weeks' gestation, which limits the usefulness of MRI assessment of fetal volume for prenatal counseling. Although there will continue to be advances in fetal imaging to assess lung volume, it will likely remain difficult to differentiate lethal from nonlethal pulmonary hypoplasia.

The management of the preterm pregnancy with premature rupture of membranes near the limit of fetal viability is beyond the scope of this chapter. However, a review by [Waters and Mercer \(2009\)](#) offers a management algorithm that illustrates the areas of controversy. For the patient who desires conservative management of premature rupture before the limit of viability, the algorithm recommends serial assessments for signs of infection or labor, as well as interval ultrasound examinations to watch for the development of pulmonary hypoplasia. If this occurs, prenatal administration of antibiotics and corticosteroids should be considered if more aggressive management is desired.

Prenatal Treatment

Treatment of oligohydramnios with amnioinfusion with saline has been proposed to improve fetal survival by increasing the latency period (the interval between premature rupture of membranes and delivery) and thus the gestational age at delivery. A metaanalysis found a reduction in neonatal morbidity, neonatal sepsis, pulmonary hypoplasia, puerperal sepsis, and latency, but

the authors recommended circumspection as the positive findings were mainly due to one trial with unclear allocation concealment ([Hofmeyr et al., 2014](#)). When it is successful, the benefit may come from restoration of back-pressure from the amniotic sac fluid to the lungs, stimulating fetal lung growth and development during the critical canalicular stage between 16 and 26 weeks' gestation.

On the basis of this same logic, there have been efforts to reverse pulmonary hypoplasia in utero by fetal tracheal occlusion with a balloon early in pregnancy. Recent research with this technique appears to be limited to fetuses with congenital diaphragmatic hernia ([Doné, 2015](#)). Whether this technique proves to be valuable for preterm premature rupture of membranes is uncertain, but pulmonary hypoplasia may be more reversible than previously assumed.

Pneumonia

Incidence and Etiology

The incidence of pneumonia and sepsis in infants with birth weight from 1500 to 2500 g is only 0.28%, whereas in patients with birth weight less than 1000 g the incidence is severalfold higher at 1.9% (see [Fig. 46.1](#); [Rubaltelli et al., 1998](#)). If only newborns with respiratory distress are considered, the overall incidence is 5% ([Table 46.1](#))—respiratory distress being the third most likely cause, after RDS (46%) and TTNB (37%). The incidence of pneumonia in newborns with respiratory distress who weigh less than 1000 g, 1000 to 1500 g, and 1500 to 2500 g is 4%, 2%, and 1%, respectively (see [Fig. 46.1](#)). The National Institute of Child Health and Human Development Neonatal Research Network reported an incidence of blood culture–proven early-onset sepsis (<72 hours after birth) in newborns with birth weight less than 1500 g of 1.9% in 1991 to 1993 ([Stoll et al., 1996](#)) and 1.5% in 1998 to 2000 ([Stoll and Hansen, 2003](#)). In the earlier period, group B streptococcus was the most frequent pathogen (31%), followed by *Escherichia coli* (16%) and *Haemophilus influenzae* (12%). In the more recent period, the most common pathogens were *E. coli* (44%), group B streptococcus (11%), coagulase-negative staphylococcus (11%), viridans streptococci and other streptococci (8%), *H. influenzae* (8%), *Citrobacter* (2%), *Listeria monocytogenes* (2%), and *Candida albicans* (2%). Preterm newborns can also acquire postnatal pertussis and viral pneumonia, commonly due to respiratory syncytial virus, adenovirus, and rhinovirus. Pneumonia can also begin in utero with infectious agents such as cytomegalovirus and herpes simplex virus (see Chapter 37).

Clinical Signs

The clinical signs of pneumonia after preterm birth are often indistinguishable from the more common problem of RDS. Bacterial pneumonia is usually accompanied by bacteremia because newborns are frequently unable to confine bacteria to the lung, and therefore some infants will exhibit clinical signs of sepsis or shock, including poor perfusion and hypotension, in addition to respiratory failure. Many premature infants with pneumonia also have surfactant deficiency from RDS, further obscuring the diagnosis of pneumonia.

Laboratory and Radiographic Signs

Blood culture findings will be positive in some premature newborns with pneumonia, but the presence of maternal antibiotics in the

blood of the newborn reduces confidence in a negative result. Leukopenia, increased percentage of immature granulocytes, and elevated levels of inflammatory markers such as C-reactive protein increase the likelihood of sepsis/pneumonia, but with poor positive predictive value. Tracheal aspirate culture (but not Gram stain) obtained immediately after placement of an endotracheal tube may help with diagnosis and guide therapy, especially when the blood culture result is negative (Booth et al., 2009).

Premature newborns with pneumonia will have pulmonary infiltrates on chest radiograph, but the radiographic appearance is difficult to distinguish from RDS (although classically in term newborns it has a more coarse and wetter appearance). Because newborns are unable to localize pulmonary infection, lobar infiltrates are rarely an indication of pneumonia—plugging of airways with secretions is more likely.

Treatment

Administration of antibiotics directed at the most common organisms (see earlier discussion) should be started immediately when pneumonia in the preterm infant is suspected. Ampicillin and gentamicin are reasonable choices, to be administered for 48 hours pending culture results. Empiric vancomycin has the disadvantage of promoting the emergence of vancomycin-resistant organisms, and a delay in treating coagulase-negative staphylococcal bacteremia until culture results are positive is rarely of consequence to the patient. If the blood culture result is negative, and the mother has been pretreated with antibiotics, a longer course of antibiotics (e.g., 5 to 7 days) may be prudent if the clinical course is strongly suggestive of pneumonia.

Among the possible adverse consequences of unnecessary empiric antibiotics are interference with the colonization of the intestinal tract with nonpathogenic bacteria, selection of antibiotic-resistant bacteria, and fungal infection. Although the development of BPD is associated with inflammation from chorioamnionitis (Speer, 2009), there is no convincing evidence that empiric antibiotics at birth reduce the incidence or severity of BPD.

Pulmonary Hemorrhage

Incidence and Clinical Signs

Pulmonary hemorrhage occurs in 3%–5% of preterm infants needing respiratory support, usually at 1 to 3 days of age, with onset of pink or red frothy fluids in the endotracheal tube and sudden respiratory deterioration, cyanosis, pallor, hypotension, or hypotonia (Bendapudi et al., 2012). The frequency is greater with increasing degree of prematurity and with intrauterine growth restriction (Scholl and Yanowitz, 2015). The chest radiograph shows widespread “whiteout” on chest radiograph, unlike mucosal bleeding of the airway, which is usually accompanied by minimal clinical or radiographic changes. The hematocrit of the tracheal fluid after pulmonary hemorrhage is much lower than from a venous sample, indicating that the underlying problem is hemorrhagic pulmonary edema.

Etiology

Pulmonary hemorrhage has long been speculated to be due to left ventricular failure and excessive left-to-right flow through a patent ductus arteriosus, with resultant disruption of the pulmonary capillaries (Cole et al., 1973). There is a threefold increased risk

after prophylactic synthetic surfactant (Soll, 2000), so some have speculated that it may have a direct cytotoxic effect that leads to impaired membrane integrity in the alveolar capillary (Findlay et al., 1995). However, it may be caused by insufficient attention to reducing mechanical ventilator pressure and volume after lung compliance improves following exogenous surfactant treatment. A recent case–control analysis (Scholl and Yanowitz, 2015) found a higher incidence of pulmonary hemorrhage in premature newborns with moderate-to-large patent ductus arteriosus who were extubated earlier than case-matched controls; the authors theorized that the decrease in pulmonary resistance associated with relief of RDS, combined with the reduction of mean airway support associated with extubation, facilitated left-to-right shunting through the patent ductus arteriosus, which increased the likelihood of pulmonary hemorrhage. This theory is supported by evidence that infants who received early indomethacin therapy to close a large patent ductus arteriosus had a lower incidence of pulmonary hemorrhage (Kluckow et al., 2014).

Treatment

The mainstay of treatment includes careful suctioning of the trachea to prevent obstruction, increased ventilator pressure if needed, and increased positive end-expiratory pressure for pulmonary edema. Although a low platelet count and coagulopathy are rarely the cause, newborns should be checked for these and treated if necessary. If anemia is severe, transfusion with packed red blood cells may be indicated, but this should be done very slowly to avoid compounding the pulmonary edema. In one small study, the trachea was “sprayed” with 0.5 mL of 1:10,000 epinephrine mixed with 1 mL of air in a 5-mL syringe through a small tube, after each suctioning of the endotracheal tube; most patients had resolution of pulmonary hemorrhage after three to five such treatments (Yen et al., 2013). Hemocoagulase, a purified enzyme mixture from the venom of the South American viper, has been used for its procoagulant effects in a small study of neonatal pulmonary hemorrhage, with a 48% reduction in mortality (Shi et al., 2005). Because red blood cells and serum impair surfactant function, there is evidence that exogenous surfactant helps (Pandit et al., 1995; Amizuka et al., 2003), but there are no randomized controlled trials to prove benefit (Aziz and Ohlsson, 2012).

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- Complete references used in this text can be found online at www.expertconsult.com*

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Respiratory Disorders in the Term Infant

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KEY POINTS

- Marked hypoxemia in the newborn can be caused by parenchymal lung disease, pulmonary vascular disease, or congenital heart defects.
- Events occurring at delivery, as well as the response to supplemental oxygen and to mask continuous positive airway pressure, can provide important clues to the pathophysiology of hypoxemic respiratory failure in the term newborn.
- Although a significant percentage of term births are complicated by passage of meconium in utero, less than 10% of such infants develop meconium aspiration syndrome.
- Transient tachypnea of the newborn is one of the most common causes of respiratory distress in the term newborn.
- The most important question to ask when one is reviewing a neonatal chest X-ray is whether the severity of hypoxemia is out of proportion to the X-ray findings.
- The syndrome of persistent pulmonary hypertension of the newborn rarely occurs without concomitant parenchymal lung disease.
- The echocardiogram has become a vital tool in the clinical management of newborns with hypoxemic respiratory failure, including the diagnosis of congenital heart defects for which inhaled nitric oxide treatment would be contraindicated.

The evaluation and management of respiratory distress in the term newborn poses unique challenges and remains one of the most vexing problems facing neonatal caregivers. Although some of the pathophysiologic features of respiratory disorders in the term infant are similar to those in preterm newborns, several disorders occur more commonly in the term newborn (e.g., meconium aspiration) and are often made more difficult to evaluate and manage because of cardiac and pulmonary vascular abnormalities that complicate the clinical course. Indeed, the traditional perspective of categorizing hypoxemia and respiratory failure in the term newborn as due to cardiac, pulmonary vascular, or air space (lung) disease is insufficient. For example, the problem of persistent pulmonary hypertension of the newborn (PPHN) is defined by severe pulmonary vasoconstriction leading to suprasystemic pulmonary artery pressure with extrapulmonary right-to-left venoarterial admixture across the fetal channels of the oval foramen and the arterial duct. However, PPHN rarely occurs without concomitant parenchymal lung disease and disturbances in cardiac performance.

In this chapter we present an algorithm for evaluation of the term newborn with hypoxemia and respiratory distress, review the syndrome of PPHN, and discuss common causes of respiratory failure in the term newborn.

Evaluation of the Term Newborn With Hypoxemia/Respiratory Distress

One of the most anxiety-provoking experiences for many clinicians (particularly those in training) is the initial evaluation and management of a term newborn with hypoxemia and respiratory distress. Traditional textbooks provide a wealth of information about individual conditions once they have been identified. However, there are few sources designed to guide the clinician in an ordered fashion through a comprehensive diagnostic evaluation. In this section, we propose an approach to the evaluation of the hypoxemic newborn that may be useful in clarifying the cause of hypoxemia/respiratory distress and in determining the proper sequence of diagnostic and therapeutic interventions.

History

Marked hypoxemia in the newborn can be caused by parenchymal lung disease with ventilation-perfusion (V/Q) mismatch or intrapulmonary shunting, pulmonary vascular disease causing extrapulmonary right-to-left shunting (PPHN), or anatomic right-to-left shunting associated with congenital heart disease. Evaluation should begin with a history that includes assessment of risk factors for hypoxemic respiratory failure. A relevant history may include the results of prenatal ultrasound studies. Lesions such as congenital diaphragmatic hernia (CDH) and congenital cystic adenomatoid malformation are diagnosed prenatally with increasing frequency. Although many anatomic congenital heart defects can be diagnosed prenatally, vascular abnormalities (e.g., coarctation of the aorta, total anomalous pulmonary venous return) are more difficult to diagnose with prenatal ultrasonography. A history of a structurally normal heart by fetal ultrasonography should be confirmed by echocardiography in the newborn with cyanosis (see later).

Other historical information that may be important in the evaluation of the cyanotic newborn includes a history of severe and prolonged oligohydramnios causing pulmonary hypoplasia. Also important is a history of prolonged fetal bradyarrhythmia

• BOX 47.1 Neonatal Respiratory Failure: History and Risk Factor Assessment

Prenatal

Prenatal ultrasound study results
History of oligohydramnios and duration
History of fetal brady/tachyarrhythmia
Maternal illnesses, drugs, medications
History of fetal distress
Risk factors for infection

Delivery

History of positive pressure ventilation in delivery room
Meconium-stained amniotic fluid
Hemorrhage
Birth trauma
Low Apgar score

and/or tachyarrhythmia and marked anemia (caused by hemolysis, twin–twin transfusion, or chronic hemorrhage) that may cause congestive heart failure, pulmonary edema, and respiratory distress. Maternal illness (e.g., diabetes mellitus), medication use (e.g., aspirin or medications containing nonsteroidal antiinflammatory drugs causing premature constriction of the ductus arteriosus, association of Ebstein malformation with maternal lithium use), and illicit drug use may contribute to acute cardiopulmonary distress in the newborn. Risk factors for infection that cause sepsis/pneumonia should be considered, including premature or prolonged rupture of membranes, fetal tachycardia, maternal leukocytosis, uterine tenderness, and other signs of intra-amniotic infection.

Events at delivery may provide clues to the cause of hypoxemic respiratory failure in the newborn. For example, if positive pressure ventilation is required in the delivery room, the risk of pneumothorax increases. A history of meconium-stained amniotic fluid is the sine qua non of meconium aspiration syndrome (MAS). Birth trauma (e.g., clavicular fracture, phrenic nerve injury) or acute fetomaternal or fetoplacental hemorrhage may cause respiratory distress in the newborn (Box 47.1).

Physical Examination

The initial physical examination provides important clues to the cause of cyanosis. Marked respiratory distress in the newborn (retractions, grunting, nasal flaring) suggests the presence of pulmonary parenchymal disease with decreased lung compliance. However, it is important to recognize that upper airway obstruction (e.g., Pierre Robin sequence or choanal atresia) and metabolic acidemia also can cause severe respiratory distress. In contrast, the newborn with cyanosis alone or cyanosis plus tachypnea (i.e., nondistressed tachypnea) typically has cyanotic congenital heart disease, most commonly transposition of the great vessels or idiopathic PPHN.

The presence of a heart murmur in the first few hours of life is an important sign in the newborn with cyanosis or respiratory distress. In this setting it is unusual for the common left-to-right shunt lesions (patent ductus arteriosus, atrial septal defect, ventricular septal defect) to produce an audible murmur because pulmonary vascular resistance (PVR) remains high and little turbulence is created across the defect. A murmur that sounds like a ventricular septal defect in the first few hours of life is most commonly caused by tricuspid regurgitation (associated with PPHN or an ischemic myocardium).

• BOX 47.2 Physical Examination

Respiratory Distress (Retractions, Grunting, Nasal Flaring)

Suggests lung parenchymal disease (compliance), upper airway disease, or metabolic acidemia

No Significant Respiratory Distress (Tachypnea Alone)

Suggests hypoxemia caused by cyanotic heart disease without lung disease

• BOX 47.3 Short-Term Response to Supplemental Oxygen (High FiO_2 by Hood, Mask)

Minimal or Transient Change in SaO_2

Cyanotic heart disease, persistent pulmonary hypertension of the newborn

Marked increase in SaO_2

Parenchymal lung disease, congenital diaphragmatic hernia with ductal-dependent systemic blood flow

FiO_2 , Fraction of inspired oxygen; SaO_2 , Arterial oxygen saturation.

The response to supplemental oxygen can also provide important clues to the pathophysiology of hypoxemic respiratory failure in the term newborn (Boxes 47.2–47.3).

Interpretation of Pulse Oximetry Measurements

The interpretation of preductal (right hand) and postductal (lower extremity) arterial oxygen saturation, measured by pulse oximetry, provides important clues to the cause of hypoxemia in the newborn. Right-to-left shunting across the ductus arteriosus (but not the patent foramen ovale) causes postductal desaturation (with a >5% preductal–postductal saturation difference). However, it is important to recognize that variability in oximetry readings may be related to differences in available devices and may be affected by local perfusion.

If the measurements of preductal and postductal arterial oxygen saturation (SaO_2) are equivalent, this suggests either that the ductus arteriosus is patent and PVR is subsystemic (i.e., the hypoxemia is caused by parenchymal lung disease with intrapulmonary shunting or cyanotic heart disease with ductal-dependent pulmonary blood flow) or that the ductus arteriosus is closed (precluding any interpretation of pulmonary artery pressure without echocardiography). It is uncommon for the ductus arteriosus to close in the first few hours of life in the presence of systemic or suprasystemic pulmonary artery pressures.

The most common cause of preductal–postductal gradients in oxygenation is suprasystemic PVR in PPHN causing right-to-left shunting across the ductus arteriosus (associated with MAS, surfactant deficiency/dysfunction, CDH, non-CDH pulmonary hypoplasia, or idiopathic [pulmonary hypertension without accompanying pulmonary parenchymal disease]). However, ductal-dependent systemic blood flow lesions (hypoplastic left-sided heart syndrome, critical aortic stenosis, interrupted aortic arch, coarctation) may also present with postductal desaturation. Moreover, anatomic pulmonary vascular disease (alveolar capillary dysplasia, pulmonary venous stenosis, anomalous venous return with obstruction) can cause suprasystemic PVR with right-to-left shunting across the ductus arteriosus and postductal desaturation.

TABLE 47.1 Role of Pulse Oximetry in Evaluation of Neonatal Hypoxemic Respiratory Failure

| | |
|--|---|
| Preductal $\text{SaO}_2 =$ postductal SaO_2 | Intrapulmonary shunt: $\text{PVR} < \text{SVR}$ Cyanotic congenital heart disease with left-to-right shunting across the PDA Ductal-dependent pulmonary blood flow: pulmonary atresia/stenosis, tricuspid atresia, Ebstein anomaly PPHN: right-to-left shunt at PFO, $\text{PVR} > \text{SVR}$, ductus closed |
| Preductal $\text{SaO}_2 >$ postductal SaO_2 | $\text{PVR} > \text{SVR}$ with right-to-left shunting across the PDA: PPHN: MAS, RDS, CDH Ductal-dependent systemic blood flow: HLHS, IAA, coarctation Anatomic pulmonary vascular disease: alveolar capillary dysplasia, pulmonary vein stenosis, TAPVR with obstruction |
| Preductal $\text{SaO}_2 \leq$ postductal SaO_2 | TGV with pulmonary hypertension TGV with coarctation of the aorta |

CDH, Congenital diaphragmatic hernia; HLHS, hypoplastic left-sided heart syndrome; IAA, interrupted aortic arch; MAS, meconium aspiration syndrome; PDA, patent ductus arteriosus; PFO, patent foramen ovale; PPHN, persistent pulmonary hypertension of the newborn; PVR, pulmonary vascular resistance; RDS, respiratory distress syndrome; SaO_2 , arterial oxygen saturation; SVR, systemic vascular resistance; TAPVR, total anomalous pulmonary venous return; TGV, transposition of the great vessels.

Finally, the unusual occurrence of markedly lower preductal SaO_2 compared with postductal SaO_2 suggests one of two diagnoses: transposition of the great vessels (TGV) with pulmonary hypertension (PH) or TGV with coarctation of the aorta (Table 47.1).

Laboratory and Radiologic Evaluation

One of the most important tests to perform in the evaluation of the newborn with cyanosis is the chest radiograph (CXR). The CXR can demonstrate the classic findings of respiratory distress syndrome (RDS) (air bronchograms, diffuse granularity, underinflation), pneumonia (diffuse parenchymal lung disease), MAS, or CDH. Perhaps the most important question to ask when one is viewing the CXR is whether the severity of hypoxemia is out of proportion to the radiographic changes. In other words, marked hypoxemia despite supplemental oxygen in the absence of severe pulmonary parenchymal disease radiographically suggests the presence of an extrapulmonary right-to-left shunt (idiopathic PPHN or cyanotic heart disease; Boxes 47.3–47.4).

Other essential measurements include measurement of arterial blood gas to determine the blood gas tensions and pH, a complete blood count to evaluate the newborn for signs of infection, and blood pressure measurements in the right arm and a lower extremity to identify aortic obstruction (interrupted aortic arch, coarctation).

Response to Supplemental Oxygen

A marked increase in SaO_2 (increase to 100%) with supplemental oxygen (by hood, mask, or endotracheal tube) suggests the presence of intrapulmonary shunt or V/Q mismatch caused by lung disease or reactive PPHN. The response to mask continuous positive airway pressure is also a useful discriminator between severe lung disease and other causes of hypoxemia. Most patients with PPHN have

• BOX 47.4 Chest Radiograph and Laboratory Evaluation

Chest Radiograph

Hypoxemia out of proportion to radiographic changes suggests congenital heart disease with ductal-dependent pulmonary blood flow or extrapulmonary right-to-left shunting with persistent pulmonary hypertension of the newborn.

Arterial Blood Gas, Complete Blood Count, Blood Pressure

Arterial blood gas: assess respiratory and metabolic acidemia

Complete blood count: for evidence of infection

Blood pressure: ductal-dependent systemic blood flow and closing of patent ductus arteriosus (e.g., coarctation)

• BOX 47.5 Role of Echocardiography in Evaluation of Persistent Pulmonary Hypertension of the Newborn and the Use of Inhaled Nitric Oxide

Extrapulmonary Shunt

Right-to-left shunting at the arterial duct and/or foramen ovale is observed in infants with suprasystemic pulmonary hypertension.

If echocardiography demonstrates adequate left-ventricular performance, consider inhaled NO use after effective lung recruitment (see functional measurements below).

Anatomy

Inhaled NO use may be contraindicated in the presence of duct-dependent systemic blood flow (e.g., hypoplastic left heart syndrome).

Left-Ventricular Performance

Inhaled NO use may be contraindicated in the presence of left ventricular systolic/diastolic dysfunction (e.g., mitral insufficiency with left-to-right atrial shunting with right-to-left ductal shunting suggesting possible right ventricle-dependent systemic blood flow).

NO, Nitric oxide.

at least a transient increase in oxygenation in response to interventions such as high inspired oxygen concentration and/or initiation of mechanical ventilation. If preductal SaO_2 never reaches 100%, the likelihood of cyanotic heart disease is high.

Echocardiography

Echocardiography has become a vital tool in the clinical management of newborns with hypoxemic respiratory failure (Box 47.5). The initial echocardiographic evaluation is important to rule out structural heart disease causing hypoxemia (e.g., coarctation of the aorta, total anomalous pulmonary venous return). Moreover, it is critically important to diagnose congenital heart lesions for which inhaled nitric oxide (iNO) treatment would be contraindicated. In addition to the lesions mentioned earlier, congenital heart diseases that can present with hypoxemia unresponsive to high inspired oxygen concentrations (i.e., dependent on right-to-left shunting across the ductus arteriosus) include critical aortic stenosis, interrupted aortic arch, and hypoplastic left-sided heart syndrome. Decreasing PVR with iNO treatment in these conditions could lead to systemic hypoperfusion, worsening the clinical course and delaying definitive diagnosis.

Echocardiographic evaluation is an essential component in the initial evaluation and ongoing management of the hypoxemic newborn. Not all hypoxemic term newborns have echocardiographic signs of PPHN. As noted earlier, hypoxemia can be caused by intrapulmonary right-to-left shunting or V/Q disturbances associated with severe lung disease. In unusual circumstances, right-to-left shunting can occur across pulmonary-to-systemic collaterals. However, extrapulmonary right-to-left shunting at the foramen ovale and/or ductus arteriosus (PPHN) also complicates hypoxemic respiratory failure and must be assessed to determine initial treatments and evaluate the response to those therapies.

PPHN is defined by the echocardiographic determination of extrapulmonary venoarterial admixture (right-to-left shunting at the foramen ovale and/or ductus arteriosus), not simply evidence of increased PVR (i.e., elevated PVR without extrapulmonary shunting does not directly cause hypoxemia). Echocardiographic signs suggestive of PH (e.g., increased right ventricular systolic time intervals, septal flattening) are less helpful.

Doppler measurements of atrial-level and ductal-level shunts provide essential information when one is managing hypoxemic respiratory failure in a newborn. For example, left-to-right shunting at the foramen ovale and ductus arteriosus with marked hypoxemia suggests predominant intrapulmonary shunting, and interventions should be directed at optimizing lung inflation.

Finally, the measurements made with echocardiography can be used to predict or interpret the response or lack of response to various treatments. For example, in the presence of severe left-ventricular dysfunction with pulmonary hypertension, pulmonary vasodilation alone may be ineffective in increasing oxygenation. The echocardiographic findings in this setting include right-to-left ductal shunting (caused by suprasystemic PVR) and mitral insufficiency with left-to-right atrial shunting. In this setting, efforts to reduce PVR should be accompanied by targeted therapies to increase cardiac performance and decrease left-ventricular afterload.

This constellation of findings suggests that left-ventricular dysfunction may contribute to pulmonary venous hypertension, such as occurs in congestive heart failure. In this setting, pulmonary vasodilation alone (without improvement in cardiac performance) will not cause a sustained increase in oxygenation. Careful echocardiographic assessment will provide invaluable information about the underlying pathophysiology and help guide the course of treatment.

The initial echocardiographic evaluation determines both structural and functional (i.e., extrapulmonary right-to-left shunting in PPHN, left-ventricular performance) causes of hypoxemia. Serial echocardiography is important to determine the response to interventions (e.g., pulmonary vasodilators) and to reevaluate cases where specific interventions have not resulted in improvement or with progressive clinical deterioration. For example, in a patient with extrapulmonary right-to-left shunting and severe lung disease, pulmonary vasodilation might reverse the right-to-left venous admixture with little increase in systemic oxygenation. These observations unmask the critically important contribution of intrapulmonary shunting to hypoxemia.

Persistent Pulmonary Hypertension of the Newborn

As described previously, PPHN is a syndrome associated with diverse neonatal cardiac and pulmonary disorders that are characterized by high PVR causing extrapulmonary right-to-left shunting of blood

across the ductus arteriosus and/or foramen ovale. The syndrome of PPHN and the role of iNO are discussed in more detail in Chapter 52. However, because its relationship to respiratory failure in term newborns is so vital to understanding the clinical pathophysiology and approaches to treatment, we briefly address some historical perspectives in this section. Indeed, to understand the acronym PPHN, it is important to consider its historical evolution in term newborns with respiratory failure and critical hypoxemia and the advances that led to current management strategies.

In the early 1960s the association of RDS with PH and right-to-left ductal shunting was described in the landmark studies of [Rudolph et al. \(1961\)](#) and the clinical observations of [Stahlman \(1964\)](#). The first clear description of the pathophysiology of PPHN is attributed to [Gersony et al. \(1969\)](#), who coined the phrase *persistence of the fetal circulation* (PFC) in describing two newborns with clear lung fields who had critical hypoxemia associated with severe PH and right-to-left shunting across the foramen ovale and ductus arteriosus. In the next decade, several authors reported small series of infants with physiology similar to that described by Stahlman and Gersony et al. ([Lees, 1970](#); [Siassi et al., 1971](#); [Haworth and Reid, 1976](#)). The phrase *persistent pulmonary hypertension of the newborn* was first used by [Levin et al. \(1976\)](#) to describe a group of newborns with severe pulmonary hypertension, clear CXRs, and right-to-left shunting across the ductus arteriosus demonstrated both by simultaneous temporal arterial and umbilical arterial sampling (i.e., postductal desaturation) and by cardiac catheterization.

These initial descriptions of PFC focused on a discreet subset of newborns who had adequate cardiac performance without structural heart disease, absence of significant parenchymal lung disease, and suprasystemic PVR causing hypoxemia through right-to-left shunting of blood across the oval foramen and/or the arterial duct. However, it soon became clear that PFC could complicate the course of other diseases of the newborn, particularly meconium aspiration—initially described by [Stahlman \(1964\)](#) and later by [Fox et al. \(1977\)](#)—and pulmonary hypoplasia/CDH ([Harrison and de Lorimier, 1981](#)).

The nomenclature for this syndrome was clearly quite varied. Although there was initial appeal of the term *persistent fetal circulation* ([Behrman, 1976](#)), this description is not quite accurate because of the absence of the placenta and onset of air breathing after delivery. Over time, most authors have embraced *persistent pulmonary hypertension of the newborn* or PPHN as the proper name for this syndrome, with the classic PFC subtype (idiopathic PPHN) representing a relatively small percentage of the cases now commonly encountered. Pathophysiologic mechanisms and etiologic classifications of PPHN described by [Rudolph \(1980\)](#) and further characterized by [Geggel and Reid \(1984\)](#) and [Gersony \(1984\)](#) provided an important framework for understanding the complex nature of this syndrome as management strategies evolved over the past 2 decades.

Because of the role of PH in newborns with hypoxemic respiratory failure (initially severe hyaline membrane disease [HMD] and subsequently PFC, as described earlier), early approaches to management included a focus on pulmonary vasodilation using tolazoline, one of the few pharmacologic agents available at the time. Its use was first described by [Cotton \(1965\)](#) in newborns with HMD and later in infants with PPHN by [Gersony et al. \(1969\)](#), [Korones and Eyal \(1975\)](#), and [Levin et al. \(1976\)](#). However, its efficacy was limited by variable responsiveness and significant complications, including systemic hypotension and gastrointestinal hemorrhage ([Goetzman et al., 1976](#); [Stevenson et al., 1979](#)).

Multiple approaches to the treatment of PPHN evolved after its first recognition as a disease marked by severe pulmonary hypertension, but therapy was limited by the lack of suitable selective pulmonary vasodilators. Intensive investigation ultimately led to the discovery of nitric oxide (NO) as the endothelium-derived relaxing factor responsible for the rapid decline in PVR at birth (Abman, 1990). This recognition finally provided investigators with a selective vasodilator that has led to striking changes in the management of PPHN in the past 20 years.

The observation that dilute NO gas could be therapeutically delivered by inhalation was first described by Pepke-Zaba, who reported that brief iNO treatment caused potent and selective pulmonary vasodilation in adults with severe PH (Pepke-Zaba et al., 1991). Frostell et al. (1991) demonstrated the selectivity of iNO in an adult animal model of hypoxic pulmonary vasoconstriction, and the first description of the potent, sustained, and selective vasodilator effect of iNO in newborn lambs was reported by Kinsella et al. (1992). The current use of iNO and other approaches to PPHN treatment as they relate to specific respiratory diseases are discussed in the following sections.

Specific Pulmonary Conditions Causing Respiratory Distress in the Term Newborn

Transient Tachypnea of the Newborn

Transient tachypnea of the newborn (TTNB) is among the most common causes of respiratory distress in the newborn period, affecting 0.5%–4% of all late preterm and term neonates. The symptoms of respiratory distress typically start within the first several hours after birth and result from failure of adequate absorption of fetal lung fluid. Studies have consistently shown that risk factors for TTNB include prematurity, birth by cesarean delivery, and male sex (Riskin et al., 2005; Jain et al., 2009; Silasi et al., 2010). Among babies born by elective cesarean delivery, a recent study suggests that delivery before 39 weeks increases the risk of TTNB by more than twofold (Doan et al., 2014).

Early theories of lung fluid clearance focused on the role of thoracic compression during vaginal delivery and were supported by the observation that TTNB is more common among babies born by cesarean delivery (Milner et al., 1978). However, more recent studies have demonstrated that the complex process of lung liquid clearance likely begins well before term birth (Brown et al., 1983). During fetal life the lung epithelium is responsible for the production of a substantial volume of alveolar fluid, a process that is essential for normal fetal lung growth (Olver and Strang, 1974). With parturition, increased levels of epinephrine, glucocorticoids, and other hormones effectively cause the lung epithelia to transition from a secretory to a resorptive phenotype (Barker et al., 1990; Baines et al., 2000). Activated endothelial sodium channels (ENaC) at the apical surface of lung type II epithelial cells transport sodium and water from the alveolar space into the type II cells (Olver et al., 1986). Sodium is then actively moved from the type II cell into the interstitium by sodium-potassium pump (Na/K-ATPase), causing passive movement of water, which is then resorbed into the pulmonary circulation and lymphatics. Supporting a possible role for abnormal activity of ENaC and Na/K-ATPase in TTNB, it has been found that genetic polymorphisms in β -adrenergic receptor–encoding genes (which regulate expression of these channels) are more common in babies with TTNB (Aslan et al., 2008).

The diagnosis of TTNB remains problematic for clinicians. The most typical presenting symptoms, tachypnea/respiratory distress and the need for supplemental oxygen, are common among most neonatal respiratory disorders, and unfortunately, there exist no reliable diagnostic tests for TTNB (Guglani et al., 2008). For those reasons the diagnosis remains one of exclusion, and vigilance for other, more severe disorders is imperative. Typically, symptoms of TTNB develop within the first several hours after birth. The degree of respiratory impairment, including the respiratory rate, use of accessory respiratory muscles, and impairment in gas exchange, differs widely. CXRs should be considered in any baby presumed to have TTNB. Although radiographs commonly show prominent perihilar markings and fluid in the fissures, clinicians and radiologists often disagree in their interpretation of these findings in TTNB (Kurl et al., 1997).

Once a presumptive diagnosis of TTNB has been made, treatment is largely supportive. Oxygen should be provided to maintain normal arterial oxygen saturations. The degree of tachypnea and respiratory distress should determine whether a baby is allowed to feed by mouth. If there is a suspicion of pneumonia or sepsis, empiric antibiotic therapy should be considered. A controlled trial of furosemide administration to accelerate clearance of lung fluid showed no benefit in attenuating the course of TTNB (Wiswell et al., 1985). A prospective study suggests that moderate fluid restriction for the first 72 hours reduced the duration of respiratory support and cost of hospitalization (Stroustrup et al., 2012). Alternative or additional diagnoses should be considered in any infant who is deteriorating or requires mechanical ventilation. With supportive care, full recovery is to be expected after TTNB. However, compared with well infants of a similar gestational age, newborns with TTNB have a significantly prolonged hospital course (Riskin et al., 2005). Moreover, recent epidemiologic studies have suggested that newborns with TTNB are at a mildly increased risk of the later development of asthma (Birnkranz et al., 1996; Schaubel et al., 2006; Liem et al., 2007).

Meconium Aspiration Syndrome

MAS is associated with inhalation of meconium and amniotic fluid during fetal life or at delivery and is often complicated by significant pulmonary hypertension. It is among the most common causes of hypoxemic respiratory failure in term newborns who require intensive care (Fig. 47.1). The incidence of MAS in babies born after 37 weeks' gestation ranges from 0.4% to 1.8% (Dargaville and Copnell, 2006; Singh et al., 2009), and a study suggests that the rate of MAS may have been declining in recent years (Vivian-Taylor et al., 2011). Among babies born after 39 weeks' gestation with lung disease requiring mechanical ventilation, more than half have MAS (Gouyon et al., 2007). Moreover, MAS is the primary diagnosis for a significant proportion of those newborns who require extracorporeal membrane oxygenation (ECMO) in the United States (26%) and the United Kingdom (51%) (Brown et al., 2010).

Although a significant percentage of term births are complicated by the passage of meconium before or at delivery, less than 10% of those exposed to meconium develop MAS. Among that 10%, fetal acidemia is believed to cause increased intestinal peristaltic activity that results in passage of meconium and fetal gasping, which draws meconium-contaminated amniotic fluid deep into the lungs. Supporting this theory, autopsy studies of babies who died of MAS demonstrate distal muscularization of small pulmonary arterioles, suggesting long-standing hypoxemia (Murphy et al., 1984). Recent work suggests that activation of inflammatory cascades



• **Fig 47.1** Chest radiograph of term newborn with severe meconium aspiration syndrome. Note the anterior pneumomediastinum and small left anteromedial pneumothorax.

may worsen the severity of MAS (Lindenskov et al., 2015; Lee et al., 2016). Particulate meconium in the distal airways causes check-valve obstruction of air passages and leads to regional hyperinflation and atelectasis. In addition, meconium inactivates surfactant, leading to secondary surfactant deficiency (Moses et al., 1991). Moreover, babies with MAS are at high risk of persistent pulmonary hypertension, which significantly increases their morbidity and complicates their management.

Historically, prevention of MAS has focused on decreasing exposure of the fetal and newborn lung to the noxious effects of intrapulmonary meconium-contaminated amniotic fluid. Infusion of saline into the amniotic cavity (i.e., amnioinfusion) during labor has been studied as a means of both diluting meconium and relieving pressure on the umbilical cord, a potential cause of fetal acidemia. In the largest trial investigating this practice, Fraser et al. (2005) found no reduction in the risk of MAS. An alternative strategy for decreasing lung exposure to meconium is intrapartum oropharyngeal and nasopharyngeal suction of fetuses born through meconium-stained amniotic fluid. Although this practice was widely adopted in the 1970s, more recent studies have failed to demonstrate benefit (Vain et al., 2004), and the practice is no longer endorsed by the American College of Obstetricians and Gynecologists (Committee on Obstetric Practice, American College of Obstetricians and Gynecologists, 2007). Moreover, the current American Academy of Pediatrics/American Heart Association guidelines for cardiopulmonary resuscitation and emergency cardiovascular care of the neonate no longer recommend intubation and tracheal suctioning for nonvigorous newborns born through meconium-stained amniotic fluid (Wyckoff et al., 2015).

The clinical signs of MAS differ widely among babies and may relate to the degree of prenatal compromise, the timing, volume, and consistency of aspirated meconium, and the presence of associated problems. Clinical signs of MAS typically present immediately after birth with tachypnea, increased work of breathing, and cyanosis. Other common associated findings include metabolic acidosis, cardiac dysfunction and hypotension, and postductal desaturation indicative of right-to-left shunting of blood at the ductus arteriosus caused by pulmonary hypertension. Because of the potential for ball-valve obstruction of small airways and failure to empty distal lung segments, pneumothorax can complicate the

clinical picture. In recent series, the risk of pneumothorax among ventilated babies with MAS ranged between 10% and 24% (Dargaville and Copnell, 2006; Velaphi and Van Kwawegen, 2008). Like the degree of clinical signs, CXR findings differ widely. The classic CXR shows diffuse, fluffy infiltrates. However, some babies have milder initial radiographic findings, and there is often progression of visible parenchymal disease over time, likely related to secondary surfactant dysfunction.

Approximately half of babies with MAS require mechanical ventilation. The ventilator strategy should be individualized to each baby and to the disease evident on the CXR. In general, because of the likelihood of increased airway resistance, a conventional strategy using slower rates with long inspiratory and expiratory times allows better gas dispersion and more adequate emptying during expiration. Gas trapping and regional or generalized hyperinflation can occur, particularly when rapid rates are used with a conventional mode of ventilation. Some babies respond better to ventilation with a high-frequency device, although there is also a high risk of hyperinflation. When severe, hyperinflation causes impaired gas exchange and hypercarbia, limits systemic venous return (adversely affecting cardiac performance), increases the risk of pneumothorax, and may exacerbate pulmonary hypertension. Surfactant lung lavage has demonstrated some promise in improving lung function in animal models of MAS (Dargaville et al., 2003), but a small clinical trial of this practice demonstrated no benefit (Gadzinowski et al., 2008). However, conventional surfactant administration in infants with MAS may reduce the severity of illness and decrease the need for treatment with ECMO (El Shahed, 2014). In addition to management of parenchymal lung disease in MAS, special consideration must be given to other associated problems, particularly pulmonary hypertension. The risk of PPHN is quite high, exceeding 50% in some series. It has been demonstrated that iNO treatment increases oxygenation in MAS and is particularly efficacious when combined with a ventilator strategy that focuses on improving lung recruitment such as high-frequency oscillatory ventilation (Kinsella et al., 1997). Treatment with antibiotics with systemic effect should also be strongly considered in babies with PPHN for a number of reasons. These include the fact that intrauterine infection might be a precipitating factor in the initial passage of meconium and that in vitro studies suggest that the presence of meconium might facilitate the growth of bacteria in the lung.

In spite of the availability of iNO treatment and high-frequency modes of ventilation, some babies do not respond to medical therapy and require treatment with ECMO. Among babies with MAS treated with ECMO, reported survival ranges between 94% and 97%, markedly higher than for newborns treated for other respiratory conditions (Gill et al., 2002; Brown et al., 2010).

Surfactant Protein Deficiency

The details of surfactant biology and of RDS in premature newborns are presented in Chapter 12. However, term newborns may rarely present with a clinical syndrome dominated by respiratory failure that may be indistinguishable from surfactant deficiency in preterm infants. In the setting of unexplained and protracted respiratory failure in term infants, genetic alterations of surfactant-associated proteins, particularly surfactant protein B (SP-B), surfactant protein C (SP-C), and adenosine triphosphate (ATP)-binding cassette subfamily A member 3 (ABCA3), must be considered and ruled out. In addition, defective signaling of granulocyte-macrophage colony-stimulating factor, which regulates alveolar macrophages

and surfactant catabolism, may cause progressive interstitial lung disease in infants (Whitsett et al., 2015).

As described later, there is considerable overlap in the clinical presentation of babies with mutations of SP-B, SP-C, and ABCA3. Although onset, severity of clinical signs, and a family history of lung disease may offer clues to the underlying disorder, a full histologic and genetic evaluation should be considered. Genetic analysis of blood or buccal swabs for mutations of the genes encoding SP-B, SP-C, and ABCA3 are the definitive tests for each disease, but analysis is both costly and time consuming. Moreover, analysis is not routinely available, necessitating transport of specimens to a small number of laboratories specializing in these analyses. In addition, bronchoalveolar lavage (BAL) aspirate should be analyzed by enzyme-linked immunosorbent assay for the presence of SP-B and pro-SP-C. Lung biopsy, perfused by both standard microscopy and electron microscopy, can also provide important clues to the underlying diagnosis.

Surfactant-Associated Protein B Deficiency

Mature SP-B is a small hydrophobic protein (79 amino acids) that plays several key roles in the processing and function of normal pulmonary surfactant (Nogee et al., 2000). Transgenic mouse models suggest that SP-B is critical for phospholipid packaging into lamellar bodies, the formation of tubular myelin, and spreading/function of the surfactant monolayer. In addition, normal SP-B appears to be essential for normal processing of SP-C (Vorbroke et al., 1995). In all babies described to date with SP-B deficiency, the genetic mutation has been inherited from the parents (autosomal recessive) rather than occurring as a spontaneous mutation (Hamvas et al., 1994). Although multiple genetic mutations have been described, approximately 70% of affected babies carry the 121ins2 mutation (Dargaville and Copnell, 2006). In a series of term babies referred for genetic evaluation of unexplained respiratory failure, 2 of 17 had detectable mutations of the SP-B sequence (Somaschini et al., 2007).

Almost all babies with recognized deficiency of SP-B develop clinical signs of severe respiratory distress, including tachypnea, grunting, and retractions, within the first several hours of life. As for preterm newborns with HMD, CXRs classically reveal diffuse, hazy air space disease with visible air bronchograms. Severe PH may be a prominent feature of the disease. Treatment with surfactant is either ineffective or unsustainable, and progressive respiratory failure is the rule (Hamvas, 2006). Lung transplant is currently the only effective long-term treatment, with both short-term and long-term outcomes similar to those for other infants undergoing lung transplant (Palomar et al., 2006).

Analysis of fluid obtained by BAL/tracheal aspirate from babies with SP-B deficiency should fail to detect any immunoreactive SP-B. The presence of pro-SP-C increases suspicion of SP-B deficiency because intact SP-B is necessary for normal posttranslational processing of the protein to mature SP-C. Histologic findings include alveolar cell hyperplasia, interstitial thickening, and variable degrees of fibrosis and alveolar proteinosis. Staining of lung tissue for pro-SP-B is variable, but staining for mature SP-B should be minimal (because of cross-reactivity with epitopes on pro-SP-B) or absent. Initial DNA analysis focuses on the 121ins2 mutation. More exhaustive testing for other known mutations is warranted if initial testing is negative.

Surfactant-Associated Protein C Deficiency

Mature SP-C is a 35 amino acid hydrophobic protein. SP-C is believed to enhance spreading of surfactant and to participate in

normal surfactant catabolism (Whitsett et al., 2015). As with SP-B, multiple mutations of SP-C have been described; however, most of these mutations of the gene that encodes SP-C arise spontaneously and result in sporadic disease. Whether the abnormal phenotype associated with SP-C deficiency arises from dysfunction of the alveolar surfactant or from accumulation of abnormal cellular SP-C and consequent type II cell injury is not known.

In contrast to babies with SP-B deficiency, babies with SP-C deficiency have a wide range of clinical presentations. Some may develop symptoms within the first several hours of life, similar to SP-B deficiency, whereas others present later in childhood or in adulthood with interstitial lung disease. In one series, half of children with SP-C deficiency presented in the neonatal period, and mortality was reported as 15% (Guillot et al., 2009). The reasons that underlie the variable onset, presentation, and severity of SP-C deficiency are not fully understood, but it is suggested that those individuals whose SP-C mutation lies within the specific BRICHOS chromosomal domain are at higher risk of neonatal presentation (Thouvenin et al., 2010). Corticosteroids are the mainstay of treatment of infants with SP-C deficiency, although many centers also administer azithromycin and/or hydroxychloroquine (Thouvenin et al., 2010).

In common with SP-B deficiency, the lung histopathologic features of patients with SP-C deficiency are nonspecific and widely variable. Common findings include accumulation of alveolar protein and macrophages and epithelial cell hyperplasia. Ultrastructural examination may reveal disorganized lamellar bodies with aggregates of small vesicles with electron-dense cores in the type II cells (Nogee et al., 2001). Allele-specific testing using the polymerase chain reaction for the most common 173T mutation provides an initial screen for SP-C deficiency. If the screen is negative, direct sequencing of the entire SP-C gene should be undertaken.

Adenosine Triphosphate–Binding Cassette Subfamily A Member 3 Deficiency

The ATP-binding cassette transporter proteins are essential for normal transport of compounds in numerous biologic systems (Mulugeta et al., 2002). Deficiencies of individual ATP-binding cassettes have been associated with clinical diseases in a number of different organ systems. ABCA3 is highly expressed in the lung and is involved in the transport of lipids. Individuals lacking the gene for ABCA3 have abnormal accumulation of surfactant-rich lamellar bodies within their type II alveolar cells, with apparent inability to transport surfactant into the alveolar space. Shulenin et al. (2004) detailed a variety of mutations within the ABCA3 gene in a substantial portion of term infants with unexplained respiratory failure and suspected surfactant protein deficiency.

The age at presentation for individuals with ABCA3 deficiency is highly variable, ranging from the immediate newborn period to later in childhood. There may be a family history of consanguinity. Clinical manifestations of disease in the neonate may be indistinguishable from those of neonates with SP-B deficiency, with onset of respiratory failure within hours of birth. The disease may be progressive and fatal. In addition, ABCA3 deficiency may also manifest itself as severe PPHN (Kunig et al., 2007). The treatment options for ABCA3 deficiency are limited. Some children undergo lung transplant, and recent case reports suggest that some patients may respond to corticosteroids, azithromycin, or hydroxychloroquine (Hayes et al., 2012; Thouvenin et al., 2013; Williamson and Wallis, 2014). It has been reported that milder, transient neonatal symptoms may not prompt a diagnostic evaluation in the newborn period, although recurrent pulmonary symptoms may lead to later investigation (Doan et al., 2007). These reports raise the possibility that

deficiency of ABCA3 may be underrecognized in infants or children with a mild or normal phenotype.

The predominant pathologic findings of neonates with ABCA3 deficiency with neonatal respiratory failure include alveolar proteinosis (Bullard et al., 2006), type II cell hyperplasia with dense lamellar bodies, and accumulation of alveolar macrophages in the distal air space. Inheritance is believed to be autosomal recessive. Unlike SP-B deficiency, a single predominant mutation has not been described; rather, multiple distinct mutations affecting different protein domains have been identified (Brash et al., 2006).

Thyroid Transcription Factor 1 Gene Mutation

Recent studies demonstrate that mutations of the gene encoding thyroid transcription factor (TTF)-1 (*NKX2-1*), which is expressed in the thyroid, lung, and brain, can result in neonatal respiratory failure. TTF-1 regulates structural lung development and plays a key role in the expression of SP-B, SP-C, and ABCA3. Histologic findings from these patients are heterogeneous and not diagnostic. Hamvas et al. (2013) reported a series of 21 patients with *NKX2-1* mutations, 17 of whom had neonatal RDS (with or without PPHN). Some of these patients also had thyroid and/or brain abnormalities, while others had only respiratory disease evident at the time of diagnosis.

Surfactant-Associated Proteins A and D

Although lung structural abnormalities have been described in mice lacking SP-A and SP-D, no phenotype attributable to abnormalities of either of these surfactant-associated proteins has been described in human infants. Recent work suggests a role for mutations in the gene that encodes surfactant-associated protein A (SP-A) in adult-onset pulmonary fibrosis in rare circumstances (Whitsett et al., 2015).

Interstitial Lung Disease

The interstitial lung diseases that occur in infancy are a heterogeneous group of disorders that overlap with the surfactant protein disorders and may involve one or more of the several components of the lung. All produce considerable morbidity and mortality. Detailing each individually is beyond the scope of this chapter, but two, alveolar capillary dysplasia with misalignment of pulmonary veins (ACD/MPV) and pulmonary interstitial glycogenosis, have attracted considerable attention in recent years (Deterding, 2010; Hamvas et al., 2013; Whitsett et al., 2015).

Alveolar Capillary Dysplasia/Misalignment of Pulmonary Veins

ACD/MPV results from severe developmental abnormalities of the structure of the pulmonary circulation. In particular, alveolar capillary density is reduced, and capillaries typically reside in the center of abnormally widened alveolar walls rather than immediately proximate to the alveolar epithelium and air space. An additional histologic hallmark of the disease is abnormally positioned pulmonary veins, which are adjacent to the terminal bronchioles (Langston, 1991). Most newborns with ACD/MPV experience respiratory failure and PPHN within the first several days of life. For reasons that are poorly understood, presentation may be delayed in some babies. The typical course of ACD/MPV is refractory respiratory failure and pulmonary hypertension, which ultimately lead to death. Some babies may respond to pulmonary vasodilators but only transiently (Bishop et al., 2011). Stankiewicz et al. (2009) documented deletions of the Forkhead Box (FOX) transcription

factor gene cluster on chromosome 16 in a series of ACD/MPV patients. Depending on the underlying chromosomal abnormality, patients with ACD/MPV may also have other malformations, most notably of the gastrointestinal, genitourinary, and cardiovascular systems.

Pulmonary Interstitial Glycogenosis

Pulmonary interstitial glycogenosis (PIG) is an interstitial lung disease thought to be unique to infants that is characterized by a thickened lung interstitium and the accumulation of intracellular glycogen in immature interstitial cells (Canakis et al., 2002; Dishop, 2011). It has been postulated that PIG represents a developmental lung disorder rather than one resulting from inflammation or other precipitants. Infants with PIG typically develop respiratory symptoms in the first few days of life, including tachypnea and hypoxemia; many require a period of assisted ventilation. Many infants with biopsy-proven PIG have been treated with pulse steroid therapy, and often respiratory symptoms abate or resolve altogether in the first year of life. Whether steroid treatment accelerates the abatement process has not been established and remains controversial (Das et al., 2011).

Congenital Diaphragmatic Hernia

CDH is a complex clinical syndrome caused by a developmental defect in the diaphragm, resulting in a spectrum of potentially severe cardiopulmonary abnormalities (Fig. 47.2). The estimated incidence of CDH ranges from approximately 1 in 2500 to 1 in 7000 liveborn babies (Prober, 2007). Approximately 80%–85% of diaphragmatic hernias occur on the left side. In rare circumstances they may be bilateral. Anatomically, CDH is classically divided by location into posterolateral (Bochdalek) defects, retrosternal anterior (Morgagni) defects, and other anterior or central defects. The vast majority, 90%–95%, are of the Bochdalek type. However, these classic anatomic distinctions cannot always be clearly differentiated (Prober, 2008). Moreover, the implications of hernia location on the severity of illness or mortality are not clearly defined (Wynn et al., 2013).



• **Fig. 47.2** Chest radiograph of an infant with left-sided congenital diaphragmatic hernia. Note the proper position of the orogastric tube and umbilical venous catheter.

As many as 30%–40% of babies with CDH have additional congenital anomalies, most commonly of the heart, central nervous system, and genitourinary system. The remaining 60%–70% of babies have isolated CDH without other identifiable major anatomic malformations. CDH may occur as an element of several well-recognized syndromes or of a chromosomal abnormality (particularly trisomy 18). In babies with CDH but without a recognized genetic syndrome, 10%–15% have heart defects, most commonly septal defects, conotruncal malformations, and obstruction of the left ventricular outflow tract (Lin et al., 2007). Coexisting cardiac disease complicates the management of babies with CDH and likely increases mortality. There is growing interest in the potential for fetal tracheal occlusion in the most severe cases diagnosed prenatally, but there is insufficient evidence to warrant routine use (Grivell et al., 2015).

Medical management of the newborn with a CDH remains one of the most complex and challenging situations for a neonatologist. A host of interrelated issues must be considered, including optimal mechanical ventilation strategies, the presence and treatment of pulmonary hypertension, evaluation of cardiac performance, and consideration of support with ECMO. Unfortunately, owing to the relative infrequency of CDH, definitive studies guiding clinical decisions in the management of these babies are lacking.

The optimal mode and strategy of mechanical ventilation in CDH have not been definitively established. Previously, aggressive hyperventilation and alkalinization as a means to lower PVR were advocated (Bohn et al., 1987). More recently, with the recognition that aggressive mechanical ventilation to the point of hyperventilation may cause significant iatrogenic lung injury and worsen outcome, this approach has generally been abandoned (Sakurai et al., 1999). However, prospective trials to clearly guide the ventilator strategy are lacking. Many experts now advocate a gentle ventilation strategy that minimizes peak inspiratory pressure, targets arterial partial pressure of carbon dioxide (PaCO_2) levels between 40 and 60 Torr, and tolerates postductal desaturation if preductal saturations are adequate. If a conventional ventilator strategy is used and the peak inspiratory pressures needed to achieve these goals are unacceptably high (generally 25 to 30 cmH_2O), transition to high-frequency oscillatory ventilation (HFOV) is undertaken (Finer et al., 1998; Bohn, 2002; Boloker et al., 2002). As an alternative, some advocate earlier or initial use of HFOV (Frenckner et al., 1997; Reyes et al., 1998; Bagolan et al., 2004). Although definitive evidence in support of this gentle ventilation strategy is lacking owing to the retrospective nature of the reports cited, mortality in several centers has fallen coincident with adoption of this approach.

PH is a well-recognized complication of CDH, and the severity of PH at 1 month of life correlates with increased mortality (Wynn et al., 2013). Decreased cross-sectional area of the pulmonary circulation, structural remodeling of the pulmonary vessels, and diminished size and function of the left ventricle have all been implicated in the PH associated with CDH. Newborns with CDH should all undergo early echocardiography to ascertain the presence of associated heart defects, to assess the degree of pulmonary hypertension, and to evaluate the function of the left and right ventricles. Treatment of early PH in babies with CDH is controversial. Treatment with iNO did not reduce mortality among a group of infants with CDH in whom aggressive medical therapy had failed (Neonatal Inhaled Nitric Oxide Study Group, 1997). Moreover, newborns treated with iNO in that study more frequently required ECMO than the control group, likely related to the adverse effects

of pulmonary vasodilation in the presence of severe left-ventricular dysfunction, potentially worsening systemic hemodynamics. Current evidence does not support the routine use of iNO in the first 24 hours after birth in infants with CDH. Rather, its use should be limited to those babies with optimized lung inflation and well-defined PH who do not have evidence of impaired left-ventricular performance and right ventricle-dependent systemic blood flow (Kinsella et al., 2005; Abman et al., 2016). Other pharmacologic pulmonary vasodilators to treat acute PH in babies with CDH have not been carefully evaluated. In babies with severe PH and a closed ductus arteriosus, consideration should be given to restoration of ductal patency with prostaglandin infusion, to allow the right ventricle to serve as a source of systemic blood flow (Kinsella et al., 2005; Abman et al., 2016).

Animal models of CDH demonstrate biochemical and physiologic evidence of surfactant deficiency (Glick et al., 1992). In these models, surfactant replacement lowers PVR and improves gas exchange and lung mechanics (Wilcox et al., 1994; O'Toole et al., 1996). Studies of human babies with CDH also suggest a delay in surfactant maturation, and postmortem studies of babies with CDH commonly demonstrate the presence of hyaline membranes (Hisanaga et al., 1984; Moya et al., 1995). Taken together, the findings of studies raise the possibility that surfactant replacement therapy might have a role in the management of babies with CDH. Although initial anecdotal evidence supported this possibility, a more recent retrospective study of more than 500 term babies with CDH suggested that surfactant treatment was associated with greater ECMO use, increased incidence of chronic lung disease, and reduced overall survival (Van Meurs and Congenital Diaphragmatic Hernia Study Group, 2004). These findings strongly argue against the routine use of surfactant replacement in babies with CDH.

Owing to the infrequency of CDH and the wide variability in therapeutic approaches (both among centers and over time at individual centers), few studies of long-term outcome have been published. Studies suggest that babies born with CDH are at risk of a number of serious long-term morbidities, including impaired neurodevelopmental outcome, wheezing/asthma, protracted pulmonary hypertension, sensorineural hearing loss, gastroesophageal reflux, scoliosis, and pectus excavatum (Peetsold et al., 2009). One study reports lower than anticipated scores on tests of intelligence and adaptive behavior among 11 CDH survivors who were not treated with ECMO (Bouman et al., 2000). Stolar et al. (1995) reported that among 51 babies treated with ECMO, the subset of those with CDH had worse cognitive outcome than those with alternative underlying diagnoses. Neurologic (physical) testing was similar between the two groups. Wynn et al. (2013) reported that CDH patients had lower developmental scores at 2 years of age and that developmental delays were associated with the need for ECMO and lower socioeconomic status. Although concerning, these findings must be interpreted with caution, because the mean age at evaluation was less than 3 years. CDH is also associated with a high rate of sensorineural hearing loss (SNHL), nearly 50% in one study (Morini et al., 2008). The causes that underlie the high incidence of SNHL in this population, and whether it represents a genetic/anatomic predisposition or is a result of treatment-related risk factors, have yet to be clarified.

As might be expected, several cardiopulmonary sequelae of CDH have been reported. In a follow-up study of adolescents born with CDH, Trachsel et al. (2005) reported several abnormalities elicited by pulmonary function testing. Compared with normal controls, CDH survivors demonstrated mild to moderate airway obstruction,

and nearly half were responsive to bronchodilators. CDH patients also had decreased inspiratory muscle strength and maximum minute ventilation. In spite of these findings, these adolescents generally had minimal compromise of daily activities. The persistence of PH in some patients with CDH remains a major concern. After CDH repair and with readiness for removal from mechanical ventilation, some infants with underlying PH develop suprasystemic pulmonary artery pressures with discontinuation of iNO treatment. Kinsella et al. (2003) reported successful ongoing treatment of these babies with delivery of noninvasive iNO treatment via a nasal cannula. Schwartz et al. (1999) report that 38% of their CDH patients who required ECMO had echocardiographic evidence of PH well beyond the newborn period, with a mean follow-up age of 3.2 years. Importantly, most of those children had no apparent physical signs or symptoms of PH, supporting the need for proactive, ongoing surveillance. Although recent studies focused on the long-term follow-up of pulmonary vascular disease in these patients are lacking, the American Academy of Pediatrics recommends serial echocardiographic evaluation of children with CDH who have persistent PH after repair (Lally and Engle, 2008). Newer pharmacologic agents for treatment of PH, such as phosphodiesterase inhibitors (sildenafil) or endothelin antagonists such as bosentan, may offer effective longer-term therapy for PH but have not been investigated in this population.

Postnatal nutrition presents a major challenge for many survivors of CDH. Among 121 CDH survivors, Muratore et al. (2001) reported that one-third had failure to thrive requiring the placement of a gastrostomy tube for the provision of adequate calories, and 25% demonstrated evidence of oral aversion. Many reports have documented the high incidence of gastroesophageal reflux after repair of CDH, and studies have reported that 12%–28% require fundoplication for severe gastroesophageal reflux (Fasching et al., 2000; Diamond et al., 2007; Su et al., 2007).

Summary

The initial evaluation of respiratory distress/hypoxemia in the term newborn presents one of the most difficult challenges faced by pediatricians and neonatologists. An ordered approach using information derived from the history, physical examination, pulse oximetry measurements, radiographic and laboratory measurements, and echocardiography can help elucidate the cause of hypoxemia

Pulmonary Hypoplasia

In the absence of a diaphragmatic hernia, pulmonary hypoplasia and respiratory failure can develop in association with a number of other conditions. An extensive list of conditions associated with pulmonary hypoplasia has been generated, but most cause either restriction of normal fetal breathing motion or compression of the developing lung. Thus among the most common causes of pulmonary hypoplasia are low amniotic fluid volumes, neuromuscular disorders, pleural effusions/chylothoraces, and space-occupying lung lesions.

Inadequate amniotic fluid volume to allow normal fetal breathing can result from a number of circumstances. Because fetal urine is a primary contributor to amniotic fluid volume, any abnormality of renal development that limits fetal urine production or urine flow can result in pulmonary hypoplasia. In addition, preterm premature rupture of membranes, particularly with persistent leak of amniotic fluid, can result in pulmonary hypoplasia. For a more comprehensive discussion of preterm premature rupture of membranes and pulmonary hypoplasia in preterm infants, see Chapter 46.

Definitive diagnosis of pulmonary hypoplasia can be made only at autopsy. Norms have been established for both the gestational age–corrected ratio of lung weight to body weight and for histologic evaluation of radial alveolar counts (Askenazi and Perlman, 1979; Lauria et al., 1995). Clinical criteria for pulmonary hypoplasia are less well defined. Neonates may have a bell-shaped chest and elevated diaphragms on CXR. Management of pulmonary hypoplasia should focus on maximizing lung inflation while avoiding pneumothorax, the incidence of which ranges widely, depending on the study (Leonidas et al., 1982; Klaassen et al., 2007). Ventilator strategies include permissive hypercapnia, to minimize lung injury, and the use of HFOV. If PH is defined echocardiographically, use of iNO should be considered. With sustained postnatal ventilatory support, the PH associated with pulmonary hypoplasia may resolve over time.

and respiratory failure and direct each step of clinical management. Recognizing the important contributions of parenchymal lung disease, pulmonary vasoconstriction, and cardiac performance is critical to successful clinical management of the term newborn with respiratory failure.

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Bronchopulmonary Dysplasia

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KEY POINTS

- Bronchopulmonary dysplasia (BPD) is a heterogeneous disorder.
- The cause is multifactorial.
- Early nutritional and ventilatory support are important preventive strategies.
- Effective pharmacologic strategies remain a challenge, although caffeine use—for prevention—has been widely adopted.
- Extremely preterm infants are at highest risk for BPD (genetic predisposition, unfavorable fetal environment, severe early cardiopulmonary illness), but there are limited preventive options.

Historical Overview

Bronchopulmonary dysplasia (BPD), also referred to as *chronic lung disease of prematurity*, is the most common chronic lung disease of childhood. BPD was first described by [Northway et al. in 1967](#) as a severe chronic lung injury in premature infants who survived hyaline membrane disease after treatment with mechanical ventilation and oxygen ([Northway et al., 1967](#)). Four distinct clinical stages were described radiographically and pathologically, progressing from typical respiratory distress syndrome (RDS), with alveolar interstitial edema as well as atelectasis, to massive fibrosis and consolidation of the lung with areas of cystic emphysema and overinflation.

BPD, as originally described, occurred predominantly in preterm infants born at 30 to 34 weeks' gestation with a history of severe respiratory distress necessitating aggressive ventilatory support and high levels of oxygen exposure for a prolonged period of time. These infants were born before the introduction of prenatal corticosteroids or postnatal surfactant replacement therapy and at a time when ventilators were first being adapted for use in the newborn.

Since that time, much has been learned about BPD, and the disorder as originally described has become rare. The infants who are more commonly affected with BPD today are those of extremely low birth weight, born at less than 26 weeks' gestation. Following exposure to prenatal corticosteroids, combined with postnatal exogenous surfactant for treatment of RDS, these infants initially frequently do remarkably well, requiring low levels of supplemental oxygen and ventilatory support. They often have a subsequent period of several days during which there is minimal or no requirement for ventilatory support, before entering into a more chronic phase of variable requirements for assisted ventilation and

supplemental oxygen. The form of BPD seen in the contemporary extremely preterm infant who has had minimal initial respiratory distress has been termed the *new BPD*, with arrested lung development affecting both alveologenesis and the pulmonary vascular bed ([Jobe, 1999; Abman, 2001, 2008](#)).

Although the initial definition of BPD was a persistent requirement for supplemental oxygen 28 days after birth, in an attempt to better define the functional implications of abnormal postnatal lung development in extremely preterm newborns, the need for oxygen at 36 weeks' postmenstrual age (PMA) was found to be a better predictor of later pulmonary illness in extremely premature infants ([Shennan et al., 1988](#)). The definition of BPD was subsequently reviewed at a National Institutes of Health (NIH)-sponsored workshop ([Jobe and Bancalari, 2001](#)). This group proposed diagnostic criteria for increasing severity of BPD, including the receipt of oxygen supplementation of varying degrees and assisted ventilation (continuous positive airway pressure [CPAP] and/or mechanical ventilation) at 36 weeks' PMA ([Table 48.1](#)). Concurrently, a physiologic challenge (protocolized withdrawal of supplemental oxygen and nasal cannula flow to room air, as tolerated) was being developed and tested ([Walsh et al., 2003, 2004](#)). In these initial studies, the use of the physiologic challenge reduced the incidence of BPD by almost 30%, with some decrease in center variability ([Walsh et al., 2004](#)). However, more recent reports from the same study group (the NIH-sponsored Neonatal Research Network [NRN]) have suggested less of an effect of the physiologic challenge on the incidence of BPD (the "physiologic definition"), only decreasing BPD rates from 42% to 40% ([Stoll et al., 2010](#)). Using the severity criteria proposed in the NIH workshop, data from the NRN demonstrated increasing frequency of respiratory morbidity and neurodevelopmental impairment at follow-up with increasing severity of BPD ([Ehrenkranz et al., 2005](#)). Furthermore, the definition of BPD using the need for supplemental oxygen alone was equally predictive of later pulmonary morbidity, as was the need for oxygen plus an abnormal chest radiograph. Similarly, increasing severity of BPD by the clinical classification was associated with persistence of respiratory morbidity over the first year after hospital discharge in the NIH-funded Prematurity and Respiratory Outcomes Program ([Keller et al., 2016a](#)). These data, along with the data from [Shennan et al. \(1988\)](#), which did not incorporate radiographic findings in their clinical definition of BPD, have largely eliminated the requirement for abnormal chest radiograph in the definition of BPD. However, with the exception of infants who undergo a physiologic challenge, infants receiving nasal cannula support (which can variably provide positive end-expiratory pressure

TABLE 48.1 Diagnostic Criteria for Bronchopulmonary DysplasiaTreatment with $\text{FiO}_2 > 0.21$ for at least 28 days

PLUS

Failure of room air challenge test at 36 weeks' postmenstrual age

Classification of Severity

| | |
|----------|---|
| Mild | Requires ≤ 0.30 effective FiO_2^a |
| Moderate | Requires > 0.30 effective FiO_2^a |
| Severe | Requires ventilatory support, usually with oxygen |

^aEffective FiO_2 is based on infant's weight and the concentration and flow of oxygen through a nasal cannula or in a hood. FiO_2 , Fraction of inspired oxygen.Data from Jobe AH, Bancalari E. Bronchopulmonary dysplasia. *Am J Respir Crit Care Med*. 2001;163:1723–1729.

[PEEP]) without supplemental oxygen (fraction of inspired oxygen [FiO_2] 0.21) are generally not given a diagnosis of BPD, which may not accurately reflect an infant's respiratory status (Sreenan et al., 2001; PoinDEXter et al., 2015).

In the presurfactant era, there was remarkable consistency in the postconceptual age at which infants surviving without later pulmonary morbidity were weaned off supplemental oxygen (Shennan et al., 1988). Subsequent studies have shown substantial proportions of infants with no BPD that do have later respiratory morbidity and relatively high proportions of infants with BPD that have no respiratory morbidity (Ehrenkranz et al., 2005; Keller et al., 2016a). Data from the Collaborative Home Infant Monitoring Evaluation Study demonstrate higher rates of apnea and desaturation in preterm infants at term-adjusted age and beyond, suggesting that persistence of oxygen supplementation in some former preterm infants may be related to dysmaturation of respiratory control (Ramanathan et al., 2001; Hunt et al., 2011). Consistent with this possibility, Coste et al. (2015) showed that former preterm infants undergoing a physiologic challenge test had increased frequency of periodic breathing. Thus as survival for the most immature newborns has improved following the introduction of surfactant replacement therapy and the broadening use of prenatal corticosteroids, it may be that later assessments of pulmonary status, at 40 weeks' PMA, for example, will better assess for differences in underlying lung function and will be better markers of respiratory morbidity through infancy and beyond (Davis et al., 2002; Keller et al., 2009).

Epidemiology

Although BPD is now relatively rare in infants born beyond 30 weeks' gestation, its incidence has been increasing in extremely low GA newborns, particularly those born at less than 26 weeks' gestation, as survival has improved. Data over a 20-year period for infants with birth weights ranging from 401 to 1500 grams and born at 22 to 28 weeks' gestation from the NRN demonstrate that the incidence of BPD at 36 weeks' postconceptional age increased from 36% in 1993–1997 to 45% in 2008–2012, while survival increased from 72%–76% over the same period (Stoll et al., 2015). In the most recent era (2008–2012), rates of BPD in infants surviving at 36 weeks' PMA were 88%, 79%, 69%, 57%, 50%, 36%, and 24% for infants of 22, 23, 24, 25, 26, 27,

and 28 weeks' gestation, respectively. Overall, survival without morbidity was unchanged for infants greater than 24 weeks' gestation but increased in less mature infants, carrying a costly chronic health burden in these most premature infants (Watson et al., 2009; Stoll et al. 2015). Among infants less than 29 weeks' gestation in the California Perinatal Quality Care Collaborative (CPQCC), rates of BPD or death before 36 weeks' PMA by birth weight were 81% among infants less than 750 grams, 49% for 751–999 grams, 25% for infants 1000–1249 grams, and 13% for infants 1250–1500 grams. As these data show, increased rates of BPD or death by 36 weeks' PMA are associated with lower GA and birth weight. Male sex and higher level of respiratory support are also consistently associated with increased rates of BPD or death (Ambalavanan et al. 2011; Laughon et al., 2011). Both the NRN and CPQCC report wide variability in the incidence of this composite outcome by center (Ambalavanan et al., 2011; Lapcharoensap et al., 2015). In CPQCC, the level of complexity of care was associated with rates of BPD or death, with centers that provide the most complex level of service demonstrating the lowest rates of adverse outcome (Lapcharoensap et al., 2015). The NRN analysis identified several practices that were independently associated with both BPD or death and center (i.e., practices that varied by center), but differences in rates of BPD persisted even when accounting for those identified center differences and infant baseline characteristics, indicating that there were other, unaccounted for center-specific or infant-specific differences (Ambalavanan et al., 2011).

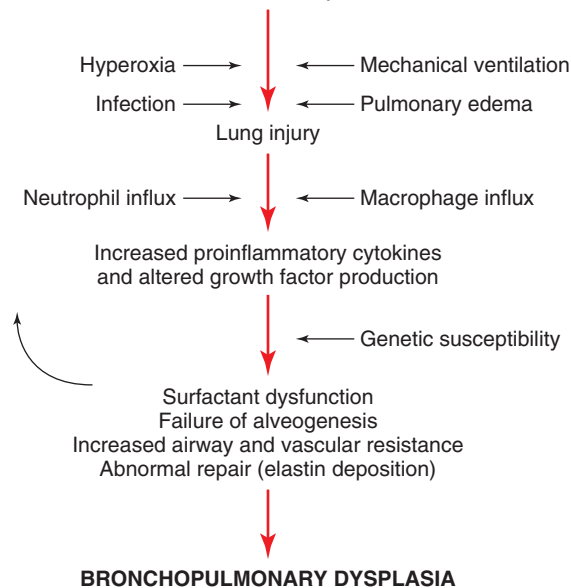
With respect to level of respiratory support, the persistence of mechanical ventilation and increasing degrees of supplemental oxygen have been associated with increased rates of BPD. Using data from the NRN, Laughon et al. demonstrated that the relative importance of the degree of respiratory support increases with respect to the outcome of BPD or death with advancing postnatal age (Laughon et al., 2011). At less than 7 days of age, GA has the most influence on this outcome, but at 7 to 21 days, the degree of respiratory support has the greatest influence. Similarly, Ambalavanan found that mechanical ventilation in the first 7 days was independently associated with BPD or death (Ambalavanan et al., 2011). In the Extremely Low Gestational Age Newborn study (infants < 28 weeks' gestation), rates of BPD in survivors at 36 weeks' PMA correlated with the pattern of supplemental oxygen exposure over the first 14 days of life (Laughon et al., 2009a). The overall incidence of BPD was 51%, but infants with a persistent low level of oxygen supplementation had the lowest rate of BPD (17%), infants with a deterioration in their pulmonary status (low initial levels of oxygen supplementation increasing in the second week) had an intermediate incidence of BPD (51%), and infants with persistently higher oxygen requirements had the highest rate of BPD (67%). Mechanical ventilation was also closely associated with these patterns of oxygen supplementation. At 7 days, 21%, 57%, and 84% of the low oxygen, pulmonary deterioration, and persistent high oxygen groups were receiving mechanical ventilation, respectively. These data are consistent with the selection of ventilated infants at 7 to 14 days of age for study of strategies to prevent BPD, given their high risk for adverse respiratory outcome (Ballard et al., 2016).

Pathobiology

The cause of BPD is clearly multifactorial and involves derangements in multiple aspects of lung function (e.g., surfactant production and function), repair from injury (e.g. elastin deposition), and growth and development (e.g., alveologensis and microvascular

development). Various factors contribute to this process, including a susceptible host with immature lung structure and developmental deficiencies of factors crucial to lung development and function such as surfactant, nitric oxide, innate immune defense, and antioxidant capability (Fig. 48.1). Interestingly, structural differences in the placentas of extremely low GA infants consistent with maternal underperfusion are associated with the development of

Premature lung with developmental deficiencies (surfactant, nitric oxide, immune defense, etc.) and immature alveoli



• **Fig. 48.1** The Pathogenesis of Bronchopulmonary Dysplasia. Lung injury in the immature lung secondary to hyperoxia, mechanical ventilation, and infection initiates an inflammatory response with an altered milieu of growth and inflammatory factors. Continuing insults contribute to the long-term changes in lung structure that characterize bronchopulmonary dysplasia.

BPD (Mestan et al., 2014). Biochemical changes that influence fetal lung development and/or some degree of fetal nutritional deprivation can accompany these structural differences. Thus both fetal and postnatal nutritional deficiencies, and the resulting growth failure, may cause or exacerbate lung structural abnormalities and impair the ability of these infants to recover from early lung injury.

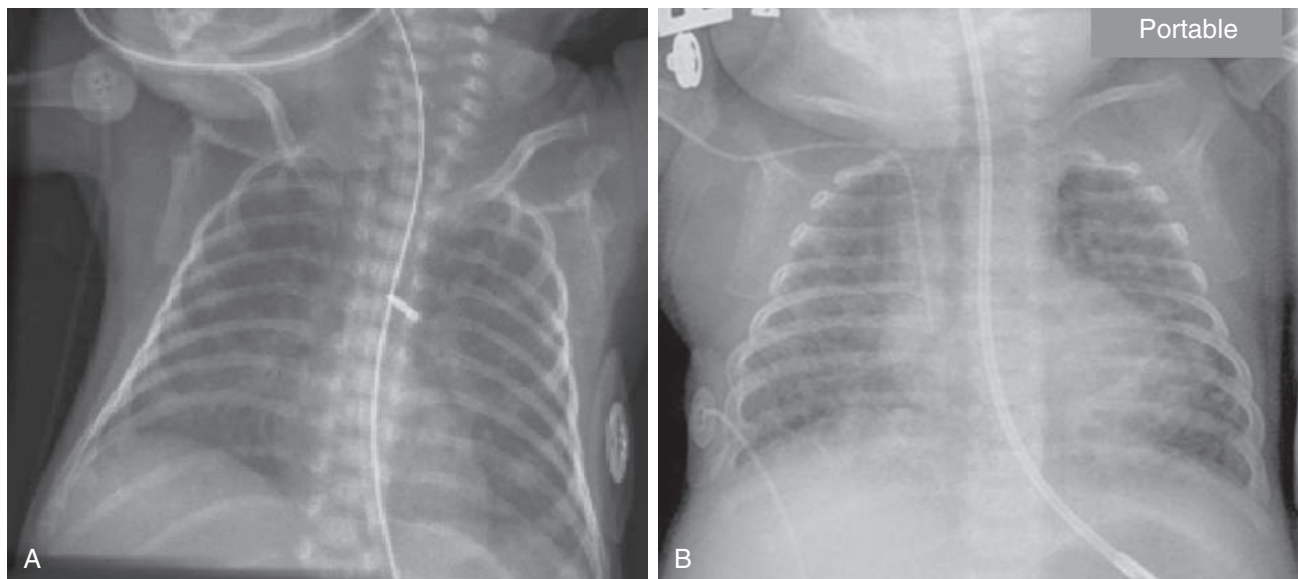
Pathologic Findings in the “New” Form of the Disease

In the chronic stage of BPD as originally described, the lung had extensive alveolar fibrosis, airway abnormalities, and pulmonary vascular remodeling (Northway et al., 1967; Bonikos et al., 1976). In contrast, the lungs are more uniformly inflated with minimal airway injury and less prominent fibrosis in the new BPD. The major abnormalities in infants dying with BPD are a decrease in alveolar number with less septation (referred to as *alveolar hypoplasia and alveolar simplification*) and dysregulated microvascular growth (Husain et al., 1998; Coalson et al., 1999; Bhatt et al., 2001; Maniscalco et al., 2002; De Paepe et al., 2006, 2008). In the very preterm infants in whom this new BPD is seen (Fig. 48.2), the lung is in the late canalicular or early saccular stage of development at the time of birth. Consistent with extremely preterm birth, investigators have noted arrested lung development in the saccular stage at autopsy in infants with prolonged ventilation, despite ongoing endothelial cell proliferation (De Paepe et al., 2006). A number of factors that interfere with postnatal alveogenesis may contribute to the disordered lung development seen in contemporary premature newborns.

Multifactorial Etiologic Factors

Host Susceptibility and Genetic Predisposition

Although the most important factor in the pathogenesis of BPD is prematurity, infants with fetal growth restriction are at increased



• **Fig. 48.2** Radiographs of Bronchopulmonary Dysplasia (BPD) Characteristic of the “New and “Old” Forms of the Disease. (A) Bilateral fine granular opacification consistent with atelectasis and/or edema, characteristic of the “new” BPD. (B) Bilateral coarse interstitial opacification consistent with fibrosis and/or edema and areas of hyperinflation, characteristic of the “old” BPD.

risk (Lal et al., 2003; Bose et al., 2009; Durrmeyer et al., 2012; Soudée et al., 2014). In addition, it has been known for some time that infants born to some families are more likely to have RDS at any given GA than those born to other families. Twin studies have supported this clinical impression, revealing that 50%–80% of the variance in the occurrence of BPD is due to genetic factors (Bhandari et al., 2006; Lavoie et al., 2008). In addition, a family history of asthma may increase the risk of BPD, although in the most recent study, this effect was present only in the absence of prenatal steroid exposure (Hagan et al., 1995; Gage et al. 2015). Several studies have described the association of BPD with single nucleotide polymorphisms for genes involved in the inflammatory response, innate immunity, angiogenesis, surfactant function, and the extracellular matrix such as interleukins, collectins, surfactant-associated proteins, and proteases. Although a recent genome-wide association study failed to replicate previous studies, the analyses suggested a dominant relationship between genetic variants and the BPD phenotype and reinforced the possibility that previous work had identified important pathways in the pathogenesis of BPD, despite the fact that the patient population had greater racial and ethnic diversity than previous studies (Wang et al., 2013). An analysis of rare variants identified three pathways as candidates, involving collagen fibril organization, embryonic epithelial morphogenesis, and regulation of the Wnt signaling pathway (involved in cell-to-cell interactions and stem cell renewal) (Li et al., 2015). The genes identified in this work were also upregulated in lungs of rats exposed to hyperoxia. These investigations may ultimately lead to more directed therapies for high-risk, susceptible infants, as variants may be associated with enhanced response to environmental insults or mitigating therapies.

Inflammation

The fetal-derived inflammatory response syndrome (FIRS) plays a major role in preterm birth and the development of BPD (Yoon et al., 1999; Kim et al., 2015). This fetal response may be triggered by microbial invasion of the amniotic cavity or sterile histologic acute chorioamnionitis. This inflammation primes the preterm lung for injury caused by neonatal inflammatory insults related to supplemental oxygen exposure (with oxidant damage) and ventilation with volutrauma. Interestingly, in a large prospective study of extremely low GA newborns with very high rates (90%) of prenatal glucocorticoid exposure, neither histologic chorioamnionitis nor the recovery of specific or multiple placental microorganisms were associated with later development of BPD (Bose et al., 2009). Study design (e.g., clinical vs histologic chorioamnionitis and lack of adjustment for GA and/or birth weight) has been implicated as the reason for the inconsistent relationship of chorioamnionitis and BPD in contemporary cohorts, with the potent antiinflammatory effects of prenatal steroid exposure potentially providing a biologic rationale for a diminished effect of chorioamnionitis (Been et al., 2009; Hartling et al., 2011).

Despite this, fetal and neonatal inflammatory responses are well documented and associated with adverse neonatal outcomes. The fetal inflammatory response has been demonstrated by cytokine and chemokine elevations in amniotic fluid and cord blood (Kim et al., 2015). Increased levels of interleukin (IL)-6 are the hallmark of FIRS, but IL-1 α and IL-1 β , IL-10, IL-8, and other chemokines are also elevated. This inflammatory response is reflected in increased numbers of neutrophils in tracheal aspirate samples as early as the second day of life (Arnon et al., 1993). Although alveolar macrophages are essential for recognition, ingestion, and elimination of

• BOX 48.1 Pro- and Antiinflammatory Markers Perturbed in the Lung Fluid (Tracheal Aspirate or Lavage) of Preterm Infants Who Later Develop Bronchopulmonary Dysplasia

Proinflammatory Markers

Neutrophils
Macrophages
Intercellular adhesion molecule-1
Interleukins-1, 6, 8
Macrophage inflammatory proteins 1 α and β
Monocyte chemoattractant proteins 1, 2
Matrix metalloproteinases
NF- κ B
Soluble L selectin
Tumor necrosis factor- α

Antiinflammatory Markers

CC10 (Clara cell protein)
Interleukin-10

lung pathogens, they also produce fibroblast, epithelial, and endothelial cell growth factors, leading to lung tissue repair. In addition, they can have deleterious effects through release of oxygen radicals and can release fibronectin, a large glycoprotein that is a growth factor for fibroblasts, as well as releasing the growth factor transforming growth factor (TGF)- β , which stimulates the growth of mesenchymal cells and inhibits proliferation of epithelial cells. Multiple markers of inflammation (cytokines, chemokines, and other inflammatory factors) have been found in high concentrations in lung lavage fluid of infants in whom BPD develops. Box 48.1 lists inflammatory response factors quantified in tracheal aspirate or lavage samples that are often correlated with each other (Merrill et al., 2011) and are associated with the development of BPD in the preterm infant; levels of IL-10, CC10, and vascular endothelial growth factor are decreased in infants that later develop BPD (reviewed in Bose et al., 2008; Ryan et al., 2008; Speer, 2009). Studies of mediators in lung fluid, compared with plasma, may have the advantage of reflecting local lung inflammation rather than the systemic fetal or neonatal inflammatory response, but they are limited to those infants who remain mechanically ventilated via endotracheal tube at any time point. Ambalavanan et al. evaluated systemic inflammatory responses and found relationships consistent with studies of lung fluid: early elevations in plasma concentrations of IL-1 β , IL-6, and interferon- γ were associated with BPD or death in preterm infants (Ambalavanan et al., 2009). A recent secondary analysis of these data, evaluating BPD in survivors, demonstrated higher levels of IL-8 and matrix metalloproteinase-9 and lower levels of granulocyte-macrophage colony-stimulating factor in infants who later developed BPD. Of infants with BPD, those with persistently higher levels of supplemental oxygen had multiple elevations of inflammatory markers (cytokines and chemokines), compared with those with BPD with lower levels of oxygen supplementation (D'Angio et al., 2016). Bose et al. also found systemic inflammatory markers in the first 14 days to be predictive of BPD (Bose et al., 2011). Some of these effects were attenuated in the multivariate model by inclusion of persistent mechanical ventilation at 7 days, suggesting an important relationship between mechanical ventilation and inflammation in the development of BPD.

Neonatal Infection

Airway microbial colonization with a variety of organisms has been associated with a diagnosis of clinical chorioamnionitis, evidence of lung inflammation, and an increased risk of BPD (Groneck et al., 1996; Bhandari et al., 1998; Cordero et al., 2001; Young et al., 2005). Postnatal bacterial sepsis with gram-positive, gram-negative, or fungal organisms has also been associated with an increased risk of BPD (Stoll et al., 2002), although this relationship is not consistent across all studies with adjustment for other clinical risk factors for BPD (Ambalavanan et al., 2011). There has been a lot of interest in the role of fastidious organisms (particularly *Ureaplasma* and *Mycoplasma* species [spp.]) in the development of BPD, with colonization and infection of the placenta, fetus, and newborn broadly studied. A metaanalysis of the recovery of *Ureaplasma* spp. from neonatal airways and BPD demonstrated a significant relationship of organism recovery to BPD (Schelonka et al., 2005). However, there was concern regarding the validity and implications of these published studies: the relationship for BPD determined at 28 days was stronger than the relationship for BPD determined at 36 weeks' PMA, and older studies, those including infants with lower rates of surfactant administration, and those with smaller sample sizes (with more potential for bias), were more likely to demonstrate a positive association with airway colonization. More recent changes in perinatal practices, including the utilization of macrolide antibiotics for latent preterm labor, may affect the application of these study findings. In fact, most recently, the administration of neonatal macrolide antibiotics demonstrates marginal effect on BPD only in infants with *Ureaplasma* colonization (which may occur in up to one-third of preterm infants who do not have intrauterine growth restriction) (Ballard et al., 2011; Ozdemir et al., 2011). Thus if the new BPD in the extremely preterm infant is a syndrome of arrested lung development, it may be that even short-term exposure to microbial organisms and the resultant inflammatory response (augmented by exposure to mechanical ventilation) contribute to the arrest of normal alveolarization and microvascular growth.

Oxygen Toxicity and Oxidative Stress

In the initial cases of BPD reported by Northway et al. (1967), it was clear that exposure to high inspired oxygen concentrations was a factor in development of the disease. Subsequent reports continue to show an association between high levels of supplemental oxygen or prolonged oxygen exposure and the development of BPD in ventilated preterm infants, an effect which may plateau in the first several weeks of life (Wai et al., 2016). Recent studies have shown that even short periods of exposure to higher levels of supplemental oxygen during resuscitation of preterm infants increase rates of BPD (Vento et al., 2009; Kapadia et al., 2013). And, although even a relatively small increase in inhaled oxygen concentration over a period of several weeks may increase respiratory morbidity when applied later in the neonatal course (STOP-ROP Multicenter Study Group, 2000; Askie et al., 2003), studies instituting lower oxygen saturation targets from birth have failed to significantly lower rates of BPD and may increase mortality in the least mature infants (SUPPORT Study Group, 2010; BOOST II United Kingdom Collaborative Group et al., 2013; Schmidt et al., 2013; Darlow et al., 2014; Manja et al., 2015).

In experimental animals, prolonged hyperoxic exposure of varying degrees recapitulates the clinical and morphometric findings of the older and newer forms of BPD (reviewed by Bhandari, 2014),

and oxidative stress and inflammation have been implicated in the pathogenesis of the morphometric changes in these models (Chang et al., 2003, 2013; Ratner et al., 2009). The damage to the lung caused by oxygen toxicity appears to be mediated by reactive oxygen species that are produced during univalent reduction of molecular oxygen. These species include superoxide anion, hydrogen peroxide, and hydroxyl radical. Evidence suggests the presence of an oxidant–antioxidant imbalance in lungs that are at risk for BPD. Both Vento et al. and Kapadia et al. found that short exposure to hyperoxia during newborn resuscitation increased markers of oxidative stress and inflammation of preterm newborns (Vento et al., 2009; Kapadia et al., 2013). Infants resuscitated with higher oxygen concentrations had an increased oxidative balance ratio and higher ratios of oxidized-to-reduced glutathione levels in blood as well as increased urine levels of oxidized proteins and DNA in the first week of life. Extremely preterm infants also had increased systemic markers of inflammation (tumor necrosis factor- α and IL-8) and a shorter duration of mechanical ventilation and supplemental oxygen exposure, as well as decreased rates of BPD, although the latter effect was not significant in a metaanalysis of the subgroup of infants less than 29 weeks' gestation (Vento et al., 2009; Oei et al., 2017). Other observational studies have found higher concentrations of lipid peroxidation metabolites, as well as excess carbonylated proteins, in preterm infants later developing BPD, compared with preterm controls without BPD, further supporting the concept of oxidant–antioxidant imbalance (reviewed in Saugstad, 2003).

Inflammation is concurrent with high oxygen exposure, and in experimental models, inflammation is mitigated by therapies that improve lung structure, such as mesenchymal stem cell transplant (Chang et al., 2013). Antioxidant defenses are decreased in preterm infants, and critical nutrients such as retinoic acid (shown to suppress both superoxide and hydrogen peroxide formation in stimulated neutrophils and macrophages) are deficient (reviewed in Shenai, 1999). Nitric oxide, either endogenous or exogenous in origin, may function as either a prooxidant or antioxidant; no significant differences were seen in plasma carbonylated or nitrosylated protein levels in infants treated with inhaled nitric oxide (iNO) versus placebo in the randomized NO CLD Trial over the first 10 days of gas administration, when iNO dose was 10–20 parts per million (ppm) (Ballard et al., 2008). An early randomized trial of allopurinol, a xanthine oxidase inhibitor studied for its potential neuroprotective effects, demonstrated no differences in the incidence of BPD at 28 days (Russell and Cooke, 1995). Similarly, intratracheal recombinant human superoxide dismutase (rhSOD) did not affect the incidence of BPD at 36 weeks, although treated infants had less pulmonary morbidity than control infants at 1-year follow-up (Davis et al., 2003).

Given the concerns raised in maintaining lower oxygen saturation targets in extremely low GA newborns, and studies demonstrating that higher cumulative supplemental oxygen exposure in this population is associated with increased rates of BPD (Laughon 2009a; Wai et al., 2016), either effective antioxidant therapy, or other strategies that can persistently improve lung function and decrease supplemental oxygen exposure, or a combination of these effects is critical for prevention of BPD. For instance, exogenous surfactant has been shown in vitro to have antioxidant activity, and it transiently decreases the degree of mechanical ventilation support (as measured by the respiratory severity score, equal to mean airway pressure \times inspired oxygen concentration) when given to high-risk preterm infants after the first week of life (Dani et al., 2009; Merrill et al., 2011; Keller et al., 2012). Similarly,

mesenchymal stem cells both provide and activate antioxidant activity in experimental systems, while also decreasing respiratory severity score in extremely preterm newborns (Chang et al., 2014; reviewed by Pierro et al., 2015).

Ventilation With Volutrauma

As is the case with exposure to high oxygen, the association between overdistension of the preterm lung and the development of BPD is well established. Overinflation produces stress fractures of the capillary endothelium, epithelium, and basement membrane. This mechanical injury causes leakage of fluid into the alveolar spaces, with additional inflammatory response and the release of additional proinflammatory cytokines. The relative contribution of high peak inspiratory pressure (PIP) (*barotrauma*) versus overdistension of the lung (*volutrauma*) to lung injury has been a source of some controversy. Hernandez et al. (1989) compared the respective roles of high tidal volume with high PIP in immature New Zealand white rabbits to look at the effect on microvascular permeability in animals that were treated with chest wall restriction with a full-body plaster. Preventing overdistension also prevented any significant increase in microvascular permeability, even when the manometer indicated pressures up to 45 cmH₂O. In contrast, when isolated excised lungs were ventilated with PIPs of only 15 cmH₂O for 1 hour (with overdistension), there was an 850% increase in microvascular permeability. This finding suggests that preterm infants with relatively healthy lungs and highly compliant chest walls may experience significant lung injury even at apparently low ventilator pressures. In addition, in the preterm lamb model, manual ventilation with as few as six very large breaths at birth may compromise the therapeutic effect of subsequent surfactant administration, leading to significant lung damage (Björklund et al., 1997; Dreyfuss and Saumon, 1998). Evidence that exposure to high tidal volumes in preterm human infants contributes to BPD comes from several sources. In particular, there is an association between hypocarbia and an increased incidence of BPD (Kraybill et al., 1989; Garland et al., 1995) as well the known association between lung overdistension and the occurrence of pneumothorax and pulmonary interstitial emphysema.

Multiple attempts have been made to decrease the incidence of BPD through improved ventilatory strategies (see also Chapter 45 on the principles of respiratory care). The use of high-frequency oscillatory ventilation (HFOV) as a primary mode of ventilation is one such strategy (Morierte et al., 2001; Courtney et al., 2002; Johnson et al., 2002). Although a statistically significant decrease in the incidence of BPD has been described in some studies, none of the studies using HFOV achieved large clinically relevant differences in outcome. A recent (2015) Cochrane Collaboration metaanalysis of randomized clinical trials of HFOV versus conventional ventilation found a modest effect of ventilation mode on the incidence of BPD (relative risk [RR] 0.86, 95% confidence interval [CI] 0.78–0.96) and on the incidence of BPD or death (Cools et al., 2015). However, heterogeneity among the study results was not explained by variation in ventilation strategies and failed to demonstrate efficacy of a strategy of lung recruitment with decreased need for supplemental oxygen. In fact, air leak syndromes were significantly increased with HFOV.

More recent advances in the delivery of synchronized ventilation for newborns have allowed for the introduction of volume-targeted ventilation (reviewed by Keszler, 2009). In contrast to pressure-limited ventilation, these strategies can limit the delivery of excessive tidal volumes in tiny preterm infants. However, the delivered tidal

volume is accurately assessed only in the “volume guarantee” mode, which measures and responds to the expiratory volume proximate to the airway. This is not available on all neonatal ventilators in common use. Although a metaanalysis did demonstrate decreased rates of BPD or death and decreased duration of intermittent positive pressure ventilation with volume-targeted ventilation, these effects were attenuated in the pragmatic studies where specific ventilators and the approach to triggering breaths were not matched in the two arms of the study, suggesting that other variables may have accounted for some of the differences seen (Wheeler et al., 2010). Episodes of hypocarbia were significantly decreased with volume-targeted ventilation. Generally, lung protection strategies have not explicitly been shown to decrease BPD. For example, a randomized trial to evaluate a minimal ventilation strategy (higher carbon dioxide partial pressure and lower pH targets compared with more standard targets) demonstrated no differences in the incidence of BPD or death (63% vs 68%) or other major morbidities (Carlo et al., 2002). However, in this study, the need for ventilatory support at 36 weeks’ PMA was significantly lower in the minimal ventilation group compared with the routine conventional ventilation treatment group (1% vs 16%). Thus the more permissive approach to assisted ventilation appears to influence the severity of BPD, although this approach alone may be insufficient to prevent the disorder.

Avery et al. (1987) published a descriptive review of treatment center differences in the incidence of BPD. Use of nasal CPAP immediately after delivery was associated with a much lower incidence of BPD at one center compared with the incidence at other centers. Subsequently, Van Marter et al. compared the practices and outcomes for neonatal units in Boston and at Columbia University and found that, overall, the incidence of BPD at Columbia was much lower, as was the use of mechanical ventilation, surfactant administration, indomethacin treatment, and sedation, while the use of nasal CPAP was higher (Van Marter et al., 2000). Infants on nasal CPAP at Columbia were supported with “bubble” CPAP, in which the end-expiratory pressure is created by an underwater seal. Bubble CPAP has been shown to deliver consistently higher intraprong pressures than ventilator-delivered CPAP set to the same end-expiratory pressure. However, variable-flow CPAP delivered by a dedicated device decreases work of breathing, in comparison with both bubble CPAP and ventilator-delivered CPAP (de Klerk and de Klerk, 2001; Pandit et al., 2001; Kahn et al., 2008).

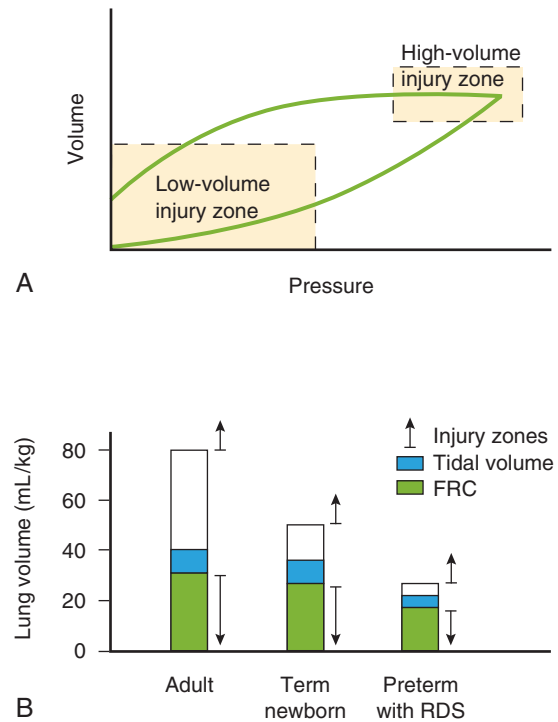
The need for early invasive ventilation identifies the least mature and sickest infants. In more recent reports, among extremely preterm infants that received mechanical ventilation despite a preferential strategy of nasal CPAP, those who were intubated were more depressed at delivery, with lower Apgar scores, lower GA and birth weight, more severe RDS by radiograph, and more impaired oxygenation (Ammari et al., 2005; Fuchs et al., 2011). Infants that required intubation were more likely to have a diagnosis of pneumothorax, severe intracranial hemorrhage, and BPD. However, a previous metaanalysis had demonstrated that infants with RDS who are intubated and rapidly extubated following exogenous surfactant after failing nasal CPAP (with inspired oxygen concentration ≤ 0.45) are less likely to have BPD (as assessed at 28 days), compared with infants who are intubated without a trial of CPAP (Stevens et al., 2007).

Beyond initial resuscitation and treatment of RDS, preterm newborns extubated to nasal CPAP are less likely to fail treatment than those supported without positive airway pressure, particularly when nasal CPAP is applied at greater than 5 cmH₂O pressure or

methylxanthines are administered (Davis and Henderson-Smart, 2003). However, this strategy has not been shown to decrease the incidence of BPD. There is no well-defined advantage to bubble CPAP over variable-flow CPAP for successful separation from mechanical ventilation (Gupta et al., 2009; Mazmanyan et al., 2015). Nasal CPAP, in comparison with humidified high-flow nasal cannula (as a strategy to apply continuous end-expiratory pressure), trends toward less treatment failure following tracheal extubation (RR 1.21, 95% CI 0.95–1.55), but there is no benefit of nasal CPAP for prevention of BPD (Wilkinson et al., 2016). Nasal intermittent positive pressure ventilation also does not provide an advantage over nasal CPAP for prevention of BPD following extubation or as a primary mode of support (Kirpalani et al., 2013).

The preferential application of nasal CPAP, as compared with early intubation and surfactant administration, to prevent BPD was studied in the COIN (Continuous Positive Airway Pressure or Intubation at Birth) and SUPPORT (Surfactant, Positive Pressure and Pulse Oximetry Randomized Trial) trials. Although both studies randomized extremely low GA newborns before birth to receive nasal CPAP or mechanical ventilation, the study designs differed. Infants eligible for COIN had to be spontaneously breathing at 5 minutes of age without previous intubation, whereas infants in the SUPPORT trial were managed from birth based on their treatment allocation (Morley et al., 2008; SUPPORT Study Group et al., 2010). Because informed consent was obtained prenatally, there were high rates of prenatal steroid exposure in these infants. There was not a significant decrease in the need for oxygen supplementation at 36 weeks' PMA in either of these trials. Furthermore, a greater number of infants assigned to the CPAP group were intubated: 46% in COIN and 83% in SUPPORT (with one-third of the CPAP group intubated in the delivery room). In both studies, there were fewer days of mechanical ventilation in the CPAP group, with a higher rate of pneumothorax in the CPAP group in the COIN trial and no difference in air leak in the SUPPORT trial. There were no increases in mortality or other comorbidities of prematurity.

The application of positive pressure in preterm infants is critical, as loss of functional residual capacity (FRC) with onset of generalized atelectasis also can be a major contributor to the development of BPD. In infants who are being ventilated below a normal FRC, repetitive opening and closing of lung units occur in the presence of maldistribution. This then leads to areas of significant overdistension. There is accumulating evidence from both animal and human newborns that the optimal use of PEEP is associated with a lower risk for BPD. Fig. 48.3 demonstrates the appearance of static pressure–volume curves for infants with either normal lungs or with RDS and also depicts the potential areas of lung injury from either high-volume ventilation or low-volume ventilation with atelectasis. Taken together, the literature supports a primary strategy of the application of nasal CPAP for extremely low gestation age newborns in the delivery room, with continuous distending pressure from birth to recruit lung and maintain FRC. However, there are probably subpopulations of infants who will still require intubation and surfactant administration, particularly those who are most immature and those who were not exposed to prenatal steroids. In a small single center study, the stable microbubble test from the gastric aspirate shortly after birth was used to quantify surfactant activity (Bhatia et al., 2013). The stable microbubble count significantly predicted CPAP failure and the need for intubation and exogenous surfactant administration with an area under the receiver operating characteristic curve of 0.80 (95% CI 0.70–0.90). For



• **Fig. 48.3** (A) Static pressure–volume curve indicating areas of lung injury from either high-volume ventilation or low-volume ventilation with atelectasis. (B) Lung volumes for a normal adult, a term newborn, and a preterm with respiratory distress syndrome. The low- and high-volume injury zones are indicated by arrows. The preterm lung is susceptible to injury with ventilation because of the small volume/kg between the two injury zones. FRC, Functional residual capacity; RDS, respiratory distress syndrome.

those infants that require intubation despite a strategy of preferential CPAP, the approach that would seem most likely to contribute to prevention of BPD is the use of an optimal PEEP to support a normal FRC combined with synchronized low-tidal-volume ventilation, with a goal of early extubation to nasal continuous distending airway pressure (CPAP or intermittent positive pressure).

Fluid Balance, Pulmonary Edema, and Patent Ductus Arteriosus

It is clear from animal studies (Bland et al., 2000) that abnormalities of lung fluid balance contribute to BPD. However, in a recent metaanalysis of studies of liberal versus strict fluid balance for prevention of BPD, the favorable effect of strict fluid balance was not significant (Bell and Acarregui, 2014). Notably, there were a limited number of extremely low GA newborns included in these trials. Patent ductus arteriosus (PDA) was decreased with fluid restriction, which is one mechanism by which fluid management may relate to BPD. In a retrospective study from the National Institute of Child Health and Human Development (NICHD) NRN of extremely preterm infants, higher fluid intake and lesser weight loss in the first 10 days of life were associated with increased odds of BPD or death (Oh et al., 2005). This study also showed that PDA was associated with increased odds of BPD or death, but this relationship is complex, as factors that predispose to symptomatic PDA are also associated with higher levels of respiratory support. In addition, the need for increased respiratory support usually prompts further evaluation for PDA (Weisz and McNamara,

2014). A post hoc analysis of a small randomized trial of prophylactic surgical PDA ligation conducted when the use of prenatal steroids was limited demonstrated increased BPD among surviving infants that underwent prophylactic ligation but no difference in the combined outcome of BPD or death (70% vs 66%) (Clyman et al., 2009). These data suggest that persistence of PDA did not affect the outcome of BPD, or those effects were offset by any adverse effects of surgical ligation. A more contemporary (1999–2009) time-series study of extremely low GA newborns who were managed with prophylactic indomethacin followed by an early, aggressive approach to PDA closure (including surgical ligation) versus a more conservative approach to closure did not demonstrate a difference in rates of BPD, despite a longer period of time with exposure to a moderate-to-large PDA in those infants with conservative management (Jhaveri et al., 2010). However, a more recent study (2006–2008) compared morbidities in infants with PDA from the Japanese and Canadian neonatal networks (Isayama et al., 2015). In Japan, the approach to PDA is one of active surveillance and treatment, whereas in Canada, it is one of selective evaluation and treatment. Infants with PDA in Japan demonstrated decreased odds of BPD. Interestingly, rates of prenatal glucocorticoid exposure were lower in the Japanese cohort than in the Canadian cohort, regardless of PDA status. Two recent studies demonstrated that a more hemodynamically important PDA (by echocardiographic markers) is independently associated with increased odds of BPD (El-Khuffash et al., 2015; Schena et al., 2015). In a multivariate analysis, El-Khuffash et al. further demonstrated that surgical ligation was not significantly associated with development of BPD, after controlling for PDA severity.

Pulmonary Vascular Abnormalities

Prospective studies evaluating early echocardiographic findings in extremely preterm newborns have demonstrated that early changes (7–14 days) on echocardiogram consistent with elevated pulmonary arterial pressure are associated with the later development of BPD (Mirza et al., 2014; Mourani et al., 2015). This right-heart pressure elevation was also associated with increased need for mechanical ventilation. Vascular changes in the placenta are also associated with increased odds of BPD, as well as BPD complicated by the clinical diagnosis of pulmonary hypertension, based on echocardiographic findings consistent with elevated pulmonary arterial pressure (Mestan et al., 2014). Other work implicating the fetal environment in the pulmonary vascular development demonstrated that among extremely low GA newborns with BPD, those with less robust fetal growth (lowest quartile of birth weight percentile for GA) were more likely to have a clinical diagnosis of pulmonary hypertension, after adjustment for other perinatal factors, along with more severe BPD (Check et al., 2013). Thus intrauterine and early neonatal differences in vascular growth and development may result in early signs of elevated pulmonary artery pressure, pulmonary vascular disease, and ongoing impairment of vascular development, with greater likelihood of BPD. Interestingly, in the largest prospective cohort of echocardiographic surveillance for pulmonary hypertension published to date, PDA on the day 7 echocardiogram was associated with both severity of later BPD and elevated right-heart pressure estimates (Mourani et al., 2015). In some cases, this increase in pressure may be due to a hemodynamically important PDA with substantial left-to-right flow, further implicating the interaction between the presence of the PDA and the ongoing need for mechanical ventilation in alveolar and microvascular growth and development.

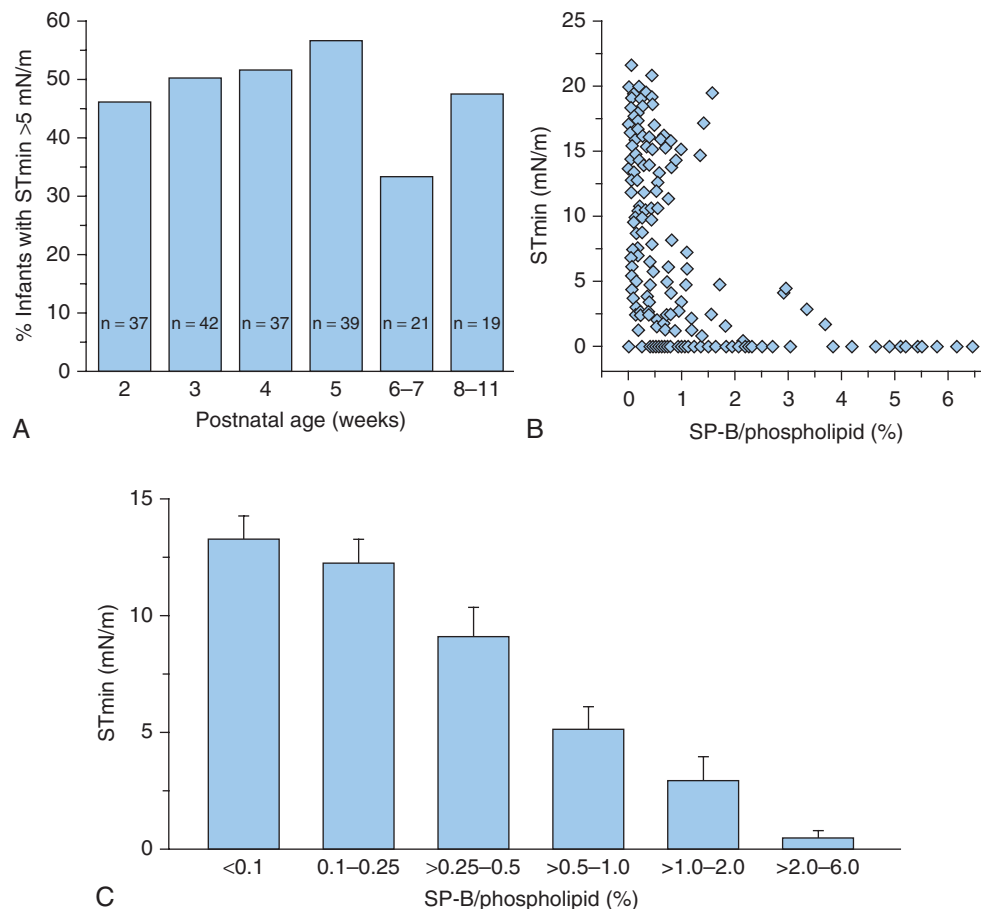
Inadequate Nutrition

Further data supporting the role of impaired fetal growth in the development of BPD come from multiple larger epidemiologic studies (Bose et al., 2009; Durrmeyer et al., 2012; Torchin et al., 2016). Although these studies applied different reference curves and different thresholds for classification of intrauterine growth restriction, the consistent findings from these and other studies suggest that there is a biologic mechanism to this relationship. It is likely that the effects of growth restriction on pulmonary vascular development are some combination of direct placental or trans-placental effects of maternal vascular disorders and the more global effects of nutritional deprivation on lung and vascular development, as newborns who are products of these complicated pregnancies that are also growth restricted have the highest risk of later development of BPD (Durrmeyer et al., 2012; Torchin et al., 2016). Regardless, the effects of the aforementioned etiologic factors and others are intensified by the inability to provide adequate neonatal nutritional support for extremely preterm newborns, particularly those who are acutely ill (Martin et al., 2009). These infants have delayed initiation and advancement of enteral feeds, inadequate parenteral nutrition because of restricted fluid intake, and a catabolic state secondary to illness and increased work of breathing. Martin et al. described growth failure in 75% of extremely low GA newborns at 28 days old (Martin et al., 2009). Growth failure at 36 weeks' PMA and beyond is common among extremely preterm infants with BPD. However, earlier enteral feeding and earlier and greater amino acid administration result in lower rates of growth failure (Theile et al., 2012; Natarajan et al., 2014; Poindexter et al., 2015). Restricted nutritional intake results in decreased alveolar number with alveolar simplification and dysregulated lung development in animal models of alveolarization (Massaro et al., 2004; Joss-Moore et al., 2016). These differences can be reversed by normalizing nutritional intake. In addition, preterm infants have vitamin deficiencies (e.g., vitamin A, vitamin E) associated with disruption of alveolar formation and diminished antioxidant activity (Abdel Ghany et al., 2016). Poor nutrition also leads to increased susceptibility to infection, which leads in turn to a further cycle of impaired defense against injury.

Surfactant Dysfunction

Surfactant dysfunction is common among extremely low birth weight infants that remain intubated beyond the first week after birth (Fig. 48.4) (Merrill et al., 2004, 2011; Keller et al., 2012). In addition, episodes of infection or respiratory deterioration (sustained increase in inspired oxygen concentration and/or mean airway pressure) have been associated with increasing surfactant dysfunction. The surfactant content of the surfactant proteins (SP) B and C is tightly correlated with surfactant function in preterm infants, with lower SP levels correlated with higher surface tension. This is particularly true of SP-B, which increases acutely with SP-B-containing exogenous surfactants and which is downregulated by TGF- β (McDevitt et al., 2007; Keller et al., 2012); other studies in animals and humans have demonstrated decreased SP content or expression following exposure to oxygen and cytokines.

Later administration of animal-based surfactants as rescue for clinical decompensation in preterm newborns has been evaluated, with a short-term decrease in ventilator settings following surfactant administration (Pandit et al., 1995; Katz and Klein, 2006; Bissinger et al., 2008). An additional study in persistently ventilated preterm newborns showed similar effects (Merrill et al., 2011). Pilot



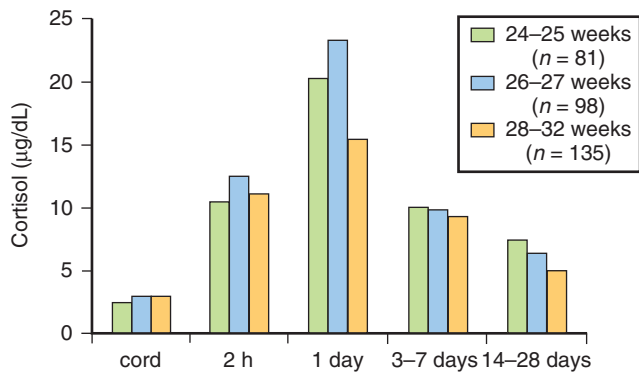
• **Fig. 48.4** Surfactant Dysfunction in Ventilated Preterm Infants. (A) Prevalence of surfactant dysfunction (minimum surface tension [STmin] > 5 mN/m) during weeks 2 to 11 of life in ventilated preterm infants. (B and C) Inverse relationship of surfactant protein B (Sp-B) content (normalized to phospholipid content) to STmin in ventilated preterm infants; low surface tension is associated with higher SP-B content. STmin, Minimum surface tension; SP-B, surfactant protein B. (Adapted from Merrill JD, Ballard RA, Cnaan A, et al. Dysfunction of pulmonary surfactant in chronically ventilated premature infants. *Pediatr Res*. 2004;56:918–926.)

randomized studies of SP-B (or an analogue)–containing surfactants in preterm infants persistently ventilated at 3 to 14 days have also demonstrated transient decreases in ventilator support compared with controls but no effect on the prevention of BPD or death at 36 weeks (lucinactant, Discovery Laboratories, Inc., Warrington, PA; calfactant, Ony Inc., Amherst, NY; poractant alfa, Chiesi USA, Inc., Cary, NC) (Laughon et al., 2009b; Keller et al., 2012; Hascoët et al., 2016). The large multicenter Trial of Late Surfactant (TOLSURF) randomized extremely low GA newborns who remained intubated at 7 to 14 days to calfactant (up to 5 doses) in combination with iNO versus iNO alone. Although there was no effect on survival without BPD at 36 weeks' PMA, there was a trend toward an increase in this outcome assessed at 40 weeks' PMA, and late surfactant administration was safe and well-tolerated (Ballard et al., 2016).

Adrenal Insufficiency

Preterm infants may have developmental immaturity of the hypothalamic–pituitary–adrenal axis, with an increased risk of BPD secondary to inadequate responses to inflammatory lung injury (Watterberg and Scott, 1995; Watterberg et al., 1996). Banks

et al. reported, however, in a study of cortisol levels in 314 preterm infants, that even the earliest gestation infants (24–25 weeks) have an increase in cortisol after delivery and that levels were not associated with GA (Fig. 48.5) (Banks et al., 2001). Using the Clinical Risk Index for Babies score to adjust for clinical risk factors, low cortisol at 3 to 7 days of age contributed minimally to increasing the risk for BPD, and there was no correlation at 14 to 28 days. A subsequent cohort of infants from the PROPHET (Prophylaxis of Early Adrenal Insufficiency to Prevent Bronchopulmonary Dysplasia) study demonstrated no relationship between cortisol levels in the first week and BPD, although higher cortisol levels were associated with increasing incidence of other acute and chronic morbidities (Aucott et al., 2008, 2010). Three randomized studies, including PROPHET, investigated “physiologic replacement” hydrocortisone dosing (total dose of 8.5–13.5 mg/kg over 10–15 days) for the prevention of BPD in extremely preterm newborns (Watterberg et al., 2004; Peltoniemi et al., 2005; Baud et al., 2016). Two of the studies were terminated prematurely due to increased rates of intestinal perforation in the hydrocortisone-treated group. No benefit on the primary outcome of survival without BPD in either study was seen (Watterberg et al., 2004; Peltoniemi et al., 2005).



• **Fig. 48.5** Plasma Cortisol Concentrations in Premature Infants During the First 4 Weeks of Life. Data are mean levels stratified by gestational age: 24–25 weeks ($n = 81$), 26–27 weeks ($n = 98$), and 28–32 weeks ($n = 135$).

The most recent study differed, as the cumulative hydrocortisone dose was lower than in the other studies, and it enrolled only inborn infants, with no requirement for mechanical ventilation (Baud et al., 2016). Other differences included limitations on use of ibuprofen and open-label glucocorticoids. This study did show a benefit of increased survival without BPD (odds ratio 1.48, 95% CI 1.02–2.16), with fewer infants intubated on mechanical ventilation at 7 days and with no increase in adverse events in the intervention group. Although the PROPHET study demonstrated a significant interaction between clinical chorioamnionitis and hydrocortisone treatment (with the infants exposed to the anti-inflammatory steroid more likely to survive without BPD), other studies have not demonstrated this differential effect. Of interest is the fact that formation of alveolar septae in animals, a critical step in alveogenesis, occurs during a period of low serum glucocorticoid levels. Furthermore, dexamethasone administered to newborn rodents results in persistent impaired septation and alveogenesis (Massaro and Massaro, 2000). Thus there is not consistent evidence that adrenal insufficiency contributes to BPD in the general population of at-risk premature infants.

Inhibition of Normal Lung Development and Vascular Development

Some of the contributing factors described above directly impair normal formation of secondary septae and therefore microvascular development and alveolarization. Others arrest alveolarization by as-yet unknown processes that also involve structural differences in the extracellular matrix. These insults, occurring during the saccular stage of lung development, include inflammation and cytokine overexpression, dexamethasone exposure, hyperoxia, hypoxia, and inadequate nutrition (Albertine et al., 1999; Jobe, 1999; Massaro and Massaro, 2000; Massaro et al., 2004). Fig. 48.6 shows imaging and pathology from an infant who died from BPD complicated by pulmonary hypertension.

Preventive Factors

The Bronchopulmonary Dysplasia Group, convened to identify strategies for investigation of therapies to prevent or treat BPD, proposed classes of drugs to prevent or treat evolving (at 7 to 14 days) BPD (Walsh et al., 2006). These included antiinflammatory

• BOX 48.2 Nonpharmacologic and Pharmacologic Approaches to Preventing Bronchopulmonary Dysplasia

Nonpharmacologic Approaches

Ventilation strategies: maintenance of FRC/low-volume ventilation/optimize nasal continuous distending airway pressure/gentle ventilation

Strict fluid balance

Early enteral nutrition

Early protein administration

Pharmacologic Approaches

Vitamin A

Postnatal steroids: systemic, inhaled, instilled

Inhaled nitric oxide

Caffeine

FRC, functional residual capacity.

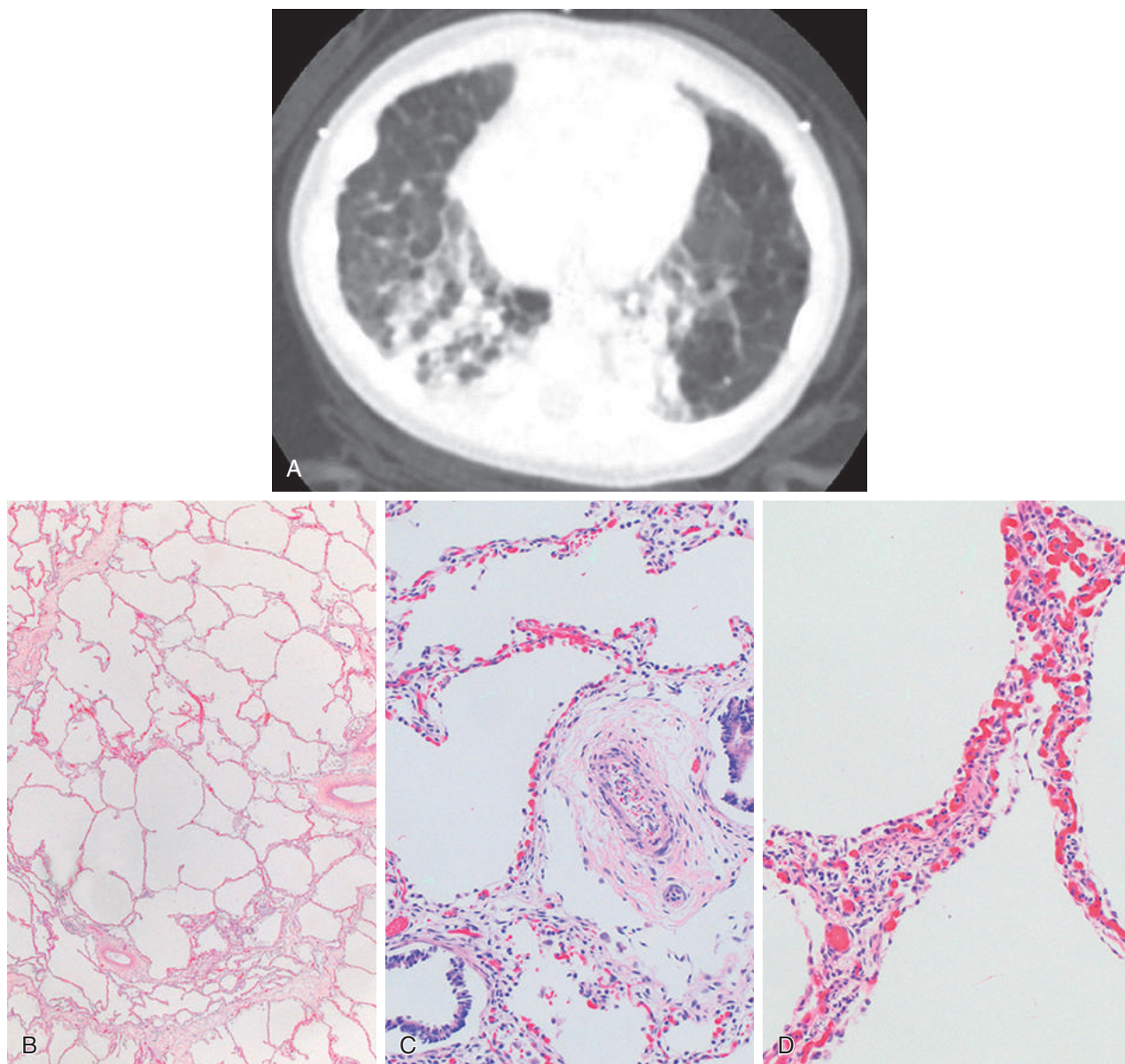
agents such as prenatal and postnatal corticosteroids (PNCS) and other small molecules, antioxidants, iNO, late surfactant replacement, and improved vitamin A preparations. Additional approaches that have been proposed and evaluated include alternative ventilation strategies and the primary use of nasal CPAP. Box 48.2 lists both nonpharmacologic and pharmacologic approaches to preventing or decreasing severity of BPD, with these approaches and others discussed further in this section.

Prenatal Steroids

Numerous clinical studies and several large metaanalyses have demonstrated that prenatal glucocorticoids administered to women at high risk for premature delivery result in decreased mortality and morbidity associated with prematurity, including RDS, intraventricular hemorrhage, and necrotizing enterocolitis (NEC) (Roberts and Dalziel, 2006). However, the benefit of prenatal glucocorticoids for prevention of BPD among survivors is not consistent. The nonsignificant effect of prenatal steroids on BPD may be due to the increased survival of higher risk, less mature preterm infants following prenatal glucocorticoid exposure.

Surfactant Replacement Therapy

Surfactant replacement therapy is clearly associated with decreased severity of RDS and its associated mortality. Although survivors do not have a decreased incidence of BPD, a decrease in BPD or death was demonstrated in a recent metaanalyses (Seger and Soll, 2009). Investigations of late surfactant replacement, during a period of secondary surfactant dysfunction (Merrill et al., 2004), for prevention of BPD in those infants that continue to require mechanical ventilation after the first week of life, have been undertaken. Several pilot randomized studies and one larger trial evaluated the effects of late surfactant replacement on the respiratory status of extremely preterm infants requiring mechanical ventilation from days 3 to 14 (Laughon et al., 2009b; Keller et al., 2012; Ballard et al., 2016; Hascoët et al., 2016). In addition to improved surfactant function and increased levels of SP-B following late surfactant replacement in these infants at high risk for surfactant dysfunction, these exogenous surfactant preparations (calfactant, poractant alfa) have antioxidant activity, which may mitigate the



• **Fig. 48.6** Pulmonary Imaging and Pathology in a Former Preterm Infant With a History of Oligohydramnios, With Severe Bronchopulmonary Dysplasia and Pulmonary Hypertension at 44 Weeks' Postmenstrual Age. (A) Computed tomography scan with contrast demonstrating patchy consolidation and mosaic perfusion, consistent with poor ventilation–perfusion matching. (B) Postmortem pathology demonstrates alveolar enlargement, consistent with alveolar simplification. (C) On higher magnification, pulmonary vascular changes indicative of pulmonary hypertension are present. (D) Patchy septal thickening is seen on higher magnification.

effects of ongoing lung inflammation and oxidative stress for these persistently intubated infants (Dani et al., 2009; Merrill et al., 2011; Keller et al., 2012). Despite transient (up to 24 hours) decreases in level of respiratory support, late surfactant failed to significantly increase survival without BPD at 36 weeks' PMA in all of these studies (Laughon et al., 2009b; Keller et al., 2012; Ballard et al., 2016; Hascoët et al., 2016). However, the two studies that evaluated later outcomes (at 9–12 months corrected age) suggested that late surfactant decreased respiratory resource utilization following neonatal hospital discharge (Hascoët et al., 2016; Keller et al., 2016b).

Gentle Ventilation and Nasal Continuous Positive Airway Pressure

As described previously, volutrauma as well as atelectasis contributes directly to lung damage and the release of cytokines, which further the cycle of damage. With respect to respiratory support strategies for extremely preterm newborns, the body of evidence suggests that a primary strategy of nasal continuous distending airway pressure (as CPAP, biphasic CPAP, or intermittent positive pressure ventilation), expeditious transition to nasal continuous distending airway pressure for those infants who require early surfactant

replacement therapy, and patient-triggered low-volume ventilation for those infants who are unable to be extubated are safe approaches to respiratory support that may decrease the incidence of BPD. These studies have been done under stringent protocols for respiratory management based on clinical status, level of respiratory support, and blood gas parameters, which is probably important for both potential risks and benefit of these approaches. Regardless, the current evidence does not support a primary strategy of high-frequency ventilation or the use of high-flow nasal cannula in lieu of nasal CPAP in extremely premature infants. Furthermore, there is no evidence to support a target oxygen saturation of 85%–89% (vs 91%–95%) to decrease supplemental oxygen exposure from birth for prevention of BPD—mortality may be increased in subgroups of preterm newborns, while rates of BPD are not significantly decreased. However, there are not currently adequate data to otherwise identify an appropriate oxygen saturation target; higher oxygen saturation targets are associated with increased rates of severe retinopathy of prematurity, and in addition to mortality, rates of NEC may be increased with lower oxygen saturation targets (Manja et al., 2015).

Vitamin A

Vitamin A is an essential nutrient for maintaining respiratory tract epithelial cells and also is stored in the septal cells of the alveoli involved in alveolar septation. Compelling animal data also support the need for vitamin A (see Fig. 48.6) (Albertine et al., 1999). Because vitamin A is accumulated predominantly in the third trimester, preterm infants have deficient liver stores of this vitamin (Zachman, 1989). These infants, who often are unable to tolerate enteral feedings, are at particular risk for vitamin A deficiency since vitamin A added to parenteral nutrition solutions is degraded by light and can adhere to the intravenous tubing, making it largely inaccessible.

A number of clinical trials have investigated whether supplementation with vitamin A, typically by intramuscular injections, would result in a decrease in BPD. The largest study to date, by the NICHD-funded NRN, also used one of the higher doses that has been studied and demonstrated a decrease in BPD or death at 36 weeks following treatment with vitamin A (55% vs 62%) (Tyson et al., 1999). A recent metaanalysis of all published trials revealed that vitamin A supplementation was associated with a modest reduction in death or BPD at 36 weeks, which was of borderline statistical—and perhaps clinical—significance (RR 0.91, 95% CI 0.82–1.00, number needed to treat [NNT] 17) (Darlow and Graham, 2011). Vitamin A is well tolerated, and there are no safety issues, although it does involve repeated intramuscular injections. A recent report found that costs associated with intramuscular vitamin A administration for prevention of BPD were favorable if multiple infants were being treated concurrently with doses drawn from the same vial but substantially higher when vials were used for single dose administration (Couroucli et al., 2016).

Interestingly, survey data have shown that a minority of centers routinely administer vitamin A supplementation to at-risk infants, with inadequate evidence or lack of substantial effect cited as the rationale for lack of supplementation (Ambalavanan et al., 2004). Furthermore, data analyzed from before and during a period of a national shortage of the vitamin A preparation (2010–2012) demonstrated a large fall in vitamin A administration in at-risk infants from greater than 30% to less than 5% of the target population (Tolia et al., 2014). However, there was no change in the rate

of death of BPD for these at-risk infants over these two epochs, data which were further supported in multivariate analysis controlling for other risk factors for BPD. Thus the efficacy of vitamin A for prevention of BPD may be lacking in the setting of contemporary strategies for the care of the extremely preterm newborn (Gawronski and Gawronski, 2016).

Systemic Postnatal Corticosteroids

Preterm infants given PNCS demonstrate some decreased inflammatory markers and suppression of cytokine-mediated inflammatory reactions in their tracheal aspirates. Other than direct antiinflammatory effects, numerous theoretical reasons have been advanced regarding why postnatal administration of steroids might decrease the incidence of BPD, including the potential for increased surfactant synthesis, enhanced β -adrenergic activity, increased antioxidant production, stabilization of cell and lysosomal membranes, and inhibition of prostaglandin and leukotriene synthesis (Watterberg et al., 1999). These potential benefits are balanced against the knowledge that dexamethasone exposure results in persistent decreases in alveolar numbers in animal models and irreversible effects on brain development (Massaro and Massaro, 2000; Kreider et al., 2006; Heine and Rowitch, 2009; Bhatt et al., 2013).

Clinical trials have demonstrated acute improvements in dynamic compliance and pulmonary resistance following treatment with PNCS, although small follow-up studies have demonstrated no differences in reported respiratory morbidity despite a trend toward improved pulmonary function in children greater than 5 years of age treated with dexamethasone (Nixon et al., 2007; Doyle et al., 2014b). Many have separated out the effects of early (≤ 7 days) versus late (> 7 days) initiation of PNCS predominantly because of differences in adverse side-effect profiles based on the timing of exposure. Based on two recent metaanalyses (Doyle et al., 2014a, 2014b), postnatal dexamethasone has similar beneficial effects on death or BPD at 36 weeks (RR 0.76–0.89, 95% CI, 0.68–0.94), decreased need for mechanical ventilation at 3 to 28 weeks after initiation of therapy, and no significant effect on survival to hospital discharge regardless of the timing of drug initiation. Aggregated studies of hydrocortisone administered in the first week of life demonstrate no effects on mortality or BPD, although this analysis was prior to the results of the most recent trial (Baud et al., 2016).

Major concerns exist regarding both short-term and long-term side effects of PNCS, including systemic hypertension, hyperglycemia and glycosuria, hypertrophic cardiomyopathy, adrenal suppression, and decreased growth. Early administration of PNCS in the first week after birth carries an increased risk of gastrointestinal perforation for both dexamethasone and hydrocortisone (RR 1.81, 95% CI 1.33–2.48) (Doyle et al., 2014a). This effect may be associated with concurrent administration of indomethacin (Watterberg et al., 2004). Gastrointestinal hemorrhage is also significantly increased (RR 1.87, 95% CI 1.35–2.58) with early dexamethasone. Individual studies have reported an increased risk of later cerebral palsy (CP) in children treated early with dexamethasone (Yeh et al., 1998; Shinwell et al., 2000). The metaanalyses support this concern for dexamethasone (with no effect seen with hydrocortisone); the relationship was not statistically significant when treatment is initiated after 7 days of age (Doyle et al., 2014a, 2014b).

In recognition of these accumulated findings, a statement from the American Academy of Pediatrics revised a previous recommendation against any routine use of postnatal dexamethasone in preterm infants (Watterberg et al., 2010). The current statement

distinguishes between high-dose dexamethasone administration, which is not recommended, and low-dose dexamethasone administration, where current evidence of benefit versus risk is lacking. Additional recent assessments illustrate overlap between the NNT (prevent BPD) and the number needed to harm (abnormal neurologic outcome, including CP) for early dexamethasone treatment, which may be related both to the cumulative dose of the medication and an individual infant's risk for BPD (Schmidt et al., 2008; Onland et al., 2009; Doyle et al., 2014c; Jensen et al., 2015). A large cohort study demonstrated a dose-dependent increased risk of death or neurodevelopmental impairment (NDI) at 18 to 22 months corrected age, regardless of PMA at the time of dexamethasone exposure (Wilson-Costello et al., 2009). However, the excess risk caused by PNCS exposure was decreased for those infants at highest risk for BPD. Similarly, in a metaanalysis of randomized trials, Doyle et al. (2014c) demonstrated that the excess risk of CP caused by dexamethasone exposure was obliterated with increasing rates of BPD in the control group. Essentially, at an underlying BPD incidence of 46% (95% CI 33%–60%), there was no excess risk of the outcome of CP or death and a diminishing risk with higher rates of BPD. With respect to dose, a separate metaanalysis suggested that higher cumulative dexamethasone dose was inversely associated with rates of BPD, for infants with treatment initiated in the first 2 weeks of life (Onland et al., 2009). With respect to NDI, earlier treatment initiation had an inverse relationship to risk of NDI. However, for later treatment, the relationship was reversed, with higher doses associated with higher risk of NDI, although this relationship was not significant. The interpretation of the cumulative data therefore is challenging, as infants who are studied outside of a randomized trial may not have appropriate controls, because of the bias regarding who is treated clinically, whereas open-label use of PNCS during clinical trials alters the ability to assess both beneficial and adverse effects of treatment (Onland et al., 2010).

An ongoing study aims to address the issue of safety and efficacy of later treatment with hydrocortisone (The Hydrocortisone for BPD study, NCT01353313). In this study, extremely preterm newborns who continue to require mechanical ventilation at 14 to 28 days are randomized to hydrocortisone or placebo. In addition to the later timing of intervention, this study protocol entails a higher dose of hydrocortisone than had been previously administered in randomized trials (18 mg/kg over 10 days). Follow-up of growth and development at 22 to 26 months corrected age is also being undertaken, to fully understand both benefits and risks of this intervention. However, it is also possible that this window of intervention will be too late to “prevent” BPD, as the underpinnings of the disorder may already be established. For instance, in the NO CLD study, infants treated with iNO initiated at 7 to 14 days had decreased BPD rates, whereas those with the drug initiated at 15 to 21 days had no effect (Ballard et al., 2006; Ballard, 2007). A recent secondary analysis from TOLSURF of cumulative supplemental oxygen exposure over the first 28 days also demonstrated that there was no additional predictive value for this index beyond 14 days (Wai et al., 2016).

Overall, the data suggest that the subset of infants at highest risk for BPD may benefit from dexamethasone. Although the optimal timing of this intervention is not clear, it seems prudent to avoid the concurrent administration of PNCS and inhibitors of prostaglandin synthesis (e.g., indomethacin, ibuprofen) early in the postnatal course. Regardless, there are insufficient data to support the use of any other systemic steroid at this point in time.

Inhaled/Instilled Corticosteroids

Because of concern for systemic effects of PNCS, there has been continued interest in local delivery of steroids to the lung. Although inhaled steroids initiated in the first 2 weeks have been studied for prevention of BPD, there were not significant improvements in either immediate or later respiratory status with this intervention, including no significant effect on death or BPD in a metaanalysis (Shah et al., 2012). There was a trend toward decreased systemic PNCS use in these infants and no increase in rates of the acute side effects seen with systemic PNCS administration (compared to placebo). A recent study of inhaled budesonide (delivered by metered-dose inhaler with spacer) in extremely low GA newborns receiving positive pressure in the first day after birth had a borderline significant effect on death or BPD (RR 0.86, 95% CI 0.75–1.00) (Bassler et al., 2015). Infants were treated until 32 weeks' PMA or off positive pressure support (whichever came first) with an average of 33.9 ± 15.9 days for infants in the budesonide group. Although BPD was decreased in survivors in the budesonide-treated group, mortality was nonsignificantly increased. There were fewer infants reintubated in the intervention group, fewer undergoing PDA ligation, and no increase in rates of potential side effects or growth parameters over the first 28 days. These data are interesting with respect to the pulmonary effects, but the higher mortality in the treated group raises concerns for systemic absorption of budesonide, as the target tissue for inhaled drugs is the airway, but proximal deposition of drug also occurs.

Instillation of budesonide by suspending it in surfactant provides another method of budesonide delivery to the lung, which may more selectively reach the lung parenchyma. In fetal lung explants, incubation with budesonide suppresses inflammatory markers by 90% 12 hours after addition of budesonide (Barrette et al., 2016). This effect was unchanged after budesonide was suspended in calfactant, and surface tension of calfactant was minimally affected. Budesonide suspended in calfactant administered intratracheally to preterm lambs demonstrates a plasma half-life of 4.76 ± 1.79 hours, with nonmetabolized budesonide and budesonide palmitate (fatty acid ester), but not the primary metabolite of budesonide, present in lung tissue (Roberts et al., 2016). Neither budesonide nor its metabolites were found in brain tissue, suggesting that the budesonide absorbed systemically did not cross the blood–brain barrier. A pilot study of budesonide (0.25 mg/kg) with Surfactant (beractant, Abbott Laboratories, Columbus, OH) compared to Surfactant alone for treatment of RDS in very low birth weight preterm infants similarly demonstrated a plasma half-life of 4.13 hours with only approximately 4% of the budesonide dose calculated to enter the systemic circulation, based on drug and metabolite levels (Yeh et al., 2008). In this study, respiratory status improved over 1 to 3 days, and treated infants were extubated earlier, compared with infants treated with beractant alone, without an increase in acute adverse effects. There was also a significantly lower rate of death or BPD. A larger multicenter study extended these findings, demonstrating decreased death or BPD (RR 0.58, 95% CI 0.44–0.77), with both lower mortality and BPD rates in the budesonide-treated group and no increase in comorbidities of prematurity (Yeh et al., 2016). Furthermore, tracheal aspirate inflammatory markers were lower in treated infants than controls: IL-1 and IL-6 were decreased at 12 hours after the intervention, and IL-8 was decreased for up to 8 days following the intervention. Neurodevelopmental follow-up from these trials did not suggest an increase in NDI at 2 to 3 years corrected age, although these studies were underpowered to detect significant differences (Kuo

et al., 2010; Yeh et al., 2016). Thus instilled budesonide may achieve local glucocorticoid (including antiinflammatory) effects on the lung, without adverse systemic and neurodevelopmental consequences. However, the appropriate dose and target population for a definitive study to prevent BPD remain to be determined, as the dose administered by Yeh et al. was substantial.

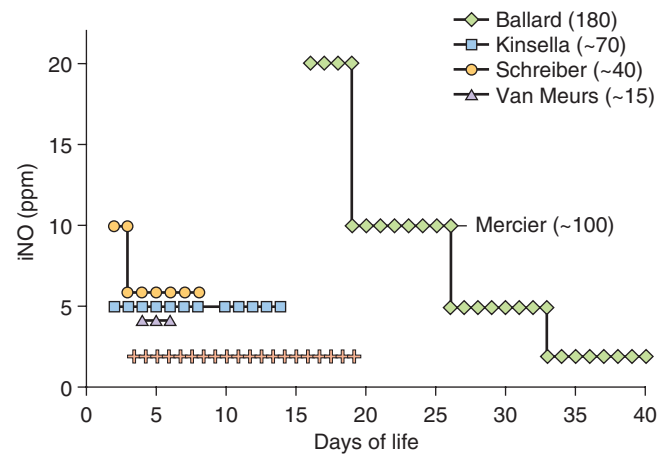
Antioxidant Therapy

Some evidence in the baboon model of BPD indicates that a catalytic antioxidant, metalloporphyrin, can protect against hyperoxia-induced lung injury (Chang et al., 2003). Superoxide dismutase is a naturally occurring enzyme that protects against oxygen free radical injury. Human studies have suggested that administration of intratracheal rhSOD is well-tolerated and might have beneficial effects on the lung; a randomized trial of rhSOD, which was stopped prematurely because of lack of efficacy for prevention of BPD, demonstrated an improvement in respiratory morbidity at 1 year corrected age (Davis et al., 2003). Other therapies proposed to prevent BPD may provide or augment antioxidant activity (e.g., mesenchymal stem cell transplant, exogenous surfactant replacement), which is one potential mechanism by which they might have an effect on the development of BPD.

Inhaled Nitric Oxide

Prolonged iNO from birth in animal models of BPD improves endogenous surfactant function as well as lung growth, angiogenesis, and alveogenesis (Bland et al., 2005; McCurnin et al., 2005). In addition, recovery from injury using iNO attenuates the impaired alveolar and microvascular development associated with prolonged hyperoxia exposure in the rodent (Lin et al., 2005). Prematurity in the baboon, and presumably in the human, is associated with developmentally deficient endogenous nitric oxide production (Shaul et al., 2002). Accordingly, iNO following preterm birth may be viewed as replacement therapy. Multiple studies of iNO initiated in the first 72 hours after birth, for prevention of BPD in ventilated preterm infants, have been conducted—with results showing variable efficacy (Kinsella et al., 1999, 2006; Schreiber et al., 2003; Van Meurs et al., 2005; Mercier et al., 2010). Safety concerns were raised in the single study that enrolled sicker infants with a higher baseline oxygenation index, because of increased severe intracranial hemorrhage (Van Meurs et al., 2005), but improved neurologic outcomes were found in less ill newborns (Mestan et al., 2005; Kinsella et al., 2006). These studies treated infants for variable duration, up to 21 days, with doses of 5–10 ppm. Kinsella et al. (2014) also studied preterm infants who were not intubated, but required respiratory support in the first 3 days, and found no benefit of iNO for prevention of BPD.

In the NO CLD Trial, which enrolled ventilated infants 500 to 1250 grams at 7 to 21 days who had evolving BPD, infants received at least 24 days of iNO therapy, with a dose of 10–20 ppm for at least 10 days (Ballard et al., 2006; Ballard, 2007) (Fig. 48.7 shows a comparison of dosing and duration of trials). In this study, the rate of survival without BPD at 36 weeks significantly increased from 37% to 44%, and fewer days of ventilation resulted in a relative cost savings with iNO (Zupancic et al., 2009). There was a significant interaction of treatment with the age at study entry, with infants that entered earlier (at 7–14 days) being more likely to benefit (increase in survival without BPD from 27% to 49% (NNT = 4). The benefit of treatment persisted at 40 weeks' PMA, with iNO-treated infants more likely to be discharged or hospitalized



• **Fig. 48.7** Summary of Large Trials of Inhaled Nitric Oxide for Prevention of Bronchopulmonary Dysplasia by Day of Life Versus Inhaled Nitric Oxide Dose. Duration of treatment and total nitric oxide dose are depicted. iNO, Inhaled nitric oxide; ppm, parts per million. (Adapted from Truog WE. Inhaled nitric oxide for the prevention of bronchopulmonary dysplasia. *Expert Opin Pharmacother.* 2007;8:1505–1513.)

without respiratory support, and both the earlier and later (15–21 days) treated groups had evidence of benefit at that point in time (Keller et al., 2009). Furthermore, pulmonary morbidity at 1 year was decreased in the iNO-treated group (fewer infants requiring supplemental oxygen and decreased use of bronchodilators, diuretics, and inhaled and systemic steroids). All subgroups of treated infants benefited (Hibbs et al., 2008). iNO therapy initiated at 7 to 21 days was also safe. There was no difference in the rate of comorbidities of prematurity between the treatment and control groups and no difference in the rate of neurodevelopmental impairment by treatment group at 2 years corrected age (Walsh et al., 2010).

A systematic review of studies in ventilated infants demonstrated an overall modest benefit of iNO that was not statistically significant (RR 0.90–0.94, 95% CI 0.80–1.02) (Barrington and Finer, 2010). However, an individual-patient data (IPD) metaanalysis did demonstrate a modification of the iNO effect by initial iNO dose: trials with a starting dose greater than 5 ppm demonstrated significant benefit (RR 0.83, 95% CI 0.74–0.95), a response which was not seen in trials with a starting dose greater than 5 ppm ($P = .02$ for interaction) (Askie et al., 2011). A more recent IPV, including newer data from an as-yet unpublished study conducted by the company that manufactures iNO (INOMax, Mallinckrodt Inc., St. Louis, MO) demonstrated that, in studies of ventilated infants with iNO starting dose of greater than 5 ppm, infants of black mothers were more likely to respond to iNO than other infants ($P = .01$ for interaction) (Ballard et al., 2016). Another potential predictor of iNO response is low endogenous nitric oxide production before therapy. The lowest quartile of nitric oxide metabolites in tracheal aspirate fluid at study entry was associated with the highest rate of survival without BPD in the NO CLD study (Posenchev et al., 2010). Thus it may be that there are subpopulations of infants that are more likely to respond to iNO, when given at an effective dose.

Caffeine

Schmidt et al. reported that the administration of caffeine, initiated in the first 10 days to facilitate extubation, or to prevent or treat apnea of prematurity, decreased the risk of BPD at 36 weeks from

47% to 36% in infants 500–1250 grams in the Caffeine for Apnea of Prematurity (CAP) Trial (Schmidt et al., 2006). Infants treated with caffeine were permanently weaned off mechanical ventilation, positive airway pressure, and supplemental oxygen approximately 1 week earlier, with less exposure to PNCS and lower rates of treatment for PDA. Caffeine was discontinued at a median of 34 weeks' PMA. There was a transient decrease in weight gain in treated infants, but no other substantial adverse effects were seen. A post hoc analysis of subgroups of infants demonstrated similar effects of caffeine on prevention of BPD, regardless of the indication for initiation of therapy and whether infants were on noninvasive or invasive positive pressure support at the initiation of therapy (Davis et al., 2010). Infants randomized early (<3 days) were more likely to benefit than those randomized at 4 to 10 days. Furthermore, caffeine-treated infants had a significantly higher rate of survival without NDI (40% vs 46%) at 18 to 21 months corrected age, with lower rates of CP (Schmidt et al., 2007). At 5 years, there was no statistically significant improvement in rates of death or disability in the caffeine-treated group, and motor outcomes were significantly better (Schmidt et al., 2012).

An economic analysis has demonstrated that caffeine therapy was cost-effective, as it resulted in lower costs and better outcomes (Dukhovny et al., 2011), and two retrospective studies from cohort data in the United States and Canada showed decreased rates of BPD, with a trend toward improved neuroimaging outcomes with early initiation of caffeine (Dobson et al., 2014; Lodha et al., 2015). Although the mechanism of the effect of caffeine on BPD is not clear, small studies demonstrated that caffeine improves diaphragmatic excursion and neural control of breathing, with increased tidal volume, which provides good rationale for its use and efficacy in extremely preterm infants with respiratory system dysfunction (Bancalari, 2006; Kraaijenga et al., 2015; Parikka et al., 2015).

Clinical Course and Treatment

As mentioned earlier, the classic BPD described by Northway et al. is now rare with the advent of prenatal steroids, exogenous surfactant, and ventilators and ventilator strategies better suited for newborn infants. Thus here we address only the clinical course of the new BPD, primarily affecting infants who are extremely low birth weight/low GA (<1000 grams and 28 weeks') that require prolonged ventilator support because of underlying lung disease complicated by apnea or poor respiratory effort. Following the use of prenatal steroids and postnatal surfactant, these infants may initially require relatively low concentrations of oxygen with fairly mild lung disease. However, their clinical course is often complicated by infection, systemic hypotension and PDA, NEC or other intestinal complications, difficulty establishing or maintaining enteral feeds, and an overall proinflammatory environment, which may result in intermittent or persistent elevations in supplemental oxygen exposure and the ongoing need for mechanical ventilation, setting up a cycle of ongoing ventilator support and further lung injury.

Nonpharmacologic Support: Nutrition, Ventilation, Noninvasive Respiratory Support, and Oxygen Saturation Targets

General nutritional recommendations for extremely preterm infants include early protein administration (through the parenteral route) and early initiation of enteral feeds (reviewed by Poindexter and

Martin, 2015). Consistent with those findings, Ehrenkranz et al. (2011) have shown that even modest decreases in energy intake in the first 3 weeks after birth increase the odds of developing BPD after adjusting for multiple other risk factors and degree of respiratory illness. Furthermore, infants with BPD have increased energy expenditure, and growth failure at 36 weeks is prevalent, progresses from 36 to 48 weeks' PMA for infants with severe BPD, and persists to 18 to 22 months corrected age for many infants with BPD (de Meer et al., 1997; Ehrenkranz et al., 2005; Natarajan et al., 2012, 2014). Thus an early aggressive approach to nutritional support, particularly for the sickest infants, mitigates the risk of BPD and its repercussions and should be balanced against the potential benefits of fluid restriction (Ehrenkranz et al., 2006; Natarajan et al., 2012, 2014).

The general approach to ventilation of the infant with evolving BPD should be one of preventing atelectasis, sustaining FRC with end-expiratory pressure, using a minimal tidal volume (usually 4–6 mL/kg) with patient-triggered ventilation, and extubating infants to noninvasive CPAP (nasal CPAP or intermittent positive pressure ventilation), optimally with variable-flow or bubble systems (Carlo et al., 2002; Ho et al., 2002; Gupta et al., 2009). The application of lower oxygen saturation targets (88%–92% vs 91%–95%) from birth did not cause harmful effects on neurodevelopment or growth at 18 to 22 months corrected age and may provide some benefit for later respiratory outcomes (Vaucher et al., 2012; Schmidt et al., 2013; Darlow et al., 2014; Stevens et al., 2014; Navarrete et al., 2016). However, lower targets from birth are not recommended because of the increased mortality seen in some randomized studies. Yet lower oxygen saturation targets later in the neonatal course (~32–35 weeks' PMA and beyond) are not associated with an increase in adverse outcomes and may decrease respiratory exacerbations (STOP-ROP Multicenter Group, 2000; Askie et al., 2003). Unresolved questions relate to the impact of higher oxygen saturation targets to minimize episodes of hypoxemia among infants with pulmonary vascular hypertensive changes and effects of higher targets on somatic growth. Many practitioners do not feel comfortable discharging infants with BPD without supplemental oxygen unless oxygen saturation is consistently greater than 92% (Abman et al., 1985; Groothuis and Rosenberg, 1987; Baraldi et al., 1997).

Pharmacologic Strategies

Caffeine

Early caffeine therapy to facilitate ventilator weaning, or to prevent or treat apnea of prematurity in extremely preterm infants, is supported by early and late follow-up from the CAP Trial and has been widely adopted (Gerull et al., 2013; Hsieh et al., 2014; Jensen et al., 2015). Caffeine is administered as a loading dose of 20 mg/kg, followed by a maintenance dose of 5 mg/kg per day, with the dose advanced up to 10 mg/kg per day as needed for ongoing apnea (Schmidt et al., 2006). It is not known if there is benefit to the initiation of caffeine in the most critically ill infants in the first days after birth, as few infants with these characteristics were enrolled in the CAP Trial (Schmidt et al., 2014). In addition, the timing of discontinuation of caffeine is unclear, as infants who are born more preterm have prolonged resolution of periodic breathing and hypoxic episodes, attenuated by prolonged caffeine therapy (Eichenwald et al., 1997; Hunt et al., 2011; Rhein et al., 2014; Coste et al., 2015). Thus the prolonged use of caffeine in these infants who are at highest risk for BPD could decrease the ongoing use of supplemental oxygen. Finally, there are no published data

regarding the effect of caffeine therapy on respiratory outcomes after neonatal discharge.

Inhaled Nitric Oxide

Multiple trials have evaluated the effect of iNO to prevent BPD. Although the metaanalysis of this preventive iNO use did not show significant benefit, only two published studies initiated iNO at a dose greater than 5 ppm, which appears to be more effective than initiation of iNO at 5 ppm (Askie et al., 2011). For instance, infants treated with iNO in the NO CLD Trial had iNO initiated at 20 ppm and received a minimum of 24 days of therapy, with iNO continued when infants were extubated, while on nasal CPAP or nasal cannula support (Ballard et al., 2006; Ballard 2007). Furthermore, the benefit of iNO in this study persisted with the assessment of respiratory outcomes at 1 year corrected age (Hibbs et al., 2008). Practitioners at many institutions have been reluctant to use iNO in preterm infants due to cost, yet iNO use is not uncommon in the highest-risk infants, although it may not provide the same benefits when used in the clinical setting (Truog et al., 2014; Ellsworth et al., 2015). The most recent data, evaluating only those studies with iNO initiated at greater than 5 ppm, suggest that there may be greater benefit of iNO on the outcome of BPD in infants of black mothers, compared with infants of nonblack mothers (Ballard et al., 2016).

Diuretics

In infants with well-developed BPD, pulmonary edema is a major component of the illness. There is clear evidence that either daily or alternate-day therapy with the loop diuretic furosemide improves lung mechanics and gas exchange in infants with established BPD (Rush et al., 1990; Hazinski, 2000). These effects may be due to diuresis or to other effects of furosemide, including pulmonary venodilation, resulting in intravascular movement of alveolar water, or to relaxation of airway smooth muscle (Cotton et al., 2012). Thiazide-type diuretics alone or in combination with spironolactone also have improved lung function in some studies.

There is no evidence for long-term benefits of diuretic therapy; nevertheless, most centers use diuretics at some point in the management of infants with BPD, particularly in infants with more severe BPD, and diuretic use in the 1 to 2 years following discharge is common (Hibbs et al., 2008; Cotton et al., 2012; Stevens et al., 2014; Guaman et al., 2015). Some metabolic effects of diuretic use are attenuated by every other day dosing, and spironolactone can counterbalance some of the electrolyte disturbances. Although an infant with chronic BPD who is chronically hypercarbic will develop a compensated respiratory acidosis, if diuretic effects on electrolyte excretion are not managed, a primary metabolic alkalosis may also develop. Additional potential complications of long-term therapy with loop diuretics include hypercalciuria with nephrocalcinosis and its sequelae and osteopenia, which can occur in these former premature infants because of decreased intrauterine mineral accretion, insufficient postnatal mineral supplementation, and the metabolic effects of the drugs. Ototoxicity is also of concern in this vulnerable population, although permanent hearing loss may be an association with more severe neonatal illness.

Bronchodilator Therapy

With established BPD, there is a significant increase in airway obstruction and persistent or intermittent wheezing in substantial proportions of infants and children with BPD (Robin et al., 2004; Fakhoury et al., 2010; Been et al., 2014; Kotecha et al., 2015).

Several studies of either short-term, inhaled, or parenteral β_2 -adrenergic agonist therapy have demonstrated improvement in infant lung function. Inhaled albuterol has been the most widely used agent, and a subset of children with BPD exhibit airway reactivity across childhood and adolescence (Robin et al., 2004; Fakhoury et al., 2010; Kotecha et al., 2015). Hazinski (2000) pointed out that there are two potential adverse effects associated with the use of β_2 -agonist drugs: (1) there can be β -agonist-induced vasodilatation, which may lead to hypoxia, and (2) β -agonist-induced augmentation of airway instability in an infant with BPD and concurrent tracheomalacia may occur. These effects may lead to clinical worsening. Hilgendorff et al. (2008) evaluated former premature infants at term-corrected age and found bronchodilator-responsive airway disease in 18/27 infants despite no clinical evidence of airway obstruction. However, an additional four infants had a “paradoxical” response to the medication, consistent with airway malacia.

As substantial proportions of infants and children with BPD exhibit airway reactivity, a trial of bronchodilator therapy may be indicated in symptomatic children and is probably a common practice, as bronchodilator exposure is frequent in both inpatient and outpatient settings (Hibbs et al., 2008; Gauman et al., 2015; Slaughter et al., 2015). Although the β_2 -agonist therapy with albuterol is the drug that has been most frequently studied, there is rationale for addition or substitution of ipratropium (an anticholinergic) for those infants without positive response to albuterol (Iyengar and Davis, 2015). Evaluation of routine bronchodilator therapy in infants with established BPD should be studied to identify if this use of bronchodilators could attenuate later respiratory morbidity.

Postnatal Corticosteroids

As discussed earlier, there is no study that convincingly supports the use of either systemic or inhaled corticosteroids for prevention or treatment of developing BPD. However, the use of inhaled and systemic steroids with established BPD remains prevalent (Hibbs et al., 2008; Stevens et al., 2014; Gauman et al., 2015). Current data are limited as to the risk versus benefit for both short-term and long-term outcomes in these infants. It may be most appropriate to trial these interventions in the setting of intercurrent infection, because increased inflammation is likely prominent in respiratory exacerbations, similar to other populations of patients with lung disease.

Pulmonary Hypertension

Although it is known that infants dying with BPD have dysmorphic pulmonary vascular development (Abman, 2008), the definitions of pulmonary hypertension used in the preterm population are variable, and the true prevalence of pulmonary hypertension in the setting of BPD is unknown. Some practitioners recommend periodic screening echocardiograms (Mourani and Abman, 2015; Nagiub et al., 2015), but the sensitivity and specificity of this technique are not adequate, in general, including infants with lung disease in an experienced center (Mourani et al., 2008). In any population, the gold standard for diagnosis of pulmonary hypertension is by cardiac catheterization. However, pulmonary hypertension as defined by echocardiogram does not universally portend a poor prognosis, with 89% of former premature newborns demonstrating improvement over time (Khemani et al., 2007). In prospective studies of at-risk infants, a relatively low proportion have elevated right heart pressures by echocardiogram near term, and these abnormalities are more common in infants with BPD than in

those without BPD (Mirza et al., 2014; Mourani et al., 2015). Although therapies for chronic treatment of pulmonary hypertension have shown efficacy in older patient populations, the risk–benefit profile for infants and young children is unknown, and empiric therapy by echocardiogram alone is not recommended (Abman et al., 2015).

Future Directions

Given the pathobiology of BPD, there are multiple potential targets for prevention of the disease, although, ultimately, the multifactorial nature of the disorder may require a multipronged strategy. Caffeine alone, in the CAP Trial, for instance, still left over one-third of infants with a diagnosis of BPD, with the sickest infants not enrolled. The timing of intervention is also likely to be important, as suggested in trials of caffeine and iNO. Selection of extremely high-risk newborns in the first week after birth may be the best strategy, and intervention beyond the second week of life may not be effective, as BPD may already be established. Treatments such as budesonide suspended in surfactant, intratracheal administration of mesenchymal stem cells, and antioxidant therapies currently hold the most promise for benefit among the highest-risk infants, given the potential to counteract inflammation and other effects of supplemental oxygen exposure, but definitive clinical trials evaluating these therapies will be challenging. Furthermore, these therapies are targeted at infants who remain intubated, while additional infants with noninvasive ventilation remain at risk of BPD and its repercussions.

Summary

BPD remains the most common form of chronic lung disease in children, with up to 10,000 new cases in the United States each year. Morbidity and comorbidity associated with the disorder are high, with chronic illness and long-term neurodevelopmental effects, accompanied by financial costs to families and society. Late pulmonary effects of the new BPD are also not yet well defined, although respiratory function impairments are present in surviving adults (reviewed by Islam et al., 2015). Broadly applied early ventilatory and nutritional approaches appear critical to overall prevention of BPD. However, for extremely preterm infants who

Outcome

Infants with severe BPD have ongoing risk of mortality; approximately 10% of infants died after the diagnosis of BPD in a study from quaternary referral centers (Murthy et al., 2014). Mortality is higher among infants with a diagnosis of pulmonary hypertension, 38% in one report, with those with severe pulmonary hypertension (systemic-to-suprasystemic) more likely to die (Khemani et al., 2007). BPD is also associated with poor neurodevelopmental outcomes in long-term follow-up assessments (Ehrenkranz et al., 2005; Wilson-Costello et al., 2009). Poor nutrition, impaired growth, and prolonged and recurrent hospitalization probably contribute to these poor outcomes (Wood et al., 2003; Watson et al., 2009). Additional morbidities associated with BPD include airway malacia, which is common among infants who ultimately undergo tracheostomy, and systemic hypertension (Sahu et al., 2013; Wai et al., 2017). Children with BPD have increased rates of pulmonary hospitalization and medication use in the first 1 to 2 years of life, a pattern that persists to at least 6 years of age (Hennessy et al., 2008). In addition, cost of care in the first year of life was 30% greater for infants with BPD than at-risk infants without BPD in one study (Watson et al., 2009). Given the multiple systems involved, it is likely that these infants would benefit from multidisciplinary follow-up care. Rhein et al. (2012) demonstrated that infants with subspecialty follow-up had similar rates of hospitalization and emergency department visits, after adjusting for risk factors, although we might expect these higher-risk infants to have greater utilization of healthcare resources.

are at greatest risk due to genetic predisposition, fetal environment, or more severe cardiorespiratory illness, there are limited early interventions that have been successfully implemented in practice to prevent BPD and its repercussions. For infants with established BPD, there is great variability in therapeutic approach, which may partially reflect the heterogeneity of the condition but also reflects a lack of data for therapies that can best decrease the respiratory morbidity associated with BPD and preserve lung growth and function later into childhood and beyond.

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Surgical Disorders of the Chest and Airways

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KEY POINTS

- Prompt diagnosis and early intervention are key in salvaging infants with life-threatening surgical disorders of the airway and chest.
- Improved prenatal ultrasound has shifted the diagnosis of disorders of the chest and airways into the fetal period.
- Increased utilization of video-assisted thoracoscopic surgery for congenital thoracic lesions has made surgical intervention on the infant chest less invasive and more tolerable.

Anomalies of the Airways

Nasopharyngeal Obstructive Disorders

Respiratory distress caused by nasal obstruction may manifest as a serious, life-threatening event shortly after birth. Because newborns are preferential nasal breathers for the first 2 to 3 weeks after birth, nasal obstruction may cause severe cyanosis, particularly during oral feedings, with airway obstruction relieved only when the mouth is open to cry (Ramsden et al., 2009). There are several causes of neonatal nasal obstruction, including congenital choanal atresia, nasal pyriform aperture stenosis, nasolacrimal duct cyst, and nasal hypoplasia. Buckling or, less commonly, dislocation of the nasal septum because of birth trauma can also cause breathing problems; most cases respond to decongestant and steroid nasal drops, but dislocations require surgical manipulation (Prescott, 1995).

Congenital Choanal Atresia

Caused by persistence of the buccopharyngeal membrane, congenital choanal atresia occurs in between 1 in 5000 and 1 in 9000 births and has a significant female preponderance. In the majority of choanal atresia cases, the obstructing membrane is of mixed bony and membranous composition (Brown et al., 1996). Choanal atresia is more frequently unilateral (~2/3 of cases) and right sided; bilateral malformations are more serious and constitute an emergency at birth (Szeremeta et al., 2007; Ramsden et al., 2009). Over half of all cases are associated with other congenital anomalies, bilateral cases more so than unilateral (Hall, 1979; Burrow et al., 2009). The most common collection of anomalies was originally termed the *CHARGE association*, consisting of some combination of colobomas of the eyes, heart defects, atresia of the choanae, retardation

of growth or development, genitourinary defects, and ear anomalies associated with deafness (Pagon et al., 1981). The association was officially named *CHARGE syndrome* in 2004, when a common mutation in the *CHD7* gene on chromosome 8 was identified in 60% of cases (Visser et al., 2004).

Because the newborn is a preferential nasal breather, there may be serious difficulties soon after birth, especially in cases of bilateral atresia. Unilateral atresia may present simply with unilateral discharge and feeding difficulties but may not present until later in childhood. The inability to pass a 5- or 6-French catheter through the nose may suggest the diagnosis. Computed tomography (CT) of the nasopharynx with intranasal contrast is the method of choice for making a definitive diagnosis and for evaluating the nature and severity of nasal obstruction (Benjamin, 1985; Crockett et al., 1987).

Emergent management of choanal atresia is focused on ensuring that the oropharyngeal airway is patent, which may necessitate endotracheal intubation. A McGovern nipple, an orogastric tube, or a modified endotracheal tube can be used to overcome the seal between the palate and the tongue (Fulton et al., 2007). Tracheostomy is rarely necessary and typically only required when associated with other anomalies (Asher et al., 1990).

Surgical repair is the mainstay of treatment and can be performed within a few days of birth. Patency can be established by various methods according to surgeon preference. Correction can be accomplished using the transnasal approach under endoscopic visualization and relieving the obstruction using dilators (Stahl and Jurkiewicz, 1985). The transnasal approach works best with thin buccopharyngeal membranes but tends to have higher recurrence and reoperation rates (Samadi et al., 2003; Hengerer et al., 2008). While temporary stenting has been used to decrease restenosis rates, the use of stents remains controversial. It may not prevent restenosis and can be associated with complications, including alar injury, columellar tear, and vestibular stenosis (Strychowsky et al., 2016). The transpalatal approach entails surgical correction of the offending defect and is typically performed for thick bony membranes. However, despite the minimal rate of reoperation, this approach is associated with a higher rate of palate growth deformities. In an attempt to avoid altering palate growth, modern endoscopic biting and drilling instruments were introduced to improve the transnasal technique (Stankiewicz, 1990; Josephson et al., 1998), and studies continue to demonstrate increasing support for the endoscopic repair of choanal atresia (Ramsden et al., 2009).

Congenital Nasal Pyriform Aperture Stenosis

Nasal pyriform aperture stenosis is a rare cause of nasal obstruction and should be suspected when encountering difficulty in passing a nasal catheter. Infants usually present with noisy breathing and respiratory distress that worsens with feeding and improves with crying (Ramadan et al., 1995). Pyriform aperture stenosis is characterized by excessive bone formation in the medial nasal processes of the maxillary bone. The condition may be isolated or associated with other anomalies, such as a solitary maxillary central incisor tooth or, more seriously, midline defects such as pituitary hypoplasia with endocrine insufficiency, diabetes insipidus, or other manifestations of holoprosencephaly and craniosynostosis (Beregszaszi et al., 1996; Godil et al., 2000; Van Den Abbeele et al., 2001). Similar to choanal atresia, an oral airway may be necessary to relieve the breathing difficulty. Although the obstruction can be suitably demonstrated by CT of the nasopharynx, because of its high association with holoprosencephaly, a karyotype analysis and brain CT and/or magnetic resonance imaging (MRI) may be required if brain abnormalities are suspected (Truong and Oudjhane, 1994; Devambez et al., 2009). In most cases, nasal obstruction is mild and may respond to nasal decongestants. In refractory cases of obstruction, usually when the pyriform aperture is less than 5.7 mm, sublabial surgery is necessary to remove excessive bone, and nasal stenting is required (Tate and Sykes, 2009; Wormald et al., 2015). Novel methods of rapid maxillary expansion to enlarge the pyriform aperture using custom palate expansion devices are being investigated with early success (Collares et al., 2015).

Pierre Robin Syndrome (Robin Sequence)

Although this pattern of upper airway problems was first described by Pierre Robin in 1923, characterization of Pierre Robin syndrome remains difficult and controversial. A constellation of anomalies within the spectrum of cleft palate, micrognathia, and glossoptosis, the Pierre Robin syndrome has relatively recently been linked to mutations in the *SOX9* gene (Jakobsen et al., 2007; Benko et al., 2009). Given the varying definitions published, the incidence of Pierre Robin syndrome is difficult to pinpoint; it has been reported to occur in anywhere from 1 in 8500 to 1 in 20,000 births (Breugem and Mink van der Molen, 2009). It is the hypoplastic development of the mandible that is of clinical significance, as the micrognathia can lead to airway obstruction and cyanosis (Cozzi and Pierro, 1985). Obstruction is common when the infant is in the supine position, during feeding, and in active sleep, when pharyngeal muscle tone is absent. Excessive air swallowing, followed by gastric distention, vomiting, and tracheal aspiration, is a frequent problem. The pharyngeal obstruction is maintained by the generation of large negative pressures in the lower pharynx during inspiration and swallowing (Fletcher et al., 1969). Chronic obstruction leads to carbon dioxide retention, failure to thrive, and development of pulmonary hypertension with right-ventricular failure (Johnson and Todd, 1980).

As with the imprecise diagnosis of Pierre Robin syndrome, the severity of respiratory obstruction and the management indicated are varied. Mild cases may present with only mild glossoptosis, and because oral feeds are tolerated without respiratory obstruction, these cases can be managed by side-to-side nursing (Cole et al., 2008; Evans et al., 2011). In the event of respiratory symptoms with feedings or failure to thrive, a nasogastric tube for feedings may be required (Cole et al., 2008). In severe cases of respiratory distress, nasopharyngeal intubation should be performed, typically by passing a 3.5-mm tube through the nose and into the hypopharynx (Stern et al., 1972; Heaf et al., 1982). This prevents the

generation of negative pressure and greatly relieves the respiratory difficulty. The nasopharyngeal tube may be left in place for weeks or even months with adequate lavage and suctioning. Other treatments include tongue–lip adhesion surgery (glossopexy) to hold the tongue forward and tracheostomy if a nasopharyngeal tube does not adequately relieve the obstruction (Gilhooly et al., 1993; Cozzi et al., 2008). Mandibular distraction and velar extension appliances have been introduced as attempts to avoid tracheostomy (Denny and Kalantarian, 2002; Buchenau et al., 2007). Nutrition can be maintained with a hypercaloric formula fed by nasogastric or gastrostomy tube. With adequate airway management and the passage of time, the problem becomes less threatening, especially after a few months, when the infant gains better control of the tongue (Mallory and Paradise, 1979). Oral feedings can then be introduced, usually with a long lamb's nipple to help hold the tongue forward. With adequate nutrition and growth of the mandible, the problem usually resolves by 3 to 12 months of age, when cleft palate repair can safely take place.

Glossoptosis–Apnea Syndrome

Pierre Robin syndrome is not the only condition characterized by mechanical obstruction by the tongue. Infants with Beckwith–Wiedemann syndrome may have considerable breathing difficulties and apnea due to the associated macroglossia (Kamata et al., 2005). Infants with a normal-sized tongue who also have conditions such as unilateral choanal atresia, choanal stenosis, or swelling of the nasal mucosa may generate considerable negative pressure in the pharynx; this, combined with inadequate muscular control over the tongue, may lead to pharyngeal obstruction with respiratory distress, cyanosis, and severe episodes of apnea (Cozzi and Pierro, 1985).

Pharyngeal Incoordination

While the tongue provides the majority of force to move a food bolus into the esophagus, weakness and incoordination of the pharyngeal musculature can lead to disordered swallowing (Massey and Shaker, 2006). This incoordination causes choking and cyanosis with feedings and may be complicated by aspiration pneumonia (Avery and Fletcher, 1974). Affected infants have difficulties in swallowing their own secretions. The condition may be seen in infants with severe hypoxic–ischemic encephalopathy and pseudobulbar palsy, Arnold–Chiari malformation, Möbius syndrome, and other facial malformations. Electromyography of the facial and pharyngeal muscles during rest, crying, and eating can aid in diagnosis (Baudon et al., 2009). Specifically, it can be used to assess the sucking and swallowing coordination in infants during bottle feeding. Drugs with antimuscarinic effects, such as atropine, can decrease secretions and may produce some relief. Although some infants may gradually improve, long-term management may require initiation of tube feedings or even gastrostomy in children who fail to improve.

Laryngeal Deformities

Laryngomalacia (Congenital Laryngeal Stridor)

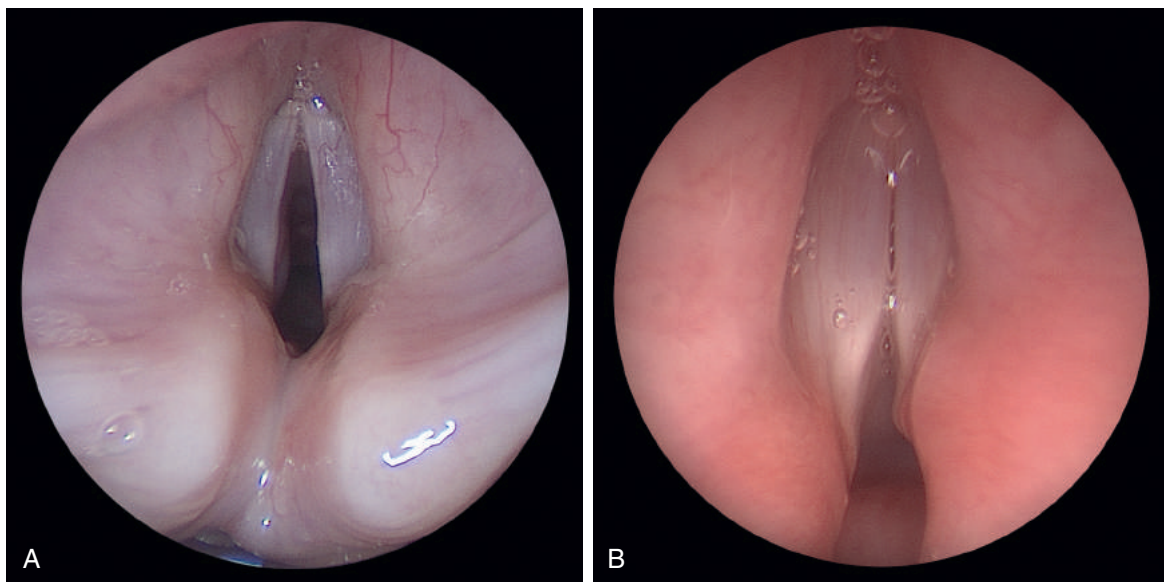
A relatively common condition, congenital laryngeal stridor or laryngomalacia is the most frequent cause of stridor in infants (Zoumalan et al., 2007). Laryngomalacia is characterized by the prolapse of poorly supported supraglottic structures—the arytenoids, the aryepiglottic folds, and the epiglottis—into the airway during inspiration, causing respiratory obstruction and difficulty with feeding (Olney et al., 1999). Despite loud, high-pitched inspiratory

stridor and significant chest retractions that typically present during the first month of life, the infant seldom has cyanosis, hypercarbia, notable feeding difficulty, failure to thrive, or an abnormal cry (Richardson and Cotton, 1984). Laryngomalacia is worse in the supine position, with the neck flexed, and subsides in the prone position, with the neck extended (Cotton and Richardson, 1981). Obstruction is worse during episodes of agitation and lessens when the infant is calmed. Severe forms of laryngomalacia may cause apneic events, pulmonary hypertension, or difficulties with feeding and/or weight gain. Although a CT scan is effective at demonstrating the abnormal prolapse of the aryepiglottic folds supporting the diagnosis (Galvin et al., 1994), confirmation should be obtained at laryngoscopy (Friedman et al., 1990; Wiatrak, 2000), with specific care to avoid fixating the supraglottic tissues with the instrument. Some practitioners prefer to pass a flexible fiberoptic bronchoscope through the nose (Berkowitz, 1998), which does not disturb the supraglottic tissues. In some cases, gastroesophageal reflux or episodes of obstructive apnea may be associated with this condition (Belmont and Grundfast, 1984). Synchronous airway abnormalities are found in up to 30% of infants, including vocal cord paralysis, tracheomalacia, and subglottic stenosis (Simons et al., 2016). Thus the evaluation of stridor must include the examination of the entire upper airway and upper digestive tract (Friedman et al., 1984). Stridor associated with laryngomalacia is usually loudest at 4 to 8 months, and most cases resolve with conservative management around 12 to 24 months (Thompson, 2007). Conservative therapy entails positioning the infant prone as much as possible, and most demonstrate improvement over roughly 18 months (Smith and Catlin, 1984). Approximately 20%, however, will have severe obstructive apnea, cor pulmonale, and/or failure to thrive and require a surgical intervention. In these cases supraglottoplasty may be indicated, with 71% having complete resolution of symptoms and 94% having symptom improvement postoperatively (Garritano and Carr, 2014). Tracheostomy is reserved for supraglottoplasty failures (Richter and Thompson, 2008).

Vocal Cord Paralysis

Unilateral cord paralysis is usually left sided and typically presents without marked stridor or retractions manifesting as aspiration (Parikh, 2004). The infant may cough and choke during feedings, as laryngeal closure with swallowing is impaired. The condition is most frequently secondary to iatrogenic injury, usually caused by excessive stretching of the neck during delivery or injury during surgery; however, it can also be due to neurologic disorders (de Gaudemar et al., 1996). Right-sided vocal cord paralysis has been reported as a complication of extracorporeal membrane oxygenation (ECMO) (Schumacher et al., 1989), presumably as a result of the surgical dissection for insertion of the catheters. Ligation of a patent ductus arteriosus (PDA) has also been associated with a high rate of left-sided vocal cord paralysis, probably secondary to recurrent laryngeal nerve injury during dissection. Recent studies have found that 40%–54% of low birth weight children who undergo PDA ligation have a left vocal cord paralysis (Benjamin et al., 2010; Røksund et al., 2010). This increases their risk of developing bronchopulmonary dysplasia (BPD), reactive airway disease, and feeding problems requiring a gastrostomy tube. Lying the infant on the paralyzed side may decrease the amount of stridor, as the affected cord falls away from the midline (Cotton and Richardson, 1981). Diagnosis can be confirmed with flexible nasolaryngoscopy but may require direct laryngoscopy in the operating room (Fig. 49.1). The condition tends to improve over a period of several weeks or months, and speech and swallow therapy can be used in cases where improvement is not seen. Generally, medialization of the vocal cord is not recommended in neonates less than 6 months of age; however, in children with gross aspiration and severe phonotory difficulty, it may be required. Tracheostomy is preferred in severe cases of respiratory obstruction (Parikh, 2004).

Bilateral cord paralysis is a much more serious condition, accompanied by high-pitched inspiratory stridor; frequently, however, the cry is normal. Usually, severe central nervous system problems are to blame, such as hypoxic–ischemic encephalopathy, cerebral hemorrhage, Arnold–Chiari malformation, hydrocephalus,



• **Fig. 49.1** Vocal Cord Paralysis. (A) Bronchoscopy of a neonate demonstrating a normal glottis. (B) Bronchoscopy of an infant with bilateral true vocal cord paralysis causing obstruction. (Courtesy of Dr. Jamie Funamura, Assistant Professor, University of California, Davis Health System, Department of Otolaryngology, Sacramento, CA.)

or brainstem dysgenesis. Associated problems may include pharyngeal incoordination with swallowing difficulty and esophageal dysfunction, recurrent apnea episodes, and tracheal aspiration of mucous secretions and formula. The stridor may resolve slowly if brain swelling subsides after birth, as is the case with ventriculoperitoneal shunt placement. The diagnosis may be suspected at laryngoscopy but should be confirmed by flexible fiberoptic bronchoscopy, rigid bronchoscopy, or ultrafast cine CT scan. Tracheostomy is frequently required in about 50%–60% of patients (Daya et al., 2000; Lesnik et al., 2015), and the prognosis usually is poor secondary to the underlying problems.

Laryngeal Atresia

Laryngeal atresia is the result of failed recanalization of the larynx during embryologic development, resulting in a newborn with complete laryngeal obstruction presenting with severe respiratory distress. In some cases the larynx may be completely obstructed by a laryngeal web, seen in the delivery room during attempts to intubate the cyanotic infant. An endotracheal tube sometimes can be forced beyond the obstruction into the trachea. Otherwise, a large-bore needle should be inserted percutaneously into the trachea to maintain marginal gas exchange while preparations for emergency tracheostomy are made. Most infants with laryngeal atresia have other lethal malformations (Smith and Catlin, 1984). Most cases are now diagnosed prenatally from ultrasound findings consistent with congenital high airway obstruction syndrome (CHAOS), such as polyhydramnios and enlarged hyperechoic lungs with an associated flattened or inverted diaphragm (Hedrick et al., 1994; Kalache et al., 1997). The mother may be evaluated for ascites or hydrops fetalis due to impaired venous return to the heart, and the amniotic fluid lecithin level may be very low in such cases. In the absence of other lethal malformations, the characteristic ultrasound diagnosis may permit preparations for emergency tracheostomy after delivery of the infant or an ex utero intrapartum treatment (EXIT)-to-airway procedure, discussed later. Survivors of fetal intervention for CHAOS have now been seen (Hirose et al., 2004; Elliott et al., 2013).

Congenital Subglottic Stenosis

Congenital subglottic stenosis, manifesting as inspiratory stridor from birth, is caused by partial obstruction of the cricoid probably due to incomplete canalization of the cricoid ring. In a full-term infant the normal subglottic lumen is 4.5–5.5 mm in diameter, whereas that of a preterm neonate is 3.5 mm in diameter. A subglottic diameter of 4 mm or less in a full-term infant or 3 mm or less in a premature infant is consistent with a diagnosis of subglottic stenosis. Subglottic stenosis is diagnosed by direct laryngoscopy supplemented with rigid bronchoscopy and chest radiography to evaluate other airway lesions and/or concomitant lung disease, as the latter may be common in the premature infant. Treatment consists of balloon dilation or endoscopic lysis with a carbon dioxide laser in cases of membranous stenosis. However, most cases severe enough to require intervention are cartilaginous and require an anterior cricoid split, obviating the need for and complications of tracheostomy in most cases (Cotton and Seid, 1980; Smith and Catlin, 1984; Schroeder and Holinger, 2008).

Congenital Subglottic Hemangioma

Subglottic hemangiomas are rare, accounting for 1.5% of all congenital abnormalities of the larynx (Pransky and Canto, 2004).

They often occur in association with a cutaneous hemangioma and may cause inspiratory stridor and expiratory wheezing that progress with enlargement of the tumor (Cotton and Richardson, 1981; Orlow et al., 1997). This diagnosis is suspected when asymmetric subglottic narrowing is seen on plain radiographs and is confirmed by flexible and rigid endoscopy demonstrating a sessile vascular lesion, most commonly in the posterolateral subglottis (Rahbar et al., 2004; Ahmad and Soliman, 2007). Although some practitioners have advocated high-dose corticosteroid therapy (Brown et al., 1972) and others have tried intralesional injections of steroids, in many cases intubation or tracheostomy is eventually required. Results of removal by carbon dioxide or potassium titanyl phosphate (KTP) laser have been encouraging, enabling treatment without tracheostomy; however, associated complications such as subglottic stenosis have been reported (Healy et al., 1984; Kacker et al., 2001; Ahmad and Soliman, 2007). Finally, case reports using beta blockers (propranolol and acebutolol) have been successful in causing regression of subglottic hemangiomas, preventing the need for tracheostomy altogether (Blanchet et al., 2010).

Laryngotracheoesophageal Cleft (Congenital Laryngeal Cleft)

In laryngotracheoesophageal cleft, a longitudinal communication is present between the airway and the esophagus, stretching from the larynx into the upper trachea or sometimes as far as the carina. This rare condition is reported in 1 in 10,000 to 1 in 20,000 births and is caused by failed fusion of the two lateral growth centers of the posterior cricoid cartilage, preventing proper fusion of the posterior cricoid lamina (Pezzettigotta et al., 2008). Affected infants have respiratory distress with inspiratory stridor and cyanosis, associated with tracheal aspiration of saliva and feedings. Chest radiographs may show evidence of aspiration pneumonia, and the cine esophagogram shows contrast material spilling into the trachea. The diagnosis can be established with direct laryngoscopy and bronchoscopy. Given the high association with other congenital anomalies and syndromes, such as tracheal atresia, tracheoesophageal fistula, and Opitz–Frias syndrome, a thorough evaluation of all organ systems and genetic karyotype are recommended.

Laryngotracheoesophageal clefts are classified, by severity of symptoms, into four groups (types I–IV), which are used to determine the management strategy and the need for surgical intervention. For all types, initial management involves adequately securing the airway with an endotracheal tube or tracheostomy (Richardson and Cotton, 1984). Mild cases can sometimes be managed by conservative therapy, including swallow rehabilitation and antireflux medication (Pezzettigotta et al., 2008). In those who fail conservative management, endoscopic therapy including injection augmentation of the cleft may be attempted (Mangat and El-Hakim, 2012). More severe cases require extensive reconstruction employing an anterior translaryngotracheal approach or even a partial upper sternotomy. Despite these reconstruction attempts, mortality remains high, at nearly 50% among all types of clefts and higher in cases with associated congenital anomalies and type IV clefts (Roth et al., 1983; Myer et al., 1990; Simpson et al., 1996).

Tracheal Deformities and Other Tracheal Disorders

Tracheal Agenesis

Tracheal agenesis is a rare condition that occurs in less than 1 in 50,000 births (Demircan et al., 2008). The trachea can be atretic

just below the vocal cords but is most often absent all the way down to the carina (Altman et al., 1972). Clinical manifestations include severe distress, absence of vocal sound, and severe cyanosis. Affected infants usually have additional congenital anomalies within the VACTERL association, including tracheoesophageal fistulas, severe cardiac malformations, and sometimes renal and anal anomalies (Mohammed et al., 2016). Prenatal diagnosis is difficult, but prenatal presentation may manifest as CHAOS. If diagnosed prenatally, delivery via EXIT should be arranged to maximize the chances for survival (Vaikunth et al., 2009). Postnatal diagnosis should be suspected in cases of respiratory distress with immediate hypoxia, no audible cry, and a mechanical inability to intubate (Demircan et al., 2008). Despite the presence of a larynx, intubation cannot be accomplished at delivery; however, if the tracheal tube is positioned in the esophagus and connected to a mechanical ventilator, reasonable gas exchange can be obtained via the tracheoesophageal fistula (Sandu and Monnier, 2007). When the tracheal atresia is high, a tracheostomy can be performed in the remnant tracheal tissue. If survival seems possible, gastric division and a gastrostomy for feeding should be performed. Reconstructive surgery is not likely to be successful, however, and the prognosis is extremely poor, if not because of poor ventilation, then because of the underlying malformations.

Congenital Tracheal Stenosis

In congenital tracheal stenosis, a segment of the trachea is narrowed, usually starting in the subglottic region. The affected segment may be short or long; occasionally, the entire trachea is hypoplastic, and the bronchi may be involved. This disorder affects 1 in 64,500 live births and is usually caused by complete or nearly complete cartilage rings that narrow the trachea (Hewitt et al., 2016). Affected patients may have inspiratory stridor, expiratory wheezing, feeding difficulties, and experience cyanotic episodes. Mild inflammation and small mucous plugs may cause life-threatening deterioration. In up to 60% of cases, other congenital malformations are also present, such as vascular ring anomalies, congenital heart defects, tracheoesophageal fistula (especially the H type), and hemivertebrae (Benjamin et al., 1981). There also is an association with pulmonary agenesis (Volland et al., 1986). A series of cases without accompanying defects have been reported in premature infants who presented with difficulties at tracheal intubation (Hauff et al., 1988).

Patients with this deformity usually can be intubated, but the endotracheal tube cannot be advanced and should not be forced. Mechanical ventilation with generous levels of positive end-expiratory pressure (PEEP) may help stabilize the infant. Tracheostomy is not indicated and interferes with making the diagnosis (Nakayama et al., 1982). Sometimes the diagnosis can be made by inspiration and expiration chest radiographs, using air as the contrast medium. However flexible fiberoptic bronchoscopy in the neonatal intensive care unit (NICU) or rigid bronchoscopy in the operating room is usually required for diagnosis (Lobe et al., 1987). Because it is important to examine the lower limits of the stenosis, it may be necessary to proceed with tracheobronchography, but this may sometimes cause acute decompensation and is controversial (Loeff et al., 1988; Hewitt et al., 2016). Today, ultrafast cine CT scan and MRI have become useful diagnostic techniques to define the lower limits of the stenosis (Galvin et al., 1994; Rimell et al., 1997). In addition, three-dimensional (3D) CT reformats provide valuable information on the trachea–cardiovascular relationship and can allow for 3D printing of models to rehearse complex surgical interventions (Hewitt et al., 2016).

In most cases, the stenosis requires treatment of some kind in the operating room. Balloon dilation alone is not likely to be successful in the case of a complete tracheal cartilage ring, because cartilage cannot be stretched. For short-segment stenosis, balloon dilation with laser may be used: at rigid bronchoscopy, the cartilage ring is split at the midline posterior aspect using the KTP laser, and the bronchoscope is advanced with the aid of serial balloon dilations (Othersen et al., 2000). Some groups have used metallic stents to prevent restenosis with long-term success; however, granulation tissue over the stent can make removal difficult (Maeda et al., 2013). For longer-segment stenosis, Longaker et al. (1990) described segmental resection of the stenosis with end-to-end anastomosis to shorten the trachea, followed by serial balloon dilations through a rigid bronchoscope. Backer et al. (1998) described the successful use of free autografts of resected trachea for this type of tracheoplasty. However, for long-segment stenosis, slide tracheoplasty has become the standard treatment (Lipshutz et al., 2000). In this procedure, the stenosis is transected in the middle, the upper segment is incised longitudinally along the anterior aspect, the lower segment is incised longitudinally at the posterior aspect, the incised segments are slid over one another, and the edges are anastomosed, effectively shortening the trachea while widening the narrowed lumen.

The use of cardiopulmonary bypass has improved treatment and is advocated by some as averting the need for complex anesthesiology techniques (Loeff et al., 1988). After midline sternotomy, tracheal resection, and tracheoplasty with shortening of the trachea, the patient may need fixation in a brace for at least 6 weeks to maintain neck flexion and prevent excessive stretching of the anastomosis (Nakayama et al., 1982). Premature infants with congenital tracheal stenosis cannot undergo tracheal resection and tracheoplasty with cardiopulmonary bypass procedures. For these patients, aggressive balloon dilations are recommended, with splitting of the weaker posterior aspect of the tracheal rings (Messineo et al., 1992). With improvements in surgical techniques and the addition of ECMO to complex repairs, survival rates have improved to greater than 80% (Butler et al., 2014). Those patients who have concurrent distal bronchial stenosis or distal bronchomalacia have slightly lower survival rates (Hewitt et al., 2016).

Tracheobronchomalacia (Tracheomalacia)

Tracheobronchomalacia is characterized by dynamic collapse of the trachea during breathing secondary to delayed development of tracheal cartilage. This condition may be primary or associated with tracheoesophageal fistula, BPD, extrinsic tracheal compression, or prolonged intubation (Sotomayor et al., 1986). Tracheobronchomalacia should be suspected in infants presenting with respiratory distress, cyanotic spells, or persistent respiratory symptoms including expiratory stridor, recurrent respiratory infections, or persistent or recurrent wet cough (Masters, 2009). Chest radiographs are usually normal, with only a 62% specificity in diagnosing tracheobronchomalacia (Walner et al., 1999). With improvements in multidetector CT scanners and ultrafast cine CT scanners, diagnosis can be made in with improved accuracy (Lee and Boiselle, 2009; Goo, 2013). Bronchoscopy remains the gold standard for diagnosis, which shows approximation of the anterior and posterior walls of the trachea during expiration (Saltzberg, 1983). The bronchoscope may support the walls of the trachea, alleviating the respiratory distress by passage of the bronchoscope to the carina, while potentially disguising the extent of the abnormalities.

Factors associated with the development of tracheomalacia include immaturity, higher mean airway pressure, and prolonged

mechanical ventilation (Downing and Kilbride, 1995). Affected infants may have significant dynamic compression of the trachea. Because the trachea of premature infants is very compliant and may be excessively stretched and injured during mechanical ventilation, very immature infants are particularly prone to tracheomalacia. Some premature infants have greatly enlarged tracheas or tracheomalacia after mechanical ventilation (Bhutani et al., 1986).

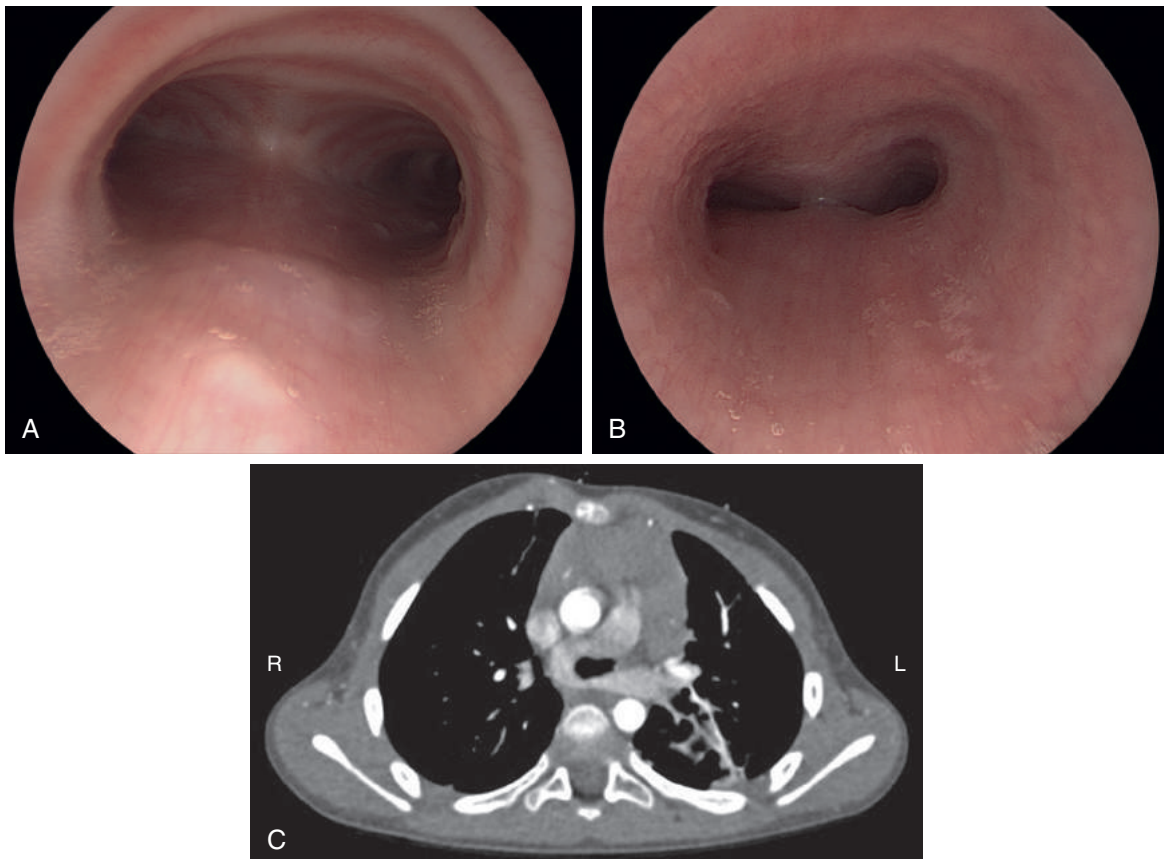
Many infants with tracheomalacia spontaneously improve by 1 to 2 years of age, when the cartilage has become strong enough to support tracheal patency and airway caliber increases (Sandu and Monnier, 2007). Severe cases with life-threatening episodes of airway obstruction necessitate tracheostomy with an elongated tracheostomy tube. However, current treatments for milder cases include tracheal intubation with continuous positive airway pressure (CPAP) or PEEP, which prevents tracheal collapse, and aortopexy (fixation of the aorta to the sternum), which has the effect of supporting the attached trachea (Wiseman et al., 1985; Jacobs et al., 1994; Dave and Currie, 2006). Many of the most severely affected patients respond well to aortopexy (McCoy et al., 1992).

Tracheal Compression by Vascular Rings

Tracheal compression can be caused by several factors: (1) a double aortic arch, (2) a right aortic arch, (3) a left-sided origin of the (right) innominate artery, (4) a right-sided origin of the left common carotid artery, or (5) an anomalous origin of the left pulmonary

artery from the right pulmonary artery (Hendren and Kim, 1978). With a right aortic arch, the trachea is compressed by the main pulmonary trunk, aortic arch, and ligamentum arteriosus. The anomalous innominate or common carotid arteries form a tight crotch, which impinges on the anterior trachea. The anomalous left pulmonary artery returns to the left by passing between the esophagus and the trachea, compressing the trachea between the right and the left pulmonary arteries. Infants with tracheal compression have inspiratory stridor and expiratory wheezing, with symptoms usually appearing later in the neonatal period. Affected infants often lie with the head and neck hyperextended to stretch the trachea and make it less compressible. If the esophagus is compressed, feeding is associated with regurgitation.

There are several methods of diagnosis. The chest radiograph may show mild overinflation, a right-sided aorta, and, with appropriate technique, evidence of tracheal narrowing. A barium swallow examination may show indentation of the esophagus. Bronchoscopy should reveal a pulsatile mass with narrowing near the carina (Fig. 49.2A–B). Cross-sectional imaging with CT and MRI has proven to be accurate in defining most vascular malformations and can give detailed imaging of the surrounding anatomy (Hernanz-Schulman, 2005)(see Fig. 49.2C). While echocardiography is less reliable in making the diagnosis of a vascular ring, it was recently demonstrated that prenatal diagnosis by ultrasound avoided unnecessary delays in the repair of symptomatic vascular rings and



• **Fig. 49.2** Pulmonary Artery Sling. (A) Bronchoscopy of a normal distal trachea just above the level of the carina. (B) Bronchoscopy of a trachea with external compression by a vascular ring; in this case a pulmonary artery sling. (C) Contrast-enhanced computed tomography demonstrating the anomalous left pulmonary artery passing behind the trachea and in front of the esophagus. Compression of the trachea can be seen. (Courtesy of Dr. Jamie Funamura, Assistant Professor, University of California, Davis Health System, Department of Otolaryngology, Sacramento, CA.)

that repair on identification of symptoms prevented the development of secondary tracheobronchomalacia (Rimell, 1997; Tuo et al., 2009). Treatment involves surgical division of the vascular ring. Recently, minimally invasive techniques including a video-assisted thorascopic approach and endoscopic robotic techniques have been employed (Al-Bassam et al., 2007; Suematsu et al., 2005). After surgical division of the vascular ring, the respiratory distress may persist for weeks or longer because of localized tracheal deformity (either stenosis or tracheomalacia), emphasizing the need for immediate repair on diagnosis. In cases of isolated vascular rings, repair provides cure with minimal postoperative complications (Ruzmetov et al., 2009).

Tracheal Compression by Extrinsic Masses

The trachea may also be compressed by a bronchogenic cyst, an enteric duplication cyst, a thoracic neurogenic tumor, or a mediastinal teratoma (Benjamin, 1980). These may be demonstrated by anteroposterior and lateral chest films and are especially apparent on CT scan. Such masses may also compress the esophagus and can be demonstrated with a barium swallow.

Congenital High Airway Obstruction Syndrome and the Ex Utero Intrapartum Treatment Procedure

CHAOS actually describes a spectrum of rare anomalies, including laryngeal web, laryngeal atresia, laryngeal cyst, and tracheal atresia or stenosis. Most cases are sporadic, with the true incidence unknown, and thus the natural history of this disease is not well known. CHAOS is characterized by enlarged lungs, dilated distal airway, everted diaphragm, ascites, and ultimately nonimmune hydrops fetalis. Prenatal diagnosis is becoming more common with the progress of ultrasound and MRI techniques. The exact nature of the airway obstruction may not be entirely clear, however, and the time required to establish a safe airway soon enough after delivery carries various risks, including anoxic brain injury.

As with any anomaly that causes either direct respiratory airway obstruction or airway compression by means of mass effect, CHAOS poses a difficult problem for the clinical team during delivery. The EXIT procedure was developed as a solution to this problem: by preserving fetoplacental circulation throughout a scheduled cesarean section delivery, a safe fetal airway can be established before umbilical cord ligation (Fig. 49.3) (Harrison et al., 1996; Hirose et al., 2004). Infants with CHAOS still require postnatal airway reconstruction after delivery, but once a tracheostomy is in place, laryngeal or tracheal reconstruction is essentially an elective procedure and can be performed once the patient's overall status is optimized (Hirose et al., 2004). With EXIT, infants with CHAOS have a chance at survival with small case series showing promising results (Moldenhauer, 2013).

Disorders of the Mediastinum

In the posterior mediastinal space, thoracic neuroblastomas and neurenteric duplication cysts are the most common disorders encountered in the newborn. Bronchogenic cysts occur in the middle mediastinal space. An enlarged thymus and a mediastinal teratoma are the masses most often seen in the anterior mediastinum.

Thymus

The thymus occupies the upper anterior mediastinum, and it is more prominent in the newborn period than at any other time of life. It may be so large as to reach the diaphragm or obscure both



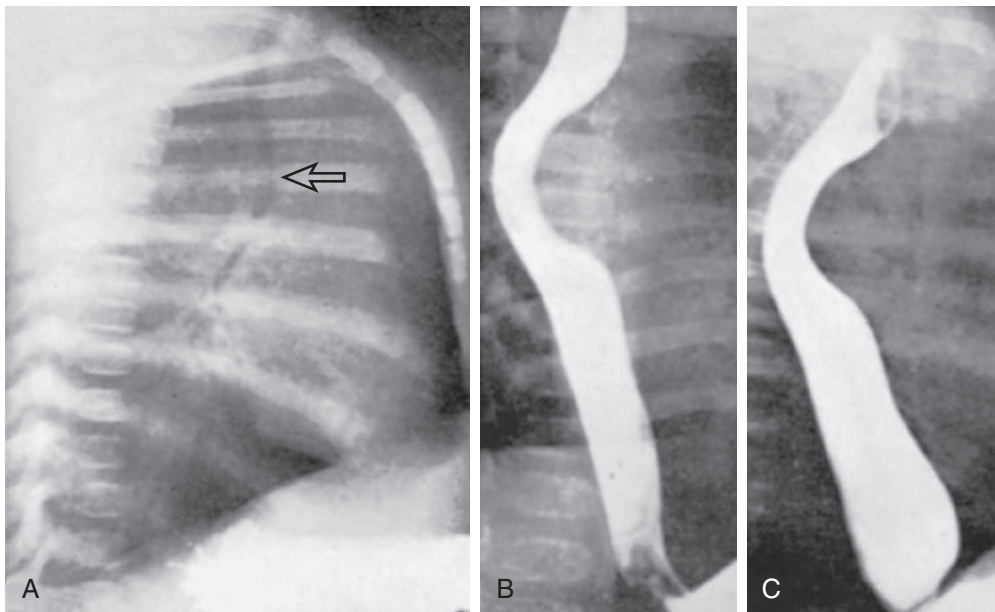
• **Fig. 49.3** Direct Laryngoscopy, Bronchoscopy, and Esophagoscopy Are Performed During an Ex Utero Intrapartum Treatment Procedure. Using these methods, airway obstruction can be overcome and endotracheal intubation can be performed. (From Hirose S, Farmer DL, Lee H, Nobuhara KK, Harrison MR. The ex utero intrapartum treatment procedure: looking back at the EXIT. *J Pediatr Surg.* 2004;39:375–380; discussion 375–380.)

cardiac borders on radiographs. The normal thymus can be distinguished from an abnormal mass by the absence of tracheal deviation or compression. The thymus changes in position with respiration and is less prominent with deep inspiration. It also involutes with stress as well as with corticosteroid therapy. Absence of the thymic shadow in an infant should alert the clinician to the possibility of severe combined immune deficiency syndrome or DiGeorge syndrome with hypocalcemia and cardiac anomalies (Collard et al., 1999).

The cardiophymic-to-thoracic ratio provides an index of thymic size. The shadow of the enlarged thymus is the most common radiopaque mass visualized in the anterior mediastinum of the newborn. The enlarged thymus causes little if any trouble in the neonatal period. Fletcher et al., as well as Gewolb et al. (1979), noted that a large thymus is present on the first day of life in infants at risk for hyaline membrane disease, presumably because of less-than-normal levels of glucocorticoids before birth (Fletcher et al., 1979). The effect that glucocorticoids have on the thymus is also supported by a study that found that prenatal steroid administration causes thymic atrophy (Michie et al., 1998). This steroid-induced atrophy, however, does not appear to have any clinical significance.

Congenital Mediastinal Teratoma

Mediastinal teratomas arise from primordial germ cells and are classified as being mature or immature. Mature teratomas are more common and contain all three embryonic cell layers (ectoderm, mesoderm, and endoderm). Immature teratomas are characterized by mature elements of all three germ layers but also have immature embryonic tissue interspersed (Thambi Dorai et al., 1998). Mediastinal teratomas rarely cause symptoms in the newborn infant. However, when an anterior mediastinal mass is associated with respiratory distress in the newborn, the strong likelihood is that the lesion is a mediastinal teratoma (Mogilner et al., 1992). Teratomas can also cause chest pain, cough, and recurrent post-obstructive pneumonia secondary to bronchial obstruction. Chest



• **Fig. 49.4** Bronchogenic Cyst. (A) The lateral film shows the trachea displaced anteriorly (arrow). (B) The anteroposterior film shows the barium-filled esophagus displaced to the right. (C) Another lateral film shows the barium-filled esophagus displaced posteriorly. (From Hope JW, Koop CE. Differential diagnosis of mediastinal masses. *Pediatr Clin North Am.* 1959;3:379.)

radiographs show an anterior mediastinal mass with calcification; however, CT and MRI are the primary modes of diagnosis. Mature teratomas are invariably not malignant, and surgical resection via median sternotomy, posterolateral thoracotomy, or thorascopy is sufficient treatment (Dulmet et al., 1993). Immature teratomas in children fortunately behave similar to mature teratomas and should be completely excised; however, there may be a role for chemotherapy before resection.

Prenatal diagnosis of a mediastinal teratoma is rare; however, large masses can cause compression of the mediastinum, leading to nonimmune hydrops and fetal demise (Sbragia et al., 2001). If hydrops does not occur and the pregnancy is carried to term, delivery can be complicated by airway compression and cardiopulmonary failure (Tsao et al., 2003a). These cases can be managed by in utero resection in the case of hydrops or via an EXIT procedure near term followed by resection after stabilization (Merchant et al., 2005).

Congenital Bronchogenic Cysts

Bronchogenic cysts arise from the foregut and are usually extrapulmonary. They can be located in the mediastinum (just above the tracheal bifurcation), pericardium, abdomen, neck, and within the diaphragm (Langston and Thurlbeck, 1986; Winters et al., 1997; Winters and Effmann, 2001). The minority of bronchogenic cysts are found within the lung, and some pathologists believe that these may not be distinct from intralobar sequestrations or type 1 congenital pulmonary airway malformations (CPAMs) (Stocker, 2009). Bronchogenic cysts can be seen on prenatal ultrasound, although they are more likely to present later as they increase in size over time, with airway compression, recurrent infection, hemoptysis, or pneumothorax (Langston, 2003). Intra-uterine airway compression can result in congenital lobar emphysema (CLE; see later discussion).

In the newborn, bronchogenic cysts are encountered infrequently, and most do not come to the attention of the practitioner until

later in infancy or during the second decade of life. Bronchogenic cysts seldom attain a large size and often contain clear fluid and debris. They are lined with columnar or cuboidal epithelium, and their walls generally contain smooth muscle, glands, and cartilage, the latter indicating their bronchial origin (Stocker, 2009). These cysts lie near the carina in the middle mediastinal space. They produce lung overdistension or atelectasis, depending on whether airway obstruction is complete or partial, and this is accompanied by respiratory distress in the newborn infant. Opsahl and Berman reported a case that showed overinflation on the left followed by clearing and then similar overinflation on the right (Opsahl and Berman, 1962). Radiographic examination often shows a mass lesion at or just above the carina and displacing the lower trachea forward (Fig. 49.4A). CT images show sharply marginated cystic masses of soft tissue or water attenuation, and specific criteria have been developed to distinguish these cysts from other mediastinal masses on CT (McAdams et al., 2000; Winters and Effmann, 2001). Barium swallow examination may reveal indentation of the esophagus, from external compression of the cyst pushing it backward at the level of the carina (see Fig. 49.4B–C). Bronchoscopy reveals compression of the trachea and sometimes of one major bronchus, usually from the posterior aspect. Sometimes a bronchogenic cyst may communicate with the airway and contain air. In the immediate newborn period, there may be retention of fetal lung fluid in the lung compromised by the bronchogenic cyst; the fluid may take days to clear. Treatment for bronchogenic cyst consists of surgical excision by partial or total lobectomy, with uniformly good results. Early excision prevents eventual development of symptoms in adulthood and malignant degeneration later in life (de Perrot et al., 2001; Jiang et al., 2015).

Neurenteric Duplication Cysts

These mediastinal cysts may be derived from the esophagus, stomach, or small bowel, so they may also be called enterogenous cysts.

Although they are not encountered frequently, they are far from uncommon in the newborn. They originate from duplicated segments of the foregut that have become partially or completely detached from the parent viscus. Neurenteric duplication cysts lie in the posterior mediastinum but with increasing size may project far into one or the other hemithorax. Their walls are composed of a mucosal layer, characteristic of their site of origin, and one or more muscular layers. They contain secreted fluid that is the same as that of their parent viscus; the fluid in a gastrogenic cyst contains pepsin and hydrochloric acid in the same concentration as in gastric juice.

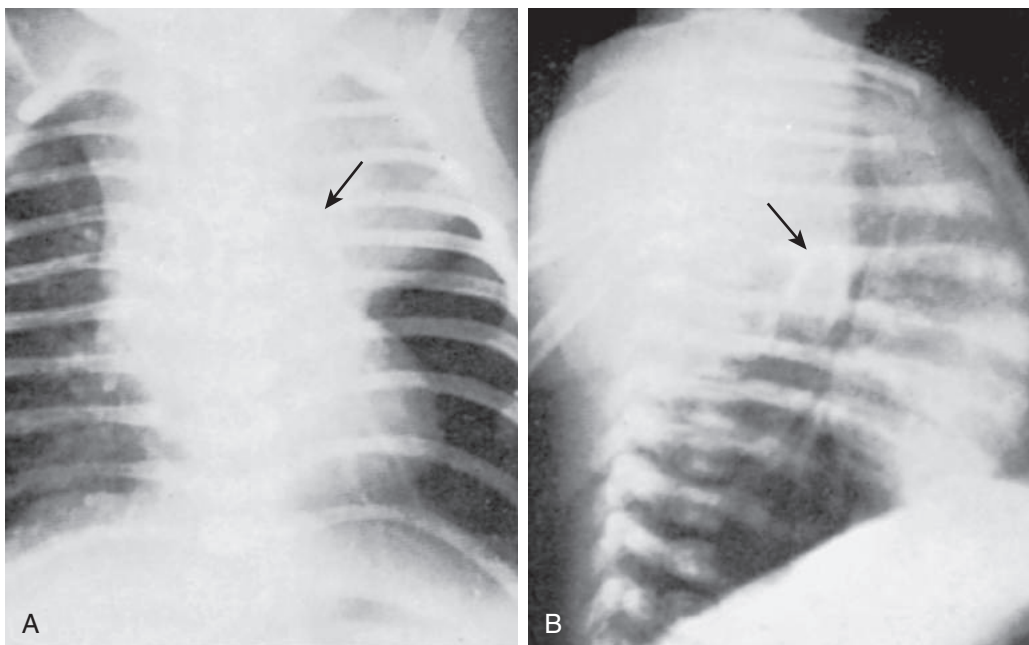
The foregut becomes duplicated in the course of embryonic development by failure of complete resorption of primitive occluding epithelium, resulting in the formation of a supernumerary wall and eventually a separate lumen and cyst. The high percentage of vertebral anomalies associated with neurenteric duplication cysts led Veeneklaas to suggest that the primary embryonic defect lies in abnormal persistence of the primitive foregut adherence to the notochord (Veeneklaas, 1952). When the foregut descends from its early position in the neck, this adhesion to the notochord causes anomalies of the vertebral bodies. As the adhesion breaks off the duplicated portion of the foregut, it prevents complete descent into the thorax and abdomen along with the mature foregut.

Clinical signs depend on the size and location of the duplication cyst. Because all of these cysts are posterior and lie close to the trachea, esophagus, and great vessels, they are seldom present without signs of abnormality. Cyanosis, tachypnea, and dyspnea often are present from birth. Swallowing difficulties and vomiting are less frequent. Recurrent lower respiratory tract infections are findings in older infants with such cysts. Frank hemorrhage from the lungs or stomach is not uncommon. In most instances, hemorrhage indicates that the cyst is of gastrogenic origin, with peptic acid erosion into the trachea or esophagus. Technetium scans are useful for delineating cysts lined with gastric mucosa. Radiographs of the chest show abnormal densities that are often difficult to

distinguish from unusual cardiac contours. The barium swallow examination commonly shows displacement of the esophagus, usually forward because the mass is in the posterior mediastinum. The cyst may partially or totally compress the bronchus, with consequent lung overdistension or atelectasis. Sometimes the symptoms are intermittent as the cyst enlarges or empties. Bronchoscopy may show signs of external compression of the trachea or bronchus, usually from the posterior aspect. Superina et al. (1984) reviewed 25 years of experience with neurenteric duplication cysts and noted that a spinal component may accompany the mediastinal cyst in as much as 20% of affected children. They recommended careful radiographic evaluation of the spinal canal with CT scan and then excision of the intraspinal cyst, if possible, before the onset of neurologic signs in later childhood. The MRI scan may give improved delineation of intraspinal cysts (Azzie and Beasley, 2003). Operative resection is indicated as soon as the diagnosis of a neurenteric duplication cyst is made. It is neither necessary nor wise to delay exploration, because all intrathoracic masses will eventually become symptomatic and have been known to cause paralysis and fatal meningitis (Philippart, 1994).

Congenital Thoracic Neuroblastoma

Neuroblastoma, the most common solid tumor in the mediastinum of infants, arises from sympathetic neural tissue along the vertebral column and is therefore located in the posterior mediastinum. Neuroblastomas can arise anywhere in the sympathetic nervous system but most commonly occur in the adrenal gland. Thoracic neuroblastomas account for approximately 20% of cases (Grosfeld, 1999). They may extend into both lungs, causing respiratory distress, and they may extend into the spinal canal, later causing neurologic signs, including Horner syndrome and paralysis. The chest mass may be obvious on routine radiographs obtained for unrelated reasons in the newborn (Fig. 49.5) or on chest radiographs taken to evaluate significant respiratory distress (Li et al., 2001). In older



• **Fig. 49.5** Congenital Thoracic Neuroblastoma. The mass (arrow) is in the left upper hemithorax (A) and in the posterior mediastinum (arrow) (B). (From Hope JW, Koop CE. Differential diagnosis of mediastinal masses. *Pediatr Clin North Am.* 1959;6:379.)

infants the diagnosis may follow chest radiography for lower respiratory tract infection, or radiographs may be taken to investigate dyspnea with physical exertion. Approximately 80% of neuroblastomas will demonstrate tumor calcification on CT scan. While CT scan can aid in diagnosis, MRI is essential to assess the degree of intervertebral tumor extension, evaluate for lymphatic spread and chest wall involvement, and for discerning liver metastases (Slovic et al., 1997; Fraga et al., 2010). Diagnosis of thoracic neuroblastomas is also possible in the prenatal period as the fidelity of fetal ultrasound has improved (Moppett et al., 1999).

Despite some characteristic imaging findings specific to neuroblastomas, differentiation from other posterior mediastinal masses may be impossible before exploration. Invasion of neighboring lung parenchyma, however, strongly supports a diagnosis of neuroblastoma. Other clinical markers of neuroblastomas, including elevations of urinary vanillylmandelic acid and homovanillic acid, may be present, but this finding is less common in the newborn, and its absence does not rule out neuroblastoma. If the patient has systemic hypertension, plasma levels of epinephrine, norepinephrine, and dopamine may be elevated, but this has not been reported in a thoracic neuroblastoma. Also, results of assays for various clinical biologic markers may be positive; for example, serum ferritin and serum lactate dehydrogenase may be elevated, but this is not usual in the newborn. A bone marrow aspirate should be obtained for cytologic evaluation, and a nuclear bone scan should be performed to exclude the possible remote spread of metastases. A number of cytogenetic biologic markers may be detected in the excised tumor tissue (e.g., cellular DNA ditetraploidy, increased *N-myc* oncogene copy number); these usually indicate malignancy in older children (Ladenstein et al., 2001), but, again, they are commonly negative in the newborn.

Upfront surgical exploration is indicated for all benign tumors and should be pursued on low-risk stage I and II neuroblastomas, as excellent survival rates have been shown with surgery alone in these patients (Fraga et al., 2010; Strother et al., 2012). Intermediate risk stage III and IV neuroblastomas should be treated with adjuvant chemotherapy, with the goal of shrinking the tumor to allow for subsequent complete resection when able. Traditionally, these excisions have been done via open thoracotomy; however, with improvements in thoracoscopic technology, several groups advocate the use of video-assisted thoracoscopic surgery (VATS) for resection of thoracic neuroblastomas and report good results with no documented recurrences and with minimal complications (Fraga et al., 2012). While some worry about inadequate margins with VATS, the zoom magnification may improve visualization, making it easier and safer to resect large thoracic neuroblastomas. Patients with high-risk tumors, including those with disseminated disease or localized disease with *MYCN* amplification, should undergo multimodal therapy with high-dose chemotherapy and hematopoietic stem cell rescue, radiation, and surgical resection if indicated (Laprie et al., 2004).

The outlook for neuroblastomas in extra-adrenal locations is better than that for their adrenal counterparts (Young et al., 1970). The outlook for neuroblastomas manifesting in the first year of life also is good. Many of these tumors are cystic in nature, and the histologic examination suggests that the neuroblasts are arranged in clumps rather than in sheets; this “neuroblastoma in situ” feature carries a high likelihood of spontaneous regression and therefore a good prognosis. Most neuroblastomas in the newborn are Evans stage A, with a good outlook. So-called stage D (S) also is quite common (Moppett et al., 1999); although there are metastases to the liver, marrow, or skin, the prognosis is still good because

metastases to the bone are rare. The clinical markers are seldom elevated, and results of assays for the cytogenetic markers are seldom positive, all of which indicate a reasonable outlook. If the lesion can be completely removed and no bone metastases are found, then most infants survive. Some clinicians suggested that the chance of spontaneous regression is so high in the newborn that even surgery may not be necessary (Morgan, 1995; Li et al., 2001). However, intraspinal spread may occur, in which case the later clinical course is more troublesome, with paraparesis and neurogenic bladder (Moppett et al., 1999); most authors consider surgery to be advisable.

Disorders of the Chest Wall

Abnormalities of the bone and muscle of the chest wall may be a mechanical hindrance to ventilation.

Skeletal Disorders

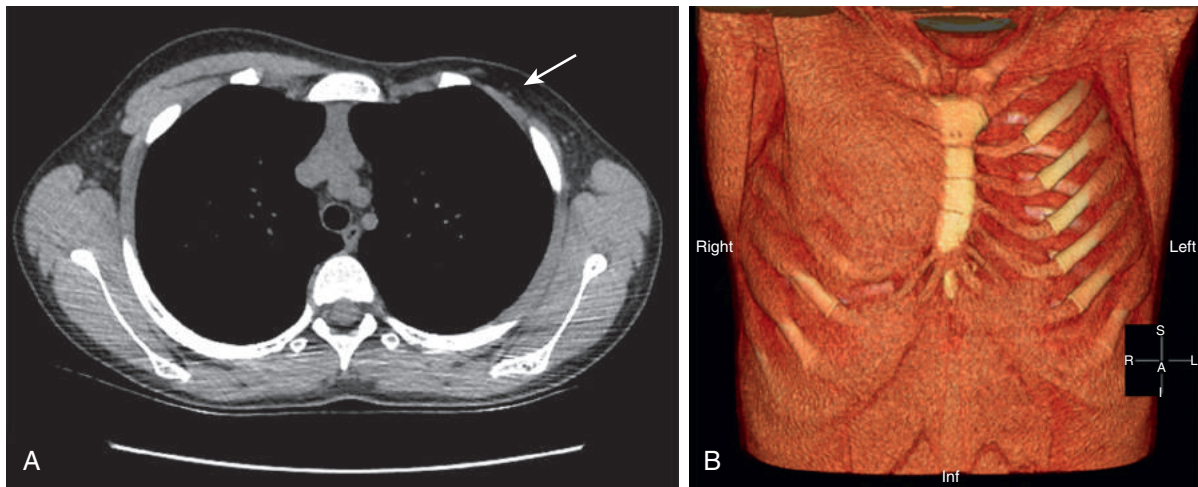
Although abnormalities involving bone are rare, they may be recognized immediately and are sometimes amenable to operative correction.

Defects of Sternal Fusion

Defects in fusion of the sternum are uncommon, occurring in less than 1 in 100,000 live births (Jabbad et al., 2010). Complete separation of the two halves of the sternum allows protrusion of cardiovascular structures, a condition known as *ectopia cordis* (Maier and Bortone, 1949). Lethal malformations of the heart are commonly associated with this condition. Upper sternal clefts are the most common type of sternal fusion defect (Shamberger and Welch, 1990). Early operation is advised to shield the underlying structures from injury, improve respiratory mechanics secondary to paradoxical chest motion, and because of the greater ease of approximating the separated parts in the first days of life compared with later (Sabiston, 1958). A lower sternal cleft and *ectopia cordis* with a congenital heart defect may be associated with congenital apertures in the upper abdominal wall, in the pericardium, and in the anterior diaphragm, with a Morgagni-type diaphragmatic hernia, the so-called pentalogy of Cantrell (Cantrell et al., 1958).

Pectus Excavatum

The most common sternal defect is pectus excavatum, occurring in 400 to 1000 live births (Fonkalsrud, 2009). It is characterized by a posterior curvature of the sternum and lower costal cartilages, resulting in a “funnel chest” appearance. Pectus is three to five times more common in males and is sometimes associated with Pierre Robin syndrome or Marfan syndrome. A family history of some type of anterior thoracic deformity was found in 37%–43% of patients (Shamberger et al., 1988; Nuss et al., 1998). Only approximately one-third of cases of pectus excavatum are present in infancy (Kelly, 2008). Even in those cases that are present at infancy, it is rarely a severe deformity, and symptoms are minimal. As the deformity worsens, the heart may be compressed between the sternum and the vertebral column and displaced to the left, impinging on the space of the left lung. This compression may result in exercise intolerance, chest pain, and shortness of breath (Nuss and Kelly, 2010). In addition to the physical symptoms, the chest deformity often leads to cosmetic concerns and psychological distress sufficient to warrant intervention. Although routine chest radiographs may suffice in evaluating the severity of chest wall deformity, chest CT is typically preferred because this modality



• **Fig. 49.6** Poland Syndrome. (A) Axial computed tomography (CT) scan and (B) three-dimensional CT reconstruction demonstrating a congenital absence of the pectoralis major and pectoralis minor muscle (arrow). There is no underlying rib deformity. (Courtesy of Dr. Chirag V. Patel, Assistant Professor, University of California, Davis Health System, Department of Radiology, Sacramento, CA.)

provides the bony and cartilaginous anatomic details as well as any information regarding cardiac compression necessary when considering surgical intervention. Using chest CT allows one to calculate the Haller index (HI), or the ratio of the transverse distance to the anteroposterior distance. A normal HI is about 2.5, and most cases of pectus excavatum that qualify for operative correction are greater than 3.25 (Haller et al., 1987). However, the indications for operative correction are debatable. Those patients with severe cardiac or pulmonary compression, abnormal cardiac or pulmonary function studies, or failed previous repairs are candidates for repair (Nuss and Kelly, 2010). Periodic evaluation of cardiovascular status with echocardiogram and electrocardiography in addition to assessment of pulmonary function is appropriate in the presence of progressive deformity and during periods of rapid growth such as adolescence. In our opinion, correction should not be undertaken until the child is several years of age and then only in those few children in whom the deformity appears to be progressing. Serial photographs are the best way to document changes in pectus excavatum. Results of both minimally invasive and open operative correction are excellent in the majority of patients; surgery is almost always associated with improved self-image and perceived functional activity (Kelly et al., 2008; Nuss, 2008; Fonkalsrud, 2009). Recurrences are possible during later active growth and may require repeat intervention, often with excellent outcomes (Sacco Casamasima et al., 2015).

Poland Syndrome

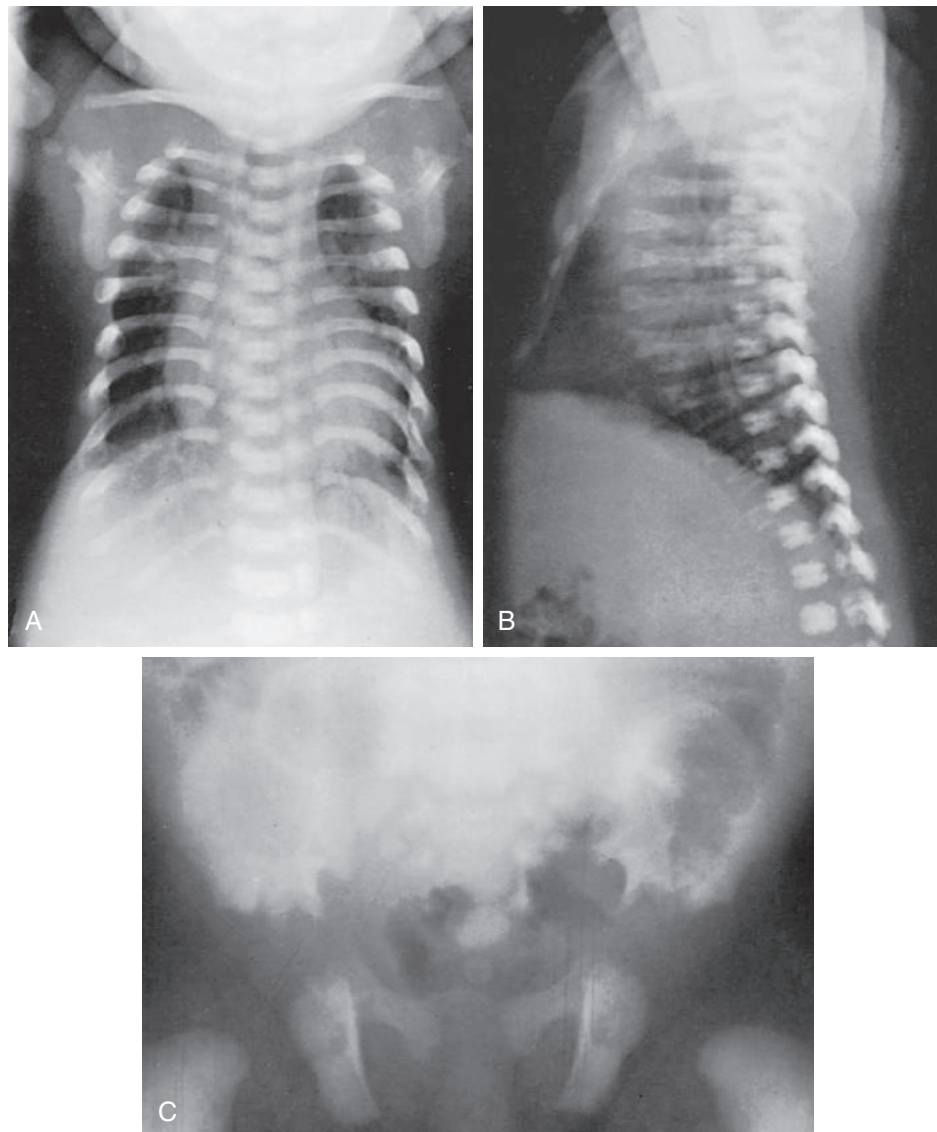
There is a great amount of diversity in the clinical manifestations of Poland syndrome; however, all children must have hypoplasia or absence of the pectoral muscles, typically only on one side. Additional manifestations include cartilage agenesis of the second through fifth ribs on the ipsilateral side, athelia, and amastia. There may be associated syndactyly, hemivertebrae, and scoliosis (Urschel, 2009). Breathing may be paradoxical and the cardiac impulse easily observed through the soft tissues, but there is rarely any severe respiratory distress that would necessitate emergent intervention. Later in childhood, although uncommon, there may be increasing respiratory symptoms with scoliosis-related lung disease and/or heart failure. CT scans are useful in assessing the configuration of the chest wall and its need for reconstruction when symptoms

develop (Fig. 49.6) (Bainbridge et al., 1991). No operative intervention is required in infancy, although complex reconstruction with autologous rib grafts, chest wall implants, and latissimus dorsi flaps may be desirable in later childhood as well as mammoplasty in affected girls after puberty (Haller et al., 1984; Hochberg et al., 1994).

Thoracic Dystrophies

Asphyxiating Thoracic Dystrophy (Jeune Syndrome)

A rare deformity of the thoracic cage in approximately 1 in 100,000 to 130,000 live births, asphyxiating thoracic dystrophy is an autosomal recessive chondrodystrophy, associated with short-limbed dwarfism and often polydactyly (Fig. 49.7). It was first described by Jeune et al. (1955). The ribs are horizontal, hypoplastic, and short, with flared costochondral junctions. The thorax is small, bell-shaped, and rigid; this results in displacement of the liver and spleen well into the abdominal cavity. Some degree of lung hypoplasia may be present and, if present, is often severe and lethal (Phillips and van Aalst, 2008). The pelvis shows flaring of the iliac wings and acetabular abnormalities (Kohler and Babbitt, 1970). Renal cystic dysplasia may be present, resulting in hypertension and renal failure. Prenatal diagnosis with ultrasonography is possible. In the past, most patients with this condition did not survive the first month. Oberklaid et al. (1977) studied 10 cases and noted that only 2 patients were alive at the time of the report: 1 of the 2 was in excellent health at 15 years of age. The more severely affected infants had respiratory distress from birth. Three patients have been described in one family; because it is an autosomal recessive trait, the expectation would be for an occurrence in 1 of 4 siblings, so mutations must be common. No parent–child occurrence has been described. Davis et al. (2001) reported an operative technique for lateral thoracic expansion in 10 patients with chest wall deformities limiting thoracic capacity—including 8 patients with classic Jeune syndrome. Three were younger than 1 year of age at the time of surgery, and 6 were ventilator dependent. All of the infants older than 1 year of age at the time of surgery improved, with measured lung volumes increasing in 2 of 3 studied and thoracic volumes by CT increasing in 4 of 5 studied. The only deaths were in 2 infants younger than 1 year of age at the time of surgery, suggesting that lateral thoracic expansion is a safe



• **Fig. 49.7** Asphyxiating Thoracic Dystrophy. On (A) anteroposterior and (B) lateral views of the chest, the thoracic dimension is seen to be reduced in comparison with the abdominal dimension. (C) Radiograph of the pelvis shows flaring of the iliac crests and bony protrusions of the acetabulae. (A and B, Courtesy of Dr. John Kirkpatrick; C, from Avery ME, Fletcher BD, Williams RG, *The Lung and Its Disorders in the Newborn Infant*. 4th ed. Philadelphia, PA: WB Saunders; 1981.)

and effective procedure for patients beyond the first year of age. A vertical expandable prosthetic titanium rib procedure has also been introduced for the purpose of progressive expansion of the chest cavity in patients with thoracic insufficiency (Waldhausen et al., 2007). However, long-term improvements in lung function, specifically in patients with Jeune syndrome, have yet to be reported (Phillips and van Aalst, 2008).

Other Thoracic Dystrophies

Severe underdevelopment of the thoracic rib cage, accompanied usually by lethal pulmonary hypoplasia, may be seen in other conditions, such as thanatophoric dwarfism syndrome, short rib–polydactyly syndrome, and camptomelic dwarfism syndrome. Affected infants do not usually survive for long after birth.

Neuromuscular Disorders

Other causes of thoracic dysfunction are diseases of the nerves and muscles, including congenital myasthenia gravis, congenital spinal muscular atrophy (Werdnig–Hoffmann disease), congenital myotonic dystrophy, glycogen storage diseases, and congenital spinal injury. Such conditions are usually recognized in the context of the associated systemic muscular weakness. Newborns with myasthenia gravis have episodes of muscle weakness, poor feeding, weak cry, hypoventilation, and apnea with a positive response to an anticholinesterase medication. In congenital spinal muscular atrophy, there is lung hypoplasia associated with absent fetal breathing, and as a result the thorax is small. Other features include severe hypotonia, muscle fasciculation, respiratory failure, and

early death; the inheritance is autosomal recessive. In congenital myotonic dystrophy, there is lung hypoplasia from absent fetal breathing; affected infants have respiratory distress at birth, rapidly need mechanical ventilation, and are soon ventilator dependent. Mothers of these infants have myotonia, difficulty in relaxing muscle contractions; the inheritance is autosomal dominant.

Disorders of the Pleural Cavity

The pleural cavity lies between the parietal pleura, lining the chest wall, and the visceral pleura, lining the lung and other structures. The blood supply to both pleural surfaces is systemic. Venous drainage of the parietal pleura is to the systemic system and the visceral pleura to the pulmonary system. The pleural surfaces filter fluid into the pleural space and the pleural lymphatics then resorb fluid from the pleural cavity (Wiener-Kronish et al., 1985). This process is hindered in the setting of abnormal lymphatic development or abnormal systemic venous pressures (because the thoracic lymphatic system drains directly to the systemic veins), resulting in chylothorax. Chylothorax can also occur as a result of surgical disruption of the thoracic duct or in the setting of lymphatic malformations. Other causes of hydrothorax in the newborn include hydrops fetalis, transudative pleural effusions associated with congenital lung lesions or group B streptococcal pneumonia, empyema (usually associated with nosocomial pneumonia), or fluid extravasation from a displaced central venous catheter.

Congenital Chylothorax

The prenatal diagnosis of an isolated pleural effusion without an associated thoracic malformation or hydrops fetalis is most likely to be a chylothorax—or lymphatic effusion, because chyle cannot be present in the absence of fat-containing enteral feeds (Beghetti et al., 2000). A large fetal chylothorax may evert the diaphragm and can be the cause of hydrops fetalis, probably because of hypoproteinemia or impaired venous return in the setting of increased intrathoracic pressure. Fetal chylothorax portends a worse prognosis for survival if it is bilateral or associated with hydrops, and prognosis is better if the effusion resolves without reaccumulation (Longaker et al., 1989). Large fetal chylothoraces can result in pulmonary hypoplasia. In utero intervention may be undertaken in cases of large or bilateral effusions, or hydrops, and has been shown to improve survival (Lee et al., 2016). Classically, these interventions include transabdominal thoracentesis (with ongoing ultrasound monitoring for fluid reaccumulation) and placement of an indwelling thoracoamniotic shunt (if the effusion reaccumulates), to allow continued drainage of fluid. More recently, in utero pleurodesis has been successfully performed using Picibanil (OK-432); however, long-term effects of this therapy are unknown (Yang et al., 2012). Fetuses diagnosed with chylothorax should be evaluated for associated conditions that may affect their prognosis, including Down syndrome, Noonan syndrome, and Turner syndrome.

Newborns with congenital chylothorax often present with severe respiratory distress, requiring immediate respiratory support and urgent drainage of pleural fluid. Chest radiographs will show either unilateral or bilateral fluid accumulation, often with shift of the mediastinum. Aspiration of the fluid can confirm the diagnosis. Before feedings have been started, the fluid will be straw colored but will change to the classic milky-white appearance after feedings have been initiated. Characteristics of a chylous aspirate include a high cell count with a lymphocytic predominance, a high

TABLE 49.1 Characteristics of Chylous Effusions

| Characteristic | Description |
|-----------------------|--|
| Appearance | Clear yellow (milky with fat-containing feeds) |
| Cell count | >1000 cells/mm ³ |
| Lymphocyte proportion | >80% |
| pH | 7.4–7.8 |
| Triglycerides | >110 mg/dL |
| Cholesterol | 65–220 mg/dL |
| Albumin | 1.2–4.1 g/dL |
| Total protein | 2.2–5.9 g/dL |

Adapted from Rocha G. Pleural effusions in the neonate. *Curr Opin Pulm Med.* 2007;13:305–311.

triglyceride level (usually above serum levels, but not present unless enteral feeds initiated), and high protein content (Table 49.1). The introduction of small-volume fat-containing feeds with resultant elevated fluid triglyceride levels can confirm the diagnosis if biochemical indices are otherwise not confirmatory. A review of 39 cases of pediatric chylothorax revealed that the composition was consistent with previously described classic characteristics (Buttiker et al., 1999). Total cell counts were greater than 1000/mm³ in 92% of cases, and 85% of effusions had greater than 90% lymphocytes (range 57%–89% in 6 additional children). Fluid triglyceride levels were greater than 1.1 mmol/L (98 mg/dL) in all but one case, with values ranging from 0.56 to 26.6 mmol/L (50 to 2358 mg/dL).

Management of Chylothorax

Neonatal management of a congenital chylothorax includes replacement of protein, clotting factors, and immunoglobulins as needed. Ongoing respiratory support may be needed, and often a chronic chest drain is required to decrease respiratory compromise. Feeds are usually restricted, either providing medium-chain triglycerides (MCTs) only (because MCTs are generally absorbed directly from the intestine without processing to chylomicrons) or a nonfat diet. If these measures fail, a period of total enteric rest is undertaken, with parenteral nutrition administered, until resolution of the chylous effusion. Some practitioners believe that a period of enteric rest is necessary to decrease strain on the lymphatic system, because lymphatic efflux is still increased even with nonfat-containing diets (Le Coultré, 2003). Once chest drainage has resolved, feeds are reintroduced with a MCT-only formula for 3 to 6 weeks before transitioning to a normal diet.

There are no controlled studies demonstrating that one strategy is superior to others to hasten resolution of chylothorax, and in one series, 50% of pediatric chylothoraces resolved with MCT-only diets (Le Coultré et al., 1991). A later series from the same institution had 80% (41 of 51) resolution with conservative, nonoperative management (MCT-only diet or enteric rest) up to 4 weeks' duration (Beghetti et al., 2000). The primary risk of this approach is infection, because these children have prolonged hospitalizations with central venous access, chest drains, no enteral feeds, and protein and immunoglobulin losses. Eight of 51 children had severe infections in this series. Children who were less likely to have spontaneous

resolution of chest drainage included those children with elevated central venous pressure, children who had more prolonged effusions, and children with higher output than those with surgical injury. There were more frequent operative interventions in this group (6 of 14 children), which were undertaken only if 4 weeks of conservative therapy failed to decrease drainage to less than 10 mL/kg per day (Beghetti et al., 2000). In one series, 4 of 11 children with drainage after 4 weeks resolved their chylothorax by 6 weeks, with 2 of the remaining children too unstable to undergo surgical intervention (Buttiker et al., 1999).

An adjunctive medical approach is the administration of somatostatin or its analogue, octreotide, to decrease pleural drainage. Somatostatin is delivered only via continuous infusion, whereas octreotide can also be given via intermittent subcutaneous injection, because of its longer half-life. The mechanism of effect is thought to be via decreased intestinal secretions and absorption and therefore intestinal efflux and abdominal lymphatic return (Kalomenidis, 2006). Some practitioners proceed quickly to the use of these medications, believing they decrease the volume and duration of chest drainage compared with conservative management; however, this has not been studied in a controlled setting, so the efficacy and risks of this approach are unknown (Das and Shah, 2010). Others use these medications once conservative measures have failed. Potential risks of therapy include hormonal effects of glucose instability and hypothyroidism and gastrointestinal (GI) effects such as cholelithiasis, hepatocellular injury, nausea, diarrhea, abdominal distention, and decreased intestinal perfusion. In this regard, monitoring of serum glucose, thyroid function, liver enzymes, and indicators of cholestasis during therapy is recommended. These serious side effects raise concerns, particularly regarding concomitant feeding during administration of the medications. Dosing is either titrated up until chest drainage is minimal (typically starting at 3.5 µg/kg per h and advancing in several steps to 10 µg/kg per h) or started at the higher dose and titrated down once drainage has abated (Helin et al., 2006; Roehr et al., 2006). The infusion is usually continued for several days after chest drainage is controlled and then weaned off over several days. Outcomes using octreotide for congenital chylothoraces have been promising, with several case reports and a recent small case series demonstrating resolution of the chylothorax without adverse effects (Bulbul et al., 2009; Shah and Sinn, 2012). There are reports of recurrence after discontinuation of the medication (Roehr et al., 2006).

Most practitioners recommend about 3 to 4 weeks of maximal medical therapy, before proceeding with an operative intervention, while others recommend intervention in patients who drain more than 100 mL/year of age per day without slowing down after 10 to 20 days (Buttiker et al., 1999). Effusions are unlikely to resolve after 6 weeks, and waiting too long for operative intervention may result in significant compromise to the child. Operative interventions include thoracic duct ligation via thoracotomy or thoracoscopy, pleurodesis, and/or placement of a pleuroperitoneal shunt (Buttiker et al., 1999; Clark et al., 2015; Resch et al., 2016).

Congenital Thoracic Masses and Cysts

Fetal lung masses vary in the degree of abnormality of parenchyma and vasculature and probably represent a spectrum of developmental abnormalities that may be difficult to distinguish prenatally (Langston, 2003; Ankermann et al., 2004). In fact, some investigators have suggested classifying these lesions solely on the basis of the vascular supply (systemic vs pulmonary) and whether or not lung structure is normal (Achiron et al., 2004), because the histology

and probably the developmental pathways leading to the individual lesions are overlapping. Some level and degree of fetal airway obstruction have been implicated as the cause of many of these lesions (Langston, 2003). In prenatal series, CPAMs of the lung are frequently the most common lesion reported (Achiron et al., 2004). However, in postnatal series, CPAMs are often less common (with various imaging modalities and pathology available to clarify the diagnosis after birth), particularly when only cystic lesions are considered (Winters and Effmann, 2001; Langston, 2003; Stocker, 2009). There are multiple reports of “hybrid” lesions, with features of both CPAM and bronchopulmonary sequestration (BPS), emphasizing that these lesions constitute a spectrum of anomalies (Winters and Effmann, 2001; Langston, 2003).

Congenital Pulmonary Airway Malformation of the Lung

CPAMs are the most common congenital lung lesions and develop from an overgrowth of abnormal lung tissue, extending from different levels of the airway. In general, they are unilateral. The lesions may communicate with the airways, allowing them to transition from being fluid filled in utero to air filled postnatally, but they do not contain normal alveoli. CPAMs were previously known as congenital cystic adenomatoid malformations (CCAMs), which were originally classified into three types based upon the size of the cysts and their cellular characteristics (Stocker et al., 1977). Subsequent revisions of this classification system have included less common proximal and distal lesions and have suggested new nomenclature based on the fact that not all lesions are either adenomatoid or cystic. The term “congenital pulmonary airway malformation” (CPAM), initially proposed by Stocker, has now become the accepted terminology for these lesions (Stocker, 2002). Table 49.2 shows the stages of lung development with the corresponding airway malformation and CPAM type. Consistent with this classification, persistent epithelial expression of the nuclear regulatory protein thyroid transcription factor 1 has been found in type 1 and 3 CPAMs (see later discussion), indicating developmental arrest in the pseudoglandular stage (Morotti et al., 2000), and targeted fetal airway overexpression of the growth factor fibroblast growth factor 10 in rats produces CPAM-like lesions (Gonzaga et al., 2008).

Congenital Pulmonary Airway Malformation Types

Type 0 CPAMs originate from the tracheobronchial tree. There is normal lung lobulation, but no formation of alveoli (Stocker, 2002). There are only bronchial-like structures present, indicating an arrest of development in the pseudoglandular stage (Davidson et al., 1998). Lungs may be normal weight or small, but, regardless, this lesion is rapidly lethal because of severe, intractable respiratory failure. The lesion has been termed *acinar dysplasia* (Rutledge and Jensen, 1986). It is diffuse and bilateral, recurs in families, and can be associated with other anomalies, suggesting a genetic cause (Moerman et al., 1998; Gillespie et al., 2004). In a large pathology series, fewer than 2% of the pulmonary airway malformations were characterized as acinar dysplasia (Stocker, 2002).

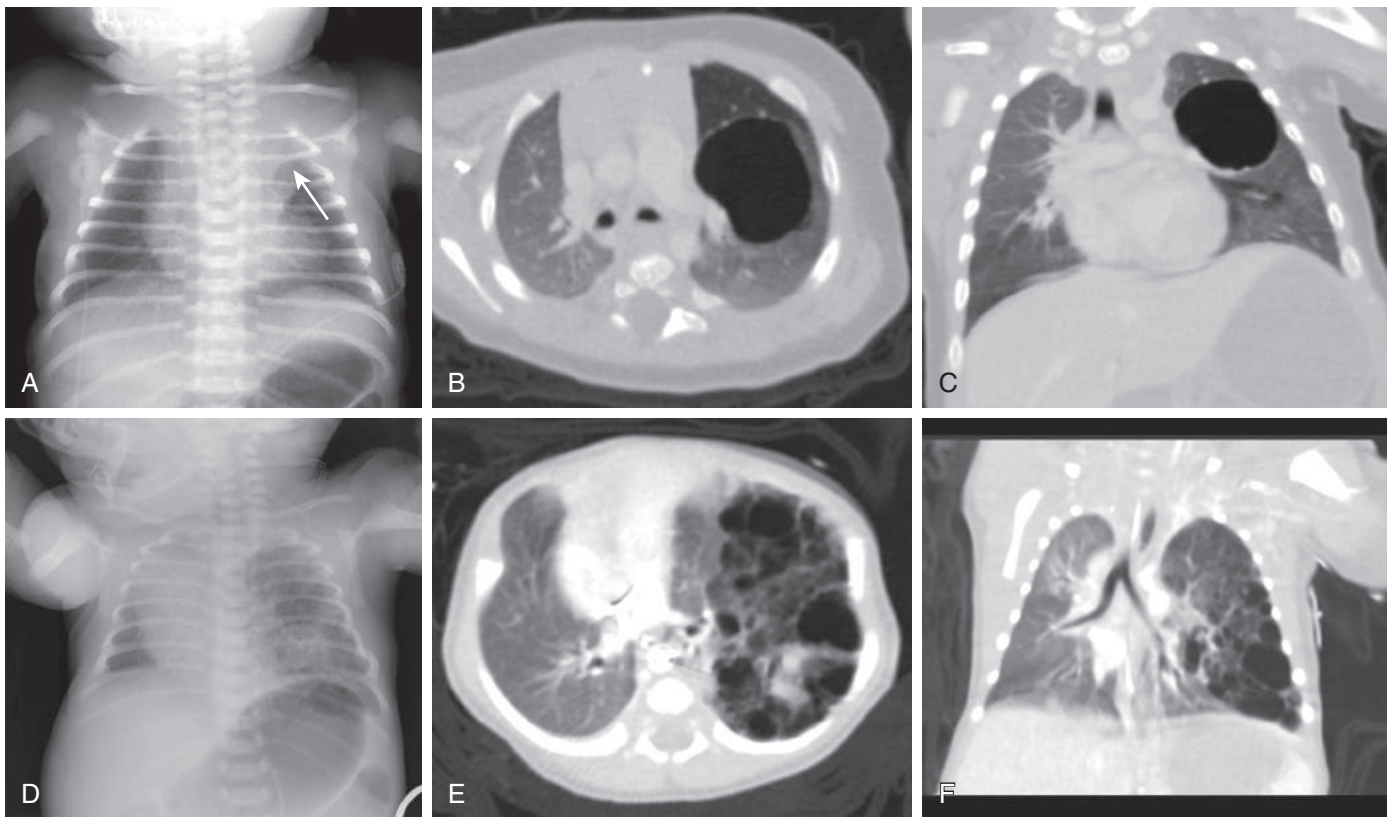
In contrast, type 1 CPAMs are the most common lesion in postnatal series of airway malformations, identified in 60%–65% of cases (Stocker, 2002, 2009). Cysts are large, usually greater than 2 cm, limited in number, and originate from the distal bronchi or proximal bronchioles (Fig. 49.8A–C). The cysts usually communicate with each other, and they may decrease in size as they drain progressively during advancing gestation. Postnatally, they are aerated, with

**TABLE
49.2****Stages of Airway Branching and Lung Development With Corresponding Congenital Airway Malformation**

| Developmental Stage | Developmental Events | CPAM TYPE | |
|-------------------------------|---|---|-------------|
| | | Stocker | Adzick |
| Embryonic 0–7 weeks | Formation of tracheal bud and growth and branching to segmental bronchi | Type 0 Tracheobronchial Type 1 Bronchial/bronchiolar | Macrocytic |
| Pseudoglandular 7–17 weeks | Completion of airway branching to terminal bronchioles (preacinar); gland formation | Type 2 Bronchiolar | |
| Canalicular 17–27 weeks | Formation of respiratory bronchioles to prealveolar structures | Type 3 Bronchiolar/alveolar duct | Microcystic |
| Saccular 28–36 weeks | Formation of secondary septae | Type 4 Distal acinar | |
| Alveolar 36 weeks–2 years | Formation of alveoli | | |

CPAM, Congenital pulmonary airway malformation.

From Cha I, Adzick NS, Harrison MR, Finkbeiner WE. Fetal congenital cystic adenomatoid malformations of the lung: a clinicopathologic study of eleven cases. *Am J Surg Pathol.* 1997;21:537–544; Hislop AA. Airway and blood vessel interaction during lung development. *J Anat.* 2002;201:325–334.



• **Fig. 49.8** Congenital Pulmonary Airway Malformation. (A) Chest radiograph of an asymptomatic child with a prenatally diagnosed congenital pulmonary airway malformation (CPAM) in the left upper lobe (arrow). (B, C) Axial and coronal computed tomography (CT) images demonstrate a single 3.1 × 2.7-cm cystic lesion in the left upper lobe consistent with a type 1 CPAM. The child underwent uncomplicated left upper lobectomy at 3 months of age. (D) A chest radiograph of an infant with a prenatally diagnosed CPAM that had rapid enlargement of the cystic component, resulting in mediastinal shift and acute respiratory compromise, is shown. (E, F) Axial and coronal CT images show a multicystic mass with cysts ranging from a few millimeters to 1.5 cm consistent with a type 2 CPAM. In the operating room, the patient was found to have a multicystic lesion confined to the left upper lobe with compressive atelectasis of the left lower lobe and underwent left upper lobectomy. (Courtesy of Dr. Chirag V. Patel, Assistant Professor, University of California, Davis Health System, Department of Radiology, Sacramento, CA.)

fluid also present in some lesions. Type 1 CPAMs are restricted to a single lobe in 95% of the cases. Bilateral lesions are rare. The lesions are lined by a spectrum of respiratory epithelial cells, ranging from cuboidal to ciliated pseudostratified columnar epithelium, and mucus-producing cells may occur in gland-type tissue. The walls of the lesions may resemble walls of bronchi. Respiratory distress depends on the size of the lesion, with some lesions detected only by incidental imaging or following infection or malignant transformation. There does appear to be a risk of malignant transformation in unresected or residual CPAM tissue. The specific link to neoplasm has not been elucidated, though the diagnosis of bronchoalveolar carcinoma (BAC) is made in relatively young patients (10–42 years), and a spectrum of malignancy has been described (MacSweeney et al., 2003; Mani et al., 2007). This is the only CPAM type that is associated with malignancy, and the young age of affected patients suggests that the CPAM lesion is the cause of the malignancy (MacSweeney et al., 2003).

Type 2 CPAMs are characterized by multiple, smaller macroscopic cysts, 0.5–2 cm in diameter, which may not be evident by chest radiograph (see Fig. 49.8E–F). These lesions account for 15%–20% of CPAMs. The cysts are lined by respiratory epithelial cells, ranging from cuboidal to columnar in morphology and appear as multiple bronchiole-type structures, although intra-acinar structures may also be interspersed (Rosado-de-Christenson and Stocker, 1991). These lesions are most commonly associated with other anomalies, in 50% of cases, which include severe bilateral renal dysplasia or agenesis, agenesis of other genitourinary structures, sirenomelia, extralobar pulmonary sequestration, and diaphragmatic hernia (Stocker et al., 1977; Stocker, 2009). Conotruncal cardiac malformations have also been described in association with type 1 and type 2 CPAMs (Stocker et al., 1977; Hüsler et al., 2007), and ventricular septal defects have been diagnosed in fetuses and infants with all CPAM types.

Type 3 CPAMs are uncommon lesions, occurring in 5%–10% of CPAM cases (Stocker, 2002). These lesions are generally solid, with microscopic cysts (although single larger cysts can be present), and involve an entire lobe or the entire lung. There is often a mass effect in the thorax, and the lesions can cause lung hypoplasia through compression of adjacent structures. The lesions themselves contain bronchiolar and alveolar duct-type morphology with cuboidal epithelium, and there are alveoli-like structures.

Type 4 lesions have been more recently described and account for 10% of CPAMs (Stocker, 2002). With large cysts (maximum diameter 7 cm) present, some of these lesions may be mistaken for type 1 CPAMs. They are usually confined to one lobe and tend to be peripheral in location. Infants and children may present with mild respiratory distress, or more severe symptoms if pneumonia occurs, or if the lesion ruptures, causing pneumothorax. However, type 4 CPAMs can also be detected by incidental imaging. These lesions are lined with type I alveolar (flat) and type II (rounded) epithelial cells. The presence of substantial portions of cells representing the more proximal areas of the lung should raise suspicion for pleuropulmonary blastoma (PPB), a distinction that is important (because of malignancy associated with PPB; see later discussion), but one that can be difficult.

The approach to diagnose CPAMs in utero has led to a different classification system, based on the natural history of these fetal lesions (see later discussion). Macroscopic CPAMs are defined when a lesion contains cysts that are greater than or equal to 5 mm (types 1 and 2 CPAMs), and microcystic CPAMs are defined as a solid lesion, with cysts less than 5 mm (type 3 CPAMs) (Adzick et al., 1985). Some lesions categorized by these criteria on fetal

ultrasound have been diagnosed as BPS after postnatal resection (Davenport et al., 2004).

Fetal Diagnosis and Natural History

Fetal diagnosis of a CPAM is often made when mediastinal shift because of mass effect from the lesion is identified and a cystic, intermediate, or solid mass is detected. This may occur during routine fetal ultrasonography or when the mother is referred for evaluation of polyhydramnios (thought to occur when the mass compresses the esophagus and thus limits fetal swallowing) (Adzick, 2009). A systemic vascular supply identified on imaging is more consistent with a diagnosis of BPS (Winters and Effmann, 2001). Fetal MRI can be used to help distinguish a CPAM from other pulmonary pathology, including BPS and congenital diaphragmatic hernias. In a recent population-based study from the United Kingdom, confirmed CPAMs (postnatal or postmortem) occurred in 9 per 100,000 births, without accounting for lesions that were not further investigated secondary to prenatal resolution (Gornall et al., 2003). In this study, 57% (21 of 37) of fetal CPAMs were confirmed, and 5 cases were missed (prenatal detection rate 21 of 26 [81%]). Generally, growth of CPAM lesions increases until about 28 weeks' gestation, after which time it plateaus and the lesion regresses in size while the fetus continues to grow (Kunisaki et al., 2007a; Adzick, 2009). Fetal and neonatal problems that arise as the result of a CPAM include the development of nonimmune hydrops and lung hypoplasia, which may be due to compression of the otherwise normally developing lung. Up to one-third of fetuses with a CPAM develop hydrops (Adzick et al., 1998). Factors associated with the development of nonimmune hydrops include an everted ipsilateral hemidiaphragm, predominantly cystic lesions, lesions that exist into the third trimester, and lesions with a CPAM volume ratio (CVR) greater than 1.6 (Vu et al., 2007). The CVR calculates the volume of the mass ($\text{length} \times \text{weight} \times \text{height} \times 0.52$) divided by the head circumference. With a CVR greater than 1.6, 75% (12 of 16) of fetuses developed hydrops, while only 17% of those with a CVR less than or equal to 1.6 (7 of 42) developed hydrops (Crombleholme et al., 2002). In a series evaluating fetal macrocystic and microcystic lesions, generally the fetuses with microcystic lesions had poorer outcomes, with intrauterine demise or early neonatal death (Adzick et al., 1985). Compared with normal lung, CPAM lesions have unregulated growth, with increased proliferation and decreased apoptosis (Cass et al., 1998), although they are relatively hypovascular (Cangiarella et al., 1995). In fetal CPAM tissue, increased Platelet-Derived Growth Factor subunit B (PDGF- β) and decreased fatty acid binding protein-7 expression have been detected (Liechty et al., 1999; Wagner et al., 2008).

Prenatal Management

Initial ultrasound evaluation of the fetus with possible CPAM should include assessment of lesion size with relevant indices, vascular supply, degree of mediastinal shift, ipsilateral diaphragmatic conformation (normal, flat, or everted), polyhydramnios, placentalomegaly, and the presence of any other fluid collections (ascites, integumentary edema, pleural or pericardial effusion) indicating the development of fetal hydrops. Additional prenatal evaluation of a fetus with a known CPAM should include a full fetal survey to identify other anomalies, a karyotype, and an echocardiogram, to evaluate for congenital heart disease and to assess cardiac inflow patterns and outputs. These cardiac indices may help identify impending hydrops, mandating closer ultrasound follow-up and repeated echocardiographic measurements. Most referral centers

recommend at least weekly follow-up ultrasound examinations evaluating lesion size and monitoring for development of hydrops until 28 to 29 weeks' gestation, at which point regression of the lesion should be occurring. Thereafter, ultrasound evaluations can be spread out to every 2 weeks. Some referral centers have advocated twice-weekly surveillance if the CVR is greater than 1.6, until 28 weeks' gestation (Adzick, 2009). In a recent series, 21 of 54 fetal lung masses decreased in size or resolved by delivery (Davenport et al., 2004), and a similar proportion of fetal CPAM cases were not detectable at birth in a population-based study (13 of 37, 35%) (Gornall et al., 2003).

The diagnosis of hydrops in a fetus with CPAM portends a poor prognosis, with either fetal demise or preterm delivery of a compromised infant (Gornall et al., 2003; Grethel et al., 2007; Kunisaki et al., 2007a). Thus impending or definite hydrops is an indication for fetal intervention. The specific intervention depends on the type of lesion. In fetuses with a macrocystic CPAM, a growing, dominant cyst, and impending or definite hydrops, placement of a thoracoamniotic shunt results in a decrease in cyst size and hemodynamic improvement, with relatively good neonatal survival (12 of 16 in two series) (Adzick et al., 1998; Wilson et al., 2004). However, shunt placement at less than 21 weeks' gestation may be associated with chest wall deformity, which can compromise later respiratory function, so other interventions may need to be taken at that early gestation (Merchant et al., 2007). Fetal thoracentesis is rarely effective as definitive treatment, because cyst fluid invariably reaccumulates, but it might serve as a temporizing measure until further treatment can be undertaken. For fetuses with impending hydrops and a microcystic CPAM (or failed decompression of a large cyst), open fetal resection (lobectomy or pneumonectomy) improves the chances of survival, although mortality remains high (10 of 23 in one series), and nonsurvivors tended to be more premature (Grethel et al., 2007). This selected approach to fetal resection has resulted in resolution of hydrops and mediastinal shift in survivors (Adzick, 2009). For fetuses greater than 32 weeks' gestation, or in the case where fetal surgery is contraindicated because of preterm labor, an EXIT-to-resection procedure may provide a chance for survival (Grethel et al., 2007; Adzick, 2009). EXIT procedures have also been undertaken in later-gestation fetuses with persistent large lesions (mean CVR 2.2) despite other fetal interventions, although the impact of this strategy on survival and other outcomes is unknown (Hedrick et al., 2005; Adzick, 2009).

Before undertaking any fetal intervention, prenatal glucocorticoid therapy to accelerate fetal lung maturation should be administered. This practice, utilizing betamethasone (12 mg intramuscularly every 24 h \times 2 doses), led to the observation that fetuses with microcystic CPAMs (type 3) and hydrops resolved their hydrops after betamethasone exposure (Tsao et al., 2003b). In this series, three fetuses with mild-to-moderate hydrops treated at 21 to 26 weeks' gestation avoided further fetal interventions, were delivered at term, and survived. This observation led to subsequent studies comparing the use of steroids with open fetal surgery in fetuses with macrocystic CPAMs that demonstrated the effectiveness of steroids in not only halting further growth of a CPAM but also causing regression (Peranteau et al., 2007; Loh et al., 2012). Since this discovery, steroids have become the first line of treatment in fetuses with large microcystic CPAM lesions.

Postnatal Management

In fetuses that do not develop hydrops, the overall prognosis is good and probably depends on the type of lesion and the presence

of other anomalies. Generally, the prognosis of a type 1 CPAM is good, particularly if fetal intervention is not required. Following resection, there is usually resolution of any symptoms associated with the lesion. Type 2 CPAMs have a worse prognosis, because of the association with additional serious malformations. Type 3 CPAMs are thought to be uniformly lethal from their earliest descriptions and can be associated with lung hypoplasia (Stocker et al., 1977). For fetuses with large microcystic CPAMs, fetal resection, if indicated, may mitigate hypoplasia. However, case series have described substantial spontaneous regression of microcystic CPAMs, with an increased rate of in utero resolution compared with macrocystic lesions (Davenport et al., 2004). In a large series from a single referral center, neonatal survival was 98% (118 of 121) (Grethel et al., 2007). Five infants underwent fetal procedures, with 1 death, and there were 2 neonatal deaths. In a population-based series, there were no postnatal deaths following 20 live births, with a single intrauterine fetal demise and 5 terminations (Gornall et al., 2003). Three fetal interventions (thoracentesis or thoracoamniotic shunt placement) were undertaken in these patients.

Approximately 75% of newborns with a prenatally diagnosed CPAM are asymptomatic at birth (Parikh and Rasiah, 2015). The remainder may present with respiratory distress with or without pulmonary hypertension, which can be severe enough to require ECMO support (Kunisaki et al., 2007a; Adzick, 2009). The likelihood of respiratory distress is best predicted by a CVR of greater than 0.84, and the severity of the respiratory distress usually increases as the size of the CPAM increases (Ruchonnet-Mettrailler et al., 2014). However, even in a series of large fetal CPAMs (at least 3 cm \times 3 cm), only 4 of 9 infants had respiratory distress requiring resection in the first week of life. For newborns with prenatally diagnosed CPAMs, lesions may be detected on chest radiograph, as solid masses, or fluid or air-filled cysts. Mediastinal shift, mass effect, or areas of air trapping due to airway obstruction may be appreciated. Some centers use ultrasound as an adjunctive modality in the neonatal period, but many surgeons prefer a CT scan as the definitive imaging study, which also can detect lesions that are no longer present on fetal ultrasound (Winters and Effmann, 2001). CT scans can also determine if there are multiple bronchopulmonary malformations, which may also require resection, and the use of intravenous contrast allows for definition of the vascular supply, helping to differentiate CPAMs from BPS (Johnson and Hubbard, 2004). For asymptomatic newborns, surgeons will often defer this study until several months of age, because surgical resection is also deferred. The usual surgical approach is lobectomy for the majority of lesions confined to a single lobe. More diffuse lesions might require bilobectomy or pneumonectomy. These surgical approaches are likely to result in the removal of normal lung but may also decrease the risk of air leak and infection after resection (Shanmugam, 2005), and compensatory lung growth does occur after lobectomy (Nakajima et al., 1998). Additional postoperative complications may include prolonged pleural effusion, pneumothorax, pneumonia, and wound infection (Waszak et al., 1999; Kim et al., 2008).

The need for resection in asymptomatic newborns remains controversial, and there is no consensus on this topic (Aziz et al., 2004; Singh and Davenport, 2015; Stanton, 2015). Those who argue for watchful waiting cite the risks of surgery and anesthesia, the overtreatment of "nondisease," and a lack of evidence of the associated malignancy risk as factors for not resecting asymptomatic lesions. Those who argue for intervention say that resection prevents complications of disease, including the risk of malignancy, the

psychological benefit of removing the lesion, and the improvement in lung volume after resection. In a national survey of Canadian pediatric surgeons, there was not even consensus on whether to resect asymptomatic lesions or not between different surgeons within individual centers (Lo and Jones, 2008). The majority of respondents in this survey (67%) endorsed resecting asymptomatic lesions present on CT, with 78% selecting 2 to 12 months as the age for operation. The concerns about not resecting a lesion are that it remains a persistent nidus for lower respiratory tract infection and that there is the potential for malignant transformation. However, the latter concern is confined to type I CPAMs. A similar study done in the UK found that 24% of surgeons never resect asymptomatic lesions, while 20% always do (Peters et al., 2013).

Generally, children who have undergone resection for CPAMs are healthy. Pulmonary function data demonstrate normal vital capacity, residual volume, and expiratory flows between 1 and 2 years post lobectomy (Nakajima et al., 1998). There are some reports of reactive airway disease and lower respiratory tract infection (Kunisaki et al., 2007a) and, in more severely affected children, chronic lung disease and pulmonary hypertension (Keller et al., 2006; Kunisaki et al., 2007a).

Bronchopulmonary Sequestration

Bronchopulmonary sequestrations are rare congenital malformations consisting of nonfunctional lung parenchyma that does not have a normal connection to the tracheobronchial tree (Rosado-de-Christenson et al., 1993). The lesions have a systemic arterial supply. Sequestrations are categorized into *extralobar* sequestrations, which are lesions that have their own pleural investment and systemic (80% of the time) venous drainage (and are therefore separate from the lung), and *intralobar* sequestrations, which are integral to the lung pleura and drain via the pulmonary venous system. Intralobar lesions usually require lobectomy for removal, because they are invested within the lung. The origin of an intralobar sequestration is somewhat controversial. Some experts believe it is not a congenital lung lesion but is rather always acquired after lung infection and injury, because inflammation, fibrosis, and cystic degeneration are its primary pathologic features (Frazier et al., 1997). In a large pathologic series, intralobar lesions usually diagnosed in adulthood and presenting as lower respiratory tract infection account for about 75% of BPS. Others believe that intralobar sequestration can be congenital in origin but is relatively rare in that setting when compared to extrapulmonary sequestration (Winters and Effmann, 2001). Thus the remainder of this discussion focuses on extralobar sequestration.

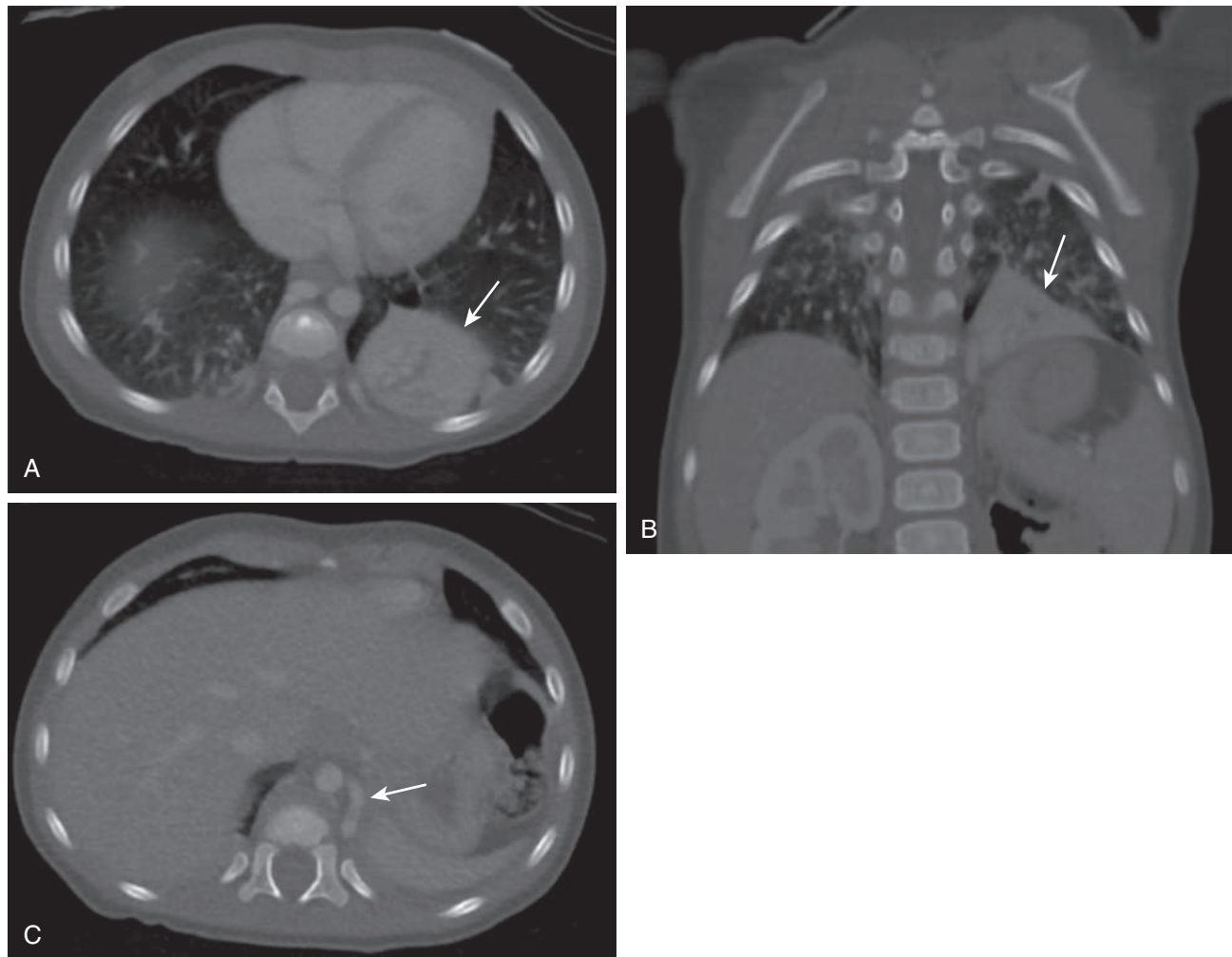
Extralobar sequestrations probably originate as an independent bud from the foregut that derives its blood supply from splanchnic vessels (Rosado-de-Christenson et al., 1993). Usually the connection to the foregut is lost during development, although some lesions have persistent connections to the esophagus or the stomach (also referred to as bronchopulmonary foregut malformations). As accessory lobes, they occur within both the thorax and abdomen. Hybrid lesions with features of both a CPAM and a sequestration can occur (Lopoo et al., 1999; Winters and Effmann, 2001). Extralobar sequestrations are usually situated on the left side (65%–90%), with the most common location between the lower lobe and the hemidiaphragm (approximately 70% of cases). Extrapulmonary BPS can also occur in the abdomen, the mediastinum, the pericardium, and the diaphragm itself. It is more common in males than females (3 to 4:1 ratio), and it also commonly occurs in association with other anomalies, including foregut duplication cysts, bronchogenic cysts, CPAMs, pericardial

defects, and ectopic pancreas. In addition, approximately 5% of children with a congenital diaphragmatic hernia will have extralobar sequestration. Histologically, these lesions appear as normal lung, except with dilated airway structures and, commonly, lymphangiectasia. A normal-appearing bronchus is present about 50% of the time. Associated pleural effusions are not uncommon (up to 10% of fetal BPS) and may be secondary to torsion of the vascular pedicle with resultant venous obstruction and elevated pressure or lymphatic abnormalities (Hernanz-Schulman et al., 1991; Johnson and Hubbard, 2004).

Fetal diagnosis of intrathoracic BPS is suspected when there is an echogenic mass, which may also contain cysts. Depending on its size, the mass may be associated with some degree of mediastinal shift (Lopoo et al., 1999). It can be difficult to distinguish BPS from a CPAM, but when a systemic arterial supply is identified, the diagnosis of BPS is highly likely. If there is also an associated ipsilateral pleural effusion, the diagnosis is almost certainly BPS (Johnson and Hubbard, 2004). If the diagnosis is still in doubt, fetal MRI can be used to help distinguish between the two (Hubbard et al., 1999). Infradiaphragmatic masses also need to be distinguished from neuroblastoma, other tumors (such as lymphangioma), and adrenal hemorrhage (Curros and Brunelle, 2001; Winters and Effmann, 2001). As with CPAM, BPSs often regress spontaneously over time, and if there are no associated anomalies, the prognosis for the fetus is good (Adzick et al., 1998; Lopoo et al., 1999). Close ultrasound follow-up is prudent, however, until regression is documented. When pleural effusion occurs, there is a risk of development of tension hydrothorax. In these cases, repeated fetal thoracentesis or placement of a thoracoamniotic shunt can avert or resolve hydrops fetalis, which may improve the chances for survival (Lopoo et al., 1999).

On postnatal imaging, BPS may be present as a radiographic density on plain film. The presence of linear or cystic lucencies within the radiopaque density suggests a persistent communication between an extralobar sequestration and the GI tract (Leithiser et al., 1986; Laberge et al., 2005). An upper GI study can demonstrate communication with the GI tract and is indicated for surgical planning if feeding difficulties are present. Ultrasound can also be useful in demonstrating the lesion (most easily seen if it is located at the lung base), and Doppler studies can identify a systemic feeding vessel and venous drainage. The use of contrast-enhanced CT provides the best visualization of the parenchymal abnormalities but has variable sensitivity for delineation of the vascular supply, although the lesion itself can often be demonstrated even when it has resolved by fetal imaging (Fig. 49.9). Magnetic resonance with angiography can also be useful in demonstrating the lesion and its blood supply. Conventional angiography can identify the vasculature, but this has been replaced by the imaging modalities discussed above (Winters and Effmann, 2001).

Symptomatic BPSs are usually identified in the first 6 months of life, with respiratory distress or feeding difficulties. Less commonly, recurrent infection, congestive heart failure (because of a high output state), or pulmonary hemorrhage is present. Distress at birth can be severe, particularly with large lesions complicated by a pleural effusion or hydrops fetalis. For neonates that present with symptoms in the first week of life, early resection is indicated (Azizkhan and Crombleholme, 2008). Because the lesion is completely separate from lung, sequestrectomy is not a complex operation and can be done thoroscopically. However, the feeding vessels can be very large in more severe cases, mandating a thoracotomy. The primary risk associated with an unresected BPS is recurrent infection, although this risk is not well quantified, as



• **Fig. 49.9** Bronchopulmonary Sequestration. (A, B) Axial and coronal computed tomography images of an infant with a prenatally diagnosed chest mass (arrows). The mass is located at the left lung base, measuring $3.6 \times 3.0 \times 2.5$ cm. (C) The arterial supply is via a prominent collateral from the descending thoracic aorta at the level of the diaphragm (arrow). The venous drainage is via the azygos vein (not shown). These findings are consistent with an extralobar sequestration. The patient underwent thorascopic resection at 18 months of age. (Courtesy of Dr. Chirag V. Patel, Assistant Professor, University of California, Davis Health System, Department of Radiology, Sacramento, CA.)

there are probably adults with persistent, small, asymptomatic lesions. Consequently, as with CPAM, there is controversy as to whether fetal lesions that are not identified on plain film should be further investigated in asymptomatic infants and, in general, whether asymptomatic lesions should be resected. However, since sequestrectomy can now be accomplished thorascopically at 3 to 15 months, with rapid recovery and short hospitalization (Albanese et al., 2003), most lesions are surgically resected. In addition, this approach may preserve rib architecture and limit later chest wall deformity (Rothenberg and Pokorny, 1992). Early resection, before infectious complications (the primary risk associated with an unresected lesion) may limit complications, because secondary changes such as emphysema might be averted (Rosado-de-Christenson et al., 1993). Some have attempted coil embolization of the feeding vessel(s), with hope of complete or partial involution of the lesion (Curros et al., 2000; Chien et al., 2009). However, coil embolization may only result in partial vascular occlusion and incomplete regression, transient lower limb ischemia (because of

distal migration of embolic material), sepsis, and other blood vessel complications. Because of this, thorascopic resection is the current standard of care (Cho et al., 2012).

Other Cystic Lesions

Additional cystic lung lesions include congenital lobar emphysema, pleuropulmonary blastoma, and acquired cysts (pneumatocoeles or emphysema, in association with infection or BPD, respectively). Extrapulmonary cystic lesions include foregut cysts (bronchogenic or enteric duplication cysts) and neuroenteric cysts, which are less common than the other lesions and usually present later in infancy or childhood (Langston, 2003).

Congenital Lobar Emphysema

CLE occurs in 1 to 20,000 to 1 in 30,000 live births (Thakral et al., 2001). Because there is no evidence of lung destruction (true emphysematous changes), the common term CLE is a

misnomer, with this lesion, which is also known as *congenital lobar over inflation* (Langston, 2003). CLE is thought to arise from an obstructed lobar bronchus, which can either be intrinsic (including malacia) or extrinsic in origin. While the cause varies, the fundamental mechanism is that air can pass into the effected bronchus but is unable to leave, causing air trapping and lobar overexpansion. The upper lobes are most commonly affected, with the left upper lobe the single most commonly affected lobe. Lesions occupying multiple lobes are infrequent (Mani et al., 2004). Although the affected lobe is larger than usual, the number of alveoli in the involved area is within normal limits. The exception to this is the subset of these lesions with *polyalveolar lobe*, which was present in 9 of 33 cases of CLE in one series and has an overlapping clinical presentation with CLE (Mani et al., 2004). In polyalveolosis, the total number of alveolar is increased severalfold from normal, but the conducting airways are normal in size and number. This form of lung hyperplasia is consistent with pathophysiology associated with fetal airway obstruction (Langston, 2003).

CLE can be detected on fetal ultrasound, although it is difficult to make the correct diagnosis (Olutoye et al., 2000; Babu et al., 2001). Clinical reports have described the appearance of the lesion by ultrasound as cystic and/or echogenic, with mediastinal shift present, and subsequent regression with advancing gestation. As expected, these lesions are suspected to be CPAM or BPS, based on these findings. Fetal MRI can be helpful in characterizing the lesion, although it is not diagnostic (Olutoye et al., 2000). The postnatal clinical presentation and histology (after resection) distinguish CLE from the other lesions. The majority of children with CLE present with respiratory distress, cyanosis, or recurrent pulmonary infections in the first 6 months of life, with 13 of 33 (39%) symptomatic on the first day of life in one series (Mani et al., 2004). The severity of symptoms depends on the size of the affected lobe, the compression of the surrounding tissue, and the extent of mediastinal shift. Chest radiographs demonstrate a hyperinflated lung (transitioning from fluid filled to air filled over the initial postnatal days), with compression of other areas of the lung and mediastinal shift (Fig. 49.10). These findings are generally diagnostic. In children with less severe presentation, bronchoscopy or CT scan can be helpful in management decisions, because some surgeons will elect to manage these patients expectantly, with resolution of symptoms in some cases (Stigers et al., 1992). After resection, prognosis is generally good, with compensatory lung growth present on the affected side (McBride et al., 1980). Airway obstruction continues to be a feature of the disease on pulmonary function tests, although these findings could be consistent with either compensatory lung growth exceeding airway growth (dysanapsis) or intrinsic, diffuse airway abnormality.

Pleuropulmonary Blastoma

Pleuropulmonary blastoma (PPB) is a rare but malignant lesion arising from the lung or the pleura. Lesions can be predominantly cystic (type I), mixed cystic and solid (type II), or predominantly solid type (III) and can occur in association with other congenital lung lesions. The average age of diagnosis of type I, II, and III PPBs is 10 months, 34 months, and 44 months, respectively, and overall survival of type I PPBs is 80%–85%, while survival in type II and III patients is 45%–50% (Priest et al., 1996). The diagnosis and resection of this lesion are essential, because of the risk of metastasis, recurrence, and associated malignancies (Priest et al., 1996). Although these lesions tend to present later in childhood than CPAMs, there is overlap in the timing of presentation (Stocker, 2002), and PPB can be detected on fetal ultrasound (Miniati et al.,

2006); thus the consideration of this diagnosis in the perinatal period is relevant for counseling and surveillance, even if a newborn is not symptomatic.

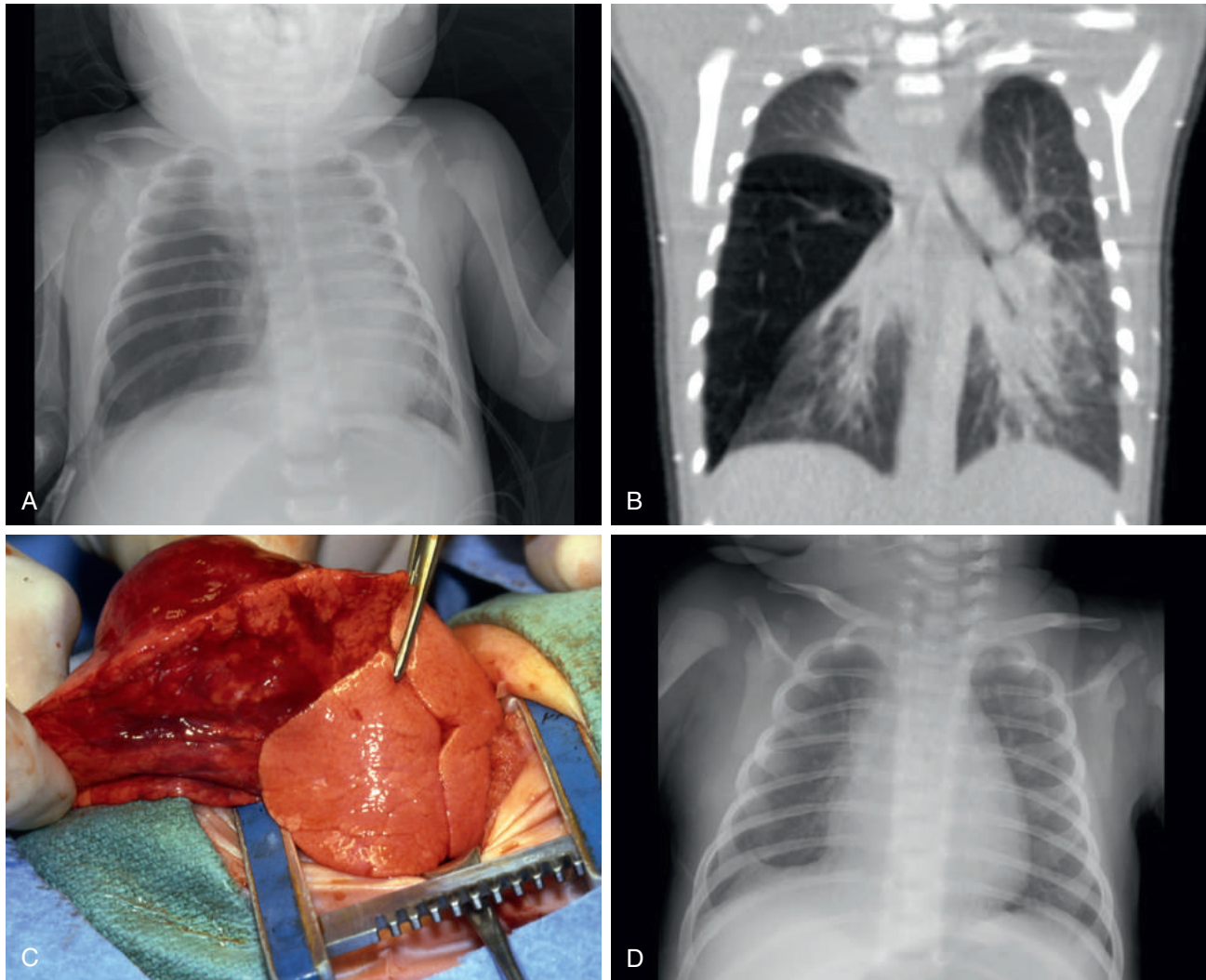
Symptomatic infants usually present with respiratory distress. Before resection, most lesions are thought to be CPAMs, based on their appearance on fetal ultrasound or postnatal CT scan (Miniati et al., 2006). The treatment of choice for PPBs is surgical resection. While type II and type III lesions have been treated with adjuvant chemotherapy given their aggressive nature, there are increasing data to suggest adjuvant chemotherapy will reduce reoccurrence in type I patients as well (Miniati et al., 2006). Patients with type II and III lesions with residual disease following resection should also be treated with radiation therapy (Parsons et al., 2001). Resected lesions contain cuboidal or columnar epithelial cells with underlying rhabdomyosarcoma cells (or other sarcomas). The malignant cells may not be widespread in the lesion, making the diagnosis of PPB challenging even by histology (Stocker, 2002, 2009; Hill and Dehner, 2004). Because of the difficulty in distinguishing CPAMs from PPBs, careful histologic evaluation of prophylactically resected CPAMs is recommended, as those with stellate and spindle cells should be followed closely (Papagiannopoulos et al., 2001). Furthermore, PPBs may require a more extensive resection than CPAMs, so this distinction is important in determining surgical approach as well (MacSweeney et al., 2003). Tumor cells often have complex chromosomal rearrangements, and affected children can exhibit other neoplastic diseases: medullablastoma, nephroblastoma, thyroid dysplasia and malignancy, and brain sarcoma (Priest et al., 1996; Stocker, 2002). Kindreds also demonstrate multiple malignancies, suggesting that familial surveillance for disease might be indicated (Priest et al., 1996).

Postinfectious Pneumatocoles

Pneumatocoles are thin-walled, air-containing cystic structures resulting from alveolar and bronchiolar necrosis. In our experience, the most common infection associated with pneumatocoles in the newborn (maybe because of its higher frequency of infection) is *Staphylococcus aureus* pneumonia (Imamoglu et al., 2005). Other infections seen in the NICU that are associated with development of pneumatocole include pneumonia caused by *Actinomyces* and *Candida* species, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* (Stocker, 2009). Pneumatocoles are often present on initial chest radiograph documenting the infiltrate, although they can also occur later in the process. Given the thin wall, pneumatocoles can rupture, resulting in a pneumothorax that may be under tension or compress the lung tissue through mass effect, resulting in worsening respiratory status (Kunyoshi et al., 2006). Acutely, urgent interventions may be needed to improve ventilation and oxygenation in the setting of rupture. Placement of chest drains into the pneumatocole(s) in the absence of rupture, either percutaneously or under direct visualization via VATS, can be considered; however, the majority of these cysts are regressive and resolve spontaneously (Kunyoshi et al., 2006; Fujii and Moulton, 2008) (Fig. 49.11). For patients with chronic ventilator dependence, in particular former premature infants with BPD, resection of the affected lobe could be considered (Al-Saleh et al., 2008).

Hyperinflation and Emphysema in Chronic Lung Disease

Similar to postinfectious pneumatocoles, frank cysts or hyperinflated lung may develop in association with chronic lung disease of prematurity (BPD) or with certain developmental lung abnormalities. This cystic hyperinflation can cause compression of more functional areas of the lung, with consequent respiratory compromise



• **Fig. 49.10** Congenital Lobar Emphysema. (A) Chest radiograph showing significant hyperinflation of the right lung with mediastinal shift to the left. (B) Coronal chest computed tomography demonstrating hyperaeration of the right middle lobe consistent with congenital lobar emphysema right sided. (C) Intraoperative photo showing the right middle lobe characteristically “popping out” of the thoracotomy incision prior to resection. (D) Chest radiograph 3 days after right middle lobe resection showing normalization of lung volume on the right with resolution of mediastinal shift. (A, B, and D, Courtesy of Dr. Chirag V. Patel, Assistant Professor, University of California, Davis Health System, Department of Radiology, Sacramento, CA. C, Courtesy of Dr. Clifford C. Marr, Clinical Professor, Sutter Medical Group, Department of Surgery, Division of Pediatric Surgery, Sutter Medical Center, Sacramento, CA.)

(Moylan and Shannon, 1979; Stocker, 2009). While this very severe chronic lung disease complication of prematurity rarely occurs today, affected infants can acutely decompensate or remain ventilator dependent despite maximal medical therapy. Evaluation of 6 infants with BPD and decompensated lobar hyperinflation in one series revealed extensive lobar bronchomalacia, with almost complete collapse of the affected airway through the expiratory phase or the entire respiratory cycle (Azizkhan et al., 1992). Lobectomy results in acute improvement, although ultimately only half of infants who are so treated survive.

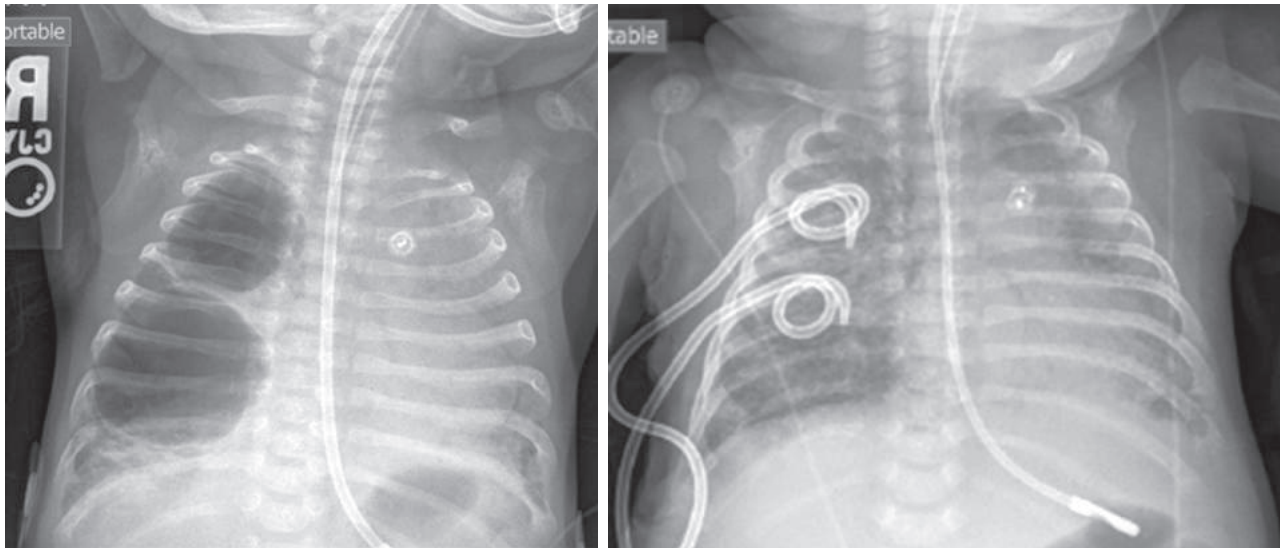
Miscellaneous Cysts

Lymphatic, lymphangiomatous, mesothelial, and parenchymal cysts can be detected in the thorax, so these lesions may need to be included in the differential diagnosis of cystic lesions (Langston, 2003).

Disorders of the Diaphragm

Congenital Diaphragmatic Hernia

Congenital diaphragmatic hernia (CDH) is a disorder of lung and pulmonary vascular hypoplasia that results from failure of formation of the diaphragm (Areechon and Eid, 1963; Kitagawa et al., 1971; Hislop and Reid, 1973). This may be due to primary deficiency of the embryonic pleuroperitoneal fold, which is one of the critical primordial diaphragmatic structures that must fuse at 6 to 8 weeks' gestation to form an intact diaphragm (Clugston et al., 2006). Failure of this event results in herniation of abdominal contents into the hemithorax, and the subsequent arrest of precapillary airway branching at 10 to 14 weeks' gestation is consistent with this early developmental defect. Hypoplasia is bilateral, although the lung ipsilateral to the hernia is most affected. Airway diameter is



• **Fig. 49.11** Postinfectious Pneumatocoeles. Chest radiographs from a former preterm infant with pneumatoceles secondary to *Staphylococcus aureus* pneumonia. Cysts are compressing the lung and causing mediastinal shift (left). Placement of draining thoracostomy tubes decompressed the cysts and decreased mediastinal shift, but the infant ultimately succumbed to respiratory failure secondary to bronchopulmonary dysplasia.

substantially decreased, but increase in airway muscle occurs as a later postnatal event (Broughton et al., 1998). Although acinar alveolar counts are normal, overall alveolar hypoplasia is present due to the branching defect ($<10 \times 10^6$ vs 50×10^6 alveoli at term).

Because of the interdependence of lung and vascular growth, both alveolar and capillary surface areas are decreased (Hislop and Reid, 1973; Kitagawa et al., 1971; Joshi and Kotecha, 2007). Vascular branching is impaired, with a decreased diameter of the vessels and increased muscle mass that is inversely related to the degree of lung hypoplasia (Kitagawa et al., 1971; Naeye et al., 1976). Some morphometric reports have demonstrated abnormal distal extension of the muscular media to the intra-acinar arteries, whereas others have not demonstrated abnormal distal muscularization (Kitagawa et al., 1971; Geggel et al., 1985). The mechanism of developmental lung and vascular hypoplasia is unknown but may include decreased static transthoracic pressure (secondary to open communication with the peritoneal cavity) and decreased phasic pressure alterations (secondary to impaired fetal breathing movements). Compensatory alveolar growth does occur in survivors, although it is more pronounced in the contralateral lung, and relative perfusion to the ipsilateral lung can be persistently diminished (Wohl et al., 1977; Thurlbeck et al., 1979; Okuyama et al., 2006). These findings are consistent with evidence of greater injury present in the more hypoplastic and vulnerable ipsilateral lung, compared with the contralateral lung in survivors of CDH, with consequent impairment of lung growth (Hislop and Reid, 1973; Wohl et al., 1977; Thurlbeck et al., 1979).

Ninety-five percent of CDHs are posterolateral (Bochdalek) hernias (Deprest et al., 2014). The remaining 5% are either anterior, retrosternal, or peristernal (Morgagni). CDH is more common on the left side (75%–80% of cases) than the right, probably because of slightly later fusion of the left-sided structures. Bilateral hernias account for 1%–2% of cases. Morgagni hernias are much less frequent in occurrence and usually are not associated with substantial lung hypoplasia, although they may be associated with

pericardial, sternal, and abdominal wall defects as part of the pentalogy of Cantrell spectrum. There is a predominance of males to females in CDH (1.4 to 1.6:1 ratio), and the occurrence of CDH (including stillbirths) is about 1 in 4000 births (Levison et al., 2006; Yang et al., 2006; Gallot et al., 2007). Additional anomalies occur in about 40% of affected infants and fetuses (Yang et al., 2006; Gallot et al., 2007). Musculoskeletal (including ribs, vertebrae, and digits) and cardiac anomalies are most common, although the patterns of malformation differ in association with right-sided CDH compared to left-sided CDH (Slavotinek et al., 2007). CDH can be associated with aneuploidy (most frequently trisomy 18), and it can present in autosomal recessive (e.g., Fryns syndrome), sex-linked (e.g., Simpson–Golabi–Behmel syndrome), and autosomal dominant (e.g., Cornelia de Lange syndrome) disorders (Slavotinek, 2007; Slavotinek et al., 2007). With the exception of these disorders, recurrence rate is quoted at 1%–2%, and more recent genetic studies have identified micro-deletions in affected infants through use of microarray technology (Slavotinek, 2005; Kantarci et al., 2006). It is unclear whether or not infants with isolated CDH (no other anomalies found by prenatal and postnatal investigation) are at increased risk for these minor chromosomal aberrations, because large-scale investigations have taken place with very limited positive findings. Single gene mutations have been identified in animal models and some humans as causal in CDH, and certain areas of the genome may be critical regions, wherein other causal genes might be found (Clugston et al., 2006; Ackerman and Pober, 2007).

In recent population-based studies, overall survival among liveborn affected infants ranged from 57% to 73% (Collin et al., 2016; Partridge et al., 2016). This is slightly improved from a 52% to 61% survival rate just a decade ago (Stege et al., 2003; Colvin et al., 2005; Yang et al., 2006; Gallot et al., 2007). Those patients with left-sided diaphragmatic hernias fare better than those with right-sided hernias, with survival rates being 73%–69%, respectively; however, this difference is not statistically significant. While the survival rate is similar, those patients with right-sided

CDH have higher rates of pulmonary morbidity, including an increased need for tracheostomy, long-term vasodilatory therapy, and greater likelihood of requiring supplemental oxygen at the time of hospital discharge (Partridge et al., 2016). Survival of liveborn infants with isolated (no additional anomalies) CDH has been higher (63%–77%) than survival in liveborn infants with other anomalies or chromosomal aberrations (19%–43%) (Stege et al., 2003; Yang et al., 2006; Gallot et al., 2007). Survival is low (<10%) in affected infants with chromosomal abnormalities, and there is a risk of intrauterine fetal demise in both isolated (2%) and nonisolated (11%) CDH. Although individual referral centers have reported survival rates ranging from 75% to 93% (Bohn, 2002; Boloker et al., 2002; Downard and Wilson, 2003), there have been studies documenting a hidden mortality, demonstrating that a proportion of liveborn neonates die within hours of birth and before arrival at a surgical center (Harrison et al., 1994; Mah et al., 2009). In a recent study from Ontario, Canada, survival decreased from 67% to 58% for multicenter compared to population-based data, after accounting for infants who never reached a referral center (Mah et al., 2009).

Both population-based and center-based studies have identified some risk factors for mortality for liveborn infants undergoing full resuscitative measures and ongoing neonatal care. All of these are probably related to the severity of the CDH, with respect to the degree of lung and vascular hypoplasia. For instance, prenatal diagnosis has been associated with increased mortality and may be due to the identification of more severe CDH (because mediastinal shift will be more pronounced and present earlier in gestation; see later discussion) (Stege et al., 2003; Colvin et al., 2005; Gallot et al., 2007; Stevens et al., 2009). A recent multicenter, retrospective Children's Hospital Neonatal Database analysis of 677 children with CDH identified six variables independently associated with mortality and hospital length of stay greater than 109 days: infants that were small for gestational age, those with major birth anomalies, 5-minute Apgar scores less than or equal to three 3, acidosis at the time of referral, those requiring ECMO, and those with bacteremia (Murthy et al., 2016). Other postnatal factors that are associated with increased mortality include prematurity (<37 weeks) and air leak, conditions associated with immature lung development, and/or increased risk for lung injury (Levison et al., 2006; Tsao et al., 2010).

Interestingly, two studies have shown somewhat conflicting data regarding the timing of term delivery and survival. For infants receiving ECMO support, late-term (40–41 weeks' gestation) infants had somewhat better survival compared with early-term (38–39 weeks' gestation) infants, although the relationship was not statistically significant, and fewer ECMO-related complications were seen in the late-term group (Stevens et al., 2002). However, overall survival was slightly better for early-term (37–38 weeks' gestation) versus late-term (39–41 weeks' gestation) infants in another study that included infants not receiving ECMO support (Stevens et al., 2009). Other data show that infants not born at a tertiary center (outborn), but subsequently transferred to a tertiary center, have higher survival rates than those that are born at a tertiary center (inborn), which may reflect the hidden mortality associated with more severe, prenatally diagnosed CDH (Boloker et al., 2002; Levison et al., 2006). For those infants who do undergo surgical repair, the need for a prosthetic patch is associated with subsequent mortality, indicating a larger diaphragmatic defect and probably more severe lung hypoplasia (Wilson et al., 1997; Lally et al., 2006; Bryner et al., 2009).

Prenatal Diagnosis and Management

CDH is usually suspected on prenatal ultrasound when mediastinal shift away from the side of the hernia is appreciated. Prenatal detection rates are higher with left-sided than right-sided hernias, and bilateral hernias can be difficult to discern because of the distorted anatomy. CDH in fetuses with additional anomalies are also detected at a higher rate than CDH in isolated cases (Gallot et al., 2007). Fetal MRI can be helpful in determining the diagnosis, if the anatomy is difficult to identify. A prenatal diagnosis of CDH mandates careful evaluation for other anomalies, including a fetal echocardiogram, because of the high rate of additional anomalies and their association with lower survival rates. Some of this evaluation may be limited by the anatomic distortion caused by the hernia. A karyotype and other genetic analysis (as indicated) are also recommended. Additional prognostic information regarding the severity of the hernia can be gathered during prenatal evaluation by ultrasound and MRI, although the prognostic ability of these measures is likely center dependent, as survival varies to some extent across centers. The most useful discriminator of CDH severity is herniation of the liver into the hemithorax (Metkus et al., 1996; Jani et al., 2006). Liver herniation can occur with both right-sided and left-sided CDH. In left CDH, the left lobe of the liver herniates in the thorax; thus the course of the hepatic vasculature is distorted and indicative of liver herniation. Herniation of the liver is also predictive, with one study showing a significantly higher survival rate in fetuses without liver herniation (74%) versus those with herniation (45%) (Mullasery et al., 2010). Further research looking at the predictive nature of prenatal ultrasound in the assessment of liver herniation has demonstrated that sonographic measurements (liver-to-thorax ratio and stomach position) are predictive of survival outcomes in those with isolated left-sided CDH (Werneck Britto et al., 2015; Sananes et al., 2016). The prenatal liver-to-thorax ratio can also be predictive of the need for ECMO.

Other prenatal discriminators of severity that have been used include stomach herniation and polyhydramnios (a later finding, secondary to GI obstruction or esophageal dysmotility). A number of ultrasound measures have been developed as intrauterine measures of lung size (Keller, 2007). The lung-to-head ratio (LHR) is widely used for this purpose (Metkus et al., 1996). It is the perpendicular area of the lung contralateral to the hernia at the level of the cardiac atria divided by the biparietal diameter. It has been used predominantly in mid-gestation (22–27 weeks' gestation), with an LHR ≤ 1.0 combined with liver herniation accepted as the most severe group of CDH. LHR has been studied in left CDH, although it has also been extrapolated to right CDH and probably has prognostic ability; LHR thresholds for severity could be lower in right CDH, since the normal right lung is larger than the left lung (Hedrick et al., 2004; Peralta et al., 2005). Investigators have developed nomograms for normal LHR over 12 to 32 weeks, which has led to an observed-to-expected (O/E) LHR measurement, employing the mean LHR at any gestational age as the "expected LHR," which increases with advancing gestational age because of a more rapid increase in lung area compared with head circumference (Peralta et al., 2005). It follows that the LHR ≤ 1.0 will represent a higher O/E LHR earlier in gestation than it does later in gestation, and the O/E LHR will be higher with right CDH than left CDH for a given LHR and gestational age (Jani et al., 2009a). In a large database of 354 fetuses with isolated CDH (including 25 fetuses with right CDH), the O/E LHR was predictive of survival, independent of liver herniation (Jani et al., 2007). Subsequent analyses of this data have stratified fetuses with and without liver herniation, because survival does differ between these groups for

a given O/E LHR range (Deprest et al., 2009). In these analyses, survival was less than 20% for fetuses with isolated left CDH and O/E LHR less than or equal to 25%. Fetuses with a higher O/E LHR of 26%–35% survived in greater numbers: 30% if liver was herniated and almost 60% if liver was not herniated. The lower bound of the 95% confidence interval (CI) for O/E LHR in unaffected fetuses is 60% (Jani et al., 2007). Decreasing O/E LHR was also related to increasing time on assisted ventilation, prolonged hospitalization, and an increased risk for prosthetic patch repair (Jani et al., 2009a).

Fetal MRI techniques have also been pursued for prognostic information in CDH. Liver herniation can be determined by fetal MRI, and at some centers it is the preferred technique for this determination. A number of different nomograms to determine lung volume as a percent of normal (based on estimated fetal size or gestational age) have been developed. Recently, Büsing et al. (2008) evaluated seven published nomograms for estimation of relative fetal lung volume in 68 fetuses with isolated left CDH evaluated at their center. They generated receiver operating characteristic curves for each of the seven equations and found high (0.800–0.900) area under the curve (AUC) for prediction of survival, regardless of technique used. In this dataset, prediction of need for ECMO was not as strong (AUC 0.653–0.739), although individual centers have found that relative fetal lung volume is a very useful predictor of the need for subsequent ECMO support (Barnewolt et al., 2007).

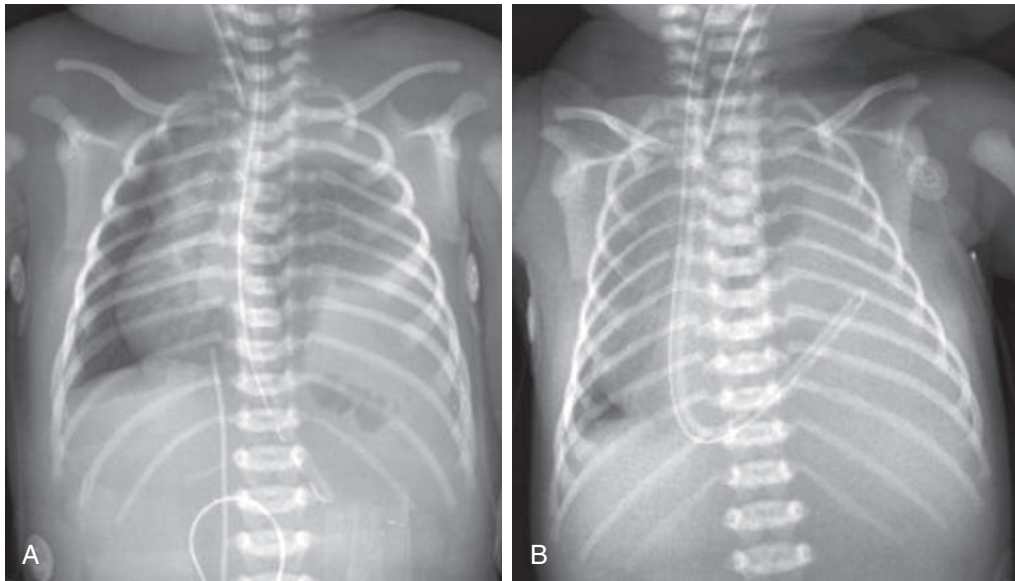
Early attempts at fetal intervention for CDH focused on an anatomic repair of the diaphragmatic defect; however, this approach was quickly abandoned after survival outcomes did not improve (Harrison et al., 1997). Fetal intervention has since shifted its focus to enhancing intrauterine lung growth via endoscopic temporary balloon tracheal occlusion. Only fetuses likely to be the most severely affected newborns are candidates, if additional anomalies are not found after careful investigation. A randomized controlled trial evaluating this technique was terminated prematurely because of unexpectedly high survival in the control group, with no difference in survival between tracheal occlusion and standard care infants (Harrison et al., 2003). The tracheal occlusion procedure was complicated by premature delivery (mean gestational age 30.8 weeks), often stemming from premature rupture of the membranes, and this may have compromised survival in the intervention group. With development of smaller instruments, this technique can now be accomplished as a fully endoscopic procedure, and the incorporation of a second procedure to remove the tracheal balloon (i.e., plug–unplug) has also decreased the need for delivery by EXIT procedure, allowing for vaginal delivery in the majority of cases (Jani et al., 2009b). The European Fetal Endoluminal Tracheal Occlusion (FETO) task group has been performing minimally invasive tracheal occlusion in fetuses with LHR less than or equal to 1.0 with promising results demonstrating an improved survival to discharge and a later mean gestational age at the time of delivery (33.5 weeks) (Jani et al., 2005). This was attributed to the less invasive nature of the procedure. With operator experience, the procedure has been accomplished more rapidly, and shorter procedures were associated with lower rates of premature membrane rupture. Elective prenatal removal of the intratracheal balloon (i.e., unplug) was accomplished at a median of 34 weeks in 70% of the cases. Survival to neonatal discharge was 47% (98 of 210); 6 deaths were fetal and 10 deaths occurred secondary to difficulty with removal of the intratracheal balloon. From their historical experience, the investigators cite an expected survival of only 24%. In a subset of these fetuses, and some expectantly managed with CDH, Cannie

et al. (2009) evaluated changes in relative lung volume over gestation by fetal MRI. They found that relative lung volume was largely unchanged in fetuses without intervention, and it tended to increase more consistently in fetuses with CDH when tracheal occlusion was undertaken at greater than or equal to 29 weeks' gestation ($n = 8$). Most recently, a small randomized controlled trial comparing FETO to standard postnatal management found that 50% of fetuses treated with tracheal occlusion survived to 6 months of life compared with 4.8% in the postnatal treatment group (Ruano et al., 2012).

Postnatal Diagnosis and Management

Most newborns with CDH will present immediately or within several hours of birth with respiratory distress, cyanosis, decreased breath sounds on the hernia side, and a scaphoid abdomen. An occasional infant will not have symptoms until several days or months of age and often will have feeding intolerance and mild respiratory distress (Kitano et al., 2005). An initial chest radiograph will show a smaller lung on the hernia side, with bowel gas in the chest and shift of mediastinal structures (Figs. 49.12–49.13). The findings of a small lung, with no mediastinal shift and usually without concern for herniated bowel, should raise suspicion for other diagnoses, which may require an alternate surgical approach or may not require surgery at all (see later discussion). Newborns with a fetal diagnosis of CDH or those presenting soon after birth are usually mechanically ventilated with an endotracheal tube, and a Replogle (Covidien - Medtronic, Minneapolis MN) is placed for continuous gastric suction to minimize accumulation of thoracic intraintestinal air and reduce lung compression.

Hypoplastic lungs with small alveoli have poor compliance, and thus ventilation is severely reduced (Keller, 2007). Combined with the restrictive pulmonary vascular bed and consequent pulmonary hypertension, this leads to severely impaired oxygenation. Because these physiologic challenges cannot be overcome without lung growth, most high-volume centers use a gentle ventilation strategy. This strategy attempts to achieve adequate oxygen delivery and preserve the potential for lung growth while minimizing oxygen toxicity and ventilator-induced lung injury (barotrauma). Actual targets for ventilation and oxygenation vary somewhat, but consistency in care within a center is important (Logan et al., 2007). The ventilation target is often an arterial partial pressure of carbon dioxide (PCO₂) of 50–65 mm Hg (permissive hypercapnia). For oxygenation, a more liberal strategy allows for a preductal (right upper extremity) and postductal (descending aorta or lower extremity) arterial saturation (SaO₂) differential, with oxygenation targets based on preductal SaO₂ (permissive oxygenation). Oxygenation targets are generally preductal SaO₂ greater than or equal to 95% in less severely affected infants and SaO₂ greater than or equal to 85% in more severely affected infants. Ventilator pressures are limited, with either positive inspiratory pressure (PIP) targeted at less than or equal to 25–28 cm H₂O or mean airway pressure on high-frequency ventilation at less than or equal to 15 cm H₂O. Aggressive weaning strategies are used to achieve gentle ventilation goals and to minimize lung injury. Lung recruitment is not an effective strategy to achieve persistent improvements in oxygenation in CDH (Kinsella et al., 1997), and increases in PIP to achieve transient improvements in oxygenation lead to further increases in ventilator support, with worsening compliance because of lung injury and edema (Kays et al., 1999). There are also some centers that advocate low PEEP and high ventilator rates (which are physiologic in lung hypoplasia). With high ventilator rates, gas trapping and auto-PEEP may be an important factor, so limitation of the PEEP set on the ventilator is important (Boloker et al.,



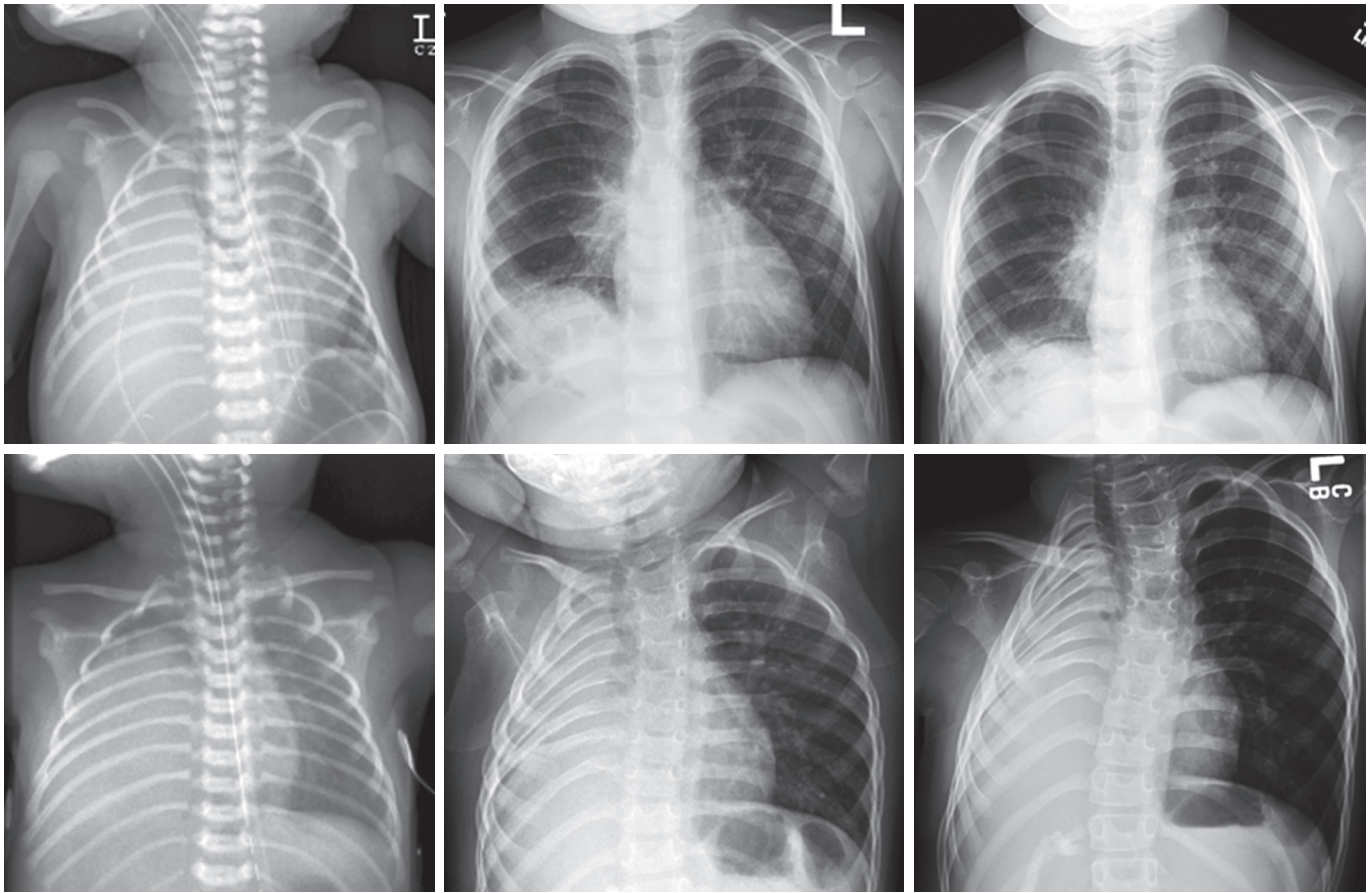
• **Fig. 49.12** Left Congenital Diaphragmatic Hernia. (A) Radiograph of an infant with congenital diaphragmatic hernia (CDH) without liver or stomach herniated into thorax. He subsequently underwent primary repair of the diaphragmatic defect, was extubated within several days of surgery, and was discharged to home without supplemental oxygen. (B) Radiograph of an infant with severe CDH with liver and stomach herniated into thorax. He subsequently required extracorporeal membrane oxygenation (ECMO) support, underwent prosthetic patch repair for diaphragmatic aplasia, and succumbed to respiratory failure and pulmonary hypertension after decannulation from ECMO support.

2002). In addition, low PEEP (2–3 cm H₂O) is associated with higher lung compliance in infants with CDH than PEEP of 4–6 cm H₂O (Dinger et al., 2000).

Employment of gentle ventilation strategies has been associated with improved survival at individual centers (Wilson et al., 1997; Kays et al., 1999); however, regardless of actual targets for ventilation and oxygenation and preferential mode of ventilation, it is likely that other aspects of care, such as infant stimulation and positioning and sedation and feeding practices, affect survival and other outcomes. Although there are a number of ancillary treatments employed in infants with CDH (prenatal glucocorticoids, surfactant replacement therapy, inhaled nitric oxide, and other pulmonary vasodilator therapies), there are no studies documenting broad efficacy of these treatments in term infants with CDH. Some have advocated the primary use of high-frequency ventilation in these infants, but there is no evidence of benefit to survival or other outcomes based on the mechanical ventilation technique alone (when similar permissive targets are used) (Snoek et al., 2016). In addition, the use of prostaglandin infusion to maintain ductal patency and improve right heart function in cases of prolonged pulmonary hypertension has been advocated (Bohn, 2002). The one ancillary treatment that has shown benefit is the use of ECMO, which improved survival to hospital discharge in the United Kingdom Collaborative study, although the effect dissipated at follow-up with later deaths among initial survivors (Mugford et al., 2008).

ECMO can be used to rescue newborns with CDH, particularly those with lung injury evident by pneumothorax (Sebald et al., 2004), and it may be used in the setting of gentle ventilation (where pneumothorax is a less frequent occurrence), to avoid prolonged high ventilator pressures or fraction of inspired oxygen (FiO₂). Although CDH has become the most common indication for neonatal ECMO (other than cardiac defects), its efficacy is difficult to assess given the absence of clinical trials. However,

those with CDH who go onto ECMO have a 40%–50% survival rate based on observational data (Rais-Bahrami and Van Meurs, 2005; Seetharamaiah et al., 2009). Because ECMO is a temporary therapy designed to allow for resolution of a reversible process, it is unlikely that all infants with CDH will benefit from ECMO support; some will have lethal pulmonary hypoplasia (Antunes et al., 1995). Thibeault and Haney described persistent pulmonary hypertensive changes in vessels of infants with CDH dying after ECMO support, and others have described recurrent pulmonary hypertension (Payne et al., 1991; Thibeault and Haney, 1998). The results of ECMO in CDH greatly depend on patient selection criteria, which varies widely among institutions. The primary indication for ECMO should be failure of conventional therapy. This failure can be characterized by persistent productal SaO₂ less than or equal to low 80s, hypotension resistant to fluid/inotropic support, peak inspiratory pressures less than 30 cm H₂O, or worsening metabolic acidosis (Downard and Wilson, 2003). For those who do go on ECMO, a duration of ECMO support greater than 2 weeks and the use of renal replacement therapy for renal insufficiency have been identified as independent predictors of mortality. Survival among both of these groups was approximately 20% (2 of 11 for prolonged ECMO and 4 of 18 for renal insufficiency) (Tiruvoipati et al., 2007). Longer ECMO runs, renal complications, and multiple complications were also independently associated with mortality in infants with CDH in an analysis from the Extracorporeal Life Support Organization database (Stevens et al., 2002). Thus failure to decannulate an infant at less than 10 to 14 days of support is probably an indicator of severe lung and vascular hypoplasia. In these severely affected infants, it often takes longer than 4 weeks to resolve pulmonary hypertension (Dillon et al., 2004; Keller et al., 2006), and therefore awaiting resolution of pulmonary hypertension in these cases before decannulation would require prolonged ECMO support. Prolonged ECMO runs are prone to mechanical complications, which may further prolong



• **Fig. 49.13** Congenital Diaphragmatic Hernia and Hepatopulmonary Fusion. Chest radiographs in two infants with respiratory distress at admission to the intensive care nursery and at 3 and 5 years of age (left to right). *Upper panel* shows infant with right congenital diaphragmatic hernia with liver herniated into thorax and diaphragmatic aplasia. Note mediastinal shift into left hemithorax, with subsequent improvement in aeration and then normalization of right lung volume. *Lower panel* shows infant with hepatopulmonary fusion. Surgical repair required resection of hypoplastic nubbin of lung. Note lack of mediastinal shift at presentation, compensatory growth of left lung with trachea deviated to the right, and scoliosis.

the ECMO run or be irrecoverable. Thus it is unclear to what degree prolonging ECMO support could increase survival in CDH, unless there is still evidence of reversibility in the infant's condition. In the case of availability of a specific therapy, such as perfluoro-carbon partial liquid ventilation, wherein lung growth might be induced, the benefits of continuing ECMO support might become evident (Hirschl et al., 2003). This therapy, however, is still controversial, with recent clinical studies showing that perfluoro-carbon ventilation does not improve pulmonary vascular remodeling and CDH-associated pulmonary hypertension (Shah et al., 2016). Otherwise, strategies to limit the duration of ECMO support in CDH could be employed, accepting ventilator settings at decannulation that are significantly higher than what might otherwise be acceptable for newborns coming off ECMO support. These strategies could include prevention of complete lung collapse with aggressive pulmonary toilet, because re-recruitment of lung volume can be difficult in these infants (which may be related to small-caliber airways that have been damaged by pre-ECMO support regimens). Also, for infants cannulated prerepair, performance of the CDH repair post-ECMO decannulation could further limit time on ECMO support. Surgical repair of the diaphragmatic defect while on ECMO is associated with higher mortality, even after adjusting

for other markers of CDH severity (Bryner et al., 2009). This phenomenon may be related to hematologic complications from the repair (which are independently associated with decreased survival) (Stevens et al., 2002). However, some centers advocate CDH repair as soon as possible after stabilization on ECMO support (Bryner et al., 2009). In addition, some centers utilize an "EXIT-to-ECMO" approach for fetuses with high-risk criteria based on fetal evaluation (low relative lung volume) (Kunisaki et al., 2007b). These fetuses are intubated and ventilated during an EXIT procedure. If they meet certain criteria for adequate gas exchange, they are delivered for conventional management. If they do not meet these criteria, they are cannulated and placed on ECMO support. Using this strategy, (Kunisaki et al., 2007b) reported a 71% (10 of 14) survival with 11 infants going directly to ECMO support and 7 of 11 surviving. Most infants required prolonged mechanical ventilation and hospitalization.

Repair of the diaphragmatic defect usually occurs after some degree of stabilization of cardiopulmonary status, either with conventional therapy or ECMO support. Nakayama et al. (1991) demonstrated that lung compliance improves before surgery after a short period (several days) of stabilization in infants with CDH, and Sakai et al. (1987) demonstrated that compliance worsens

with early surgery in almost all infants. Keller et al. (2004) studied infants with severe CDH (liver herniation and LHR <1.4) and found that, although compliance did not improve before surgery after a period of stabilization, compliance did improve within 24 hours after surgery. In low-risk infants that do not require ECMO, the Congenital Diaphragmatic Hernia Study Group found that the timing of surgery did not seem to affect survival and that those who underwent surgery at age 0–3 days, 4–7 days, or greater than 8 days had similar mortality rates when adjusted for known risk factors (Hollinger et al., 2014). Thus the rationale for delayed surgery is sound with respect to lung function, but specific clinical parameters to guide the timing of elective CDH repair remain unknown. Some centers advocate very delayed surgery, while awaiting complete resolution of pulmonary hypertension (Wung et al., 1995). However, this approach can be problematic, because pulmonary hypertension may require weeks for resolution (Dillon et al., 2004; Keller et al., 2010). Subsequent failure to reduce the hernia contents is likely to delay establishment of enteral nutrition, with a consequent increased risk of infection and complications from parenteral nutrition. The achievement of even modest reduction in FiO_2 before surgery allows for a transient increase, if needed, after surgery, and even in cases where FiO_2 remains high, the modest improvement in lung function that occurs with surgery may help with further recovery for the infant in the most severe cases. Another issue with respect to timing of surgery arises when an infant also has congenital heart disease that requires neonatal surgery. Most centers will undertake CDH repair and then proceed to the cardiac surgery once the infant meets reasonable hemodynamic criteria for that surgical intervention (Cohen et al., 2002). In newborns who might require urgent cardiac surgery, survival is unlikely unless the lung hypoplasia and pulmonary hypertension are very mild. In a large series of 280 infants with CDH and congenital heart disease, overall survival was 41% but only 5% for infants with single ventricle physiology and 18% (2 of 11) for infants with total anomalous pulmonary venous return (Graziano, 2005).

Surgical repair of CDH involves reduction of the hernia contents and closure of the diaphragmatic defect. The surgical approach traditionally has been via laparotomy (subcostal incision on the side of the hernia), to abrogate the detrimental effect of thoracotomy on lung function. However, some surgeons will preferentially do a thoracotomy for a right CDH repair. With the advance in endoscopic technology, thoracoscopic and laparoscopic repairs are being performed in selected patients (Zani et al., 2014). These patients include infants who have achieved low ventilator settings and FiO_2 before surgery. Despite both thoracoscopic and laparoscopic repairs being feasible, there are questions about the safety and efficacy of these approaches, with minimally invasive techniques being linked to higher rates of reoccurrence, as well as intraoperative acidosis and hypercapnia, particularly with thoracoscopic repair (Kim et al., 2009; Tsao et al., 2011; Bishay et al., 2013). Additional randomized controlled trials are needed to determine the efficacy of minimally invasive repairs and to define the specific patient populations who are most appropriate for a minimally invasive repair.

Regardless of the surgical approach, when the diaphragmatic defect cannot be closed primarily, a prosthetic patch is used to bridge the gap. There is significant variability among surgeons in the need for patch repair, however. Clinical series have demonstrated that it is more common in the case of liver herniation and right CDH (which may also be an association with herniated liver) (Fisher et al., 2008; Kunisaki et al., 2008). Patch repair can be accomplished with use of a polytetrafluoroethylene (PTFE/Gore-Tex, W.L. Gore & Associates, Inc., Flagstaff, AZ)

or a bioabsorbable intestinal mucosa (SurgiSIS, Cook Medical Inc., Bloomington, IN) material. Polypropylene (Marlex, Bard Davol, Warwick, RI) and other materials have been used sporadically (Riehle et al., 2007). Use of an abdominal silo and/or prosthesis to close the abdominal wall is sometimes necessary and may decrease the need for a prosthetic diaphragm (Rana et al., 2008; Bryner et al., 2009). Temporary abdominal closures with skin or vacuum-assisted closures have also been used, most commonly in children who are repaired while on ECMO (Laje et al., 2016). Some surgeons advocate construction of a latissimus dorsi flap for initial CDH repair when primary closure of the diaphragmatic defect cannot be accomplished (Barbosa et al., 2008). This technique allows for some potential for diaphragmatic function in the innervated flap, which usually remains abnormal at late follow-up even with primary repair (Arena et al., 2005). However, the technique is time consuming and may not be tolerated in patients during the acute neonatal period, so others have reserved this technique only for hernia recurrence (Sydorak et al., 2003). Generally, use of a prosthetic patch for diaphragmatic closure is associated with increased risk of hernia recurrence, although the actual risk varies widely and may be dependent on multiple factors, including surgical technique and the type of patch used (Jancelewicz et al., 2010). Recurrent herniation may be associated with small bowel obstruction. It is also associated with persistent chest wall deformity, which might be due to the severity of the underlying disease or the complication of re-herniation (requiring multiple surgical procedures). Some surgeons have moved toward use of composite patches (more than one material), which may decrease the risk of recurrence by allowing for both durability and accommodation of rapid growth in infancy (Riehle et al., 2007; Jancelewicz et al., 2010).

Another area of variable practice is related to the intraoperative placement of a thoracostomy tube. Some of this controversy is related to the application of negative pressure, because that creates additional transpulmonary pressure and potential for barotrauma and lowers lung compliance (Dinger et al., 2000). However, negative pressure drainage is not necessary. Because the ipsilateral thorax will fill with fluid after reduction of the hernia contents as a result of the hypoplastic lung, placement of an anterior thoracostomy tube in a supine infant will prevent the accumulation of excess pleural fluid (which can occur with chylothorax). Chylous pleural effusion will cause respiratory compromise. However, it may be difficult to ascertain the cause of this deterioration because mediastinal structures remain shifted for some period of time postoperatively and may be modestly more exaggerated with tension hydrothorax. Chylothorax is not uncommon after CDH repair. In a recent series, it occurred in 7% of infants (10 of 152), and it was more common in infants who required prosthetic patch repair (8 of 10) (Gonzalez et al., 2009). Both surgical trauma and underlying hemodynamics due to right heart failure may contribute to the cause of this problem in infants with CDH (see Chylothorax earlier, for diagnosis and management).

Long-Term Morbidity

Survivors of CDH have substantial pulmonary and GI morbidity at follow-up (Muratore et al., 2001a, 2001b). Early gastroesophageal reflux following repair occurs in up to 40% of CDH patients and is more common in patients who undergo patch closure or have an intrathoracic stomach (Peetsold et al., 2010). In addition, failure to thrive is common with many patients requiring supplemental enteral feedings via gastrostomy tube. Although low lung volumes and restrictive lung disease may be seen in the first months of life, obstructive lung disease is the most common finding in early childhood and at later follow-up (Vanamo et al., 1996; Koumbourlis

et al., 2006). Children requiring prosthetic patch repair and prolonged mechanical ventilation are most likely to have later morbidity, but the relative contribution of anatomic abnormalities, physiologic derangements, and secondary injury to these outcomes is unknown. Developmental delay and hearing loss also occur and require careful follow-up for early identification and intervention (Jaillard et al., 2003; Cortes et al., 2005; Friedman et al., 2008; Keller et al., 2008).

Hepatopulmonary Fusion

Hepatopulmonary fusion probably represents a severe form of CDH. It has been described variably in the literature as both CDH and severe eventration (Rais-Bahrami et al., 1996; Keller et al., 2003; Robertson et al., 2006). All reported cases have occurred on the right side, although there is a report of a late-presenting left CDH with fusion of liver and lung tissue (Baeza-Herrera et al., 2000). It is not clear if this is the same entity that other authors have described, because these infants usually present in extremis, with respiratory failure and pulmonary hypertension (Rais-Bahrami et al., 1996; Keller et al., 2003; Robertson et al., 2006).

The embryology of this defect is speculative, and the distinction between severe CDH versus eventration is probably not critical. However, the diaphragm is not intact, it is usually moderately to severely hypoplastic, and there is fusion of hepatic and pulmonary tissue without pleura or liver capsule present, no dissectable plane, and a fibrous membrane adherent to the liver and the lung (Slovic et al., 2000). In cases where hepatopulmonary fusion is determined or suspected, there is a lack of mediastinal shift, consistent with severe right lung hypoplasia rather than a mass effect from a large right CDH with herniated liver (Slovic et al., 2000; Keller et al., 2003).

The suspicion of this diagnosis is important, because the surgical approach may differ. The need to separate the liver and the lung (usually requiring ligation or resection of part of the hypoplastic lung because of the risk of air leak and hemorrhage) will require a thoracotomy. MRI has been utilized preoperatively to help make this distinction and found enhanced lung tissue adherent and conforming to the dome of the liver in a case of hepatopulmonary fusion, suggestive of the anatomy subsequently encountered at the time of surgical repair (Keller et al., 2003). The goal of surgery is to separate the thoracic and abdominal cavities, often requiring placement of a prosthetic patch. If these children survive, later growth of the right lung is much more restricted than it is in children with severe right CDH (see Fig. 49.13). Whether this is due to more pronounced ipsilateral lung hypoplasia, less pronounced contralateral lung hypoplasia, or the need for resection at the time of surgical repair is unclear. However, chest wall deformity and scoliosis are significant problems in these children.

Congenital Eventration of the Diaphragm

Congenital eventration of the diaphragm occurs when the hemidiaphragm is partially or completely replaced by fibroelastic tissue, leading to a thinned, pliable portion of the diaphragm. Unlike CDH, the diaphragm is intact with normal insertion points. However it is elevated, and thus intra-abdominal organs are present in the thorax but still confined below the diaphragm. It can be difficult to distinguish eventration from a diaphragmatic hernia with a sac. The right and left hemidiaphragms are equally affected, and 13% (6 of 48) of affected infants had bilateral eventration in an early series (Wayne et al., 1974; Tsugawa et al., 1997).

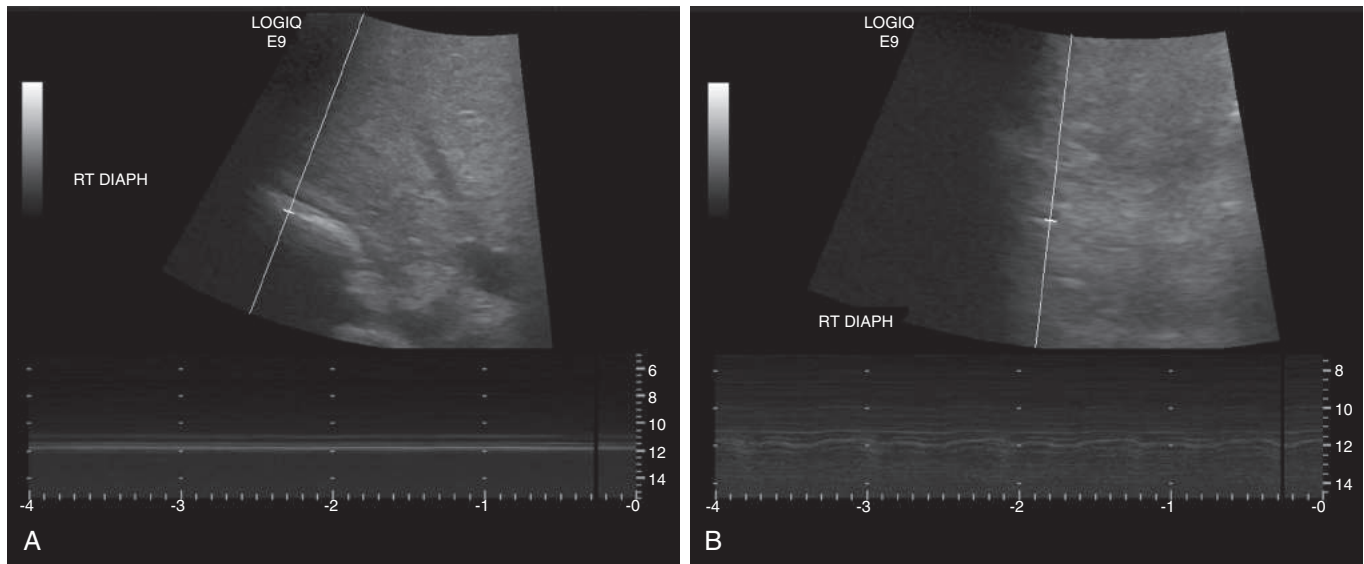
Approximately 18% of children with eventration have associated anomalies, with the most common being hypoplastic lungs, congenital heart disease, and pectus excavatum (Wu et al., 2015). With diaphragmatic eventration, movement of the hemidiaphragm is paradoxical, leading to respiratory distress, atelectasis, and recurrent pulmonary infections. Despite this, 50% of children with congenital eventration are without symptoms, making diagnosis difficult (Wu et al., 2015). Infants presenting later may have GI symptoms, and therefore early diagnosis and treatment are essential. Diagnosis is usually made by fluoroscopy of the chest, ultrasound of the diaphragm, or dynamic contrast-enhanced MRI. Small eventrations that are asymptomatic can be observed. Surgical intervention for symptomatic eventration involves plication of the diaphragm to eliminate the paradoxical movement. The diaphragm can be approached via the abdomen or chest using either open or laparoscopic/thoracoscopic techniques (Lao et al., 2013; Borruto et al., 2014). Results are generally good, with severe lung hypoplasia uncommon and improvement in symptoms without recurrence. However, infants with associated conditions, particularly neuromuscular disorders, may not survive. Some surgeons advocate plication, even when symptoms are not present, to preserve potential for lung growth (Tsugawa et al., 1997).

Diaphragmatic Paresis

Phrenic nerve injury because of birth trauma or surgical trauma can result in temporary paresis of the diaphragm, with an elevated hemidiaphragm and paradoxical movement evident by ultrasound or fluoroscopy (Fig. 49.14). If infants are persistently symptomatic, diaphragmatic plication may allow for weaning from mechanical ventilation or to lesser levels of respiratory support, but these injuries should also recover on their own over time (Wayne et al., 1974; Tsugawa et al., 1997).

Neonatal Scimitar Syndrome

Scimitar syndrome consists of three findings of right lung hypoplasia, partial anomalous pulmonary venous return, and unilateral pulmonary sequestration. These features may not all be present, and additional cardiac anomalies, including hypoplasia of the left heart or aorta, may also be detected (Gao et al., 1993). Scimitar syndrome can be suspected on fetal ultrasound, because of findings of cardiac dextroposition with a small right pulmonary artery (Abdullah et al., 2000). The presentation of scimitar syndrome in the first days after birth is usually severe, with tachypnea and pulmonary hypertension (Gao et al., 1993; Huddleston et al., 1999). Some infants will present later with failure to thrive. Chest radiograph reveals an elevated right hemidiaphragm with the mediastinum shifted to the right, consistent with primary lung hypoplasia. The classic finding of the scimitar vein is often not appreciated. Because these infants have a normally developed diaphragm, the surgical approach will depend on hemodynamic effects of the anomalous venous return and the sequestration (if present). Thus careful hemodynamic and vascular assessment are critical and may be accomplished through cardiac catheterization and angiography or, in some cases, by magnetic resonance angiography. Some infants will require pneumonectomy, others ligation or coil embolization of systemic feeding vessels, and others no intervention because the hypoplastic lung may have very little blood flow. Pulmonary vein stenosis can also complicate the hemodynamic status, and medical or surgical treatment for additional cardiac lesions may need to be addressed.



• **Fig. 49.14** Diaphragmatic Paresis. M mode ultrasound images of a newborn with tachypnea and moderate respiratory distress. (A) The absence of right diaphragmatic excursions is consistent with complete diaphragmatic paralysis. (B) The left diaphragm has low-amplitude excursions with normal inspiratory and expiratory motion. (Courtesy of Dr. Chirag V. Patel, Assistant Professor, University of California, Davis Health System, Department of Radiology, Sacramento, CA.)

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50

Developmental Biology of the Heart

ELLEN DEES AND H. SCOTT BALDWIN

KEY POINTS

- The heart forms from cardiac mesoderm as a symmetric linear tube with connections to the primitive arterial and venous systems. The heart tube is formed by 3 weeks' gestation in the human.
- The heart tube loops, establishing laterality and undergoes septation into four chambers with connections to the systemic and pulmonary arteries and veins. The heart is fully formed by 9 weeks' gestation.
- External populations of cells migrate into the developing heart, making important contributions to the cardiac valves, coronary arteries, and cardiac chambers.
- Congenital heart malformations arise when these processes are altered or incomplete.

Overview of Cardiac Developmental Anatomy

This chapter will begin with a review of embryonic and fetal cardiac anatomy and of the cell types that make up the heart and will then discuss the morphology and physiology of the developing heart. We will mention certain genes that are important in cardiac development, although this is not a comprehensive review of the genetic regulation of heart development. Rather we will focus on aspects of heart development of particular interest to clinical neonatologists. These include the creation of inflow and outflow poles of the heart, alignment of these structures with the cardiac chambers, septation of the cardiac chambers, and the physiology of blood flow in the embryo, fetus, and newborn.

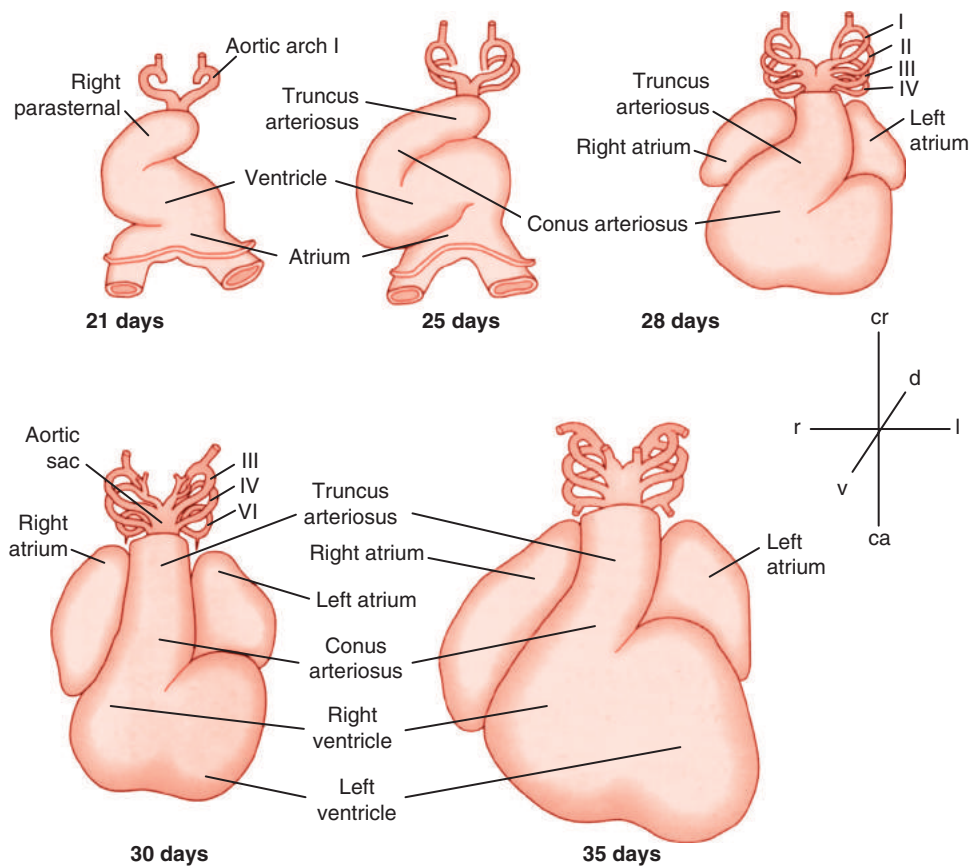
The heart begins as a field of cells (cardiac mesoderm) that moves ventrally and midline within the embryo, fusing into a tube. This primitive heart tube is formed in the human embryo by 3 weeks' gestation (Fig. 50.1, 21 days). At the posterior (dorsal) end of the tube is the venous pole, or sinus venosus. In mammals this includes connections to the yolk sac (vitelline veins), placenta (umbilical veins), and embryo proper (cardinal veins). At the anterior (ventral) end of the tube is the arterial pole, or bulbus cordis, which will pass flow from the heart tube back into the body. In the primitive heart this connection is to the aortic sac, which is connected to paired dorsal aortae. The heart tube itself is divided into an atrial segment adjacent to the venous pole, an atrioventricular

canal, and a ventricular segment adjacent to the arterial pole. Within the primitive heart tube, faint constrictions can be seen between these segments. Each of these segments is histologically and functionally distinct from the beginning: atrial and ventricular myocytes have distinct characteristics even when isolated from the primitive mesoderm. By the time the heart tube is formed, differentiating cardiomyocytes have generated functional contractile units mature enough to begin spontaneous contractions. These are initially peristaltic in nature, moving blood from venous inlet to arterial outlet.

Thus as soon as the heart forms, its primitive connections to the body are in place and its contractile function is operational, such that it pumps fluid to the rapidly growing embryo. Red blood cells begin to enter the circulation from blood islands in the yolk sac by the time the heart starts to beat at 22 days' gestation (Carlson, 2004). For the first 6 weeks the yolk sac remains the exclusive source of hematopoietic cells, until the liver (and to a lesser extent spleen) takes over. The bone marrow gradually becomes populated with hematopoietic cells beginning in the second trimester of pregnancy and by birth is the major source of blood cells (Carlson, 2004). Development of a circulatory system early is critical to maintaining nutrition and oxygen delivery to developing tissues, which are at this point beyond the ability to depend on simple diffusion of nutrients.

From the primitive heart tube state the heart undergoes significant growth and morphologic alterations. These include establishing laterality, such that the symmetry of the heart tube is lost and there are well-defined left and right structures within the heart and vessels. As laterality is being established the heart undergoes a dramatic change in shape, via a process known as *cardiac looping*. This occurs in the human embryo during week 4 of gestation (Fig. 50.1, 25–28 days). From this stage, the heart realigns its inflow and outflow segments, undergoes septation, and forms valves in processes that will be detailed in this chapter. The “final product” will be a mature heart (Fig. 50.1, bottom) with the following structures:

1. A *venous pole* or *sinus venosus* that is now connected to systemic veins from the upper body (superior vena cava [SVC]), lower body (IVC), liver (hepatic veins), and coronary circulation (coronary sinus), all of which pass into the *right atrium*.
2. A separate *left atrium*, connected to the venous system separately by ingrowth of *pulmonary veins* from the lungs.



• **Fig. 50.1** (Top) Morphology of the human heart at 3 to 5 weeks' gestation. Orientation in the body as shown, with cranial–caudal (*cr*, *ca*), dorsal–ventral (*d*, *v*), and left–right (*l*, *r*) planes. The heart tube is initially linear, with the inflow region dorsal/caudal and the outflow region ventral/cranial. During week 4 the heart undergoes looping, realigning the segments such that the inflow is shifted dorsal/leftward, and the outflow is shifted ventral/rightward. After looping the atrial and ventricular segments are approximately on the same plane in the cranial/caudal axis. (Bottom) A scanning electron micrograph of a human heart at 9 weeks' gestation, after looping, and after septation of the atria, ventricles, and great arteries. ([Top] From Carlson BM. *Human Embryology and Developmental Biology*. 4th ed. Philadelphia, PA: Mosby; 2009:457. [Bottom] From Steding G. *The Anatomy of the Human Embryo*. Basel, Switzerland: S. Karger; 2009:215.)

3. An *AV canal* that begins as a single unrestricted opening and undergoes septation into two AV valves. The *tricuspid valve* opens from the right atrium into the right ventricle (RV), and the *mitral valve* opens from the left atrium into the left ventricle (LV).
4. A *primitive ventricle* that has expanded and undergone septation into two distinct and separate chambers, a *right* and an *LV*. By processes known as *compaction* and *trabeculation*, the working myocardium of each ventricle has become highly organized and adapted to the unique requirement of a pulmonary (right) or systemic (left) ventricle.
5. An *arterial pole*, or *bulbus cordis*, that forms the two separate outflow tracts of the heart. The distal part of the bulbus cordis is the *arterial trunk*, which undergoes septation in a spiral fashion, creating an anterior *pulmonary artery* connecting to the lungs and a posterior *aorta* connecting to the body. The proximal part of the bulbus cordis is the *conus*, which is retained by the RV. At the junction between the conus and the pulmonary artery, the *pulmonary valve* forms. The *aortic valve* forms at the junction between the LV and the aorta.
6. Paired *dorsal aortae* connected to the *aortic sac* that have undergone extensive remodeling into a single leftward *aortic arch* and *descending aorta*. This connects proximally to the *coronary arteries*, the head and neck arteries, and to a temporary structure important in fetal life, the *ductus arteriosus*.
7. Networks of coronary arteries that have formed within the myocardium of the heart. These connect to the aorta as the right and left coronary arteries.
8. Networks of myocytes that have differentiated into the conduction system of the heart, including the sinoatrial node, the AV node, and the His–Purkinje system of the ventricles.

Cell Types Within the Heart and Their Origins

The mature heart has three cell layers: the endocardium, an epithelial lining one cell layer thick; the myocardium, a precisely oriented network of myocytes that perform the contractile work of the heart; and the epicardium, an epithelium that covers the external surface of the heart. Beyond this the heart resides within a pericardial sac, similar in composition and function to the pleura, which covers the lungs.

Much, but not all, of the heart has its embryonic origin from a field of lateral plate mesodermal cells referred to as the *cardiogenic mesoderm* (Fig. 50.2; orange/red). The primitive heart tube forms as a two-layer structure, with an endocardium and a myocardium, both cell types coming from cardiac mesoderm. The endocardium remains as an epithelium, but a subset of cells delaminates and invades the proteinaceous matrix between the endocardium and myocardium to form the endocardial cushions. These cells will be vital to proper valve development and to complete septation of the atria and ventricles. The myocardium primarily remains muscle, but a subpopulation of the cells also change course and differentiate into Purkinje fibers of the conduction system.

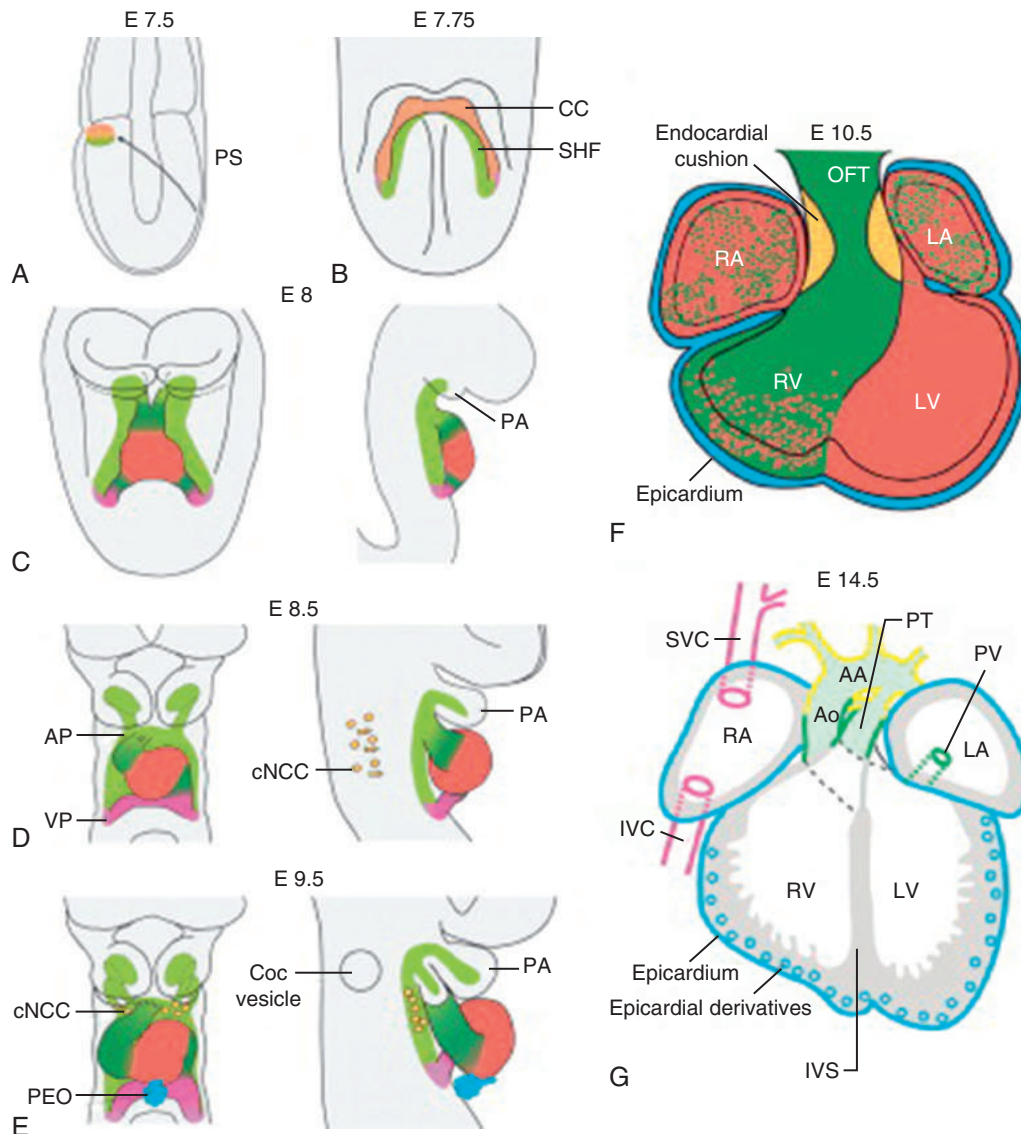
The outflow tracts of the heart are arguably the most complex genetically and morphologically and thus most prone to developmental defects. The secondary heart field is a population of cardiac mesoderm anterior and medial to the original heart field that migrates to the heart after formation of the primitive heart tube (see Fig. 50.2; green). It contributes significantly to the RV and outflow region of the heart, as well as to the ventricular septum

and probably parts of the atria (Mjaatvedt et al., 2001; Waldo et al., 2001; Kelly and Buckingham, 2002; Christoffels et al., 2006). The secondary heart field was primarily discovered in cell lineage tracing studies. In chick hearts marked just after fusion of the heart tube, a population of unmarked cells interposed themselves between marked cells of the distal part and middle part of the heart tube 1 day later (Mjaatvedt et al., 2001). Studies in the mouse compared with the chick suggest that the anterior heart field in the mouse forms most of the RV (Kelly and Buckingham, 2002; Zaffran et al., 2004). What differences there are in humans remain unknown. Genetically, the secondary heart field is characterized by markers, including *Tbx1*, *Isl1*, *Mef2c*, *Tbx20*, and *Aldh1a2*; knockout of any of these genes in the mouse causes outflow tract septation defects (Jerome and Papaioannou, 2001; Merscher et al., 2001; Niederreither et al., 2001; Cai et al., 2003; Dodou et al., 2004; Takeuchi et al., 2005). Further, it has been shown that as the secondary heart field cells move into the outflow tract of the heart, they lose many of their original genetic markers and express myocardial markers such as *Hand1*, *Nkx2-5*, *Tbx5*, *Gata4*, and *Mef2c* (Dodou et al., 2004; Verzi et al., 2005; Waldo et al., 2005b; Ward et al., 2005; Zeisberg et al., 2005; Dyer and Kirby, 2009). These are factors already well studied in the primary heart field known to contribute to myogenesis and cardiac looping (Srivastava et al., 1997; Hiroi et al., 2001; Jamali et al., 2001b; Fijnvandraat et al., 2003; Garg et al., 2003; Han and Olson, 2005; McFadden et al., 2005; Han et al., 2006; Maitra et al., 2009). Defects in expression of these genes in the secondary heart field can lead to right-ventricular hypoplasia and outflow tract abnormalities, including tetralogy of Fallot (Ward et al., 2005; Zeisberg et al., 2005).

In addition to cells from the secondary heart field, neural crest cells are an important population that migrate into the developing outflow tract (see Fig. 50.2; yellow). Neural cells originate in the anterior rhombencephalon and migrate as a sheet through the pharyngeal region and into the aortic arches (Waldo and Kirby, 1993; Waldo et al., 1996; Creazzo et al., 1998; Epstein et al., 2000; Li et al., 2003). The neural crest cells surround the epithelia of the arch arteries and extend into the truncus and proximal conus. Here they interact with endocardial cushion cells to cause the great arteries to undergo septation and close the conal septum (Kirby et al., 1983). These neural crest cells are also important to development of the nearby parathyroid, thyroid, and thymus glands. They also innervate the heart and form much of the smooth muscle of the proximal aorta (Creazzo et al., 1998).

Yet another external population of cells that makes important contributions to the mature heart is a cluster of cells that forms dorsal and inferior to the heart tube known as the *proepicardial organ* (see Fig. 50.2; blue) (Nahirney et al., 2003; Ishii et al., 2007). The origin of these cells is a subject of debate; one leading theory is that they are derived from liver primordium (Ishii et al., 2007). These cells expand as an epithelial sheet covering the surface of the heart to form the epicardium. From the epicardium, subgroups of cells delaminate and migrate into the myocardium beneath in a process known as *epithelial to mesenchymal transformation*. These cells differentiate into vascular smooth muscle, vascular endothelial cells of the coronary arteries, and cardiac fibroblasts, which make up a sizable population of cells residing within the myocardium, between myofibers.

Thus in addition to cardiomyocytes arising from at least two populations of mesoderm, the heart is composed of cells from epithelial and neural crest origins that migrate in with spatial and temporal precision during cardiac development. The next several sections will highlight the steps of this process.

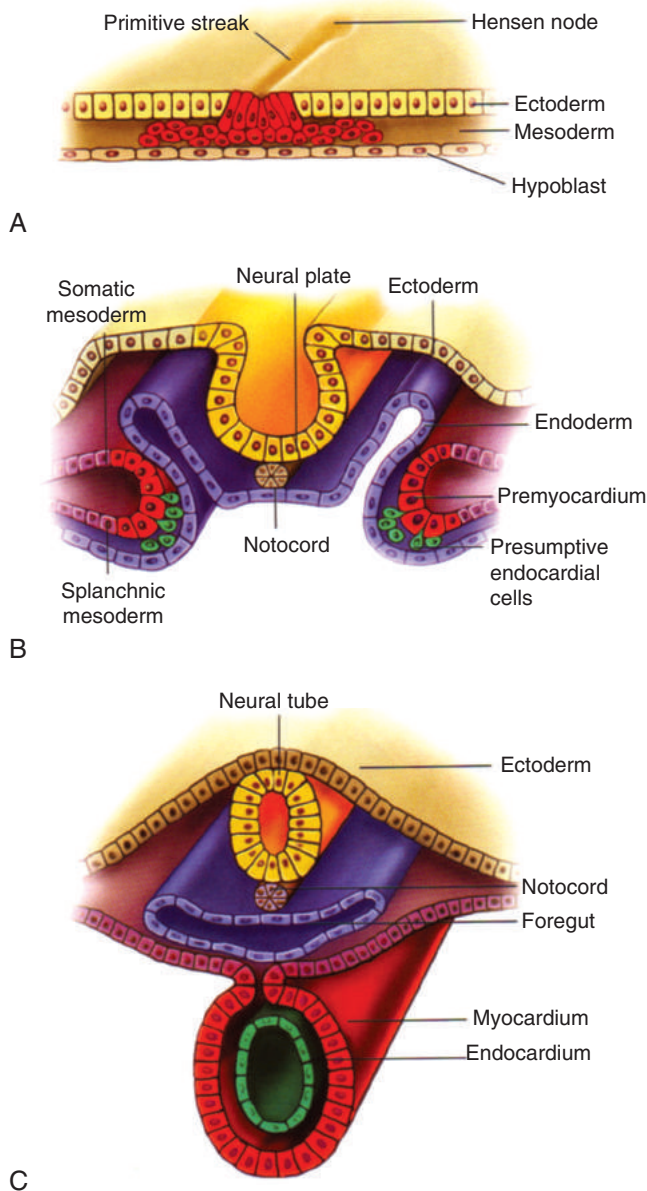


• **Fig. 50.2** The cell types of the heart. The cardiac crescent (first heart field, *orange*) forms as cells migrate anteriorly from the primitive streak at gastrulation. The second heart field (*green*) forms medial to the cardiac crescent (A, B). These cells go on to form the four chambers of the heart and inflow and outflow tracts as shown (C–F); primary heart field derivatives are in *red* and secondary heart field derivatives in *green*. There are also important contributions to the developing heart from cardiac neural crest cells (*yellow*) and the proepicardium (*blue*) (G). Embryonic stages shown are for the mouse. AA, Aortic arch; Ao, aorta; AP, arterial pole; CC, cardiac crescent; cNCC, cardiac neural crest cells; E, embryonic day; IVC, inferior vena cava; IVS, interventricular septum; LA, left atrium; LV, left ventricle; OFT, outflow tract; PA, pharyngeal arch; PEO, proepicardial organ; PS, primitive streak; PT, pulmonary trunk; PV, pulmonary vein; RA, right atrium; RV, right ventricle; SHF, secondary heart field; SVC, superior vena cava; VP, venous pole. (From Vincent S, Buckingham M. How to make a heart: the origin and regulation of cardiac progenitor cells. *Curr Top Devel Biol.* 2010;90:1–41.)

Formation of the Embryonic Cardiac Crescent and Heart Tube

Most of what is known about the early stages of embryonic development comes from extensive studies of avian and mouse models (Garcia-Martinez and Schoenwolf, 1992, 1993; Melnik et al., 1995; Yutzey and Bader, 1995; Baldwin, 1996; Fishman and Chien, 1997; Baldwin, 1999; Redkar et al., 2001; de Lange et al., 2004; Abu-Issa and Kirby, 2007; Combs and Yutzey, 2009; Cui et al., 2009; Dyer and Kirby, 2009). This is extended, with

some acknowledged gaps, to human development. In its earliest stages the embryo exists as a bilayer disk of two epithelial sheets of cells suspended between two fluid-filled cavities, the yolk sac and the amniotic cavity. The ventral layer (facing the yolk sac) is the hypoblast, which will eventually be relegated to extraembryonic structures. The dorsal layer (facing the amniotic cavity) is the epiblast, which will actually form all three embryonic germ layers: the ectoderm (nervous system and skin), mesoderm (heart, skeleton, muscle, and connective tissue), and endoderm (gut). Mesoderm and embryonic endoderm separate from the primitive ectoderm



• **Fig. 50.3** Gastrulation and the formation of the three germ layers, A–C. Cardiomyocytes originate from mesodermal precursors (red and green). The heart tube fuses as it is displaced ventrally and to the midline by the folding gut tube (blue). (From Mikawa T. Cardiac lineages. In: Harvey R, Rosenthal N, eds. *Heart Development*. New York, NY: Academic Press; 1999:19–33.)

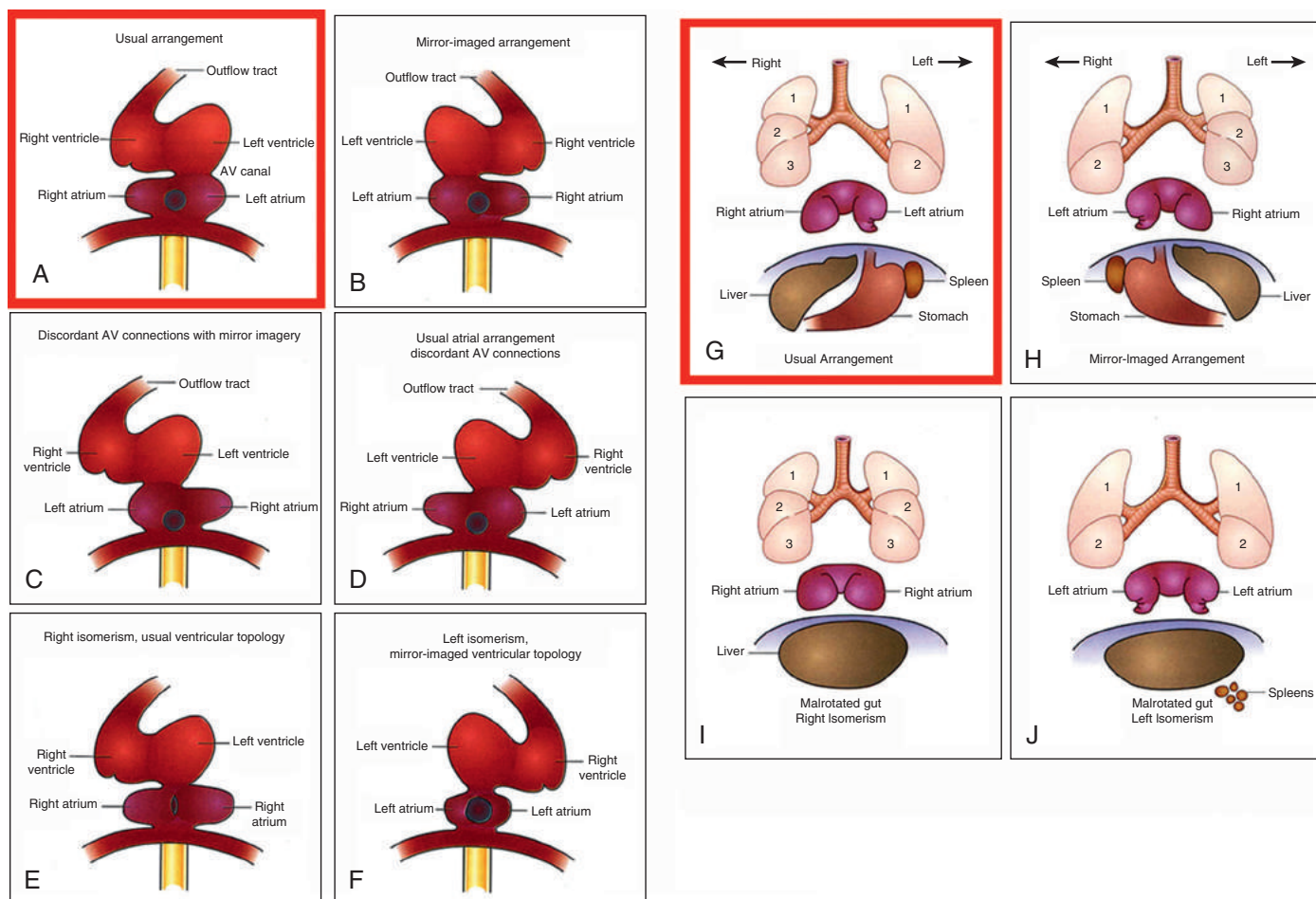
by a process known as *gastrulation* (Fig. 50.3A). Gastrulation begins as a groove forms in the epiblast starting at the tail end (caudal) and gradually extending cranially to an end point known as the prechordal plate. The groove is known as the *primitive streak*, and its leading edge is known as the *primitive node*. Along the primitive streak, epiblast cells delaminate from the epithelial sheet and invade the potential space between the epiblast and hypoblast. The heart forms as a crescent of cells cranial to the prechordal plate and extending along both sides. Fate-mapping studies suggest that many cell fate decisions may be set during gastrulation or even before (Garcia-Martinez and Schoenwolf, 1993; Fishman and Chien, 1997). For example, within the cardiac crescent, the apex of the crescent is formed from cells that have migrated through the

primitive streak closest to the primitive node and contains precursors of the outflow tract myocardium. The cells on either side of this region have migrated through the midportion of the streak and are ventricular myocyte precursors. Finally, the most lateral and caudal cells are those that have migrated through the most posterior part of the streak and will become atrial myocytes. The exact details of when and how this occurs are still under debate and refinement as better techniques become available (Ehrman and Yutzey, 1999; Abu-Issa and Kirby, 2007; Cui et al., 2009). Fate-mapping studies using live-cell tracking and time-lapse imaging demonstrate that these cells move as a cohort (tissue motion) rather than migrating individually; this is a way to maintain cells' relative positions within a tissue and, eventually, an organ (Cui et al., 2009).

Looping and Laterality of the Heart Tube

The cardiac crescent is a symmetric structure and fuses into an initially symmetric appearing heart tube. This occurs as the ectoderm dorsal to the crescent is folding to create the neural canal, and the endoderm initially ventral to the crescent is folding to create the foregut (see Fig. 50.3B). As the foregut forms, it moves dorsally, pushing the cardiac crescent ventrally and medially, such that the two arms of the crescent fuse into a tube sitting directly in front of the foregut (see Fig. 50.3C). As fusion of the heart fields occurs, the heart is already beating. In a human fetus this happens at approximately 22 days' gestation. During the next week there is tremendous growth of the head, such that the heart goes from being the most "cranial" structure in the embryo to a position tucked underneath the developing head of the embryo.

As the head grows up and over the heart tube, the heart tube itself begins to undergo a dramatic change in shape known as *looping*. Here the heart tube loses its symmetry, and distinct left and right morphology can be identified (Steding, 2009). Much is known about the molecular signaling and gene expression patterns involved in looping. The basic left–right asymmetry of the embryo is set during gastrulation by concentration gradients of the factors sonic hedgehog and fibroblast growth factor 8. This gradient is created by ciliary motion at the primitive node and causes a cascade of downstream genes to be activated, including *Nodal*, *Lefty1*, and *Pitx2* (Tsukui et al., 1999; Capdevila et al., 2000; Rodriguez-Esteban et al., 2001). Within the heart tube, several cardiac transcription factors have been shown to be important to looping, including *Nkx2-5*, myocyte enhancer factor 2, and heart and neural crest derivatives expressed 2 (Srivastava et al., 1997; Tsukui et al., 1999; Hiroi et al., 2001; Jamali et al., 2001a, 2001b; Yamagishi et al., 2001; Han and Olson, 2005; McFadden et al., 2005; Christoffels et al., 2006; Han et al., 2006; Karamboulas et al., 2006; Cui et al., 2009). Disruption of any of these factors in mice by gene knockout causes embryonic lethality, with a block in heart development at the looping stage. Morphologically, cardiac looping involves differential growth, with higher proliferation of myocytes along the outer curvature than along the inner curvature (Moorman and Christoffels, 2003), along with a substantial migration of cells from the secondary heart field (Kioussi et al., 2002). All of this serves to elongate the outflow tract and enlarge the ventricles relative to the atria (Moorman and Christoffels, 2003). In addition, there are mechanical forces pulling, twisting, and realigning structures of the primitive heart tube. This is thought to involve the cytoskeleton, including nonmuscle myosin (Linask and Vanauker, 2007), the motor protein dynein (Supp et al., 1997; Brueckner, 2001; Basu and Brueckner, 2008), microtubules (Icardo and Ojeda, 1984), and nonmuscle actin bundles (Itasaki et al., 1991).



• **Fig. 50.4** (A) Normal cardiac D-looping with normal atrioventricular (AV) connections. Also shown are several common abnormal looping patterns, including mirror-image L-looping with normal AV connections (B), discordant AV connections with atrial situs inversus (C), and discordant AV connections with normal atrial situs (D). Both discordant AV connections with atrial situs inversus and discordant AV connections with normal atrial situs are usually associated with discordant ventriculoarterial connections, creating congenitally corrected transposition. Right- and left-atrial isomerism, or bilateral right sidedness (E) and bilateral left sidedness (F), generally found along with anomalies of the systemic and pulmonary veins, of cardiac septation, and of the inflow and outflow tracts. Right and left isomerization syndromes also go along with abnormal sidedness on other asymmetric organs of the chest and abdomen, or heterotaxy. (G) Normal visceral organ arrangement, (H) mirror image, or situs inversus, (I) right isomerism, and (J) left isomerism. The numbered segments 1,2 and 1,2,3 refer to the lobes of the left and right lungs. (Modified from Brown NA, Anderson RH. Symmetry and laterality in the human heart: developmental implications. In: Harvey R, Rosenthal N, eds. *Heart Development*. New York, NY: Academic Press; 1999:447–461.)

Normal looping occurs to the right (D-looping); that is, the sinus venosus and atria move toward the left and posteriorly, and the ventricles and bulbus cordis move to the right and anteriorly. Importantly, the venous pole also moves cranially such that it lines up with the arterial pole along the horizontal body axis. This is seen in Fig. 50.4A. The bulbus cordis remains to the right of the primitive ventricle. This repositioning is critical for proper AV and ventriculoarterial connections. Also with looping, an outer curvature and an inner curvature of the heart are established. The anterior outer curvature is an area of rapid myocardial growth and expansion to form the ventricular chambers. The posterior inner curvature undergoes slower growth and acts rather like a fulcrum for the looping process. The inner-curvature myocardium contributes to the AV canal and is critical to the formation of the endocardial cushions.

Abnormalities in Cardiac Looping

Sometimes, looping can occur to the left, with the venous pole and atria moving rightward and the ventricles and arterial pole moving leftward (see Fig. 50.4B). This is referred to as *L-looping*. If there is complete mirror imagery, the cardiac connections are completely normal with dextrocardia. Often, however, there are both AV and ventriculoarterial discordance, an entity referred to as *congenitally corrected transposition*. Here the atrial situs is usually normal, but the ventricles loop to the left, and the bulbus cordis ends up to the left of the primitive ventricle (see Fig. 50.4D). When the great arteries undergo septation, there is L malposition of the great arteries, so the aorta is to the left of the pulmonary artery (parallel, not crossed). The resulting circulation is as follows: right atrium to LV to pulmonary artery; left atrium to RV to aorta.

In isolation, this defect allows normal circulation, although with the RV, which is structured to pump to the low pressure pulmonary circulation, as the systemic ventricle. Alternatively, this physiology can occur with atrial situs inversus and D looping of the ventricles with D malposition of the great arteries (see Fig. 50.4C). Often there are additional cardiac defects, ranging from a ventricular septal defect (VSD) with pulmonary valve stenosis to more complex defects involving hypoplasia of one of the ventricles or hypoplasia, aplasia, or malformation of one or more valves.

L-looping may also be associated with more global defects known as *heterotaxy syndromes*. Heterotaxy syndromes are a heterogeneous group of congenital defects, with the commonality being abnormal left–right asymmetry of the embryo (Ghosh et al., 2009). In heterotaxy, left–right assignments along the body axis are randomized. Thus it is possible to have mirror-image asymmetry, or bilateral symmetry, with either two right sides or two left sides. This is often termed *right isomerism* or *left isomerism* (see Fig. 50.4E–F and I–J). This can affect the abdomen, lungs, and heart. In the abdomen the gut, liver, and spleen may be reversed from normal (abdominal situs inversus). There may be malrotation of the bowel and anomalies of the biliary tree. The spleen, being a normally left-sided structure, can be duplicated in left isomerism (polysplenia) and absent in right isomerism (asplenia). Asplenic patients require antibiotic prophylaxis in childhood to protect against bacterial infections. Asplenic patients usually have left isomerization of the lungs and heart as described later; polysplenic patients have right isomerization of the lungs and heart. Lung abnormalities in heterotaxy manifest themselves in the bronchial and lung anatomy. The normal left lung is bilobed, and the left main bronchus travels beneath the left pulmonary artery (hyparterial). The normal right lung is trilobed, and the main bronchus travels above the right pulmonary artery (eparterial). Thus careful examination of a chest X-ray in left isomerism reveals bilateral hyparterial bronchi and in right isomerism shows bilateral eparterial bronchi.

In the heart the atrial sidedness is randomized in heterotaxy. Here the atria may be normal (situs solitus), left–right reversed (situs inversus), or unclear (situs ambiguous; including bilateral right atria or bilateral left atria). The atria are most practically defined by their venous return, systemic to the right atrium and pulmonary to the left. But in heterotaxy this is not always possible, as the pulmonary or systemic veins may return to both atria. The pulmonary veins grow in from the developing lungs normally joining the back wall of the left atrium. When the left–right cues are scrambled, the pulmonary veins often return ipsilaterally: left-sided veins to left-sided atrium and right-sided veins to right-sided atrium. Likewise, the systemic veins may return to either atrium or both atria in heterotaxy (e.g., a right SVC to the right atrium and a left SVC to the left atrium). This is because the systemic veins start out as paired symmetric structures (see [Systemic and Pulmonary Vein Development](#)), with involution of certain structures occurring as part of the establishment of left–right asymmetry in the embryo. Finally, certain patterns of additional cardiac defects often accompany asplenia/right isomerism. These include failure of septation of the ventricles, resulting in a single ventricle, pulmonary underdevelopment (stenosis or atresia of the pulmonary valve), bilateral superior venae cavae, and anomalous pulmonary venous return. For polysplenia/left isomerism, commonly associated defects include endocardial cushion defects (see later), left-sided obstructive lesions such as coarctation of the aorta, and interruption of the inferior vena cava (IVC) with azygous continuation to the SVC.

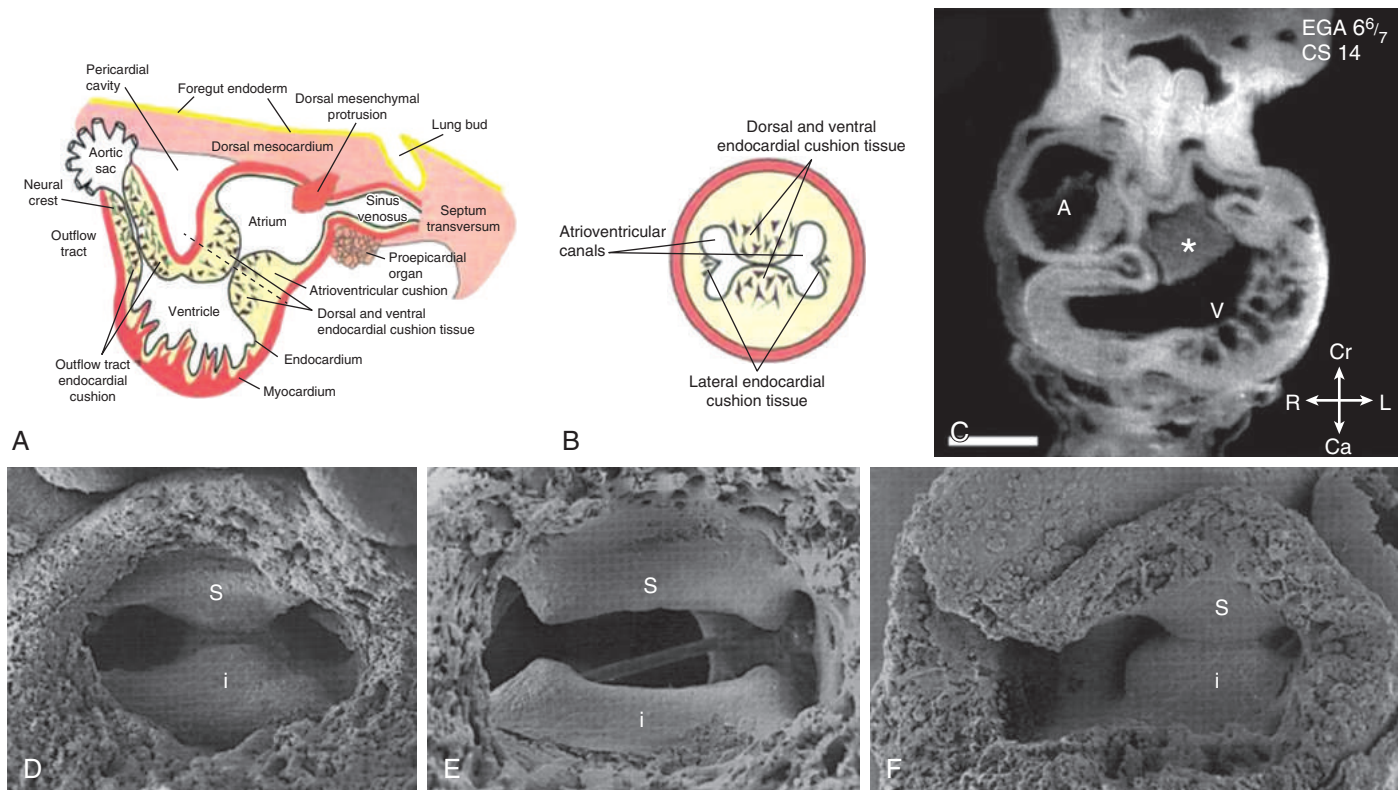
Ventricular Inlet Septation: Endocardial Cushions

After cardiac looping has occurred, the orientation of the heart tube has changed drastically, but the progression of blood flow through it remains in essentially the same sequence: into the heart from the venous pole, through the common atrium into the common ventricle, and out the conotruncus to the aorta. Inflow and atrial segments are leftward, and the ventricle and outflow are to their right. If the process arrests near this point, a heart may form with its entire inlet portion aligned over the leftward ventricle (a double-inlet LV) or with its entire outlet portion aligned over the rightward ventricle (a double-outlet RV). Both of these entities are seen in humans. What is very rarely seen is a double-inlet RV, or a double-outlet LV: even if there is L-looping and/or dextrocardia, this basic sequence is maintained.

During the next phase of heart development, the AV (inlet) and ventriculoarterial (outlet) structures will realign and undergo septation, such that there is a valved inlet and a valved outlet for each ventricle. In human embryos this occurs during week 7 of gestation (Dhanantwari et al., 2009; Steding, 2009). These valves are formed primarily from the endocardial cushions. The endocardial cushions are also critical to complete ventricular and atrial septation, completing the portions of septum adjacent to the AV valves. This will be detailed further in sections on atrial and ventricular septation. Thus the endocardial cushions form the crux of the heart: a point in the center of the heart at which separate AV canals are to the left and right, and atrial and ventricular septa are aligned above and below.

Within the primitive heart tube, even before looping, there are faint constrictions at the AV groove and in the forming conotruncus (see Fig. 50.4). The endocardial cushions initially appear as swellings in the AV and conotruncal segments of the primitive heart, by 6 weeks' gestation (Fig. 50.5A and C). The swellings are caused as cells from the inner lining of the heart (endocardium) delaminate and migrate into the extracellular matrix in between the endocardium and the myocardium. Signaling involving the transforming growth factor β (TGF- β) family between the myocardium and endocardium plays a crucial role in initiating and maintaining this process (Brown et al., 1996, 1999; Jiao et al., 2006). The delaminating cells change phenotype in a process known as *epithelial to mesenchymal transformation*. The cells lose their epithelial, or sheetlike, characteristics and acquire a mesenchymal phenotype, losing junctions with neighboring cells, invading the matrix, and proliferating faster (Kim et al., 2001; Lincoln et al., 2004; Shelton and Yutzev, 2007; Combs and Yutzev, 2009). Why some cells respond to such signals to undergo epithelial to mesenchymal transformation but neighboring cells remain epithelial is unclear.

In both the AV canal and the conotruncus, four distinct cushions form, named by their anatomic locations. For the AV canal these are superior (ventral), inferior (dorsal), left lateral, and right lateral (see Fig. 50.5B). In the conotruncus they are right superior, right dorsal, left inferior, and left ventral. The AV and conotruncal cushions are separate, with one important exception: the left ventral conal cushion and the superior AV cushion are in continuity along the inner curvature of the heart (see Fig. 50.5A). This proximity will persist in the fully septate heart as aortic–mitral valve continuity. The AV canal undergoes septation as the superior and inferior cushions grow and extend (see Fig. 50.5D–F), finally fusing in the midline. This results in complete separation of the AV canal into left (mitral) and right (tricuspid) sides. The AV valves form from remnants of the cushions: a leftward mitral valve with two leaflets and a rightward tricuspid valve with three leaflets.



• **Fig. 50.5** Formation of the endocardial cushions. (A) Cushion formation in the inflow and outflow tracts by epithelial to mesenchymal transition in the looped heart tube. Also shown are the neighboring tissues and cell populations contributing to the developing heart at this stage. (B) Atrioventricular canal undergoing septation. (C) An episcopic fluorescence image capture image of a sectioned paraffin-embedded human embryo at 6½ weeks. Seen are atria (A), ventricles (V), and atrioventricular cushions (asterisk). Scale bar 0.515 mm. (D–F) Scanning electron micrographs of human embryos at 6 weeks' gestation, showing the superior (S) and inferior (i) atrioventricular cushions, with the tricuspid (left) and mitral (right) orifices. Ca, Caudal; Cr, cranial; L, left; R, right. ([A, B] From Schoenwald GC, Bleyl SB, Brauer PR, Francis-West PH. *Larsen's Human Embryology*. 5th ed. Philadelphia, PA: Churchill Livingstone; 2015:267–303. [C] From Dhanantwari P, Lee E, Krishnan A, et al. Human cardiac development in the first trimester: a high-resolution magnetic resonance imaging and episcopic fluorescence image capture atlas. *Circulation*. 2009;120:343–351. [D–F] From Steding G. *The Anatomy of the Human Embryo*. Basel, Switzerland: S. Karger; 2009:221.)

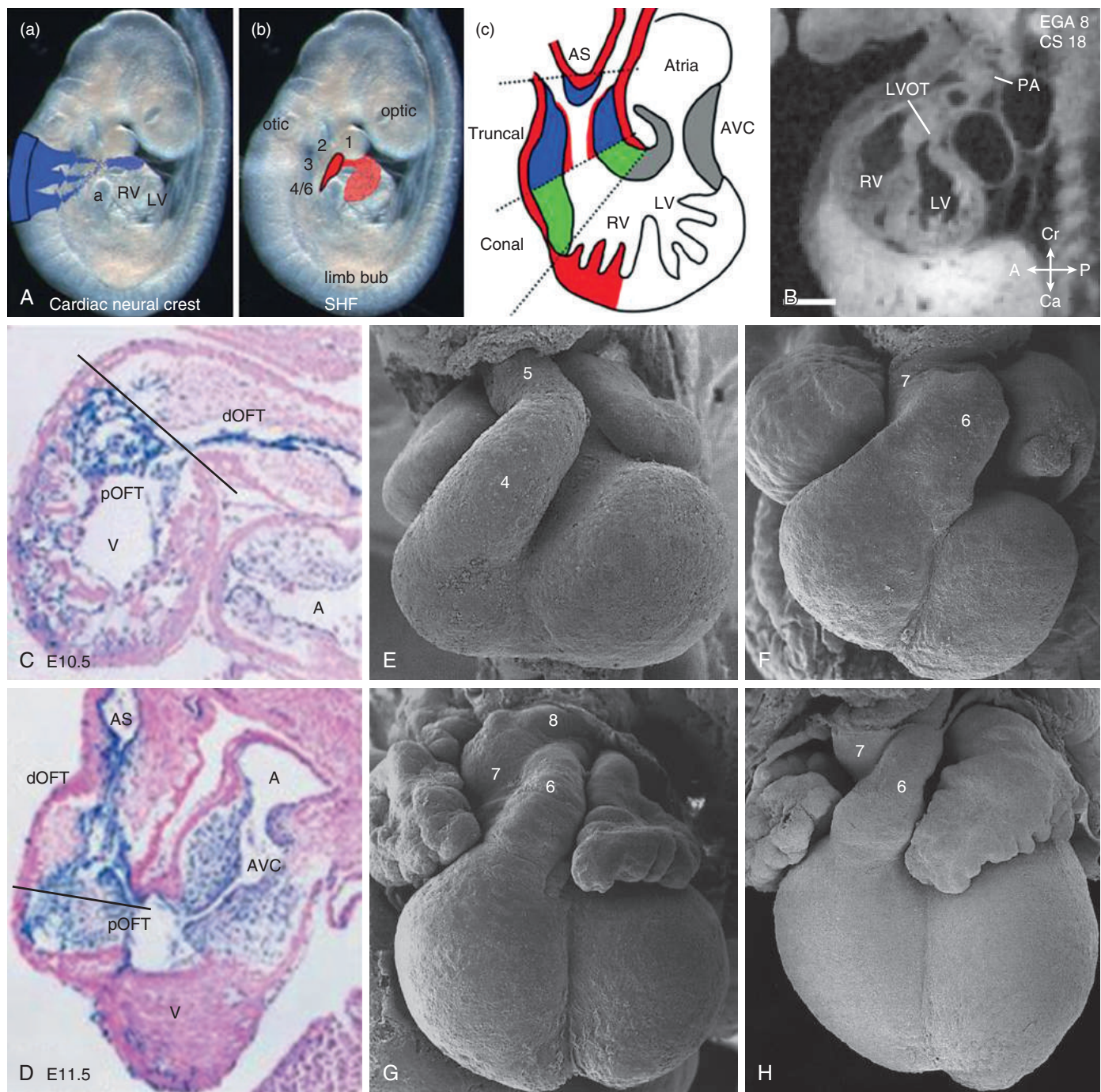
Failure of complete fusion between the superior and inferior cushions in the midline results in a cleft within the anterior leaflet of the mitral valve. This is a mild form of an endocardial cushion defect, often associated with a primum atrial septal defect (ASD) (see [Development of the Atria and Atrial Septum](#)) and known as a *partial AV canal*. The septal leaflet of the tricuspid valve also forms from these superior and inferior cushions and can be abnormal as well although this is less often clinically significant. More profound failure of proper AV cushion expansion and fusion results in a complete AV canal. Here the crux of the heart is unformed. There remains a common orifice overlying both ventricles, with defects in the adjacent atrial and ventricular septa. The common AV valve has a leaflet structure based on the nonseptate AV canal, with four or sometimes five separate leaflets corresponding to the two lateral leaflets, the superior (referred to as *anterior bridging*) and inferior leaflets.

The AV canal myocardium, part of the primitive heart tube, does not persist in the adult heart. There is no muscular connection between the mature atria and ventricles. The process for isolation of the atrial and ventricular muscle appears to occur as the

epicardium of the AV sulcus establishes continuity with the developing endocardial cushions beneath; this occurs all along the ventricular margin of the AV canal (Wessels et al., 1996). The only remaining connection that remains between the atria and the ventricles is within the conduction system, the AV node (Anderson and Ho, 1998; Anderson et al., 2000; Anderson and Ho, 2003). Occasional muscular bridges of tissue remain as accessory muscle connections, which can be clinically important as a substrate for arrhythmias (specifically AV reciprocating tachycardia, a common form of supraventricular tachycardia).

Ventricular Outflow Tract Septation: Endocardial Cushions and Neural Crest

While the AV cushions form a three-dimensional crux of the heart, the conotruncal cushions form a three-dimensional spiral, completing an almost 180-degree rotation (Ya et al., 1998; Carlson, 2004). During septation of the outflow tracts, the primitive bulbus cordis is separable into two segments, the truncus adjacent to the aortic



• **Fig. 50.6** Septation of the outflow tracts. (A) Contribution of neural crest (blue, *a*) migrating into secondary heart field (SHF; red, *b*) in developing outflow tracts in the mouse embryo; the arches are numbered 1–6. The schematic (*c*) shows the extent of these tissues, as well as the contribution from conal endocardial cushions (green). (B) Episcopic fluorescence image capture image of an embryo at 8 weeks' gestation showing left ventricle (LV) and developing left ventricular outflow tract (LVOT) and the anterior right ventricle (RV) and a portion of the pulmonary artery (PA). Scale bar 1.35 mm (C, D). Tie2 Cre LacZ staining in mouse embryos showing extent of endocardial cushions, first in the conal myocardium at embryonic day 10.5 and extending into the outflow tracts undergoing septation by embryonic day 11.5. (E–H) Septation of the outflow tracts, as viewed externally by scanning electron microscopy. 4, bulbus cordis; 5, aortic sac; 6, pulmonary trunk; 7 ascending aorta; 8, descending aorta. (E) A human embryo at 5 weeks' gestation; the conus and truncus of the common outflow tract are labeled. (F) An embryo at 6 weeks' gestation. (G, H) An embryo at 7 weeks' gestation and 8 weeks' gestation, respectively. Now completely separate pulmonary (P) and aortic (A) outflow tracts are identified. A, Anterior in (B), atrium in (C, D); AS, aortic sac; AVC, atrioventricular canal; Ca, caudal; Cr, cranial; dOFT, distal outflow tract; E, embryonic day; pOFT, proximal outflow tract. ([A] From Neeb Z, Lajiness JD, Bolanis E, Conway SJ. Cardiac outflow tract anomalies. *Wiley Interdiscip Rev Dev Biol.* 2013;2:499–530. [B] From Dhanantwari P, Lee E, Krishnan A, et al. Human cardiac development in the first trimester: a high-resolution magnetic resonance imaging and episcopic fluorescence image capture atlas. *Circulation.* 2009;120:343–351. E–H, modified from Steding G. *The Anatomy of the Human Embryo.* Basel, Switzerland: S. Karger; 2009:211, 213–215.)

sac and the conus adjacent to the ventricular myocardium (Fig. 50.6A). The right superior and left inferior cushions are within the truncal region and will contribute to the aorticopulmonary septum. The right dorsal and left ventral cushions are within the conus region and are important for pulmonary and aortic valve formation and for completion of septation between the ventricles at the level of the pulmonary and aortic valves (Waldo et al., 2005b; Brown and Baldwin, 2006). The interface between the two sets of cushions is a critical region for formation and remodeling of the ventricular outflow tracts (Waldo et al., 2005a; Brown and Baldwin, 2006; Wu et al., 2011).

Again for this process to occur correctly, the continuity of the superior AV cushion and left ventral conus cushion is critical. To align the septate great arteries directly over the ventricles, the arterial trunk must shift to the left. There is evidence from mouse models that failure to achieve continuity of the superior AV cushion and left ventral conus cushion results in malalignment of both great arteries over the RV, along with a VSD (van den Hoff et al., 1999; Waller et al., 2000). Clinically, this is an entity known as *double-outlet right ventricle* (DORV). Part of the clinical definition of a DORV is the presence of a conus segment of both ventricles (bilateral conus). Anatomically, the normal conus is a muscular “neck” within the RV between the tricuspid and pulmonary valves. Embryologically, this is a remnant of the conus of the primitive heart tube that is retained by the RV. In the LV the fusion of the superior AV and left ventral conal cushions results in mitral–aortic continuity and lack of a conal segment. If this fusion of cushions and leftward shifting of the truncus do not occur, then a conus is retained in the left-ventricular outflow tract, which is malpositioned to the right.

In addition to their role in development of the conus of the heart and the semilunar valves, neural crest cells play a more global role in development of the head and neck structures. Migrating neural crest cells are also important to development of the parathyroid, thyroid, and thymus glands (Creazzo et al., 1998). Extensive work has been done in chick models with use of neural crest ablation, and these show a very high prevalence of persistent truncus arteriosus, interrupted aortic arch, tetralogy of Fallot, double-outlet RV, and VSDs, along with abnormal parathyroid, thyroid, and thymus development (Nishibatake et al., 1987; Waldo and Kirby, 1993; Kirby and Waldo, 1995; Waldo et al., 1996; Creazzo et al., 1998). In human DiGeorge syndrome, cardiac conotruncal and arch defects are associated with developmental defects in each of these three glands as well. A deletion of up to 3 megabases in chromosome 22 (22q11) has long been known to be associated with DiGeorge syndrome, on the basis of kindred studies in families with the syndrome (Epstein, 2001; Merscher et al., 2001; Beaujard et al., 2009; Portnoi, 2009). In humans the defect is autosomal dominant, with variable penetration, meaning that one copy of the gene deletion is sufficient to cause disease and that the disease severity varies from family member to family member. A similar deletion generated experimentally in mice yielded a model of the cardiovascular features of DiGeorge syndrome (Lindsay et al., 2001). *Tbx1* has been identified as the gene responsible in these mice (Jerome and Papaioannou, 2001; Lindsay et al., 2001; Merscher et al., 2001). T-box 1 is a transcription factor in a family known to be important in embryonic patterning. Homozygous mutations of *Tbx1* caused obliteration of the third, fourth, and sixth pharyngeal arches with embryonic lethality and caused most of the extracardiac defects as well, along with an abnormal ear, jaw, and pharynx (Jerome and Papaioannou, 2001). Heterozygous mutants, which are analogous to the human disease state, showed more variable

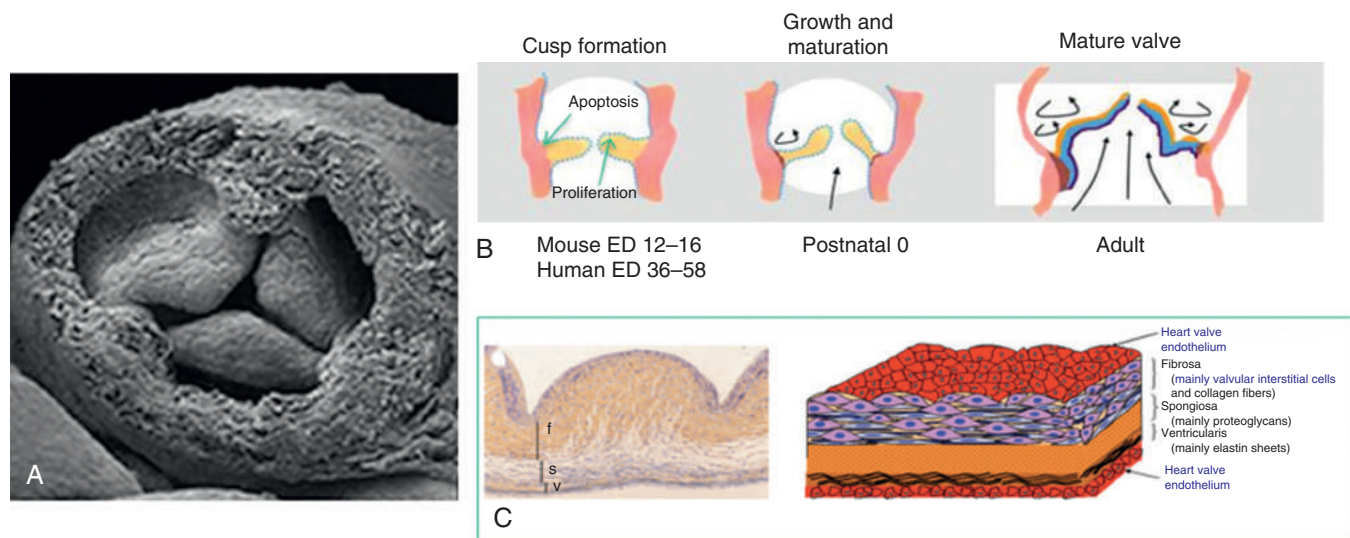
disease. The cardiac defects primarily affected the fourth pharyngeal arch and caused abnormal patterning of the great arteries in 50% of embryos in one study (Lindsay et al., 2001). This group had one heterozygous mouse mutant among 14 that also exhibited parathyroid and thymic insufficiency. Possibly, humans are more sensitive to gene dosage and exhibit the full range of defects in the haploinsufficient state. There may be other modifying genes as well, as there seems to be an important contribution of genetic background to the phenotype in mice, with more severe arch anomalies occurring in more inbred strains (Jerome and Papaioannou, 2001). This is a confounding factor of many gene targeting strategies and perhaps a clue as to why significant phenotypic variation can occur in humans with identical mutations. Isolated *TBX1* mutations appear to be very rare as causes of clinical DiGeorge syndrome (Beaujard et al., 2009; Portnoi, 2009; Rauch et al., 2009), most patients having a larger megabase deletion. Furthermore, *TBX1* expression is not found in neural crest cells themselves but rather in the secondary heart field, further highlighting the importance of interaction between these two cell populations in outflow tract development (Vitelli et al., 2002; Liao et al., 2008). Overexpression of *Tbx1* in mouse models is also associated with conotruncal defects, suggesting that there are critical levels of *Tbx1* expression necessary for proper outflow tract development (Liao et al., 2004; Liao et al., 2008).

Separation of Aorta and Pulmonary Artery: Normal Dextroposition

Within the truncus segment, septation also occurs during remodeling of the conus. This normally occurs as the truncal cushions fuse in a spiral fashion. Starting at the level of the AV valves, the newly forming pulmonary artery is directly anterior to the aorta. Further along the outflow tract, the pulmonary artery and aorta are more left–right to one another, as the spiral extends. As the aorta arches to the left, the proximal transverse arch passes anterior to the pulmonary artery, completing a 180-degree turn of the spiral. This sequence is seen in Fig. 50.6C–F. This rotation involves not only the truncal cushions but also the rotation of the myocardial tube (Lomonico et al., 1986; Bajolle et al., 2006). If this 180-degree twist does not occur or is incomplete, the result is transposed great arteries. This is defined by the aortic valve being anterior to the pulmonary valve. The left–right orientation of the valves is most commonly preserved (aortic rightward), although it can be within a 90-degree range from directly anterior–posterior to directly side-by-side. L-transposition, aortic leftward, is most often associated with L-looped ventricles or heterotaxy syndromes and is rare in isolated transposition. TGF- β signaling, important in left–right patterning and extracellular matrix remodeling and many other processes in normal development, appears to play an important role in this process both in mouse models and in human disease (Oh and Li, 1997; Rankin et al., 2000; Goldmuntz et al., 2002; Karkera et al., 2007).

Cardiac Valve Formation

Cardiac valve development begins with the endocardial cushions during week 7 of gestation, but formation of mature AV valves (mitral and tricuspid) and semilunar valves (aortic and pulmonic) takes several weeks for completion (de Lange et al., 2004; Lincoln et al., 2004; Kanani et al., 2005; Hinton et al., 2006; Combs and Yutzey, 2009; Dhanantwari et al., 2009; Steding, 2009). As the



• **Fig. 50.7** (A) Semilunar valve formation, as seen by scanning electron microscopy. This is a transverse section through the pulmonary trunk, revealing the pulmonary valve leaflets in a human embryo at week 9. (B) Semilunar valve remodeling showing thinning and elongation of the valves. (C) Layers of the mature valve, in histologic section and as a schematic with layers and their compositions as labeled. ED, Embryonic day; f, fibrosa; s, spongiosa; v, ventricularis. ([A] From Steding G. *The Anatomy of the Human Embryo*. Basel, Switzerland: S. Karger; 2009:239. [C] From Schoen, FJ. Evolving concepts of cardiac valve dynamics: the continuum of development, functional structure, pathobiology, and tissue engineering. *Circulation*. 2008;118:1864–1880; and Kidane AG, Burriesci G, Cornejo P, et al. Current developments and future prospects for heart valve replacement therapy. *J Biomed Mater Res B Appl Biomater*. 2009;88:290–303.)

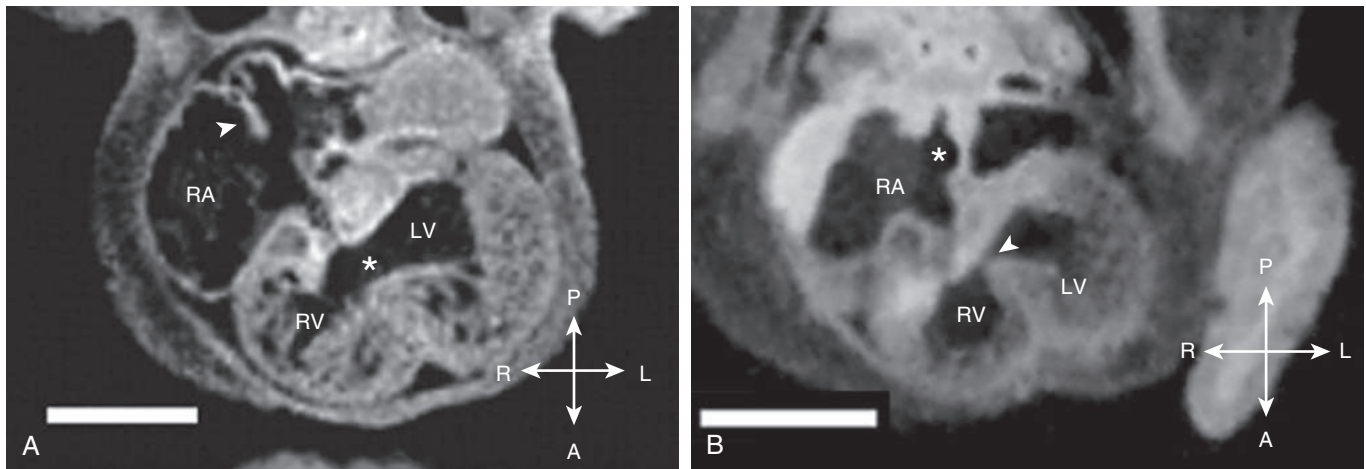
endocardial cushions enlarge, they protrude into the lumen of the heart, as shown in Fig. 50.7A–B. Here, subject to constant flow of blood, the protrusions condense and elongate. Endocardial cells overlying the cushions proliferate slowly, and there is programmed cell death (apoptosis) of mesenchymal cells underneath. The valves undergo extensive remodeling of the extracellular matrix; in the case of the semilunar valves, this results in a fibrous layer (primarily collagen) on the arterial side, a spongiosa layer (glycosaminoglycans) in the middle, and an elastic layer (elastin) on the ventricular side (see Fig. 50.7C; Hinton et al., 2006; Shelton and Yutzey, 2007; Combs and Yutzey, 2009). In the case of the semilunar valves, this remodeling of the endocardial cushions continues through the last trimester of pregnancy and into the neonatal period (Aikawa et al., 2006; Hinton et al., 2006).

Recent studies have shed some light on the genetics of cardiac valve disease. For example, Noonan syndrome is characterized by cardiac malformations, including a dysplastic pulmonary valve. Linkage studies of families with Noonan syndrome have identified a candidate gene on chromosome 12, the *PTPN11* gene (Tartaglia et al., 2001). *PTPN11* encodes SHP-2, a nonreceptor protein tyrosine phosphatase important to growth factors, cytokines, and hormones (Tartaglia et al., 2001). A specific role in mediating growth factors during semilunar valve formation had been proposed (Chen et al., 2000), and several human mutations in the gene were recently shown to cause overactivation of SHP-2 (Tartaglia et al., 2002). This suggests a gain-of-function mechanism for the pathogenesis of Noonan syndrome, an interesting distinction from the more familiar concept of the loss of functioning protein causing disease. Likewise, mutations in the Notch1 receptor and various components of the Notch signaling pathway have been associated with the formation of aortic valves and with multiple stages of cardiac development (Garg et al., 2005; High and Epstein, 2008).

Development of the Ventricles and Ventricular Septum

Most of the ventricular septum is muscular and is made up of a protrusion of ventricular myocardium that starts at the apex of the primitive ventricle and extends into the ventricular cavity. The ventricle in the early stage of septation appears as a bilobed structure (Dhanantwari et al., 2009; Steding, 2009); see Fig. 50.8. As the septum extends upward, the separation becomes nearly complete. There is controversy as to how the septum extends so quickly to form a wall between the left and RVs. Part of the answer seems to be rapid proliferation in these myocytes, which retain the ability to divide even as a working myocardium. This property is lost soon after birth, as the mature myocardium is for the most part incapable of proliferation. Small muscular VSDs are very common in newborns. One study screening asymptomatic newborns found an incidence of 53 per 1000 neonates with small muscular VSDs; only 1/10 of these had physical examination signs of their VSD. Nearly 90% of these small defects were closed by 10 months of age (Roguin et al., 1995). These data and other data suggest continued low-level proliferation in human ventricles after birth.

We have said that as the muscular septum grows and extends from the ventricular apex, the separation between the left and RVs becomes nearly complete. There are two important regions of the complete ventricular septum that are completed by endocardial cushions, as discussed earlier. First, the AV endocardial cushions form the posterior septum adjacent to the AV valves. Second, the conal cushions form the conal septum below the great arteries. An important feature of this process is that these regions of septum contain muscle as well as cushion tissue. Muscle cells of the inner curvature of the heart invade the conal cushion and superior AV cushions as they form the septum, in a process termed



• **Fig. 50.8** Atrial and ventricular septation. Human fetal magnetic resonance imaging images in the transverse plane, showing the four chambers of the heart. Orientation as shown by the arrows. (A) Fetus at 7½ weeks' gestation. The atrial septum primum is marked by the arrowhead, with the foramen primum below allowing shunting of blood from the right atrium to the left atrium. Between the ventricles, the muscular intraventricular septum is forming but is not yet complete (asterisk). Scale bar 1.25 mm. (B) Fetus at 8 weeks' gestation. Here the atrial septum primum (asterisk) is fused with the endocardial cushions inferiorly; not seen is the foramen ovale, which allows for continued right to left atrial shunting in the fetus. The arrowhead shows a small residual ventricular septal defect in the inlet (posterior) septum, not yet closed by endocardial cushion tissue. Scale bar 1.5 mm. A, Anterior; L, left; LV, left ventricle; P, posterior; R, right; RA, right atrium; RV, right ventricle. (Modified from Dhanantwari P, Lee E, Krishnan A, et al. Human cardiac development in the first trimester: a high-resolution magnetic resonance imaging and episcopic fluorescence image capture atlas. *Circulation*. 2009;120:343–351.)

myocardialization (Mjaatvedt et al., 1999). These migrating myocytes are nonproliferative, and the entire inner curvature is involved. This produces fibrous continuity between the mitral and aortic valves, completely devoid of muscle. The posterior AV septum and the conal septum, initially nonmuscular, become muscular structures by the process of muscularization. This process is considered by some investigators to complete the looping process. A DORV can be considered a failure of this final stage of looping.

The left and RVs differ not only by their relative positions to one another but also in their basic muscular structure and in their function. Each ventricle can functionally be subdivided into inflow and outflow regions. The RV, as we have discussed, has a conal segment as well and is therefore referred to as a *tripartite ventricle*. In the mature heart the LV is a high-pressure system, connected to systemic pressures, while the RV is connected to the lower-pressure pulmonary circulation. The form of the LV follows its function, with concentric rings of myofibrils that contract with a slight twisting motion, which allows an efficient ejection of blood. In the embryo the myocardium thickens and arranges itself in processes known as *trabeculation* and *compaction*. *Trabeculation* refers to projections of muscular tissue into the lumen of the ventricle, such that the inner surface is no longer smooth walled but is ridged. *Compaction* refers to the alignment of myocytes from random and loosely packed, as they are in the immature myocardium, into bundles of tightly packed and well-coordinated myocytes working as a unit in the mature myocardium (Moorman and Christoffels, 2003).

Development of the Atria and Atrial Septum

Like the ventricular septum, the atrial septum begins as a ridge of muscular tissue, posterior and midline, which expands to divide the right side of the atrium, containing the orifice of the sinus

venous, from the left side of the atrium, containing the orifice of the common pulmonary vein. This structure is known as the *septum primum*, and it grows toward the fusing AV cushions (see Fig. 50.8A). As discussed earlier, the fusing AV cushions will form the inferior portion of the atrial septum (see Fig. 50.8B). However, before the septum primum and the cushions complete atrial septation, small perforations appear, enlarge, and coalesce within the primum septum. The impetus for this is not known. Soon afterward, a new crescent of atrial septum forms on the right-atrial side of the septum primum and begins extending alongside the septum primum. During fetal life, blood flow from the right atrium into the left atrium prevents fusion of these two septa. The septum secundum is significantly more rigid than the septum primum, which flaps open to allow continued right to left flow. After birth and separation from the low-resistance placenta, the left atrial pressure rises significantly, and the atrial shunt reverses. This causes the septum primum to effect a seal against the septum secundum, often leaving a small opening known as the *foramen ovale*. A patent foramen ovale, generally 3 mm or less with a trivial degree of left to right flow, is a normal finding in a newborn and even in many older children and adults. A defect larger than 5 mm may constitute a secundum ASD, a true deficiency in atrial septal tissue in the region of the foramen ovale.

Failure of the cushions to form the most inferior part of the atrial septum results in a primum ASD. Such defects do not close spontaneously and may be associated with other endocardial cushion defects, most commonly a cleft mitral valve. Finally, there is a small contribution to the atrial septum near the junctions of the SVC and the IVC (see later). Defects in this process result in a sinus venosus ASD, and there are superior or inferior types. Such defects again result from deficiency of atrial septal tissue and do not close spontaneously.

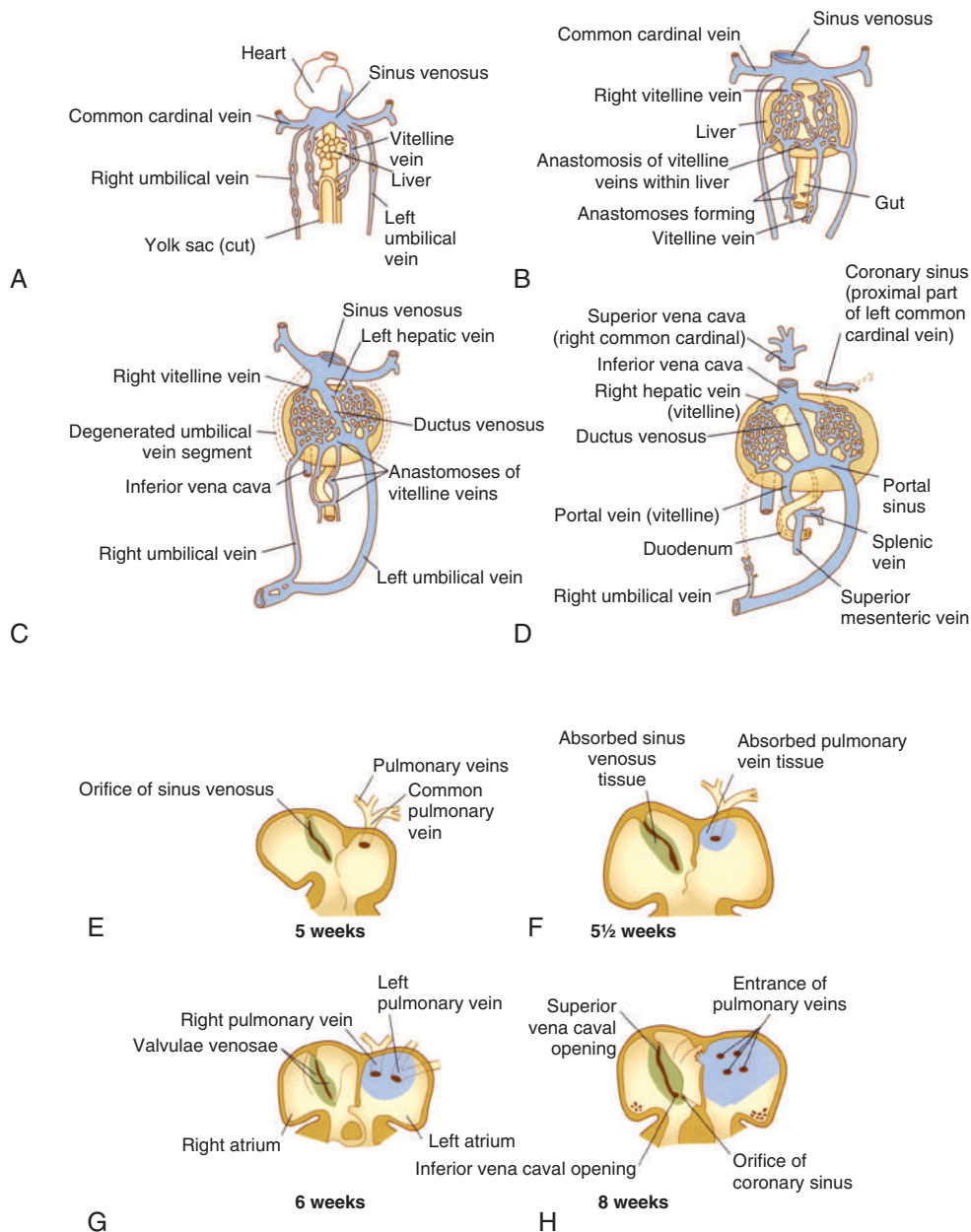
Systemic and Pulmonary Vein Development

The sinus venosus of the primitive heart tube is a symmetric structure and receives three sets of paired (left and right) systemic veins: the vitelline, umbilical, and common cardinal veins (Fig. 50.9A). This is the situation at 4 to 5 weeks' gestation (Minniti et al., 2002).

The vitelline veins return from the yolk sac, a structure that communicates with the primitive gut via the vitelline duct. The duct normally regresses completely, but an occasional remnant can be seen as a Meckel diverticulum (Carlson, 2004). Distally, the vitelline veins regress along with the duct and proximally lose their connection to the sinus venosus proximally by 6 weeks. The midportions of these vessels, however, expand within the liver to contribute to the hepatic and portal veins and contribute a small segment to the IVC (Fig. 50.9B).

The umbilical veins return from the placenta carrying oxygenated blood. These are the first to develop, by 3 weeks' gestation. As the liver develops, the umbilical veins develop connections to the liver venous plexus, and the connection to the sinus venosus involutes. Outside the embryo within the umbilical cord, the left and right umbilical veins fuse into one; thus there is a single umbilical vein in the newborn. Within the embryo the left and right segments remain separate, and the left umbilical vein becomes connected to the right hepatic veins via a new channel that forms, the ductus venosus (Fig. 50.9C, D). This circulation is formed by the 7th week of gestation. Again the umbilical veins are carrying oxygenated blood to the right atrium. This flow is primarily directed toward the atrial septum and therefore primarily through the foramen ovale into the left atrium and systemic circulation.

The cardinal veins carry the venous return from the embryo proper. The left and right common cardinal veins are relatively



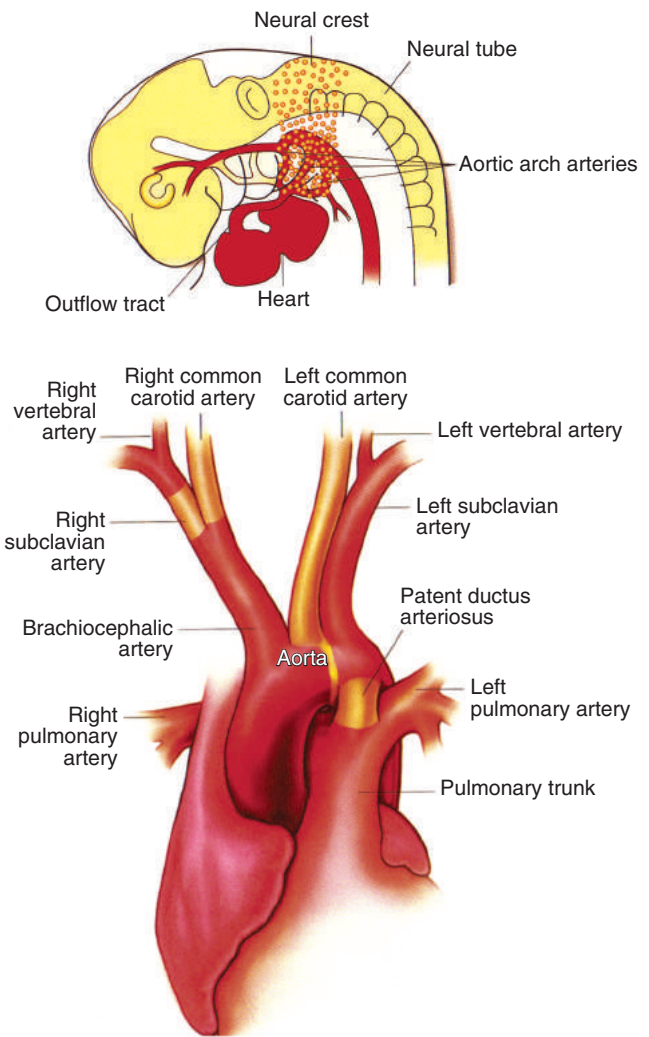
• **Fig. 50.9** “Stages of development of the systemic veins (A–D) and pulmonary veins (E–H). (From Carlson BM. *Human Embryology and Developmental Biology*. 4th ed. Philadelphia, PA: Mosby; 2009:452–453.)

short segments that connect to the sinus venosus. On each side, anterior and posterior cardinal veins join to form the common cardinal vein—the anterior cardinal veins carrying the venous return from the upper body and the posterior cardinal veins carrying the venous return from the lower body. The right anterior and right common cardinal veins will form the SVC in the mature circulation. The left common cardinal vein and left segment of the sinus venosus form the coronary sinus, which receives venous return from the coronary system. These structures are pulled rightward as the IVC forms, such that the opening (os) of the coronary sinus ends up in the right atrium once atrial septation is complete. The IVC is more complex and is made up of five segments, coming from urogenital, mesenteric, and hepatic venous channels fusing together to the right of the spine as their leftward paired counterparts involute. As numerous vessels are involved in the making of the IVC, interruption of the IVC can sometimes occur at various segments. This is most often seen in the setting of heterotaxy but can occur in isolation. When the IVC is interrupted, one of two major vessels that connect the lower and upper body venous systems generally enlarges to receive the flow and shunt it into the SVC. These are the rightward azygous vein, which runs from the suprarenal segment of the IVC to the SVC, and the leftward hemiazygos vein, which takes a more tortuous course from the leftward lumbar and renal veins up into the thoracic cavity, where it turns rightward to join the azygous vein. Thus an interrupted IVC generally has either azygous or hemiazygos continuation to the SVC.

The pulmonary veins grow in progressively from the developing lung vasculature, first as a common pulmonary vein from both lungs. This vein fuses into the back of the left atrium (Wessels et al., 2000). As the atrium expands, the common pulmonary vein becomes increasingly absorbed into the back wall, such that first two and then four of its distal branches eventually enter independently into the atrium, two from the left lung and two from the right lung (see Fig. 50.9E–H). The original atrial segment, the heart tube, is not part of the functioning atria in the mature heart, instead relegated to the right and left atrial appendages.

Aortic Arch Development

The primitive heart tube connects to the aortic sac, the precursor to the ascending aorta, and begins pumping to the developing systemic circulation of the embryo as soon as it is formed. The aortic sac connects to paired dorsal aortae posteriorly via an aortic root, which gives rise to a series of arches, the branchial arches (Fig. 50.10A). It is along these arches that the neural crest migrates into the conotruncus and supports development of the arch arteries. Again these are paired structures, and there are six pairs, although not all are patent at the same time. Arches 1–4 carry the blood flow in the 4-week embryo. Arches 1 and 2 mostly regress completely, although parts contribute to some of the arteries of the face. Arch 3 does not persist as an arch but contributes to the formation of the carotid arteries, both left and right. The left limb of arch 4 remains in continuity with the aortic root. Together they make up the true aortic arch and its first branch, the brachiocephalic (innominate) artery. The right limb of arch 4 becomes part of the right subclavian artery, which retains its proximal connection with the brachiocephalic artery (Waldo et al., 1996). This arch remodeling has occurred by 7 weeks' gestation (see Fig. 50.10B). In some cases, either independently or as part of another developmental cardiac defect, the right limb of arch 4 remains patent, and the left forms the left subclavian artery—this forms a right aortic arch with mirror-image branching. Arch 5 is small and never fully

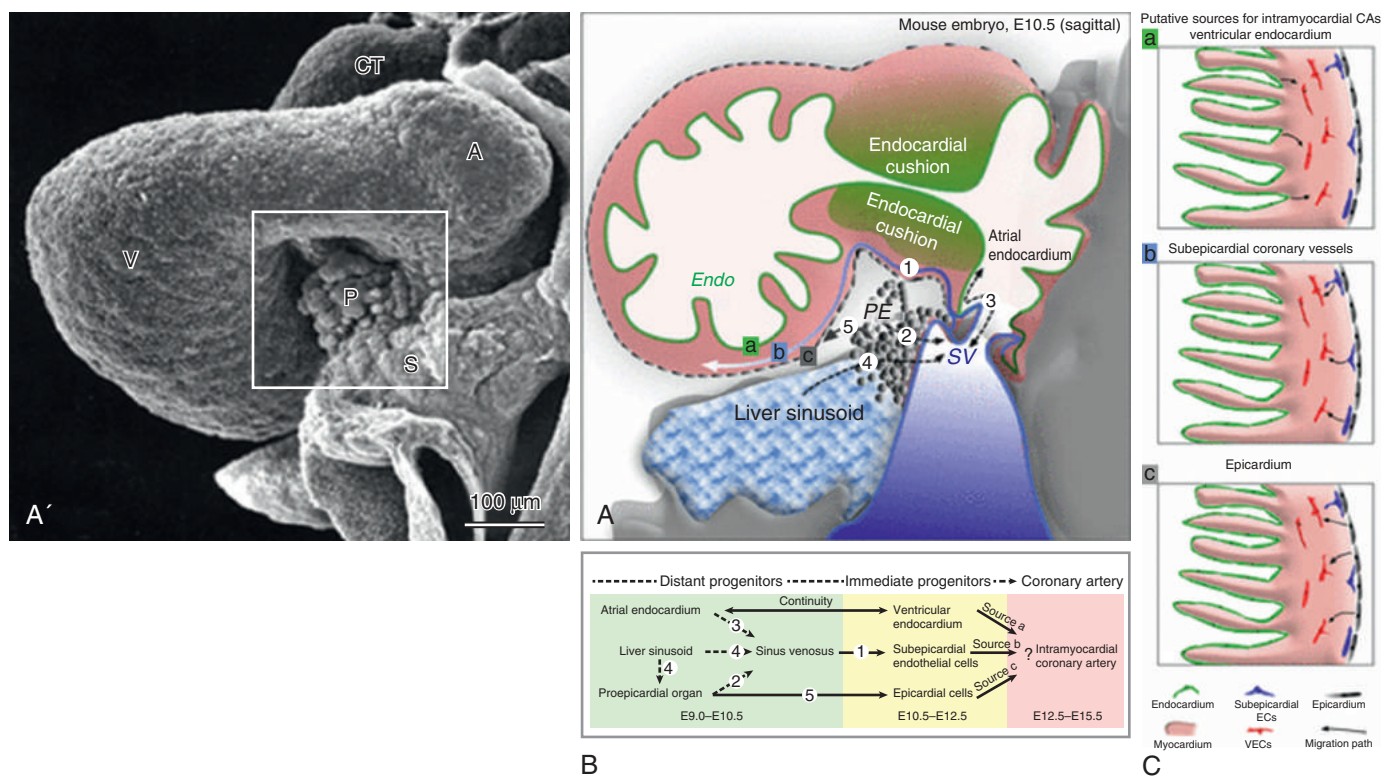


• **Fig. 50.10** (Top) Migration pattern of neural crest cells into pharyngeal arches. (Bottom) Normal aortic arch at term. Most of the pharyngeal arch segments have regressed. The third arch persists in part as the left and right common carotid arteries. The left fourth arch persists as the true aortic arch, while a portion of the right fourth arch makes up the proximal right subclavian artery. The sixth arch persists as the ductus arteriosus (ductal arch). (From Kirby ML. Contribution of neural crest to heart and vessel morphology. In: Harvey R, Rosenthal H, eds. *Heart Development*. New York, NY: Academic Press; 1999:179–193.)

develops. Arch 6 forms with the developing pulmonary artery vasculature and makes up the proximal left and right pulmonary arteries and the ductus arteriosus (Waldo and Kirby, 1993). The ductal arch persists throughout fetal life as another means of right-to-left shunt, allowing blood from the RV to bypass the pulmonary circulation and cross over into the descending aorta.

Coronary Arteries

The coronary arteries were for many years assumed to “sprout” from the ascending aorta and grow over the surface of the heart. This is now known to be incorrect. The coronary arteries form in situ within the myocardium and then connect to a coronary stem that forms within the aorta from peritruncal capillaries (Mikawa and Fischman, 1992; Mikawa and Gourdie, 1996; Tian et al., 2013a; Liu et al., 2015; Tian et al., 2015). The coronary vessels



• **Fig. 50.11** Coronary vessel origins. (A) Scanning electron micrograph showing the developing proepicardial organ (P), sinus venosus (S), atrium (A), ventricle (V), and conotruncus (CT). (A') A mouse embryo at the same stage (embryonic day 10.5) showing the various origins of the coronary plexus, including endocardium (endo; green), proepicardial organ (PE; gray), and sinus venosus (SV; blue). Dashed arrows and numbers denote the migration paths between these cell populations. a, b, and c are the sources within the heart of coronary artery (CA) precursors (a, endocardium; b, subepicardium; c, epicardium). (B) Differentiation path of the various cell types with timing in the mouse embryo; numbers correspond to the migration paths shown in (A). (C) Arrows show migration paths of each cell type into the myocardium (pink), using the same color coding as described above; epicardium arises from the proepicardial organ (black), and subendocardial endothelial cells arise from the sinus venosus (blue). The vascular epithelial cells (VECs) that form the coronary vessels are in red. E, Embryonic day. ([A'] From Hiruma T, Hirakow R. Epicardial formation in embryonic chick heart: computer-aided reconstruction, scanning and transmission electron microscopic studies. *Am J Anat.* 1989;184:129–138. [A–C] From Tian X, Pu, WT, Zhou B. Cellular origin and developmental program of coronary angiogenesis. *Circ Res.* 2015;116:515–530.)

themselves form from several sources. Early in development, bilateral clusters of cells appear near the septum transversum, where the liver bud approaches the heart as the proepicardial organ (Mikawa and Gourdie, 1996; Reese et al., 1999, 2002). This is in close proximity to the sinus venosus, and there appears to be some movement of cells from one population to the other (Tian et al., 2015). The proepicardial organ is a transient structure that spreads out over the surface of the heart as an epithelium, forming the epicardium. Some of the epicardial cells delaminate and migrate into the myocardium, contributing to coronary vasculature, both endothelial cells and arterial smooth muscle, and cardiac fibroblasts. Early studies using lineage tracing methods in chick embryos suggested that most of the coronary arteries, both endothelium and smooth muscle, were formed from this population (Mikawa and Gourdie, 1996; Reese et al., 2002). More recent studies have shown that while this is true for the smooth muscle component of the coronary arteries, the endothelium is more complex in origin. These studies have used detailed transgenic lineage tracing methods in the mouse to show important contributions from the endocardium (Wu et al., 2012) and sinus venosus (Red-Horse et al., 2010; Tian et al., 2013b, 2014). The endocardium-derived

cells invade the myocardium from the inner surface of the heart, migrating toward the epicardial surface and differentiating into coronary artery endothelial cells. The cells from the sinus venosus migrate and expand within the subepicardial space, invading the myocardium as a distinct population from the epicardium. The current understanding of the overall process of coronary endothelial formation is shown in Fig. 50.11. To add to the complexity of cell lineages, this process is not uniform throughout the ventricular myocardium. The coronary vessels within the ventricular septum appear to form differently from the ventricular free wall (Wu et al., 2012; Tian et al., 2015). Regardless of the ultimate origin, the coronary vessels form, grow, and fuse with one another to form a working vasculature. This corresponds to the process of growth and trabeculation of the ventricles. With increasing complexity and thickness of the myocardium, simple diffusion of nutrients from the blood inside the heart is no longer adequate. It is at this point that the coronary artery plexus grows into the aorta just above the aortic valve. Signaling from neural crest, epicardial-derived cells and recently discovered intra-aortic cardiomyocytes appear to play an important guiding role (Erulp et al., 2005; Arima et al., 2012; Chen et al., 2014). Occasionally this process can go awry,

and a coronary artery (usually the left) can join into the pulmonary artery instead of the aorta. This does not damage the fetal myocardium given the low oxygen state of the fetus, with little difference in oxygen tension between the aorta and the pulmonary artery. After birth, however, ischemia sets in rapidly, causing myocardial ischemia and infarction in infancy.

Conduction System

There has also been controversy regarding the origin of the conduction system, and this controversy is largely resolved. The conduction system was suspected to originate from the neural crest by many investigators, migrating in from outside the heart. However, lineage tracing studies show conclusively that the cells of the conduction system actually differentiate *in situ* from myocytes (Waldo et al., 1994; Moorman, et al., 1997; Gourdie et al., 1998; Cheng et al., 1999; Hyer et al., 1999). There is evidence that the developing coronary vasculature is a source of signaling for this transdifferentiation to occur; the conduction system tends to develop alongside coronary vessels (Hyer et al., 1999). Conduction system myocytes express different markers and junctions and have different action potential profiles that allow automaticity (Wessels et al., 1992; Alyonycheva et al., 1997; Moorman et al., 1997; Takebayashi-Suzuki et al., 2001; Hoogaars et al., 2007). Under normal conditions the automaticity of the remaining conduction system is suppressed as the dominant pacemaker of the heart forms, the sinoatrial node. Recently, lineage tracing studies in early chick embryos showed that the sinoatrial node arises from a population of cardiac mesoderm outside the primary and secondary heart fields that coalesce in the heart near the right sinus horn (Bressan et al., 2013). Activation mapping of embryonic chick heart conduction shows that the primitive sinoatrial node begins to function soon after cardiac looping (the site of earliest activation before this being in the left sinus horn) (Bressan et al., 2013). Once active, the sinoatrial node maintains the fastest depolarization and “sets the pace” for the heart rate. This starts at about 1 month of gestation in humans (Phoon, 2001). If the sinoatrial node slows for any reason, other “pacemakers” substitute. Atrial myocytes tend to have the next most rapid automatic rates, followed by the His bundles, then ventricular myocytes. The heart rate in a 5-week fetus is 100 beats per minute (Phoon, 2001). This increases gradually to a rate of 160 beats per minute by 8 weeks’ gestation and then declines slightly during gestation to the 120–130 beats per minute range (Phoon, 2001).

Physiology of Transition

The fetal heart and circulation at the end of 9 weeks are completely formed (Phoon, 2001). As depicted in Fig. 50.12, there are four chambers of the heart, two atria in communication via a patent foramen ovale and two ventricles with complete septation. The left ventricular myocardium is configured differently from the right such that it is capable of working under higher pressure (afterload). There are fully competent AV valves (the mitral and tricuspid) and fully competent semilunar valves (the aortic and pulmonary) that ensure one-way flow through the heart. The venous return from the systemic circulation and the pulmonary circulation enters the right atrium and the left atrium, respectively. The arterial blood is pumped to the body and to the lungs via the great arteries, with a communication between the two via the patent ductus arteriosus (PDA).

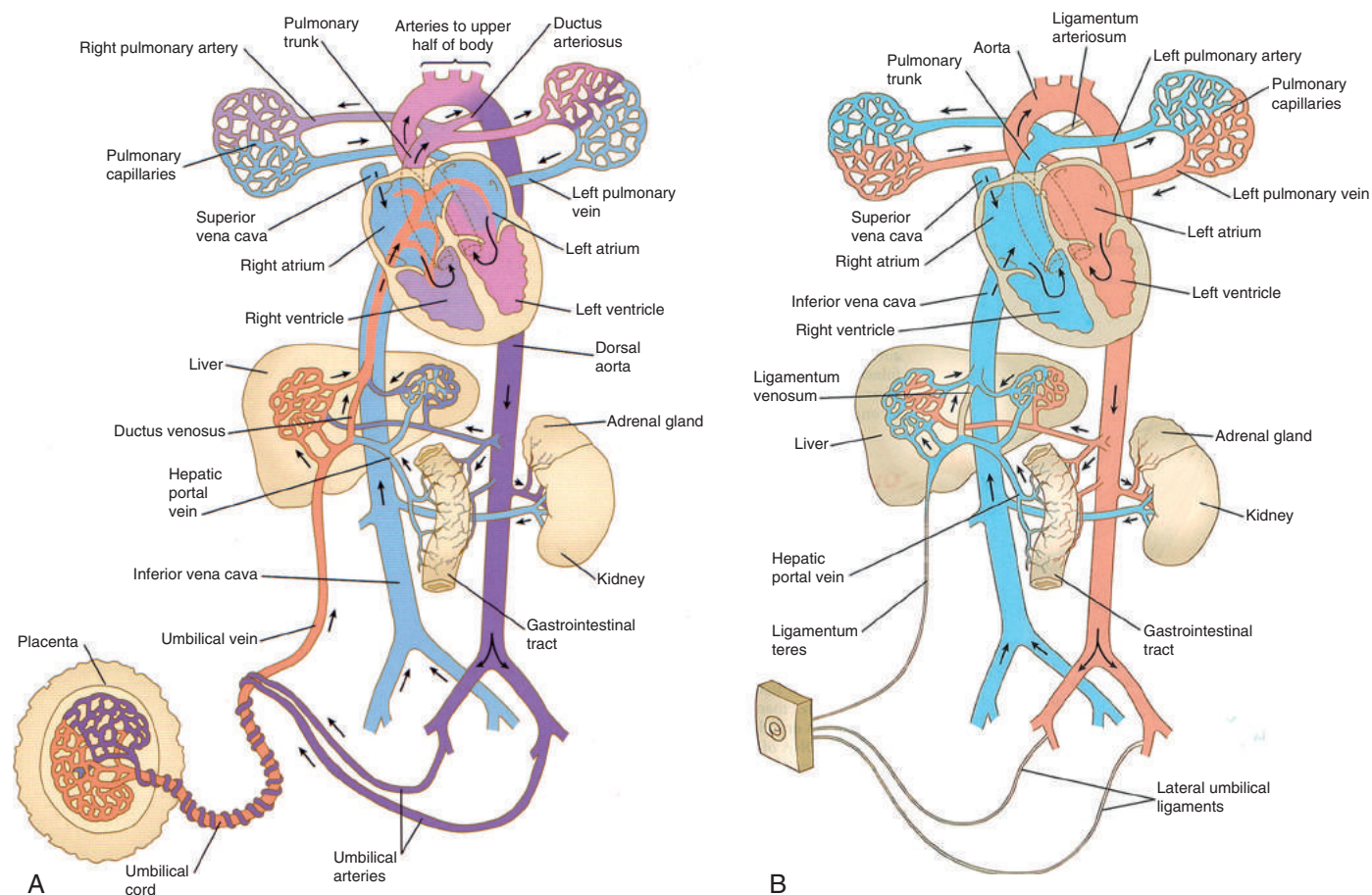
Before birth the placenta is a critical component to the fetal circulation, with a single umbilical vein and two umbilical arteries

(see Fig. 50.12A). A full 50% of the fetal blood volume is in the placenta, and 50% is in the body. In the fetus the systemic cardiac output is combined from the left and RVs, with the RV contributing more than the LV to the total output (approximately 65% vs 35%) (Teitel, 1992). This is via the “ductal arch” connecting the main pulmonary artery to the ductus arteriosus and descending aorta. The aortic arch contributes about 60% of its flow to the head and neck vessels, and the rest combines with the ductal flow to reach the lower body and return to the placenta. Return of blood from the placenta (oxygenated) is via the umbilical veins through the ductus venosus to the IVC and right atrium. This blood is under higher pressure than the systemic return from the fetus and is streamed such that at least 25% is directed across the foramen ovale into the left atrium and systemic circulation. The remainder combines the SVC return to cross the tricuspid valve into the RV. Only about 10% of the right-ventricular output goes to the lungs; this small amount of blood returns to the heart via the pulmonary veins into the left atrium, mixes with the oxygenated blood that has crossed the foramen ovale from the right atrium, and passes on to the systemic circulation (Teitel, 1992).

After birth two major factors trigger changes in circulation (see Fig. 50.12B). These are the initiation of respirations with lung expansion and alveolar gas exchange and cutting of the umbilical cord. The result is a drastic reduction in IVC return to the right side of the heart as the placental flow ceases; at the same time there is a drastic increase in capacity of the pulmonary circulation. Thus return to the right atrium decreases and return to the left atrium increases, as does left-atrial pressure. This forces the flap of the foramen ovale up against the atrial wall, reducing the volume and reversing the direction of atrial-level shunting. Thus the fetus has a large patent foramen ovale with right to left flow, which after birth becomes a smaller orifice with left to right flow. Approximately 20% of older children and adults retain a small patent foramen ovale, with a trivial left to right shunt. Also immediately after birth, the increased oxygen tension of the blood that occurs with breathing causes the ductus arteriosus to begin constricting (functional closure). This is a function of smooth muscle within the wall of the PDA and is prostaglandin sensitive. After functional closure the permanent process of anatomic closure, involving apoptosis of smooth muscle and proliferation of connective tissue, converts the PDA into a fibrous ligament (the ligamentum arteriosus). A similar but slower process occurs in the ductus venosus, also causing it to be closed by fibrosis.

Conclusion

We have reviewed heart development, with attention to the morphology, cell biology, genetics, and physiology of the heart as it forms and undergoes remodeling. The field of developmental biology of the heart continues to advance rapidly on all of these fronts. New technologies allow earlier and earlier visualization of the developing human heart, recently as early as 6 weeks’ gestation by cardiac magnetic resonance imaging (Dhanantwari et al., 2009) and 10 to 11 weeks’ gestation by cardiac ultrasonography (Gembruch et al., 2000; Weiner et al., 2002; Cook et al., 2004; Becker and Wegner, 2006; Neuman and Huhta, 2006; Vimpelli et al., 2006; Marques Carvalho et al., 2008; Bennasar et al., 2009). Early ultrasonography has the additional benefit of assessment of cardiac physiology in the embryo, using Doppler technology (Phoon, 2001). Novel genetic techniques allow us to apply what is learned from animal models to humans and to recreate human disease in animal models with greater and greater specificity. The common



• **Fig. 50.12** The fetal circulation at term: (A) before birth and (B) after birth. In (A) the path of oxygenated blood returning from the placenta is shown in *orange*. It mixes with deoxygenated blood returning from the fetal systemic veins shown in *light blue*. There is intracardiac mixing of blood as shown. The upper body receives a higher oxygen content than the lower body, as deoxygenated blood enters the descending aorta via right-to-left flow at the ductus arteriosus. After birth the pulmonary and systemic circulations are completely separated as shown in (B). (From Carlson BM. *Human Embryology and Developmental Biology*. 5th ed. Philadelphia, PA: Mosby; 2014:436, 469.)

goals to be applied to human disease are early recognition of congenital heart malformations, prevention where feasible, and state-of-the-art intervention to allow an abnormal heart to function as normally as possible. Currently such intervention is surgical and cardiac catheterization based and continues to evolve. In the future, gene therapy, cardiac stem cell grafting, and in vitro tissue engineering will likely be added to the therapeutic potentials for patients with congenital heart malformations.

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Cardiovascular Compromise in the Newborn

SHAHAB NOORI, TIMUR AZHIBEKOV, BEN LEE, AND ISTVAN SERI

KEY POINTS

- It is difficult to diagnose neonatal shock in its *uncompensated phase* during the immediate transitional period, while it is even more challenging to diagnose it in its *compensated phase* with use of a standard clinical monitoring and clinical approach.
- It is unclear what gestational age- and postnatal age-dependent blood pressure and systemic and organ blood flow values represent hypotension and poor tissue perfusion respectively warranting timely intervention in the neonate. In addition, there is still some uncertainty about which cardiovascular medication or combination of medications to use in neonatal shock with different causes (hypovolemia, myocardial dysfunction, vasoregulatory disturbance, or a combination of two or all of these factors).
- There is very little evidence that management of neonatal hypotension and/or systemic blood flow affects clinically relevant outcomes.
- A thorough understanding of the principles of developmental cardiovascular physiology and the cause, pathophysiology, and clinical presentation of neonatal shock along with the mechanisms of action and the pharmacokinetics and pharmacodynamics of the medications used in the treatment of cardiovascular compromise is essential for the neonatologist to apply the most reasonable approach to treatment of neonatal cardiovascular compromise on the basis of the collective experience and little evidence available in the literature.
- Recent advances in bedside monitoring technologies such as near-infrared spectroscopy, electrical impedance velocimetry, heart rate variability-based early warning systems, and amplitude-integrated electroencephalogram (EEG) along with the use of point-of-care ultrasonography will enable us to systematically and more thoroughly study neonatal circulatory compromise. These developments, along with the introduction and mathematical modeling of genotypic and phenotypic markers that identify subpopulations at higher risk of developing shock before it happens, will undoubtedly lead to earlier diagnosis and evidence-based management of neonatal shock, resulting in improved short-term and long-term outcomes.

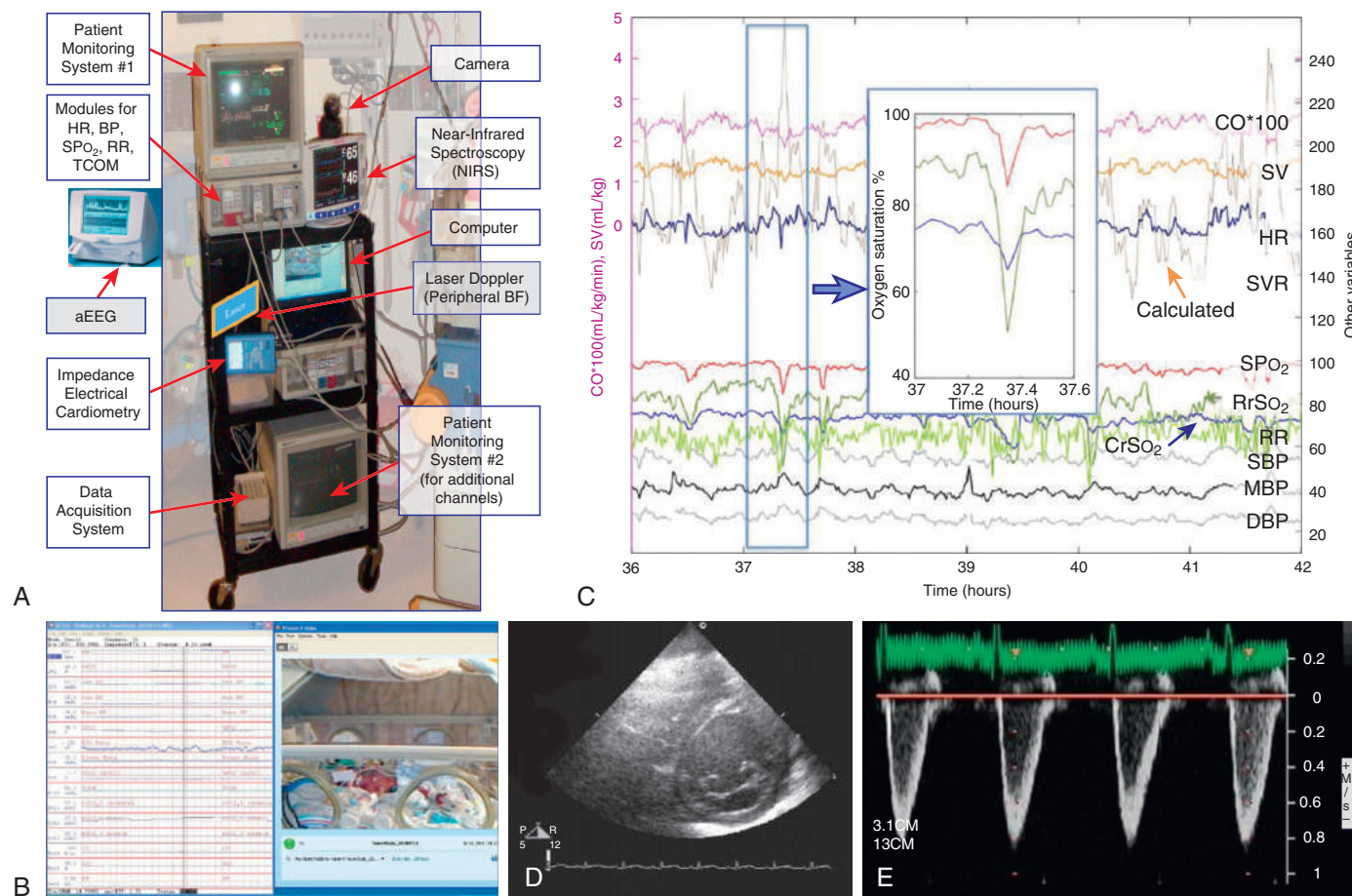
Although the prevalence of hypotension in neonates admitted for intensive care is unclear, up to 50% of very low birth weight (VLBW) neonates present with blood pressure values considered to be low in the immediate transitional period (McLean et al., 2012). However, VLBW neonates account for only approximately 25% of all patients diagnosed with hypotension in neonatal intensive care units. The lack of clear data on

the prevalence of neonatal hypotension is primarily due to the uncertainty about the lower limit of the gestational age- and postnatal age-dependent normal blood pressure range in neonates (Engle, 2012). This is illustrated, among other factors, by the significant differences in the prevalence of the use of vasopressors/inotropes in preterm neonates during the transitional period among different intensive care units across the United States (Al-Aweel et al., 2001; Rios et al., 2014; Wong et al., 2015).

As long as pulmonary gas exchange is adequate, shock is caused by hypovolemia, cardiac failure, vasoregulatory failure, or a combination of these etiologies. Shock has been defined as a “state of cellular energy failure resulting from an inability of tissue oxygen delivery to satisfy tissue oxygen demand” (Singer, 2008). According to this definition, when oxygen delivery is inadequate to meet oxygen demand, the organs start failing and, if corrective measures are not effective, will result in irreversible organ damage and ultimately death. Although oxygen delivery to the organs is dependent on several factors, it is fundamentally driven by both the oxygen content of the blood and the volume of blood flowing to those organs. Because oxygen content is primarily determined by the hemoglobin concentration and oxygen saturation, with less contribution from the dissolved oxygen (see later discussion), it is relatively easily evaluated and monitored in the neonatal intensive care unit. However, reliably assessing systemic and organ blood flow and tissue oxygen delivery and consumption at the bedside is challenging because to provide adequate information on the rapidly changing hemodynamic status of the critically ill neonate, it requires continuous monitoring of key hemodynamic parameters in absolute numbers (Fig. 51.1).

Recent advances in our ability to monitor systemic and organ blood flow and tissue oxygenation as well as vital organ (brain) function at the bedside will likely lead to a better understanding of the complex hemodynamic changes associated with neonatal cardiovascular compromise (Cayabyab et al., 2009; Azhibekov et al., 2015). These advances should lead to the development of treatment modalities more appropriately based on the cause, pathophysiology, and phases of shock, thereby improving clinically relevant outcomes.

At present in clinical practice, tissue perfusion is routinely assessed by the monitoring of heart rate, blood pressure, capillary refill time (CRT), acid-base status, serum lactate levels, and urine output. However, recent Doppler ultrasonography and near-infrared spectroscopy (NIRS) data have highlighted that these parameters



• **Fig. 51.1** Comprehensive, Real-Time Monitoring and Data Acquisition System. Comprehensive monitoring and data acquisition systems allow real-time, continuous and simultaneous assessment of cardiovascular, respiratory, and organ blood flow data, enabling researchers and clinicians to more appropriately evaluate the condition of the patient and follow the changes over time and in response to therapeutic interventions. (A) The comprehensive monitoring and acquisition system developed by the authors' group. The system has the capability to continuously monitor, in absolute values, selected cardiovascular parameters (systolic blood pressure [SBP], diastolic blood pressure [DBP], mean blood pressure [MBP], and heart rate [HR]) and, by the use of electrical impedance velocimetry (Noori et al., 2012c), stroke volume (SV) and cardiac output (CO). Total systemic vascular resistance (SVR) is also continuously calculated by the computer from the pressure and flow data. In addition, with use of near-infrared spectroscopy (NIRS) (Azhibekov et al., 2015), indices of organ blood flow and tissue oxygenation (cerebral regional tissue oxygen saturation [CrSO₂] and renal regional tissue oxygen saturation [RrSO₂]) as well as a number of respiratory parameters (arterial oxygen saturation [SpO₂], respiratory rate [RR], and transcutaneous carbon dioxide tension) are also being obtained. The system includes a motion-activated video camera; a snapshot of the image obtained by the camera is shown (B). The camera allows assessment of patient movements and activities (e.g., seizure) potentially affecting cardiorespiratory functions. In addition, it aids in the postacquisition filtering of motion artifacts. (C) A representative, real-time continuous recording of hemodynamic variables during a period of 6 hours with an oxygen desaturation episode highlighted in the box in a 1100-g, 28 week-gestation preterm neonate. The recording is an example of a preterm infant with a mature response to a hypoxic episode (red line) in which tissue oxygen saturation in the kidney (a nonvital organ; green line) dramatically decreases, while the change in brain (a vital organ; blue line) tissue oxygen saturation is significantly attenuated. Although the system provides a more complete picture of the hemodynamic status, assessment of cardiac function by echocardiography complements the hemodynamic evaluation. Among other factors, echocardiography reveals potential structural heart defects and the presence of shunts between the systemic and pulmonary circulations through a patent ductus arteriosus and/or foramen ovale and can shed light on the underlying cause of circulatory failure. For example, by assessing the movement of the septum and posterior wall of the left ventricle in the short-axis view (D), one can better understand the status of myocardial contractility. In addition, by estimating blood flow velocity (E) and measuring the diameter of the aorta, one can spot-check the validity of the continuous cardiac output data obtained by the electrical impedance velocimetry monitor used in the system. aEEG, Amplitude-integrated electroencephalography; BF, blood flow; TCOM, transcutaneous CO₂ monitoring.

are relatively poor indicators, although at present they are the only routinely available indicators of acute changes in organ blood flow and tissue oxygen delivery in critically ill neonates (Kluckow and Evans, 1996; Lopez et al., 1997; Tysczuk et al., 1998; Kluckow and Evans, 2000a). These observations and the lack of evidence that treatment of neonatal cardiovascular compromise improves outcomes (Seri and Noori, 2005; Barrington et al., 2006) call for a paradigm shift in our thinking about the pathophysiology, diagnosis, and treatment of neonatal shock. This suggests that the assessment of the hemodynamic status in critically ill neonates should include the complex interactions among blood flow and blood pressure as well as tissue oxygen delivery and consumption (Cayabyab et al., 2009; Noori et al., 2012). A comprehensive, real-time hemodynamic monitoring and data acquisition system has been developed recently (Soleymani et al., 2012; Azhibekov et al., 2014, 2015), although its use is limited to clinical research (Fig. 51.2).

Principles of Developmental Cardiovascular Physiology and Pathophysiology, Phases, and Causes of Neonatal Shock

Principles of Oxygen Delivery

Oxygen is essential for mitochondrial respiration but is not stored in the body. Thus interruption of oxygen supply to cells can result in irreversible damage (sometimes within minutes), particularly in vital organs such as the brain and myocardium.

The primary function of the cardiorespiratory system is to provide adequate oxygen delivery to *tissues*. Accordingly, shock is defined as inadequate systemic tissue oxygen delivery (see earlier). Oxygen delivery can be expressed as

$$\text{DO}_2 = \text{Cardiac Output} \times \text{Arterial Oxygen Content (CaO}_2\text{)},$$

where

$$\text{Cardiac Output} = \text{Heart Rate} \times \text{Stroke Volume}$$

and

$$\begin{aligned} \text{CaO}_2 = & (1.34 \times \text{Hemoglobin Concentration} \\ & \times \text{Arterial Oxygen Saturation [SaO}_2\text{]}) \\ & + (0.003 \times \text{Arterial Partial Pressure of Oxygen [PaO}_2\text{]}). \end{aligned}$$

Stroke volume is the result of complex interplay among preload, afterload, and contractility (Fig. 51.3), all three of which are, at present, impossible to monitor reliably and continuously at the bedside. Although these parameters are more fully described later, in brief, preload is the end-diastolic volume of the ventricle (a three-dimensional reflection of precontractile myocardial cell fiber length), and, up to a point, the greater the preload, the larger the stroke volume (the Frank–Starling relationship). Afterload is the force the ventricle must generate against the systemic or pulmonary vascular resistance. As long as appropriate perfusion pressure is ensured, the lower the afterload, the greater the cardiac output. Contractility (the intrinsic ability to generate force per unit time) may be assessed noninvasively but not continuously by echocardiogram. Considering at present most of the measures of cardiac contractility are both preload and afterload dependent, contractility is not truly an independent variable. In addition, cardiac output in neonates is considered more heart rate–dependent than contractility-dependent because the ability of neonates to augment

their stroke volume is limited when compared with the ability of children or adults.

From this simple model it is easily appreciated that if there is an acute decline in CaO_2 , caused by a decrease in either the hemoglobin concentration or SaO_2 or both, the cardiac output will increase in response to maintain DO_2 . On the other hand, because neither hemoglobin concentration nor SaO_2 can be physiologically increased rapidly, there is no acute compensation for a low cardiac output due to decreases in myocardial contractility and/or preload.

The purpose of oxygen delivery is to provide oxygen for oxygen consumption (VO_2), which can be expressed as

$$\text{VO}_2 = \text{Cardiac Output} \times (\text{CaO}_2 - \text{CvO}_2),$$

where CvO_2 is the mixed venous oxygen content.

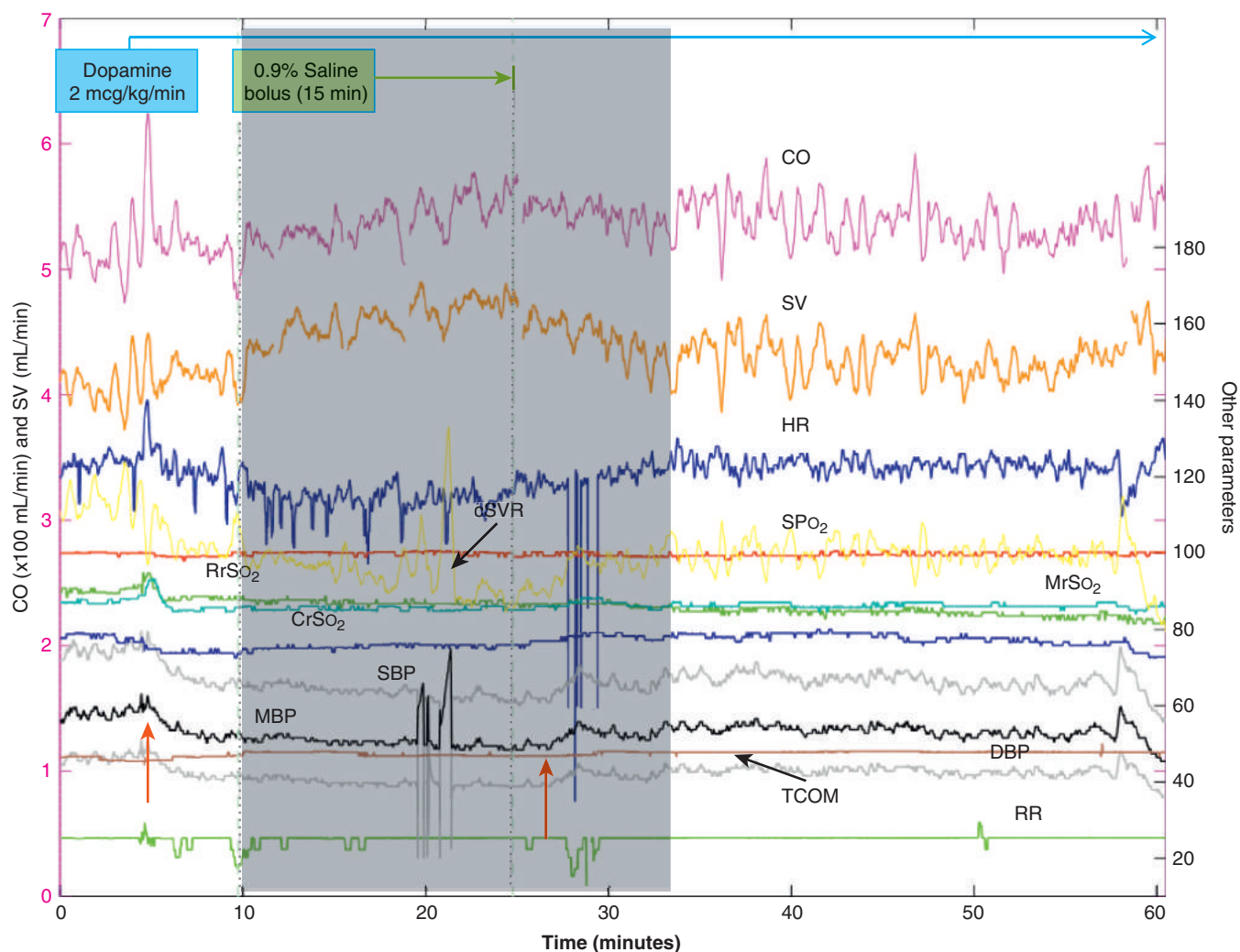
This relationship is based on the Fick principle, from which, knowing the flow rate and the arterial–venous content difference of a trace element (in this case oxygen), one can calculate the rate of uptake or removal of the tracer.

Normally DO_2 and VO_2 are well matched with O_2 extraction, being approximately 25%. Accordingly, if SaO_2 is 100%, SvO_2 would be expected to be 75%. If cardiac output falls, VO_2 may be maintained constant by capillary bed vasodilation and recruitment and/or by increased O_2 extraction by the tissues. Increased O_2 extraction is manifested as lower CvO_2 and therefore a greater CaO_2 and CvO_2 difference. The relationship between DO_2 and VO_2 may be graphically displayed as in Fig. 51.4. Once oxygen extraction is maximal, at the critical DO_2 threshold, anaerobic metabolism ensues, resulting in lactic acidosis. If not reversed, the oxygen debt accumulates, and organ failure and death will ensue. In general, during aerobic metabolism 38 mol adenosine triphosphate (ATP) is produced per mole of glucose, whereas during anaerobic metabolic conditions, 2 mol ATP and 2 mol lactate are produced per mole of glucose.

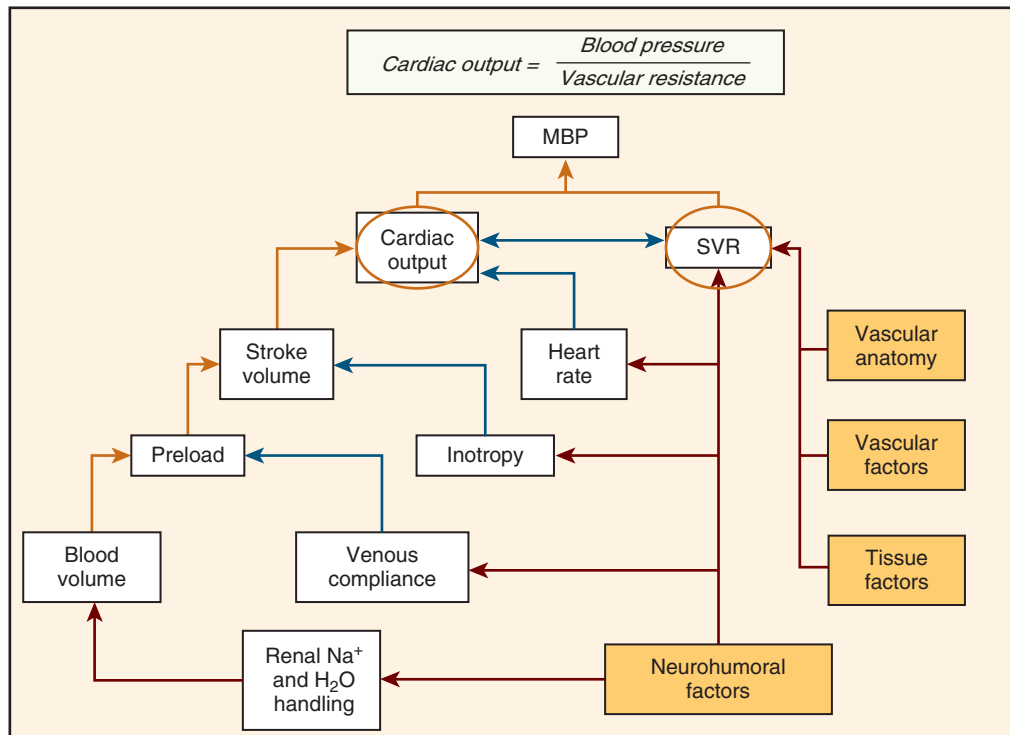
Before the stage of delivery-dependent VO_2 is reached, SvO_2 can be used as a proxy for DO_2 . Assuming VO_2 , hemoglobin concentration, and SaO_2 are constant over a short period, a decline in SvO_2 represents a decrease in cardiac output. This relationship is clinically important, as SvO_2 can be measured intermittently via a venous catheter, ideally placed in the pulmonary artery in a patient without intracardiac shunts so as to obtain a true mixed venous sample. Central venous oxygen saturation (ScvO_2) may also be used as a proxy for DO_2 , measured via a venous catheter with its tip at the superior vena cava (SVC)—right atrial junction. It is of note that the location of the catheter tip is critical; if the tip is located lower in the right atrium, it will sample more desaturated blood streaming from the coronary sinus and/or hepatic veins. However, for several reasons, measurement of ScvO_2 is not done routinely in neonates in neonatal intensive care units except for in some neonates with congenital heart disease in the postoperative period following surgical correction of the underlying cardiac condition.

In general, these principles are valuable guides to understanding and managing global DO_2 and VO_2 , but they do not readily assist with the assessment of individual organ DO_2 . Furthermore, the limitations of measuring VO_2 , DO_2 , and even just ScvO_2 are often daunting. However, advances in noninvasive regional tissue oxygen saturation (rSO_2) monitoring via NIRS have increasingly allowed such assessments in different tissues, including the brain, kidneys, intestine, and muscle (see later discussion).

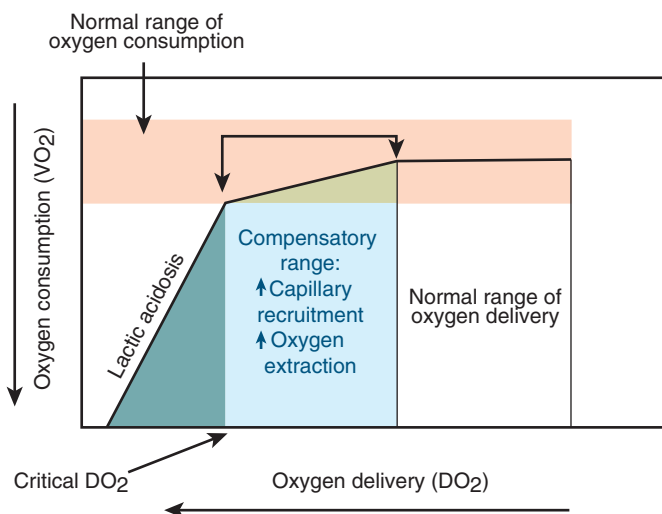
Finally, there is a class of neonates where calculations using the Fick principle can be critical in directing therapy. Newborns with



• **Fig. 51.2** Real-time Recording of 12 Hemodynamic Parameters in a Neonate. Real-time, 60-minute recording of 12 hemodynamic parameters in a 1-day-old, 3000-g, 36-week late preterm neonate receiving dopamine at 2 $\mu\text{g}/\text{kg}$ per minute. Recording was blinded to the clinical team. The patient's blood pressure decreased (left red arrow), and a 10 mL/kg physiologic saline bolus was started at the 10-minute mark of the recording to be administered in 15 minutes. No changes occurred in systolic blood pressure (SBP), mean blood pressure (MBP), diastolic blood pressure (DBP), cerebral regional tissue oxygen saturation (CrSO_2), renal regional tissue oxygen saturation (RrSO_2), and muscle regional tissue oxygen saturation (MrSO_2) measured by near-infrared spectroscopy, arterial oxygen saturation (SPO_2), transcutaneous CO_2 tension (TCOM), and respiratory rate (RR). However, stroke volume (SV; monitored by impedance electrical cardiometry) increased and heart rate (HR) decreased probably because of improved preloading conditions during the volume bolus. As the magnitude of the increase in SV was greater than the decrease in HR, cardiac output (CO) also increased ($\text{CO} = \text{SV} \times \text{HR}$). There is great variability in the trend of CO and SV values shown that may be attributed to beat-to-beat variations in SV, HR variability, or other physiologic effects and/or the limitations of the technology. Considering MBP did not change, calculated systemic vascular resistance (cSVR) also decreased (systemic vascular resistance = MBP/CO). In addition, considering CrSO_2 , RrSO_2 , and MrSO_2 did not change while SPO_2 also remained stable, it is likely that cerebral, renal and muscle blood flow also remained basically unchanged during the volume bolus. The effect on cardiac output lasted for only 10 minutes after the discontinuation of the volume bolus, and thereafter all but one of the monitored hemodynamic parameters remained unchanged. The only exception was the increase in SPB, MBP, and DBP occurring approximately 3 to 5 minutes after the volume bolus was completed (right red arrow). The change in blood pressure appears to be unrelated to the changes in the other hemodynamic parameters or the administration of the volume bolus. However, on return of the clinical team 30 minutes after the initiation of the volume bolus, and without knowledge of the changes in the hemodynamic parameters, it must have been tempting for the clinical team to speculate that the volume administration "worked" as the blood pressure returned to the previous levels. The shaded area highlights the period of hemodynamic changes during and 10 minutes after the volume bolus. The left y-axis depicts SV (mL) and CO (100 mL/minute) and the right y-axis shows the values for SPO_2 (%), CrSO_2 (%), RrSO_2 (%), MrSO_2 (%), HR (1/minute), RR (1/minute), TCOM (mmHg), SPB (mmHg), MBP (mmHg) and DBP (mmHg), and cSVR (millimeter of mercury-minutes per liter). Min, Minute; mL, milliliter. (Courtesy Soleymani S, Borzage M, Noori S, Seri I. Neonatal hemodynamics: monitoring, data acquisition and analysis. *Expert Rev Med Devices*. 2012;9:501–511.)



• **Fig. 51.3** Factors Regulating Cardiac Output, Blood Pressure, and Systemic Vascular Resistance. From a physiologic standpoint, systemic vascular resistance (SVR) and cardiac output are the regulated (independent) variables and mean blood pressure (MBP) is the dependent variable. (Modified from Klabunde RE. Factors Regulating Arterial Blood Pressure; 2016. <http://www.cvphysiology.com/Blood%20Pressure/BP022>.)



• **Fig. 51.4** Relationship Between Oxygen Consumption and Delivery. In the normal range of oxygen delivery, oxygen consumption is unaffected by changes in the rate of delivery of oxygen to the tissues. As oxygen delivery decreases below the normal range, tissue oxygen consumption remains in the normal range for a while because of activation of local compensatory mechanisms such as capillary recruitment and increased oxygen extraction. However, when oxygen delivery decreases to the "critical" point, compensatory mechanisms can no longer satisfy tissue oxygen demand, and anaerobic metabolism commences, resulting in significantly decreased ATP and increased lactate production.

congenital heart disease and intracardiac shunts may have perturbations in the usual pulmonary-to-systemic blood flow ratio (Q_p/Q_s). Normally, of course, in patients with the two circulations in series and no shunts, $Q_p/Q_s = 1$. By comparing the oxygen used by the body with the oxygen taken up by the lung, one can estimate Q_p/Q_s :

$$Q_s = VO_2 / (CaO_2 - CvO_2)$$

and

$$Q_p = O_2 \text{ Uptake} / (C_{pv}O_2 - C_{pa}O_2),$$

where pv and pa represent pulmonary vein and pulmonary artery, respectively.

After substitution and elimination of common terms, we have

$$Q_p/Q_s = (SaO_2 - SvO_2) / (SpvO_2 - SpaO_2).$$

This formula requires two assumptions, unless saturation values are determined directly, as done in the cardiac catheterization laboratory: first, $SpvO_2$ is 95% to 100%, and second, SvO_2 measured through a central venous line reflects a mixed venous sample. A Q_p/Q_s ratio of less than 1 would suggest the presence of a right-to-left shunt and, typically, decreased pulmonary circulation, both of which result in less oxygenated blood entering the systemic circulation with resultant cyanosis. A Q_p/Q_s ratio greater than 1, in turn, would suggest left-to-right shunting, with resultant pulmonary overcirculation. Of note is that changes in Q_p/Q_s in either direction will affect oxygen content and/or oxygen delivery to the organs.

The value of this calculation can be illustrated with the following example. A newborn with hypoplastic left-sided heart syndrome

is found to have SaO_2 of 95% and SvO_2 of 80%. Using the formula just given, assuming SpvO_2 of 100% and recognizing that SaO_2 and SpaO_2 are the same in this patient, we arrive at a Qp/Qs ratio of 3:1. If systemic blood flow is to be preserved, the single right ventricle will need to sustain a fourfold increase in cardiac output, whereas the significant pulmonary overcirculation will lead to the development of congestive heart failure. The inability of the right ventricle to sustain such an increase in cardiac output will then lead to inadequate systemic blood flow, resulting in shock. Importantly, both conditions can be present at the same time. However, if SaO_2 is maintained within the target range of 75% to 85% in these patients, the Qp/Qs ratio will shift closer to 1:1, providing a simplistic rationale for the use of such an SaO_2 target range. However, in patients with hypoplastic left-sided heart syndrome, the effects of shunting on blood oxygen content and/or delivery are far more complex as systemic oxygen availability is determined, along with other factors, by the relationship among SpvO_2 , SaO_2 , SvO_2 , and cardiac output (Barnea et al., 1994). Accordingly, in addition to a targeted SaO_2 range, management of these patients must be guided by the difference in systemic arterial and venous oxygen saturations and other indicators of tissue perfusion (see also Chapter 55).

Developmental Regulation of Cardiac Output and Its Determinants

Cardiac output is the product of stroke volume and heart rate and is determined by the amount of blood returning to the heart (preload), the strength of myocardial contractility, and the load against which the heart must pump (afterload). Unlike preload, afterload is in general more difficult to conceptualize, and therefore *afterload* is often used interchangeably with the simpler but less accurate term *vascular resistance*. However, although afterload is altered by changes in vascular resistance, other factors also determine its magnitude. Afterload is the load or force the heart faces during contraction and is affected by the impedance of the central vasculature, the resistance of the peripheral vascular beds, the ventricular mass, blood pressure, and the inertia of the blood. In addition, it is affected by myocardial contractility and preload. If myocardial function is intact, cardiac output depends solely on preload and afterload according to the relationships described by the Starling curve.

Therefore low cardiac output and thus low systemic blood flow can result from various combinations of abnormalities of the three determinants of cardiac output: low cardiac preload, poor myocardial contractility, and high cardiac afterload. In addition, extremes of these variables in the opposite direction (i.e., high cardiac preload, increased myocardial contractility, and low cardiac afterload) can contribute to cardiovascular insufficiency, albeit not as commonly. This is because these three variables, as well as heart rate, affect one another. For example, in an infant of a diabetic mother with hypertrophied cardiomyopathy, increased contractility and low afterload can further compromise systemic flow by reducing preload and worsening left ventricular outflow tract obstruction.

Preload

Decreases in preload lead to diminished stroke volume and cardiac output and are most often caused by low effective circulating blood volume. This can be due to loss of circulating blood volume following hemorrhage (absolute hypovolemia), or the circulating volume may be inadequate for the vascular space as in vasodilatory shock or as a side effect of administration of lusitropes (relative

hypovolemia). Because approximately 75% of the circulating blood volume is on the venous side of the circulation at any given time, the increases in venous capacitance caused by venodilation significantly contribute to relative hypovolemia under these circumstances. Because preload is also augmented by the negative intrathoracic pressure generated at each spontaneous inspiration, the positive intrathoracic pressure associated with positive pressure mechanical ventilation reduces venous return and hence preload and cardiac output (Henning, 1986; Biondi et al., 1988).

Contractility

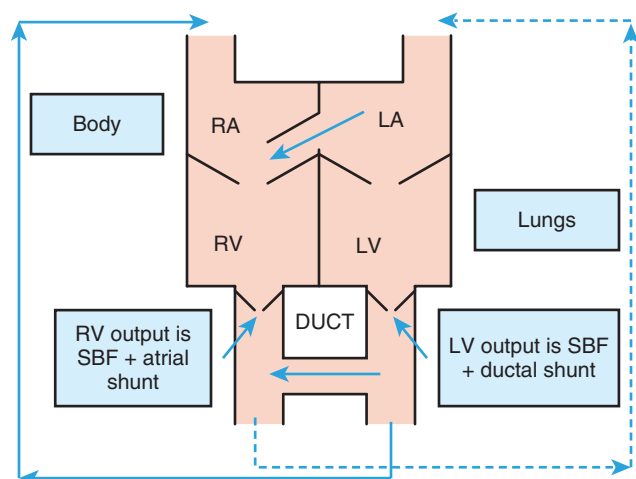
The strength of myocardial contractility depends on the filling volume and pressure and the maturity (Friedman, 1972) and integrity of the myocardium. Thus decreases in preload (hypovolemia, cardiac arrhythmia), prematurity (especially extreme immaturity), hypoxic insults, and infectious (viral or bacterial) agents (Walther et al., 1985a) all negatively affect the ability of the myocardium to contract, with resultant decreases in cardiac output.

Afterload

If cardiac afterload is too high, the ability of the myocardium to contract and pump may become compromised, and cardiac output may fall (Roze et al., 1993; Osborn et al., 2002). Such increases in afterload are associated with enhanced endogenous catecholamine release during the period of immediate postnatal adaptation along with loss of the low-resistance placental circulation. Similar increases in afterload are seen in hypovolemia, hypothermia, or when inappropriately high doses of vasopressors/inotropes are being administered to a patient with intact cardiovascular adrenoceptor responsiveness (Seri, 2006). High afterload can affect either ventricle, and if the output of one of the ventricles is reduced, this will affect the function of the other ventricle, especially when the fetal channels are closed. For instance, if the right ventricular output is low because of high pulmonary vascular resistance, the amount of blood traversing the lungs to the left ventricle will be reduced, leading to low systemic blood flow, with blood pooling in the systemic venous system.

Changes in Preload, Contractility, and Afterload During Transition

With delivery and the separation of the placenta, the fetal circulation begins its transition to the mature (adult-type) circulation in which the systemic and pulmonary circuits are in series and, with the closure of the fetal shunts between the two circulations, the right and left cardiac outputs are equal. However, some component of the normal transition (e.g., removal of placental circulation) is rather abrupt, whereas other components (cessation of flow through the ductus arteriosus and foramen ovale) are more gradual (Kiserud and Acharya, 2004). With the initiation of breathing resulting in lung expansion and the separation of placenta, the pulmonary vascular resistance drops precipitously and systemic vascular resistance increases, respectively. The resultant increase in the left ventricular afterload could lead to a decrease in myocardial contractility, which in selected populations of vulnerable preterm infants may result in decreased cardiac output. In addition, the aforementioned changes in the pulmonary and systemic vascular resistance lead to an evolution in ductal flow pattern from a purely right-to-left to bidirectional and, eventually, purely left-to-right ductal flow. In healthy term infants, left ventricular stroke volume and output increase in the first few minutes after birth. This change coincides with increasing net left-to-right ductal shunting (Noori et al.,



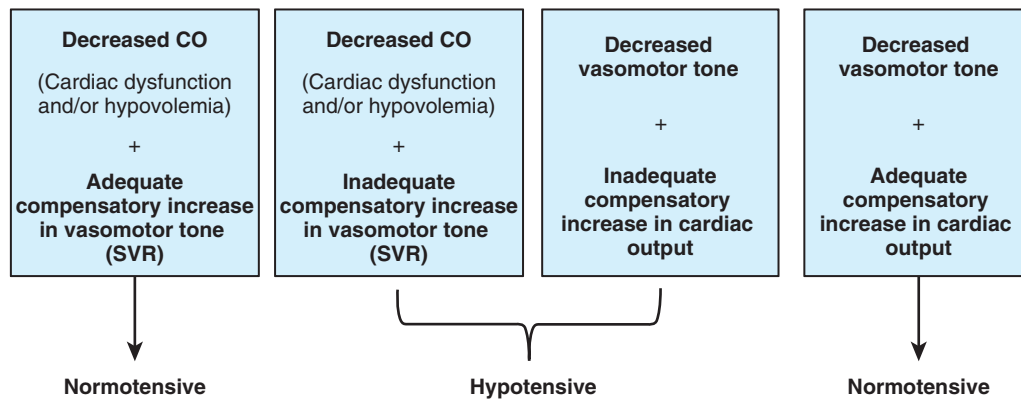
• **Fig. 51.5** Impact of Left-to-Right Shunting Across the Patent Ductus Arteriosus and Patent Foramen Ovale on Left Ventricular Output and Right Ventricular Output Measurements. Effect of left-to-right shunting across the ductus arteriosus and patent foramen ovale on left ventricular output and right ventricular output. Under these circumstances, left ventricular output represents the sum of total pulmonary venous return and ductal blood flow, whereas right ventricular output measures the sum of systemic venous return and left-to-right shunting across the patent foramen ovale. LA, Left atrium; LV, left ventricle/left ventricular; RA, right atrium; RV, right ventricle/right ventricular; SBF, systemic blood flow. (Reproduced with permission from Kluckow M, Evans N. Low systemic blood flow in the preterm infant. *Semin Neonatol.* 2001;6:75-84.)

2012b). The increase in left ventricular preload from ductal shunting likely explains the increase in left ventricular output, and the increase in left ventricular output may offset the potential impact of the left-to-right ductal shunt on the systemic circulation. Therefore, there is usually no evidence of circulatory compromise in the healthy neonate. Subsequently, the ductus arteriosus constricts and then closes in the vast majority of term neonates within 24 to 48 hours. Therefore in the healthy term neonate, the rapidly constricting ductus arteriosus prevents the development of hemodynamically significant left-to-right shunting across the ductus. However, the transition to adult-type circulation is prolonged in preterm infants and especially in extremely preterm infants and can result in circulatory compromise (Kluckow and Evans, 2000b). In 50% to 70% of VLBW neonates, the ductus arteriosus remains open (Reller et al., 1993; Clyman and Noori, 2012), and, as the right-sided pressures fall, blood will shunt left to right from the systemic circulation back into the pulmonary circulation. In most VLBW neonates, pulmonary vascular resistance initially decreases relatively rapidly for physiologic and nonphysiologic reasons (Clyman and Noori, 2012). The physiologic mechanisms most important in the postnatal decrease of pulmonary vascular resistance include the mechanical effects of initiation of breathing on pulmonary vascular resistance and the increased postnatal oxygenation-associated direct, paracrine, and endocrine vasodilation (Faro et al., 2007). Iatrogenic causes include surfactant administration and the inappropriate targeting of higher arterial oxygen saturations (Kluckow and Evans, 2000b; Clyman and Noori, 2012). With the left-to-right ductal shunting, pulmonary overcirculation develops, and left ventricular output, the gold standard of bedside assessment of systemic perfusion, cannot be used as a measure of systemic perfusion (Fig. 51.5; Kluckow and Seri, 2012). Under

these circumstances, left ventricular output measures systemic perfusion and ductal blood flow. In earlier studies investigating the posttransitional changes in systemic perfusion and/or the effects of vasoactive agents on cardiovascular function, this fact was often not acknowledged (Roze et al., 1993; Lundstrom et al., 2000). Therefore the conclusions drawn from some of these studies (Roze et al., 1993) need to be reevaluated. More recent studies have acknowledged this hemodynamic paradigm and used right ventricular output to assess systemic perfusion in the VLBW neonate during the transitional period (Kluckow and Evans, 2000a; West et al., 2006; Abdel-Hady et al., 2008; Bouissou et al., 2008). However, right ventricular output represents systemic perfusion only as long as left-to-right shunting across the foramen ovale does not become significant. In many preterm neonates, however, as left-to-right shunting across a nonconstricting patent ductus arteriosus (PDA) increases during the first 12 to 36 hours, left atrial volume and pressure increase, often leading to the development of a significant left-to-right shunt across the foramen ovale (Evans and Iyer, 1994). The left-to-right shunt through the patent foramen ovale will then render the use of right ventricular output as a measure of systemic blood flow inaccurate, because right ventricular output now represents systemic inflow and patent foramen ovale flow (Kluckow and Seri, 2012). This hemodynamic scenario results in the lack of an acceptable conventional measure of systemic blood flow in these neonates. To circumvent this problem, SVC flow has been used as a measure of upper body blood flow in preterm neonates with the fetal channels open (Kluckow and Evans, 2000a; Kluckow, 2005). The use of SVC flow has provided novel insights into the mechanisms of transitional hemodynamics, such as the observation that intraventricular hemorrhage develops in many VLBW neonates as systemic blood flow increases, resulting in reperfusion of the brain (Kluckow and Evans, 2000c) (see later discussion). However, the vulnerability of SVC flow measurements to error and the technical difficulties associated with their use as a surrogate measure of systemic blood flow have forced these measurements to remain primarily as a research rather than a clinical tool (Evans, 2012; Ficial et al., 2013).

Developmental Regulation of Systemic Blood Pressure

Systemic blood pressure is the product of systemic blood flow and systemic vascular resistance. There is an association between low blood pressure and central nervous system injury in the preterm neonate (Miall-Allen et al., 1987; Bada et al., 1990; Goldstein et al., 1995; Watkins et al., 1999). Yet, blood pressure correlates only weakly with blood flow in this patient population during the period of immediate postnatal adaptation when the fetal channels are open (Kluckow and Evans, 2000a). Thus in preterm infants during the first postnatal day, blood pressure may be low because resistance (vasomotor tone) is low even in the presence of normal or high blood flow (Fig. 51.6). Alternatively, blood pressure may be normal or high because resistance is high in the presence of normal or low blood flow (Evans and Kluckow, 1996). The uncertainty surrounding the nature of the relationship between blood pressure and systemic blood flow during the transitional period results from our inability to appropriately define the normal blood pressure range (Engle, 2012) and systemic blood flow (see earlier) and to characterize the developmental regulation of organ blood flow and vital organ assignment (see later discussion) in the preterm neonate.



• **Fig. 51.6** Pathophysiology of Neonatal Cardiovascular Compromise in Primary Myocardial Dysfunction and Primary Abnormal Vascular Tone Regulation With or Without Compensation by the Unaffected Other Variable. Illustration of why blood pressure can remain in the “normal” range when there is an appropriate compensatory increase in either vasomotor tone or cardiac output (CO). In the hypotensive scenarios, the compensatory mechanisms have been exhausted. SVR, Systemic vascular resistance. (Courtesy Wu TW, Noori S, Seri I. Neonatal hypotension. In: Polin RA, Yoder MC, eds. *Workbook in Practical Neonatology*. 5th ed. Saunders: Philadelphia, PA; 2014:230–243.)

Developmental Regulation of Organ Blood Flow and Its Autoregulation and Vital Organ Assignment

Cerebral Blood Flow Autoregulation

Even very immature preterm neonates autoregulate their cerebral blood flow (CBF) (Seri et al., 1998; Tyszczuk et al., 1998; Tsuji et al., 2000). However, the autoregulatory blood pressure range in this patient population is believed to be narrow, and thus “normal” blood pressure is very close to the lower elbow of the autoregulatory curve (Greisen, 2005, 2012). Organ blood flow autoregulation is impaired in preterm neonates who are sicker and/or more immature (Tsuji et al., 2000; Wong et al., 2008; McLean et al., 2012). In these patients, changes in blood pressure are mirrored by changes in CBF with a high coherence, and these babies are at higher risk of cerebral injury (Pryds et al., 1989; Tsuji et al., 2000; Wong et al., 2008; O’Leary et al., 2009). Factors that impair CBF and other organ blood flow autoregulation include birth asphyxia, acidosis, infection, hypoglycemia, tissue hypoxia and ischemia, and sudden alterations in arterial carbon dioxide tension (PaCO₂) (Greisen, 2012). It is of clinical importance that the CO₂–CBF reactivity is more robust than the blood pressure–CBF reactivity, as a 1-mmHg change in PaCO₂ results in an approximately 4% change in CBF, whereas a 1-mmHg change in blood pressure is associated with an approximately 1% to 2% change in CBF only (Müller et al., 2002; Greisen, 2005). The impairment of CBF autoregulation in the preterm neonate during the immediate postnatal period has been proposed to contribute to cerebral injury with loss of vascular reactivity to both blood pressure and CO₂ (Pryds et al., 1989; O’Leary et al., 2009). However, the finding that impaired autoregulation may also be a consequence of a preceding ischemic insult (Greisen, 2012) makes clarification of this question particularly difficult.

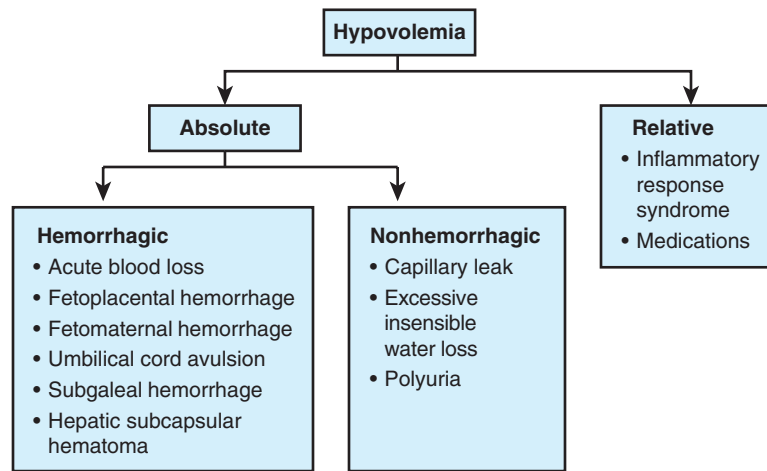
Vital Organ Assignment

The vessels of the vital organs respond to decreased perfusion pressure and/or oxygen delivery with vasodilation (i.e., high-priority vascular beds), whereas the vessels of the nonvital organs, with low-priority vascular beds, vasoconstrict. Several lines of

evidence in human neonates and developing animals suggest that the assignment of the forebrain circulation to a high-priority vascular bed may not be complete at birth (Hernandez et al., 1982; Ashwal et al., 1984; Victor et al., 2006a). For instance, the vessels of the forebrain of dog pups vasoconstrict like those of a nonvital organ, whereas the vessels of the hindbrain vasodilate in response to hypoxia (Hernandez et al., 1982). The finding that CBF autoregulation also appears in the brainstem first and in the forebrain only later in gestation (Ashwal et al., 1984) supports the notion that there are developmentally regulated differences in the timing of the blood flow autoregulatory functions and vital organ assignment characteristics between the forebrain and the hindbrain. The cellular mechanisms responsible for the assignment of vital and nonvital organ status from a blood flow regulatory standpoint are poorly understood. From these findings it is tempting to speculate that the diminished capacity of the forebrain vessels to vasodilate in the very preterm neonate during the complex process of cardiovascular transition after delivery may contribute to hypoperfusion of the forebrain. These neonates may have blood pressures in the perceived normal range while being in the compensated phase of shock. Because this early phase of shock is difficult to recognize immediately after delivery, and because the forebrain is a nonvital organ in very preterm neonates immediately after delivery (Noori et al., 2009), forebrain hypoperfusion can go unnoticed. Similarly, the subsequent evolution to the reperfusion phase as a result of eventual adaptation of the cardiovascular system to the extrauterine environment may not be detected clinically. The proposed nonvital organ assignment–associated hypoperfusion–reperfusion cycle might then contribute to cerebral injury in the very preterm neonate (Noori et al., 2009, 2014a; see later).

Developmental Regulation of Cerebral Oxygen Demand–Delivery Coupling

Very little is known about the regulation of oxygen demand–oxygen delivery coupling in neonates, especially in the transitional period. Yet, several lines of evidence indicate that the very preterm neonate is unable to couple cerebral oxygen demand with cerebral blood flow and instead increases oxygen extraction when oxygen demand is increased (Victor et al., 2006a, 2006b). This phenomenon may



• **Fig. 51.7** Causes of Hypovolemia in Neonates. Etiological factors responsible for absolute (hemorrhagic and nonhemorrhagic) and relative hypovolemia.

be linked to the developmental delay in the vital organ assignment of the forebrain immediately after delivery (see earlier discussion).

Phases of Shock

From a pathophysiologic standpoint, three phases of shock depicting advancing severity have been identified (Zaritsky and Chernow, 1984; McLean et al., 2012).

In the “compensated phase,” complex neuroendocrine and autonomic compensatory mechanisms maintain perfusion and oxygen delivery in the normal range to the vital organs (brain, heart, and adrenal glands) at the expense of decreased perfusion to the remaining organs (nonvital organs). This is achieved by vasodilation and vasoconstriction of the vessels to vital and nonvital organs respectively in response to a fall in perfusion pressure and/or oxygen delivery (Sheldon et al., 1979; Iwamoto, 1993). Blood pressure is maintained within the normal range, and heart rate increases. As perfusion of nonvital organs is decreased because of the compensatory vasoconstriction of their vascular beds, there are often clinical signs of compromised nonvital organ function, such as decreased urine output. In addition, signs of poor peripheral perfusion can often be detected, such as cold extremities and prolonged CRT.

If adequate treatment is not commenced, compensatory neuroendocrine and autonomic mechanisms begin to be exhausted, and hypotension develops as the shock enters its “uncompensated phase.” Systemic perfusion (cardiac output) will decrease, perfusion of all organs including the vital organs becomes compromised, and lactic acidosis develops (Zaritsky and Chernow, 1984).

If treatment is ineffective in the uncompensated phase of shock, multiorgan failure develops, and shock may enter its “irreversible phase,” where permanent damage to the various organ systems occurs, and further interventions will be ineffective in reversing the patient’s condition.

Pathogenesis of Neonatal Shock

Etiologic Factors

The etiologic factors leading to the development of neonatal shock include hypovolemia, myocardial dysfunction, abnormal peripheral vasoregulation, or a combination of two or all three of these factors.

Hypovolemia

Hypovolemia may be absolute (loss of intravascular volume), relative (increased venous capacitance), or combined, such as is often seen in septic shock (Fig. 51.7). Hypovolemia results in cardiovascular compromise primarily by the decrease in cardiac output (systemic blood flow) caused by the decrease in preload. In addition, if blood loss is the primary cause of hypovolemia, the associated decrease in oxygen-carrying capacity contributes to the development of the circulatory compromise. Because of the weak relationship between blood pressure and blood volume in hypotensive preterm neonates, hypovolemia was traditionally thought to be a relatively uncommon primary cause of circulatory compromise, especially during the first postnatal day (Barr et al., 1977; Wright and Goodhall, 1994). However, given the difficulty in assessing intravascular volume especially during the transition, hypovolemia can be difficult to detect clinically. Therefore the true contribution of hypovolemia to circulatory failure is uncertain. Recent studies comparing the effects of delayed umbilical cord clamping or cord milking with immediate cord clamping found increased blood pressure and decreased use of vasopressors/inotropes, suggestive of improved hemodynamic status in the delayed cord clamping and cord milking groups (Rabe et al., 2012; Hooper et al., 2015; Katheria et al., 2015). In addition, patients with delayed cord clamping have higher blood volume, and fewer of them receive transfusions (Farrar et al., 2011; Rabe et al., 2012; Backes et al., 2014). These findings imply that hypovolemia might be a more common presentation in preterm neonates who receive standard care with immediate cord clamping than previously thought.

Absolute hypovolemia in the newborn can be due to several conditions. Intrapartum fetal blood loss is usually caused by open bleeding from the fetal side of the placenta, and therefore it is likely to be detected. More difficult to diagnose is the closed bleeding of an acute fetomaternal hemorrhage or an acute fetoplacental hemorrhage. The latter can occur during delivery where the umbilical cord comes under some pressure (breech presentation or nuchal cord). Because the umbilical vein is more compressible, it is occluded before the artery, and blood continues to be pumped into the placenta. If the cord is clamped early, the excess blood remains trapped in the placenta. This probably happens to some degree in all babies with tight nuchal cords, who, as a group, have lower hemoglobin levels (Shepherd et al., 1985). However, in some

neonates a tight nuchal cord may also cause severe circulatory compromise (Vanhaesebrouck et al., 1987). Postnatally, acute blood loss may occur from any site and is frequently associated with perinatal infections or severe asphyxia-induced endothelial damage and the ensuing disseminated intravascular coagulation. Finally, acute abdominal surgical problems and conditions associated with the nonspecific inflammatory response syndrome and subsequent increased capillary leak with loss of fluid into the interstitium can lead to significant decreases in the circulating blood volume. Iatrogenic causes of absolute hypovolemia include inadequate fluid replacement in conditions of increased insensible losses in the very preterm neonate and gastroschisis before closure of the defect and the inappropriate use of diuretics.

Relative hypovolemia (i.e., a decrease in the effective circulating blood volume) may occur in pathologic conditions leading to vasodilation such as those associated with the nonspecific inflammatory response syndrome (sepsis, necrotizing enterocolitis, asphyxia, major surgical procedures, use of extracorporeal membrane oxygenation). In addition, the use of afterload-reducing agents (e.g., milrinone, prostaglandin E2) may cause significant vasodilation (especially venodilation), thereby decreasing the effective circulating blood volume.

Finally, absolute and relative hypovolemia most frequently occur in conditions associated with nonspecific inflammatory response syndrome, such as sepsis, asphyxia, and major surgical procedures.

Myocardial Dysfunction

Both systolic and diastolic cardiac dysfunction can cause circulatory failure. As echocardiographic assessment of diastolic function is complex and not well established, except in extreme cases, diastolic dysfunction often goes undetected. Nevertheless, diastolic dysfunction is recognized to be the primary cause of circulatory failure associated with hypertrophic cardiomyopathy in infants of diabetic mothers. In addition, extrinsic factors such as pericardial effusion evolving to tamponade and tension pneumothorax can lead to diastolic dysfunction. Systolic dysfunction, on the other hand, is easier to diagnose by echocardiography.

Acquired heart disease presenting as circulatory compromise includes cardiomyopathies, postasphyxial myocardial dysfunction due to hypoxic–ischemic injury, viral myocarditis, and myocardial dysfunction in the late stages of septic shock. For more details on structural heart disease and cardiomyopathies, see Chapter 55.

Among the different types of congenital heart disease, structural heart defects that produce a ductus arteriosus–dependent systemic circulation, such as hypoplastic left-sided heart syndrome, critical coarctation, and critical aortic stenosis, if not diagnosed prenatally or immediately after delivery, classically present as acute circulatory compromise with pallor, tachypnea, impalpable pulses, and hepatomegaly as the ductus starts closing. The presentation may be initially misdiagnosed as sepsis.

Abnormal Peripheral Vasoregulation

Peripheral vasodilation causes circulatory compromise by resultant decreases in perfusion pressure. However, patients with intact myocardial function usually have normal or high cardiac output as they attempt to compensate for the decrease in organ blood flow. Pathologic peripheral vasodilation in neonates occurs primarily in conditions associated with nonspecific inflammatory response syndrome, such as sepsis, necrotizing enterocolitis, severe asphyxia, major surgical procedures, use of extracorporeal membrane oxygenation, or respiratory distress syndrome of prematurity. It is of clinical importance that preterm neonates born to mothers with

chorioamnionitis, especially if they have evidence of funisitis (fetal vessel inflammation), frequently have hypotension and hyperdynamic, vasodilatory cardiovascular compromise at birth or shortly afterward (Yanowitz et al., 2002, 2004, 2006).

Clinical Presentations of Shock in Neonates Associated With Multiple Etiologic Factors

Transitional Circulatory Compromise of the Very Preterm Neonate

The transitional circulatory changes at birth and in the first 12 to 24 hours after birth denote a period of unique circulatory vulnerability especially for the extremely preterm infant. As mentioned earlier, the timing of umbilical cord clamping has a significant effect on volume status and systemic hemodynamics. During normal postnatal adaptation, pulmonary vascular resistance falls, systemic vascular resistance rises with removal of the placenta from the circulation, the ductus arteriosus closes, and the foramen ovale is closed by the reversal of the atrial pressure gradient. During this time frame, the left ventricle must double its output. Given that the very preterm infant's cardiovascular system is adapted to the low-resistance intrauterine environment and its myocardium is immature, it is not surprising that it has difficulties during this critical period. In addition, as discussed earlier, developmentally regulated factors such as the state of vital organ assignment of the forebrain and cerebral oxygen demand–flow coupling make cardiovascular adaptation of the very preterm neonate an even more complex process. It is important to note that there is much more to understand about the complex interactions between immediate postnatal cardiovascular adaptation and immaturity, organ development, myocardial and vasoregulatory function, and vital organ assignment.

Low Preload and Immediate Umbilical Cord Clamping

Following delivery of a preterm infant, immediate clamping of the umbilical cord has been the standard of clinical care since the 1960s. However, as this practice is associated, among other factors, with inadequate transfer of blood from the placenta to the newborn, the approach to the timing of cord clamping has started to change. Recent findings have demonstrated that delayed umbilical cord clamping increases blood volume (preload), confers a more gradual rise in left ventricular afterload, and allows a smoother cardiorespiratory transition in the first few minutes after birth. These beneficial effects are likely responsible for the observation that preterm infants delivered with delayed cord clamping have higher brain tissue oxygen saturation and indices of CBF compared with those delivered with immediate cord clamping (Baenziger et al., 2007; Sommers et al., 2012). Initiation of breathing seems to be a key factor in aiding effective placental transfer of blood to the infant (Bhatt et al., 2013; Boere et al., 2015). However, as cord milking also improves hemodynamics and indices of CBF (SVC flow) (Hosono et al., 2009; Katheria et al., 2015), it appears that the most important factor in enhancing smoother hemodynamic transition and reducing the incidence of circulatory compromise during the transitional period is placental transfusion itself rather than the process of maintaining placental connection to preterm infants for a period immediately after birth.

The improvement in systemic hemodynamics with placental transfusion, including CBF, explains, at least in part, the finding of lower incidence of overall periventricular/intraventricular

hemorrhage (P/IVH) in preterm infants who undergo delayed cord clamping or cord milking compared with those who undergo immediate cord clamping (Mercer et al., 2006; Rabe et al., 2012; Backes et al., 2014). This finding along with a host of other benefits has initiated support for the use of delayed cord clamping by the American College of Obstetricians and Gynecologists (Committee on Obstetric Practice, American College of Obstetricians and Gynecologists, 2012) as well as by the Neonatal Resuscitation Program (Perlman et al., 2015). However, little is known about the optimal timing of cord clamping after delivery, the populations who benefit most from it, the position of the baby relative to the placenta, and the preferred mode of respiratory support before the cord is clamped. Furthermore, as a recent study showed some evidence of better response to milking compared with delayed cord clamping in preterm infants born by cesarean delivery (Katheria et al., 2015), more needs to be understood about the most appropriate way or ways to promote placental transfusion.

Myocardial Dysfunction and High Afterload

As mentioned earlier, the abrupt increase in left ventricular afterload following the sudden removal of the low-resistance placenta can be detrimental to the immature myocardium. There is a normal inverse linear relationship between the heart rate–corrected velocity of circumferential shortening (an index of contractility) and wall stress (an index of afterload). Even in term infants the myocardium is particularly sensitive to a rise in afterload, as demonstrated by a steeper inverse relationship between the heart rate–corrected velocity of circumferential shortening and wall stress compared with that in mature heart of older children (Rowland and Gutgesell, 1995). As preterm infants have anatomically and functionally immature myocardium, they are especially prone to systolic dysfunction. Indeed, the ensuing systolic dysfunction is thought to be one of the key factors in the development of low cardiac output in a subset of high-risk preterm infants during the transitional period (Osborn et al., 2007; Noori and Seri, 2015b).

Patent Ductus Arteriosus

As mentioned earlier, a significant proportion of preterm infants fail to constrict and close their ductus arteriosus. The impact of prolonged left-to-right shunting on the pulmonary circulation and the resultant systemic hypoperfusion are discussed in Chapter 54. However, even a short exposure to significant left-to-right PDA shunting in susceptible preterm infants can result in pulmonary hemorrhage and systemic hypoperfusion and hypotension during the transitional period. Furthermore, it is important to recognize that ductal shunting can have significant hemodynamic effects before it can be clinically detected. In healthy term neonates, PDA shunting changes to predominantly left to right within the first few minutes after birth (Noori et al., 2012). Although no data are available on PDA shunting at the time of delivery in preterm infants, by 5 hours after birth the vast majority of these patients have a completely or predominantly left-to-right shunt across the ductus arteriosus (Kluckow and Evans, 1995). The heart significantly increases its output in an attempt to compensate for the left-to-right ductal shunt. There is some evidence that, at least in a subset of preterm neonates, this compensation may be inadequate in the early postnatal period (Kluckow and Evans, 2000c; Noori et al., 2012). Indeed, preterm infants with low SVC flow have a larger PDA compared with those with normal SVC flow but only during the first 6 to 12 hours postnatally (Kluckow and Evans, 2000c). This suggests that inadequate adaptation to ductal shunting early after birth may contribute to cerebral hypoperfusion immediately

after delivery. Therefore with regard to the negative effect of PDA shunting on CBF, the most vulnerable period might be the first 6 to 12 hours of postnatal life (Noori and Seri, 2015b).

Respiratory Support and Hemodynamics

Extremely preterm infants commonly require respiratory support during the transitional period. However, positive intrathoracic pressure associated with the provision of respiratory support could potentially adversely affect venous return and result in decreased preload and cardiac output. In poorly compliant lungs (e.g., in patients with respiratory distress syndrome before administration of surfactant) the effect on venous return is minimal (de Waal et al., 2009). However, in compliant lungs, venous return can be severely impaired (Hausdorf and Hellwege, 1987). These findings highlight the importance of understanding the possible adverse effects of provision of respiratory support on pulmonary and systemic hemodynamics so that the most appropriate settings and weaning strategies can be used.

Permissive hypercapnia is a lung-protective strategy commonly used in an attempt to reduce the need for ventilator support in preterm infants (Miller and Carlo, 2007; van Kaam et al., 2013). However, little is known about the safety and the optimal carbon dioxide (CO₂) range of permissive hypercapnia (Thome et al., 2006; Noori et al., 2014b). In addition to its direct effects on cerebral hemodynamics (see later) and because of the immature renal compensatory mechanisms of the preterm neonate, permissive hypercapnia invariably leads to severer respiratory acidosis during the transitional period. The effects of acidosis on the cardiovascular system in the neonate in general and the preterm infant in particular are largely unknown (Noori et al., 2013). In adults, respiratory acidosis decreases myocardial contractility and systemic vascular resistance but, presumably because of the resultant lower afterload, also increases cardiac output (Weber et al., 2000). In hemodynamically stable preterm infants, a recent study showed no association between acidic pH and indices of myocardial function and contractility and systemic vascular resistance during the first 3 days postnatally (Noori et al., 2013). While this finding might be considered reassuring, it is important to note that the cardiovascular effects of an acidic pH in hemodynamically unstable neonates are not known.

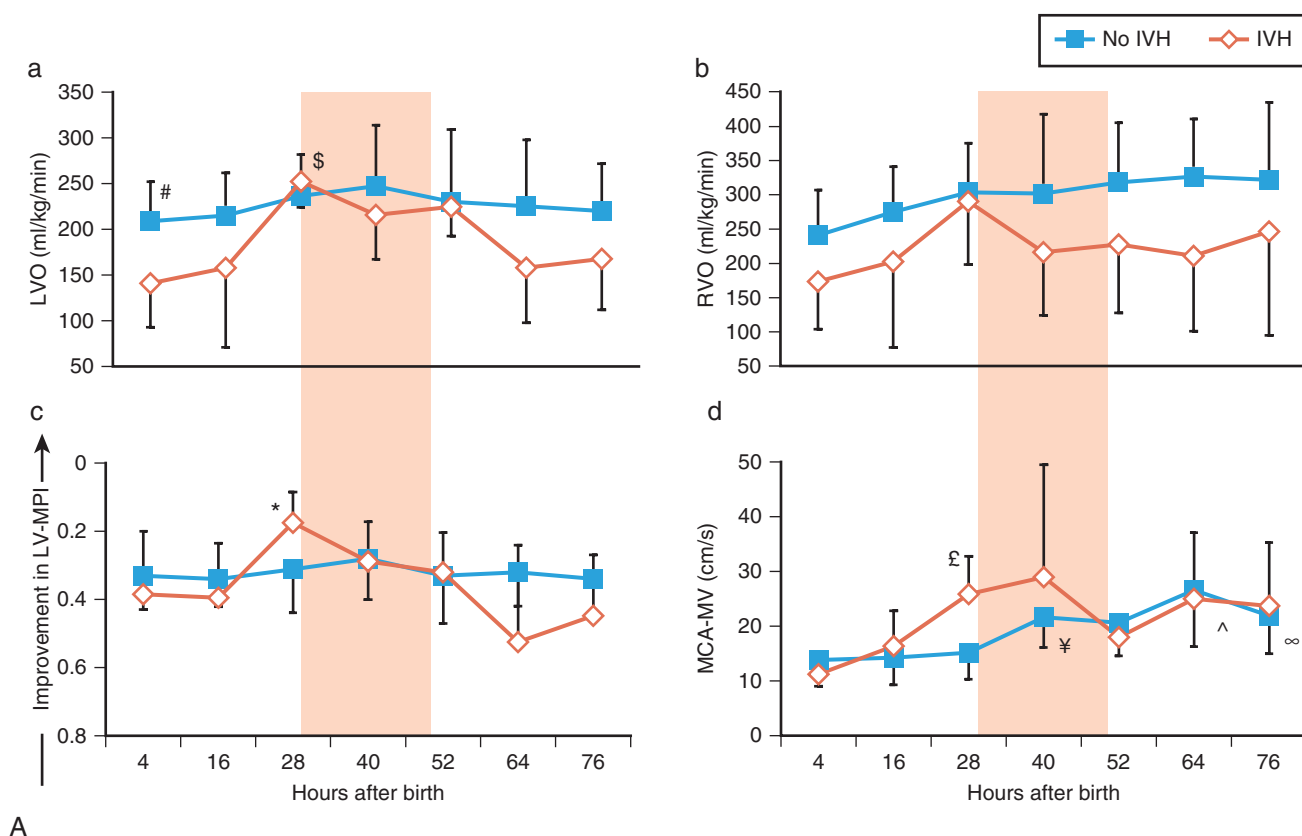
Ischemia–Reperfusion

It has long been recognized that preterm infants with low CBF during the immediate postnatal period are at greater risk of developing P/IVH (Meek et al., 1999b). The decrease in CBF in these patients has been documented with use of different techniques (Kluckow and Evans, 2000c; Kissack et al., 2004). In addition, in some patients with low SVC flow, it was observed that P/IVH occurred after increases in SVC flow (Kluckow and Evans, 2000c). Similarly, higher cerebral regional oxygen saturation (CrSO₂) and lower oxygen extraction were recently reported in preterm infants during 24 hours before detection of P/IVH (Alderliesten et al., 2013). These findings might suggest that cerebral reperfusion leads to development of P/IVH. However, these observations are not universal as others have documented only persistently low CBF for more than week after birth (Verhagen et al., 2010). Recent advancement in NIRS technology has made continuous monitoring of indices of CBF feasible (see section on *diagnosis*). When, during the first 3 days postnatally, extremely preterm infants were comprehensively evaluated by, among other methods, continuous monitoring of CrSO₂ by NIRS and periodic cardiac function, systemic perfusion, and cerebral perfusion assessments by

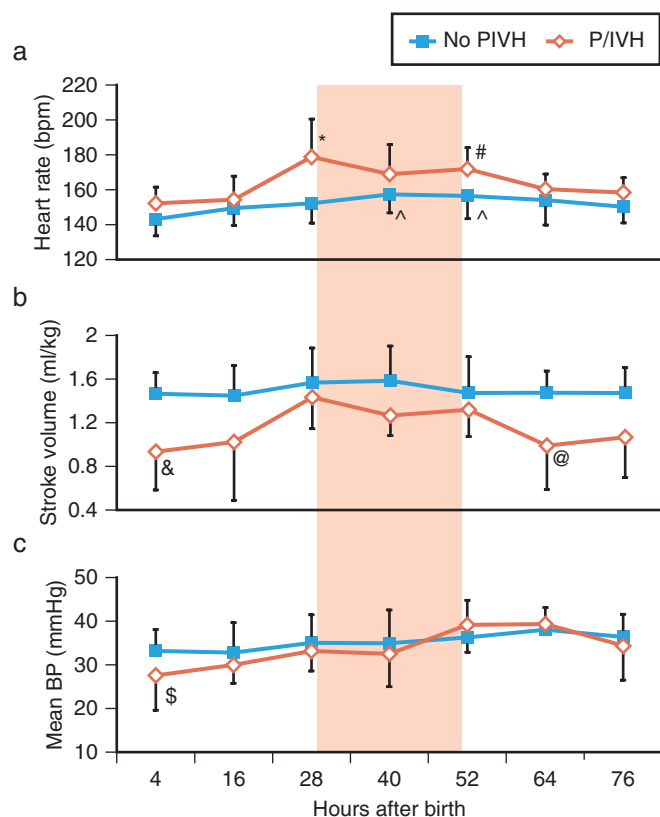
ultrasonography, a phase of initial cerebral ischemia and lower systemic perfusion (Fig. 51.8) followed by a reperfusion phase preceding the detection of P/IVH was documented (Fig. 51.9; Noori et al., 2014a). The cerebral hypoperfusion and reperfusion coincided with low cardiac output and improvement in cardiac output, respectively.

Although, as mentioned earlier, myocardial dysfunction may contribute to the occurrence of low cardiac output, this finding has not been consistently documented (Osborn et al., 2007; Noori et al., 2014a). The initial low cardiac output is likely multifactorial, and decreased preload, especially with immediate cord clamping and/or with the use of inappropriately high mean airway pressure, poor myocardial contractility in the setting of myocardial immaturity and high afterload, and uncompensated ductal shunting are among the suggested pathogenic factors (Noori and Seri, 2015a). On the other hand, the reperfusion phase could also be a consequence of

the recovery of cardiac function potentiated by hypercapnia and/or the inappropriate titration of medications used in the management of neonatal shock. As increases in CO_2 result in increases in CBF, permissive hypercapnia may exaggerate the reperfusion phase. Epidemiologic data reveal an association between hypercapnia and P/IVH (Kaiser et al., 2006; Fabres et al., 2007; Ambalavanan et al., 2015). Furthermore, PaCO_2 values above the low-to-mid 50 mmHg strongly correlate with indices of increased CBF suggesting a significant rise in CBF with the use of more extreme levels of permissive hypercapnia (Noori et al., 2014b). Hypercapnia also attenuates CBF autoregulation, likely putting preterm infants at higher risk of uncontrolled increases in CBF (Kaiser et al., 2005; Noori et al., 2014a). As hypotensive preterm infants also have impaired CBF autoregulation (Munro et al., 2004), excessive and abrupt increases in blood pressure with the inappropriate use of vasopressors/inotropes can result in sudden, excessive increases in



• **Fig. 51.8** Changes in Selected Hemodynamic Parameters (Left and Right Ventricular Output, Left Ventricular Myocardial Performance Index, Middle Cerebral Artery Mean Velocity, Heart Rate, Left Ventricular Stroke Volume, and Mean Blood Pressure) During the First 76 Hours in Very Preterm Neonates With and Without Periventricular/Intraventricular Hemorrhage. (A) Changes in left ventricular output (LVO; a), right ventricular output (RVO; b), left ventricular myocardial performance index (LV-MPI; c), and middle cerebral artery mean velocity (MCA-MV; d) in the two groups during the study. There was a trend for a lower LVO in the periventricular/intraventricular hemorrhage (P/IVH) group at the baseline with a trend for an increase before the occurrence of P/IVH (highlighted in pink). Lower LV-MPI (i.e., better function) and higher MCA-MV in the P/IVH group preceded the occurrence of P/IVH. The pattern of changes in LVO between the two groups tended to be statistically significant ($P = .068$), whereas that in RVO, LV-MPI, and MCA-MV did not reach statistical significance between the two groups. Statistically significant differences between groups, $P = .04$ (asterisk) and $P = 0.016$ (pound sign); no-P/IVH group compared with the baseline, $P = .02$ (yen sign), $P < .0001$ (caret), and $\infty P = .044$ (infinity sign). Differences approaching statistical significance and suggesting a difference between the groups, $P < .055$ (number sign), and within the P/IVH group compared with the baseline, $P = .07$ (dollar sign). The values represent the mean \pm standard deviation of the data obtained on entry into the study and every 12 hours thereafter.



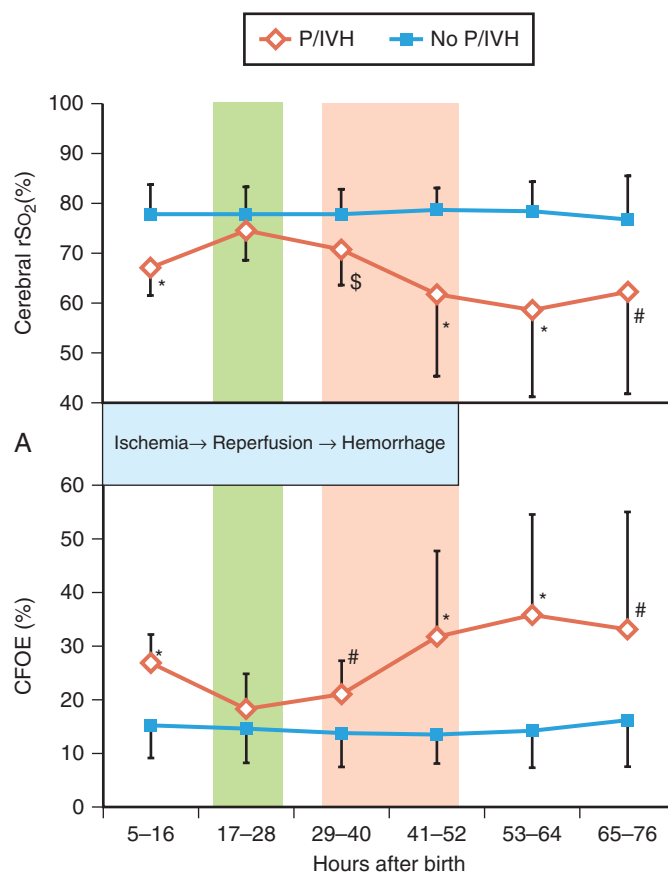
B

• **Fig. 51.8, cont'd** (B) Changes in heart rate (a), left ventricular stroke volume (b), and mean blood pressure (BP; c) in the two groups during the study. Heart rate significantly increased in the no-P/IVH group (ANOVA $P = .004$), while there was only a trend for an increase in the P/IVH group (ANOVA $P = 0.051$) during the study. Compared with the no-P/IVH group, left ventricular stroke volume in patients in the P/IVH group was lower at the baseline but similar before the occurrence of P/IVH. Mean blood pressure (c) tended to increase in the P/IVH group (ANOVA $P = 0.052$), while it remained unchanged and relatively stable in the no-P/IVH group (ANOVA $P = 0.2$). In addition, mean blood pressure at baseline also tended to be lower in patients in the P/IVH group. The pattern of changes in heart rate and stroke volume, but not in mean blood pressure, was different between the two groups. See the text for details. No-P/IVH group compared with the baseline, $P = .007$ (caret); Between groups, $P = .004$ (asterisk), $P = 0.03$ (number sign), $P = .007$ (ampersand), $P = .048$ (at symbol), and $P = 0.085$ (dollar sign). The values represent the mean \pm standard deviation of the data obtained on entry into the study and every 12 hours thereafter. The area highlighted in pink represents the period when P/IVH occurred. bpm, Beats per minute; IVH, intraventricular hemorrhage. (Courtesy Noori S, McCoy M, Anderson MP, et al. Changes in cardiac function and cerebral blood flow in relation to peri/intraventricular hemorrhage in extremely preterm infants. *J Pediatr.* 2014;164:264–270.)

CBF, which in turn might potentiate the reperfusion injury (Noori and Seri, 2015b).

Vital Organ Assignment

As discussed earlier, compared with blood supply to most other organs, blood supply to the brain is relatively protected during hypoxia by its vascular property; hence the brain is considered a vital organ. However, this vascular capacity undergoes a maturation process, with the vessels of the forebrain (including cerebral cortex and basal ganglia) reacting like those of a nonvital organ in very preterm infants during the early postnatal period (Hernandez



B

• **Fig. 51.9** Changes in cerebral regional oxygen saturation (rSO₂) and cerebral fractional oxygen extraction (CFOE) in two groups with (red) and without (blue) periventricular/intraventricular hemorrhage (P/IVH) during first 3 days postnatally. The no-P/IVH group exhibited stable cerebral rSO₂ (A) and CFOE (B) values, while the P/IVH group exhibited a characteristic pattern of changes. The P/IVH group had lower cerebral rSO₂ and higher CFOE during the first 12 hours of the study, followed by normalization of these parameters (highlighted in green) just before the two study periods when P/IVH was detected (highlighted in pink). These findings suggest initial cerebral hypoperfusion followed by a period of reperfusion before the occurrence of the bleeding. After the second study period, cerebral rSO₂ decreased and CFOE increased, suggesting a decrease in cerebral blood flow during and after the development of P/IVH. Statistically significant differences between the two groups, $P < .005$ (asterisk), $P < .04$ (number sign), and $P < .05$ (dollar sign). The values represent the mean \pm standard deviation of the data obtained in each 12-hour data collection period. (Courtesy Noori S, McCoy M, Anderson MP, et al. Changes in cardiac function and cerebral blood flow in relation to peri/intraventricular hemorrhage in extremely preterm infants. *J Pediatr.* 2014;164:264–270.)

et al., 1982; Ashwal et al., 1984; Victor et al., 2006a; Noori et al., 2009). This developmentally regulated phenomenon has significant implications as most extremely preterm infants are in compensated or uncompensated shock, with lower systemic flow, immediately following delivery. In these infants, instead of vasodilating, the vessels of the forebrain vasoconstrict just like those of the nonvital organs (e.g., kidneys or intestine). Accordingly, immaturity of vital organ assignment in the forebrain likely contributes to the observed cerebral ischemia even in nonhypotensive extremely preterm neonates during the immediate postnatal period (see earlier).

Vasopressor-Resistant Hypotension

Although the pathologies of transitional hemodynamics described earlier may or may not present with low blood pressure (compensated versus uncompensated phase of shock), there is a group of preterm neonates in whom persistent hypotension that is resistant to conventional vasopressor/inotropic support develops (Ng et al., 2001; Seri et al., 2001; Watterberg, 2002; Ng et al., 2006; Noori et al., 2006b). The underlying systemic hemodynamic changes in this condition have also been characterized (Noori et al., 2006a). These babies are more likely to be extremely preterm babies (≤ 27 weeks) and/or have been critically ill or had a degree of perinatal asphyxia. The problem may be apparent on the first postnatal day (Ng et al., 2006) but may persist beyond that and represent a state of vasodilatory shock with normal to high systemic blood flow and possibly supranormal cardiac output (Lopez et al., 1997). There are striking analogous features of this presentation in preterm neonates to those of vasodilatory shock described in adults, particularly the lack of responsiveness to vasopressors/inotropes (Landry and Oliver, 2001). Potential mechanisms for the uncontrolled vasodilation include dysregulated cytokine release, excess nitric oxide synthesis, overactivation of the ATP-sensitive potassium channels in the vascular smooth muscle cell membrane in response to tissue hypoxia, and downregulation of the cardiovascular adrenergic receptors (Hausdorff et al., 1990; Seri et al., 2001). In the preterm infant, the foregoing mechanisms are exacerbated by the immaturity-associated relative adrenal insufficiency (Watterberg et al., 1999; Watterberg, 2002; Cole, 2008), are exacerbated by preceding asphyxia, or may be secondary to the transitional circulatory failure as described earlier. In the term neonate an association between congenital diaphragmatic hernia and relative adrenal insufficiency has also been reported (Pittinger and Sawin, 2000).

Sepsis

Although clinical evidence of circulatory compromise is a leading feature of many infectious processes in the newborn, only limited data are available on the hemodynamics in neonatal septic shock (de Waal and Evans, 2010; Saini et al., 2014). In older individuals, two distinct hemodynamic patterns occur: warm shock, characterized by loss of vascular tone, increased systemic blood flow, and low blood pressure, and cold shock, characterized by increased vascular tone, low systemic blood flow, and eventually falling blood pressure. Cold shock has been well described in the newborn (Meadow and Rudinsky, 1995), whereas the warm shock pattern is more difficult to recognize clinically unless the blood pressure and the cardiovascular status in general are being closely monitored. A recent study in preterm infants demonstrated high cardiac output and low systemic vascular resistance consistent with the presence of warm shock (de Waal and Evans, 2010). Another study also found higher left ventricular output in preterm infants with septic shock compared with a control group, suggestive of vasodilation as the dominant pathophysiologic factor (Saini et al., 2014). However, because a significantly higher proportion of neonates in the septic group had a PDA, the increase in left ventricular output in that study could have been due, at least in part, to the presence of the left-to-right shunt rather than high systemic flow *per se*. The mediators of neonatal warm septic shock remain unclear, but in adult sepsis, dysregulated cytokine release and upregulated nitric oxide production as well as deficiency of vasopressin production play an important role (Landry and Oliver, 2001). The significance of this to newborn sepsis remains unclear, but it may have relevance

to the vasopressor-resistant hypotension seen in preterm babies (Ng et al., 2001; Seri et al., 2001; Ng et al., 2006; Noori et al., 2006a, 2012).

Pulmonary Hypertension With or Without Meconium Aspiration Syndrome

Term neonates with severe respiratory failure and pulmonary hypertension have a high incidence of low ventricular outputs (Evans et al., 1998). The low-output state is commonest in the early course of the disease, resolving spontaneously with time and/or clinical improvement. The causes of such circulatory compromise are probably multifactorial (Jain and McNamara, 2015) but include abnormal postnatal cardiovascular adaptation secondary to perinatal hypoxic-ischemic insult, the negative effects of positive pressure ventilation, and the systemic effect of raised pulmonary vascular resistance. In one study, low left ventricular output was a significant predictor of the need for extracorporeal membrane oxygenation in this patient population (Kinsella et al., 1992).

Diagnosis of Circulatory Compromise

There is no agreement regarding what constitutes the gold standard in diagnosing circulatory compromise in the neonate. However, blood pressure remains the most commonly used criterion for diagnosis and initiation of treatment in this patient population (Batton et al., 2013; Stranak et al., 2014). As mentioned earlier, available data suggest that sole reliance on blood pressure can lead to inaccurate or, in other cases, significantly delayed diagnosis of circulatory compromise, especially in the very preterm infant during the immediate postnatal period. On the other hand, it is clear from the observations regarding vasodilatory shock that continuous blood pressure monitoring is imperative. The other commonly used clinical signs of circulatory compromise, such as increased heart rate, slow skin CRT, increased core-peripheral temperature difference, low urine output, and acidosis due to increased lactate production, aid in establishing the diagnosis of circulatory compromise in the preterm or term infant but also have significant limitations.

With recent advancements in biomedical technology, the armamentarium of devices for bedside cardiorespiratory monitoring has broadened, enabling the clinician to perform intermittent and/or continuous assessment of systemic and regional perfusion in neonates (Table 51.1). Although they are used mostly for research applications, these devices have also started to be increasingly used for routine clinical care (Azhibekov et al., 2015).

Heart Rate and Blood Pressure

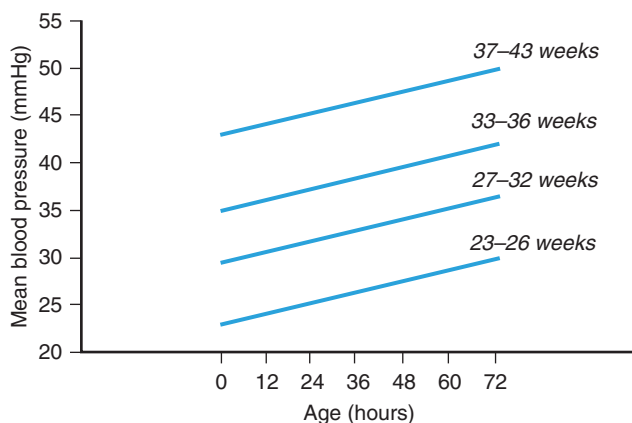
Heart rate is continuously, accurately, and routinely monitored in neonates requiring admission to neonatal intensive care units. Because many factors other than those regulating the cardiovascular system affect heart rate, it has a limited yet widely used role in the diagnosis of circulatory compromise. In babies with invasive arterial access, continuous and accurate measurement of blood pressure is routinely done. The accuracy of the noninvasive oscillometric method is less certain, especially when severe hypotension develops. Normal ranges for blood pressure in babies of different gestations have been defined in the literature (epidemiologic definition of hypotension), and it is clear that gestation and postnatal age are the dominant influences on blood pressure (Lee et al., 1999; Nuntnarumit et al., 1999; for a comprehensive review, see

TABLE 51.1**Systemic and Regional Hemodynamic Parameters Most Commonly Monitored in Neonates**

| | Parameter | Technology/Method | Purpose and Acquisition (C, I, or C/I) |
|----------------------------------|--|--|--|
| Systemic perfusion (BP and CO) | Heart rate | ECG (electrodes) | In conjunction with stroke volume gives flow status (C) |
| | BP | Arterial line/cuff (oscillometry; Doppler ultrasonography) | Perfusion pressure (C/I) |
| | Stroke volume/CO | Echocardiography IEC | Systemic, pulmonary (CO), and organ blood flow, cardiac function (I) Systemic blood flow (CO) (C) |
| Systemic oxygenation | SPo ₂ | Pulse oximetry | Oxygenation on the arterial side (C) |
| CO ₂ status | TCOM | CO ₂ diffusion through skin | Effect on cerebral vasculature (changes in CBF) (C) |
| Regional perfusion | Regional O ₂ saturation | NIRS | Tissue oxygenation and (indirectly) organ perfusion (C) |
| Peripheral perfusion | Microcirculation (oxygenation; blood flow velocity; capillary recruitment) | Visible light technology | Peripheral perfusion (C) |
| | | Laser Doppler flowmetry | Peripheral perfusion (C) |
| | | OPS imaging and SDF imaging | Peripheral perfusion (C) |
| Indirect assessment of perfusion | Capillary refill time | Visual | Systemic perfusion (indirectly) (I) |
| | ΔT (C–P) | Temperature | Systemic perfusion (indirectly) (I) |
| | Color | Visual | Peripheral perfusion (I) |
| Organ function | Brain electrical activity | aEEG | Assessment of brain activity (C) |
| | Urine output | Urinary catheter | Assessment of renal function (I) |

Various components of systemic perfusion (blood pressure [BP] and cardiac output [CO]) and oxygenation, carbon dioxide production and elimination, regional (organ) and peripheral (microcirculation) perfusion, and organ function (amplitude-integrated electroencephalography [aEEG]) that can be monitored at the bedside along with the indirect methods used in clinical practice to assess perfusion and organ function. Acquisition can be continuous (C), intermittent (I), or both (C/I). CBF, Cerebral blood flow; ΔT (C–P), core and peripheral temperature difference; ECG, electrocardiogram; IEC, impedance-based electrical cardiometry; NIRS, near-infrared spectroscopy; OPS, orthogonal polarization spectral; SDF, side-stream dark field; TCOM, transcutaneous CO₂ monitoring.

Modified from Azhibekov T, Noori S, Soleymani S, Seri I. Transitional cardiovascular physiology and comprehensive hemodynamic monitoring in the neonate: relevance to research and clinical care. *Semin Fetal Neonatal Med.* 2014;19:45–53.



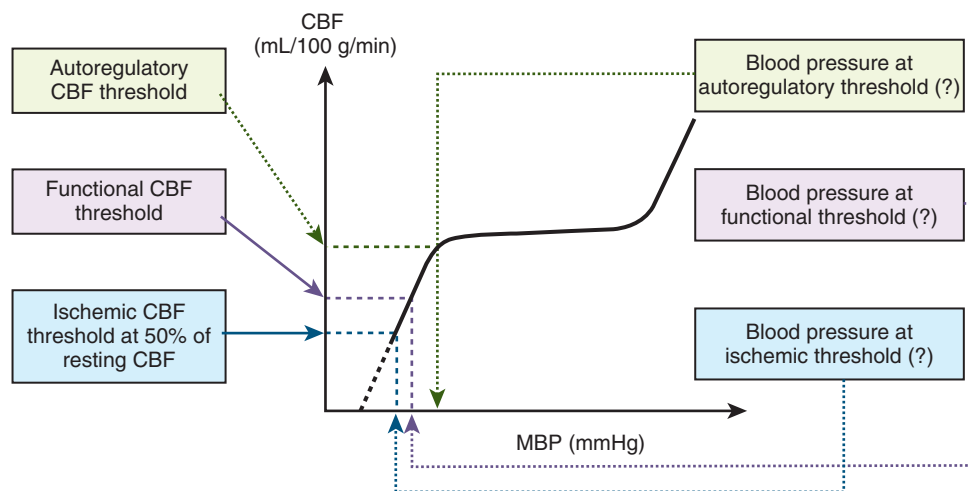
• **Fig. 51.10** Gestational Age- and Postnatal Age-Dependent Nomogram for Mean Blood Pressures in Neonates During the First 3 Days of Postnatal Life. The nomogram is derived from continuous arterial blood pressure measurements obtained from 103 neonates with gestational ages between 23 and 43 weeks. Each line represents the lower limit of the 80% confidence interval of the mean blood pressure for each gestational age group. Thus 90% of infants for each gestational age group are expected to have a mean blood pressure equal to or greater than the value indicated by the corresponding line (the lower limit of the confidence interval). (Courtesy Nuntnarumit P, Yang W, Bada-Ellzey HS. Blood pressure measurements in the newborn. *Clin Perinatol.* 1999;26:981–996.)

Engle, 2012). The nomogram from the data of Nuntnarumit et al. in Fig. 51.10 shows the 10th percentile for mean blood pressure of babies from different gestations at different postnatal ages.

The normal gestational age- and postnatal age-dependent blood pressure range is not known, primarily because it is affected by

additional factors, such as disease severity, history of perinatal insult, presence of infection or ductal shunting, and interindividual variations (Engle, 2012; McLean et al., 2012). In addition to the epidemiologic definition of hypotension, increasing severity of hypotension can be defined from a pathophysiologic standpoint as the blood pressure at which CBF becomes pressure passive (autoregulatory blood pressure threshold; the definition used for the pediatric and adult patient population), brain function is affected (functional blood pressure threshold), and tissue ischemia develops (ischemic blood pressure threshold; Fig. 51.11) (McLean et al., 2012). Some data are available for the autoregulatory and functional blood pressure threshold in very preterm neonates during the first few postnatal days (Munro et al., 2004; Victor et al., 2006b). However, there is no information on the ischemic threshold in very preterm neonates, and very little is known about these pathophysiologic measures of hypotension in the term neonate or in any neonate beyond the transitional period. Perhaps the best method to define hypotension would be the demonstration of causation between gestational age- and postnatal age-dependent blood pressures and clinically relevant outcome measures such as mortality or long-term neurodevelopmental disability. However, because there are no data available on this interaction, there is uncertainty about the normative values. Besides, treatment may have its own side effects; hence there is controversy about when and how to intervene in the critically ill neonate with suspected hypotension and circulatory compromise (Barrington and Dempsey, 2006; Noori et al., 2012; Batton et al., 2016).

In clinical practice, hypotension during the first postnatal day is usually defined in one of three ways: as a mean blood pressure less than 30 mmHg, a mean blood pressure in millimeters of mercury below the patient's gestational age in weeks at the time



• **Fig. 51.11** Definition of Hypotension by Three Pathophysiologic Phenomena of Increasing Severity: Autoregulatory, Functional, and Ischemic Thresholds of Hypotension. CBF, Cerebral blood flow; MBP, mean blood pressure. (Courtesy McLean CW, Noori S, Cayabyab R, Seri I. Cerebral circulation and hypotension in the premature infant—diagnosis and treatment. In Perlman JM, ed. *Controversies in Neonatal Neurology*. Philadelphia, PA: Saunders; 2012:3–25.)

of birth (Lee et al., 1999), or, more recently, a blood pressure that is accompanied by clinically detectable evidence of circulatory compromise (decreased urine output, poor peripheral perfusion, and/or lactic acidosis) (Dempsey et al., 2009). As mentioned earlier, there is only a weak relationship between mean blood pressure and SVC flow, a surrogate measure of systemic blood flow in preterm neonates with the fetal channels open in the immediate postnatal period (Kluckow and Evans, 1996; Lopez et al., 1997; Kluckow and Evans, 2000a; Pladys et al., 1999). Thus if blood pressure alone is used to guide treatment, patients in the unrecognized compensated phase of shock may not be treated appropriately. For instance, in the extremely preterm neonate with mean blood pressure between 20 and 40 mmHg in the immediate postnatal period, the state of systemic blood flow and CBF is unclear based on the blood pressures alone. Recent advances in our ability to apply complex, continuous cardiovascular monitoring providing data in absolute numbers using impedance-based electrical cardiometry, NIRS, and amplitude-integrated EEG combined with the intermittent use of Doppler flow measures hold the promise of gaining a better understanding of the hemodynamic changes occurring during transition and beyond in the neonatal patient population (Noori et al., 2009; Soleymani et al., 2012).

Capillary Refill Time

Several studies have attempted to validate this widely used clinical tool for accuracy. However, there is no validated standard for assessing decreased peripheral perfusion and its relationship to systemic flow in the newborn. Further, in the different types of shock (warm shock vs cold shock) the state of peripheral perfusion is different (vasodilation vs vasoconstriction). Hence it is impossible to objectively assess the practical utility of this clinical tool in neonates. Accordingly, in VLBW neonates a poor correlation was found between CRT, assessed on the forehead, sternum, and toe, and mean blood pressure, urine output, and SVC flow (Miletin et al., 2009). An earlier study documented a similar lack of tight relationship in VLBW neonates; only when CRT was more than 5 seconds did it have any clinically relevant degree of specificity

(Osborn et al., 2001). Although a CRT of 3 seconds or less is traditionally accepted as normal, a CRT of 4.23 ± 1.47 seconds with a range of 1.63 to 8.78 seconds was reported in a large population of healthy newborns during the first 72 hours postnatally. CRT does not appear to change during the first 72 hours postnatally in healthy term newborns (Raju et al., 1999). Environmental, axillary, hand, and foot temperatures have been reported to indirectly relate to CRT (Raju et al., 1999). The duration of the pressure applied when CRT is being measured affects the measurement in term neonates less than 4 hours after delivery (LeFlore and Engle, 2005). That study also found an unanticipated moderate, direct correlation between blood pressure and CRT, with a prolongation of CRT at higher blood pressures in this patient population (LeFlore and Engle, 2005). In general, interobserver variability has been reported to be fair, but a variation in the measurement among the different sites (sternum, forehead, hand, and feet) may be significant, although this issue needs to be more systematically studied.

Core–Peripheral Temperature Difference

There are few data to support the accuracy of this test in older infants (Tibby et al., 1999). In preterm neonates of less than 30 weeks' gestational age, there was also no relationship between this measure and SVC flow in the immediate postnatal period (Osborn et al., 2001). However, because SVC flow measurements have their limitations, these studies need to be repeated when a more accurate and continuous measure of systemic blood flow assessment becomes available for neonates with fetal channels open.

Low Urine Output and Hyperkalemia

Urine output is a frequently used clinical measure to assess renal perfusion and function. However, because urine output is low during the first postnatal day as a result of delivery-associated increases in the levels of stress hormones (catecholamines, vasopressin, renin–angiotensin) causing renal vasoconstriction and increased tubular reabsorption of sodium and water, its value in assessing compensated shock during the transitional period is limited. After

the first 2 to 3 days, however, a decrease in urine output may be the earliest clinical sign of compensated shock in neonates of all gestational ages. In addition, in very preterm infants a strong relationship has been documented between low SVC flow and subsequent low urine output and hyperkalemia (Kluckow and Evans, 2001). However, because hyperkalemia may occur in the very preterm neonate without oliguria (i.e., nonoliguric hyperkalemia of the extremely preterm neonate) (Vemgal and Ohlsson, 2007), and because there could be other causes of hyperkalemia (acidosis), hyperkalemia alone should not be used as a measure of poor systemic perfusion.

Lactic Acid, pH, and Base Excess

In very preterm infants, the relationship between low SVC flow and pH (and/or base excess) remains unclear (Holberton et al., 2012). This is likely due to lack of a strong relationship between pH, base excess, and lactic acid levels in neonates (Deshpande and Platt, 1997). However, because serum lactate levels can be sequentially followed routinely by blood gas measurements and because changes in serum lactate levels are informative of changes in the cardiovascular status, this indirect measure of cellular oxygen delivery and consumption has been used in clinical practice. Combining a CRT of more than 4 seconds with an elevated serum lactate concentration of more than 4 mmol/L has a specificity of 97% for detecting a low SVC flow state in VLBW neonates during the first postnatal day (Miletin et al., 2009). One needs to keep in mind though that a given serum lactate level primarily represents past hemodynamic events and not necessarily the present state of cardiovascular function. Thus as with urine output, by the time lactic acidosis develops and is detected, the initiating event may or may not be present anymore. It is not a surprise therefore that rising lactate levels are more predictive of adverse outcome than a high value early on followed by a subsequent decline (Deshpande and Platt, 1997).

Organ Blood Flow

With the use of Doppler ultrasonography and more recently NIRS, blood flow to various organs can be assessed at the bedside. For CBF, several methods have been studied in both preterm and term neonates, including Doppler ultrasonography, xenon clearance, and NIRS (Greisen et al., 1984; Seri et al., 1998; Tysczuk et al., 1998; Tsuji et al., 2000; Toet et al., 2005, 2006; Lemmers et al., 2006). Because peripheral arteries tend to be too small for size measurement, Doppler studies in such vessels are limited to parameters of velocity from which it is not possible to derive blood flow. Consequently, peripheral artery Doppler ultrasonography tends to be more useful for assessing changes over a time frame in which it is unlikely that the vessel size will have changed (Seri et al., 1998). Xenon clearance is not practical outside a research setting. However, advances in NIRS technology have allowed continuous assessment of brain, renal, mesenteric, and muscle oxygenation (i.e., blood flow) at the bedside in the critically ill neonate (Fortune et al., 2001; Lemmers et al., 2008; van Bel et al., 2008) (see the next section).

Near-Infrared Spectroscopy

NIRS uses the difference in the absorption spectra of oxygenated and deoxygenated hemoglobin to indirectly assess flow. The technology has been very useful as a research tool (Tysczuk et al., 1998;

Meek et al., 1999b; Tsuji et al., 2000; Fortune et al., 2001; Lemmers et al., 2008; van Bel et al., 2008; Caicedo et al., 2011; Alderliesten et al., 2013; Noori et al., 2014a; Verhagen et al., 2010). In addition, with better understanding of biophysical principles that govern light behavior in tissues, and the associated advances in software algorithms and sophisticated NIRS probe development, continuous assessment of regional tissue oxygenation (rSO_2) has become available and gained evidence-based application in neonatal intensive care (Adcock et al., 1999; Andropoulos et al., 2004; Lemmers et al., 2008; Nagdyman et al., 2008; van Bel et al., 2008; da Costa et al., 2015).

Commercially available monitors with up to four channels can be now applied to infants, typically on the forehead to gauge cerebral rSO_2 and over the flank, felt to be measuring renal rSO_2 . Although more validation is needed with use of invasive measurements (Nagdyman et al., 2008; Rhee et al., 2012), it is evident that when cardiac output and/or mean arterial blood pressure fall, renal rSO_2 declines. First, cerebral rSO_2 may be preserved, but interference with global CBF can also be detected with this monitor, provided that certain conditions are met. These monitors are useful during cardiac surgical procedures on infants (Andropoulos et al., 2004; Azakie et al., 2005; Farouk et al., 2008), where precipitous drops in cerebral rSO_2 may be the only warning of a malpositioned venous or arterial cannula while cardiopulmonary bypass is being used. In addition, emerging findings suggest that the monitoring of rSO_2 in critically ill term and preterm neonates without congenital heart disease during transition to extrauterine life (van Bel et al., 2008) and during treatment of various conditions, such as a PDA in preterm neonates (Lemmers et al., 2008; Cayabyab et al., 2009) or asphyxia in infants with or without cooling (Meek et al., 1999a; Toet et al., 2006; Ancora et al., 2009), and in term neonates receiving extracorporeal membrane oxygenation (van Heijst et al., 2004; Fenik and Rais-Bahrami, 2009) provides clinically useful information (van Bel et al., 2008; da Costa et al., 2015). Whether titration of therapy based on rSO_2 measurements in neonates with critical conditions other than congenital heart disease requiring surgery (Andropoulos et al., 2004) improves patient outcomes remains unknown (Hyttel-Sorensen et al., 2015). In our experience, however, renal rSO_2 reliably declines well before signs of uncompensated shock, such as hypotension or acidosis, develop (see later discussion), enabling earlier intervention (Azhibekov et al., 2014).

Echocardiographic Systemic Blood Flow Measures

In the mature circulation, systemic blood flow is the cardiac output. Although the output of both ventricles will be the same, cardiac output is traditionally measured from the left ventricular output. In clinical practice, Doppler ultrasonography offers a noninvasive but noncontinuous method to measure cardiac output. Measuring blood flow directly in the pulmonary artery and ascending aorta enables us to evaluate outputs from the right ventricle and the left ventricle respectively (Alverson et al., 1982; Walther et al., 1985; Evans and Kluckow, 1996). However, as discussed earlier, in transitional circulation of the newborn, neither ventricular output will consistently reflect systemic blood flow because of the shunts across fetal channels (Evans and Iyer, 1994; Evans and Kluckow, 1996; Kluckow and Seri, 2012). Use of SVC flow as a surrogate for systemic blood flow has been a valuable research tool offering insights into hemodynamic events unfolding during the immediate postnatal period when shunting across fetal channels precludes

the use of traditional measures of systemic blood flow assessment (see earlier discussion) (Evans, 2012).

Doppler measures of left ventricular output have been validated against more invasive measures in neonates and older individuals (Mellander et al., 1987). Right ventricular output and SVC flow are less well validated but correlate well with left ventricular output in neonates with no confounding shunts (Evans and Iyer, 1994; Kluckow and Evans, 2000a; Tsai-Goodman et al., 2001). Despite this, the validity of these measures has been questioned on the basis of comparison with cardiac output thermodilution measures in critical care patients (Notterman et al., 1989). However, thermodilution itself has an intrinsic error in that the volume of the required cold saline injection influences cardiac output (Tournadre et al., 1997).

Recently, functional magnetic resonance imaging (MRI) has become the gold standard for cardiac output measurements. However, this method is used primarily for research applications, and its use for bedside assessment in neonates remains limited because of a number of technical and logistic difficulties (Groves et al., 2011). Thus finding an ideal gold standard method capable of continuous bedside cardiac output measurements in absolute numbers remains elusive.

There are significant intrinsic errors in Doppler flow measurements as well. Intraobserver variability rates of around 10% and interobserver variability rates up to 20% are common (Hudson et al., 1990; Kluckow and Evans, 2000a). Most of the error relates to the vessel size measurement that is derived from its diameter. Thus small differences are magnified in the conversion to a cross-sectional area. The other major problem of Doppler flow measurements is that there is reliance on ultrasound technology and echocardiographic skill that may not often be available 24 hours a day in many neonatal intensive care units.

In neonates, most studies quote a normal range for left and right ventricular output between 150 and 300 mL/kg per minute (Alverson et al., 1982; Walther et al., 1985; Evans and Kluckow, 1996; West et al., 2006). It is important to note that in the transitional neonatal circulation, left ventricular output can be affected by ductal shunting to a greater extent than the right ventricular output is affected by atrial shunting. Therefore right ventricular output is a better measure of low flow than left ventricular output during the first 1 to 2 days postnatally. Accordingly, a relationship has been documented between indirect measurements of systemic (and thus cerebral) perfusion such as right ventricular output and blood pressure (and cerebral function) in preterm infants during the first 2 days postnatally. This is a period when ductal shunting significantly decreases the accuracy of left ventricular output measurements reflecting systemic blood flow (West et al., 2006). SVC flow may also be used to estimate systemic flow in the early postnatal period. The normal range in well preterm babies is between 40 and 120 mL/kg per minute, with the median rising from 70 mL/kg per minute at 5 hours of age to 90 mL/kg per minute at 48 hours (Kluckow and Evans, 2000a).

In summary, the mainstay of diagnosing neonatal circulatory compromise has been a combination of blood pressure measurement and evaluation of the previously described clinical parameters. However, none of these parameters have a sufficient degree of accuracy to allow them to be relied on as the sole evaluator of systemic blood flow and tissue perfusion. Therefore the addition of echocardiographic and NIRS hemodynamic assessment to blood pressure monitoring and thorough continuous clinical evaluation of the patient are necessary to better understand changes in organ blood flow and tissue perfusion, especially in preterm neonates during the vulnerable period of immediate transition to extrauterine

life. The goal should be to maintain normal systemic blood flow and thus oxygen delivery in the presence of an acceptable blood pressure with use of the normal range for blood pressure that controls for gestational age and postnatal age and following the indirect clinical and laboratory signs of tissue perfusion (urine output, serum lactate levels, CRT). If there is no immediate access to echocardiography or NIRS technology, the clinician will have to rely on blood pressure monitoring, while recognizing the limitations of this approach. In the past few years, training in functional echocardiography for neonatologists has become available in a structured format in several programs in the United States and other countries (Kluckow et al., 2008; Mertens et al., 2011), although the medicolegal implications of such training remain largely unknown (Nguyen et al., 2016).

Measurement of Systemic Blood Flow by Electrical Impedance Velocimetry

Electrical velocimetry measures left cardiac output by continuous, noninvasive measurement of thoracic electrical bioimpedance (Osypka and Bernstein, 1999). It has also been validated against invasive methods of cardiac output measurements, with very good correlations in animals (Osthaus et al., 2007) and children (Norozi et al., 2008) and against echocardiography in healthy term neonates during the first 2 days postnatally (Noori et al., 2012c). However, its evidence-based value in pediatric clinical practice in general (Coté et al., 2015) and neonatology in particular remains to be established (Azhibekov et al., 2015).

In summary, advances in biomedical technology and development of newer sophisticated methods for comprehensive hemodynamic monitoring may provide additional insights into the pathophysiology of the cardiovascular instability and, potentially, early diagnosis of circulatory compromise; however, their use remains limited mostly to research applications, and they have yet to be validated for routine clinical use (Azhibekov et al., 2015).

Treatment of Neonatal Shock

Selection of the most appropriate treatment strategy for neonatal shock requires identification of its pathogenesis (Seri, 2001; Kluckow, 2005; Seri and Noori, 2005; Kluckow and Seri, 2012; Noori and Seri, 2012). As described earlier, the most frequent etiologic factors responsible for neonatal cardiovascular compromise are inappropriate peripheral vasoregulation, resulting in vasodilation or vasoconstriction, and dysfunction of the immature myocardium (Gill and Weindling, 1993; Seri, 1995; Osborn et al., 2002; Noori and Seri, 2005; Kluckow and Seri, 2012; Noori and Seri, 2012). Although absolute hypovolemia was thought to be a less frequent primary cause of neonatal hypotension, especially in preterm infants in the immediate postnatal period (Barr et al., 1977; Wright and Goodhall, 1994), recent findings suggest that this assumption is incorrect (Baenziger et al., 2007; Hosono et al., 2009; Sommers et al., 2012; Katheria et al., 2015).

Association Between Systemic Hypotension, Hypoperfusion, and Their Treatment and Mortality or Neurodevelopmental Impairment

The impact of hypotension and/or its treatment on mortality, brain injury, or neurodevelopmental impairment is unclear, primarily

because it is assumed that treatment will improve outcomes, and therefore the common practice has been to treat hypotension (Noori et al., 2009). Therefore there are no prospective studies evaluating the impact of untreated hypotension on clinically relevant short-term and long-term outcomes. The only presently available prospective randomized controlled trial (RCT) with a no-treatment arm found that such a trial is not feasible, at least in the United States, primarily due to difficulty in obtaining consents but also because of the clinicians' bias favoring treatment (Batton et al., 2012). To address the consent issue, some have suggested alternative approaches to traditional prerandomization consent procedures, such as waiver of informed consent with an opt-out option after randomization and inclusion (Vain and Barrington, 2012). In addition to the paucity of data from RCTs on the effect of treatment on outcome, a gestational age- and postnatal age-dependent definition of hypotension based on physiology, pathophysiology, and clinically relevant outcome measures is lacking (Engle, 2012).

One prospective RCT found that hypotensive VLBW neonates overall had a higher rate of severe P/IVH than their nonhypotensive counterparts (Pellicer et al., 2009). Hypotensive infants responding with an increase in blood pressure to targeted titration of vasopressors/inotropes had similar rates of P/IVH when compared with nonhypotensive controls. The study authors also did not find an association between abnormal ultrasound findings and the use of dopamine or epinephrine. At long-term follow-up at 2 to 3 years of age, no difference was found in the rate of abnormal neurologic status and/or developmental delay between the formerly hypotensive but successfully treated patients and the control patients. Although the results suggest that carefully titrated use of dopamine or epinephrine in hypotensive preterm infants might be safe and even have potential benefits, the small sample size and the lack of untreated hypotensive controls do not allow generalization of the findings. Of note is that although dopamine was found to be neuroprotective in the asphyxiated lamb model in a recent study (Brew et al., 2016), evidence in the human neonate is needed to establish clinical relevance of this finding.

Finally, the observation that hypotension as currently defined is unlikely to be predictive of brain injury (Limperopoulos et al., 2007; McLean et al., 2012) is important in examining the association between hypotension and its treatment and neurodevelopmental impairment.

Volume Administration

Volume administration is the most commonly used intervention for treatment of hypotension in neonates (Stranak et al., 2014). However, observations that low blood pressure is frequently associated with normal or even high ventricular output and low index of resistance (Pladys et al., 1999) and that dopamine is more effective in increasing blood pressure than volume administration (Lundstrom et al., 2000) question the effectiveness of volume administration primarily when isotonic saline is being used. Therefore particularly in the preterm infant during the immediate postnatal period, only cautious, limited fluid resuscitation has been recommended (Noori and Seri, 2015a). In addition, and as discussed earlier, expansion of blood volume by delayed umbilical cord clamping or cord milking has been shown to improve systemic hemodynamics in preterm infants (Baenziger et al., 2007; Hosono et al., 2009; Sommers et al., 2012; Katheria et al., 2015).

There are no studies yet on the safety and efficacy of the use of whole blood transfusion for treatment of hypotension and shock in neonates in the transitional period or beyond. Furthermore,

because myocardial dysfunction frequently contributes to the development of neonatal hypotension (Gill and Weindling, 1993) and aggressive volume administration in this patient population increases pulmonary, cardiovascular, gastrointestinal, and central nervous system morbidity and mortality (Van Marter et al., 1990; Kavvadia et al., 2000; Lundstrom et al., 2000), judicious use of fluid administration is warranted. On the other hand, combined relative and absolute hypovolemia is clearly a major etiologic factor contributing to neonatal shock in neonates with sepsis and/or in the postoperative period in patients undergoing major surgery. Indeed, early and aggressive fluid resuscitation of children and neonates with sepsis has been documented to decrease mortality (Han et al., 2003) and has been recommended by the American College of Critical Care Medicine (Brierley et al., 2009).

Further controversy has emerged concerning the type of fluid administered to preterm neonates with cardiovascular compromise. Most studies have shown that isotonic saline is as effective as 5% albumin in increasing the blood pressure (So et al., 1997; Oca et al., 2003). In addition, albumin may impair gas exchange and induce fluid shift from the intracellular compartment (Ernest et al., 1999) and is associated with increased mortality (Nadel et al., 1998). Therefore given the documented comparable efficacy of isotonic saline and albumin in the face of differences in cost and the suggested increased mortality and morbidity associated with albumin administration (Van Marter et al., 1990; Kavvadia et al., 2000; Lundstrom et al., 2000), isotonic saline has been the initial choice of treatment in the neonatal patient population. However, an RCT found that in hypotensive, mostly preterm neonates, albumin administration resulted in a greater likelihood of achieving normotension and decreased the subsequent use of vasopressors when compared with isotonic saline (Lynch et al., 2008). The findings of that study need to be replicated before the routine use of albumin can be considered as the initial treatment for neonatal cardiovascular compromise. Therefore unless evidence of serum or blood loss or hypoalbuminemia is present, volume support in hypotensive preterm and term infants is provided in the form of 10 to 20 mL of isotonic saline per kilogram (Seri, 2001; Noori and Seri, 2012). It is also important to note that, because of the unbalanced nature of isotonic saline, caution should be exercised with administration of large amounts in a short period, as this may worsen the metabolic acidosis (Mirza et al., 1999; Prough and Bidani, 1999).

Should the limited-volume administration be ineffective, pharmacologic cardiovascular support with a vasopressor-inotrope or an inotrope is recommended (Seri, 1995, 2001; Osborn et al., 2002; Kluckow, 2005; Seri and Noori, 2005; Noori and Seri, 2012). However, based on the recent findings of the aforementioned studies on the use of delayed cord clamping or cord milking, it is likely that once clinical practice changes as per the recommendations of the expert panels (Committee on Obstetric Practice, American College of Obstetricians and Gynecologists, 2012; Perlman et al., 2015), the need for volume administration in the immediate transitional period will decline.

If there is an identifiable volume loss, the type of fluid lost should be replaced. In cases of blood loss, transfusion with packed red blood cells after the initial crystalloid or colloid bolus or packed red blood cells suspended in fresh frozen plasma with a hematocrit around 55% may be used. In cases of increased transepidermal water losses, higher free water administration without an increase in sodium supplementation is indicated. When polyuria is present, the composition and volume of the replacement fluid may be

adjusted to the urinary sodium and free water losses. However, replacement of half of the excessive urinary losses with 0.45% saline will usually suffice.

Dopamine and Dobutamine

Dopamine treatment and dobutamine treatment were introduced in the management of neonatal hypotension in the early 1980s and mid-1980s, respectively, without appropriately designed randomized and blinded clinical trials. Thus as mentioned earlier, we have no clear evidence that the use of these sympathomimetic amines (or any other sympathomimetic amine) reduces neonatal mortality or morbidity. By examining the drugs' effect on neonatal myocardial contractility, systemic blood flow, and organ blood flow, a number of studies have extended their focus beyond the dopamine- and dobutamine-induced heart rate and blood pressure changes (Roze et al., 1993; Seri et al., 1998; Zhang et al., 1999; Lundstrom et al., 2000; Osborn et al., 2002; Seri et al., 2002; Pellicer et al., 2005; Noori et al., 2006a; Bouissou et al., 2008; Bravo et al., 2015). Some of the earlier studies, however, used left ventricular output to assess the impact of these medications on systemic blood flow even when shunting across fetal channels occurred (Roze et al., 1993; Zhang et al., 1999; Lundstrom et al., 2000). Therefore the conclusions drawn in these studies need to be carefully reevaluated (Kluckow and Seri, 2012).

Hemodynamic Effects of Dopamine

Dopamine, an endogenous catecholamine, is the sympathomimetic amine most frequently used in the treatment of hypotension in preterm infants (Seri, 1995; Stranak et al., 2014). It exerts its cardiovascular actions via the dose-dependent stimulation of the cardiovascular dopaminergic, α -adrenergic, β -adrenergic, and serotonergic receptors. In addition, by stimulating epithelial and peripheral neuronal dopaminergic and adrenergic receptors, the drug exerts significant renal and endocrine effects independently of its cardiovascular actions (Seri, 1995). Although dopamine affects all three major determinants of cardiovascular function (preload, myocardial contractility, and afterload), the drug-induced increases in myocardial contractility (Zhang et al., 1999; Lundstrom et al., 2000) and peripheral vascular resistance (afterload) (Roze et al., 1993; Zhang et al., 1999; Lundstrom et al., 2000) are the most important factors in increasing systemic blood pressure and improving the cardiovascular status.

The original dosage range recommendation of 2 to 20 μg dopamine per kilogram per minute was based on pharmacodynamic data obtained in adults without cardiovascular compromise. However, changes in cardiovascular adrenergic receptor expression caused by critical illness (Hausdorff et al., 1990) and relative or absolute adrenal insufficiency and immaturity (Watterberg and Scott, 1995; Watterberg, 2002), as well as the dysregulated production of local vasodilators during severe illness, decrease the sensitivity of the cardiovascular system to dopamine, resulting in the emergence of hypotension resistant to conventional doses of the drug (Ng et al., 2001; Seri et al., 2001; Ng et al., 2006; Noori et al., 2006a). Thus with the advancement of the disease process, increased doses of dopamine and other sympathomimetic amines may be needed to exert the same magnitude of cardiovascular response. Therefore dopamine administration should be tailored to the drug's pharmacodynamic effects in a given patient at the bedside rather than driven by the conventional dose recommendations based on data obtained in healthy adults. Indeed, although many neonatologists do not advance the dosage of dopamine beyond 20 $\mu\text{g}/\text{kg}$ per

minute, there is no evidence that, when required to normalize blood pressure, high-dose dopamine treatment with or without additional epinephrine administration has detrimental vasoconstrictive effects (Perez et al., 1986; Seri et al., 2001). However, there are no data available on changes in cardiac output and organ blood flow in response to high-dose catecholamine treatment in vasopressor-resistant neonatal shock, and close attention should be paid to signs of inappropriate vasoconstriction when this therapy is applied. Administration of low-dose hydrocortisone in the past decade has been shown to reduce the need for high-dose vasopressor administration in most patients (Ng et al., 2001; Seri et al., 2001; Noori et al., 2006a; Seri, 2006; Cole, 2008).

As hypotension and circulatory failure are most common in the first few postnatal days, a period in which significant shunting at the PDA level is often present in VLBW infants, assessment of the cardiovascular effects of vasopressors/inotropes by echocardiography is challenging. This is especially true with regard to estimation of the magnitude of PDA shunt. Therefore one needs to be cautious in interpreting these studies. With that caveat in mind, several studies suggest that dopamine does not worsen left-to-right PDA shunt. Rather, in hypotensive preterm neonates with a significant left-to-right shunt across the PDA, dopamine administration increased systemic blood pressure, pulmonary pressure, and SVC flow, used as a surrogate of systemic blood flow in these patients (Bouissou et al., 2008). Similarly, dopamine administration has not been associated with evidence of increased pulmonary vascular resistance and decreased right ventricular output in neonates without a significant left-to-right shunting across the PDA (Wardle et al., 1999; Clark et al., 2002). This finding suggests that when pulmonary blood flow is increased, vasoconstrictive mechanisms may be upregulated in the pulmonary circulation, resulting in more effective α -receptor-mediated, dopamine-induced pulmonary vasoconstriction. The findings of an earlier study demonstrating a variable pulmonary resistance response to dopamine in preterm neonates with a hemodynamically significant PDA support this notion (Liet et al., 2002).

The vasodilatory dopamine receptors are primarily expressed in renal, mesenteric, and coronary circulations (Seri, 1995). Dopamine has been shown to selectively decrease renal vascular resistance (Seri et al., 1998, 2002) and increase glomerular filtration rate (Seri et al., 1993) in preterm infants as early as the 23rd week of gestation. However, dopamine appears to decrease mesenteric vascular resistance in preterm infants only beyond the first postnatal day (Hentschel et al., 1995; Seri et al., 1998, 2002; Robel-Tillig et al., 2007), and the effect may be variable (Zhang et al., 1999). Similarly, there are some differences in the reported magnitude of the drug-induced increases in ventricular function, cardiac output, and systemic vascular resistance (Roze et al., 1993; Zhang et al., 1999; Lundstrom et al., 2000; Clark et al., 2002). These findings may be best explained by differences in intravascular volume status, gestational and postnatal age, developmentally regulated expression of cardiovascular adrenergic and dopaminergic receptors, and the severity of adrenergic receptor downregulation among different populations of critically ill infants studied. It is important to note that none of the studies found evidence for a direct effect of dopamine on CBF as long as blood pressure was in the autoregulatory range (Seri et al., 1998; Zhang et al., 1999; Lundstrom et al., 2000; Seri et al., 2002). Thus dopamine administration appears to be devoid of potentially harmful selective hemodynamic effects in the brain. As expected in hypotensive neonates, dopamine (and epinephrine) increases both blood pressure and CBF (Pellicer et al., 2005).

Hemodynamic Effects of Dobutamine

Unlike dopamine, dobutamine is a relatively cardioselective sympathomimetic amine with significant α -adrenoreceptor-mediated and β -adrenoreceptor-mediated direct inotropic effects and limited chronotropic actions (Ruffolo, 1987). Dobutamine administration is usually also associated with a variable decrease in total peripheral vascular resistance and, at least in adults, with improved coronary blood flow and myocardial oxygen delivery (Ruffolo, 1987). Furthermore, unlike dopamine, dobutamine increases myocardial contractility exclusively through the direct stimulation of myocardial adrenergic receptors. Because myocardial norepinephrine stores are immature and rapidly depleted in the newborn, and because dobutamine may decrease afterload, newborns with primary myocardial dysfunction and elevated peripheral vascular resistance are most likely to benefit from dobutamine treatment (Martinez et al., 1992; Osborn et al., 2002). However, dobutamine may offer little hemodynamic benefits if the primary underlying pathophysiology is not poor myocardial contractility. A recent placebo-controlled pilot trial showed dobutamine to have no effect on SVC flow (Bravo et al., 2015). Although addition of dobutamine to dopamine treatment in preterm infants with respiratory distress syndrome was effective in increasing blood pressure, it was associated with supranormal cardiac output states and low systemic vascular resistance (Lopez et al., 1997). Whether the benefits of supranormal cardiac output by providing adequate tissue oxygen delivery throughout the body outweigh the risks of sustained hypercontractility, potentially resulting in myocardial injury, remains to be investigated.

There are only few data available on direct renal, cerebral, or pulmonary hemodynamic effects of dobutamine in the newborn. A nonrandomized study comparing the effects of dopamine and dobutamine on blood pressure and mesenteric blood flow in preterm infants found that both drugs increased blood pressure and were equally effective in decreasing mesenteric vascular resistance (Hentschel et al., 1995). Because dobutamine does not stimulate the dopaminergic receptors, β -adrenoreceptor-induced selective vasodilation may be responsible for the observed mesenteric vasodilation in dobutamine-treated patients.

Dopamine Versus Dobutamine

Randomized studies have uniformly demonstrated that dopamine is more effective than dobutamine in increasing blood pressure in the preterm infant, and a metaanalysis of these studies confirmed that dopamine was more successful than dobutamine in treating hypotension, with fewer infants in the dopamine group facing treatment failure (Subdehar and Shaw, 2000). However, there was no difference in short-term adverse neurologic outcome between the two groups. In the absence of long-term outcome data, no firm recommendations can be made regarding the choice of drug in treating hypotension in preterm infants in the immediate postnatal period. In addition, no information was forthcoming on changes in systemic blood flow. However, with careful titration to a predetermined "optimum" hemodynamic effect, dopamine (and epinephrine) increased blood pressure and CBF in hypotensive VLBW neonates during the first postnatal day (Pellicer et al., 2005). Neurodevelopmental follow-up of patients enrolled in this study at 2 to 3 years of age did not reveal evidence of an independent vasopressor-inotrope-associated increase in morbidity (Pellicer et al., 2009; see earlier). Although the findings of this study provide some reassurance against the possibility of a dopamine (or epinephrine)-associated increase in neurodevelopmental

morbidity in VLBW neonates during the first postnatal day, the original study (Pellicer et al., 2005) was not sufficiently powered to put these concerns to rest.

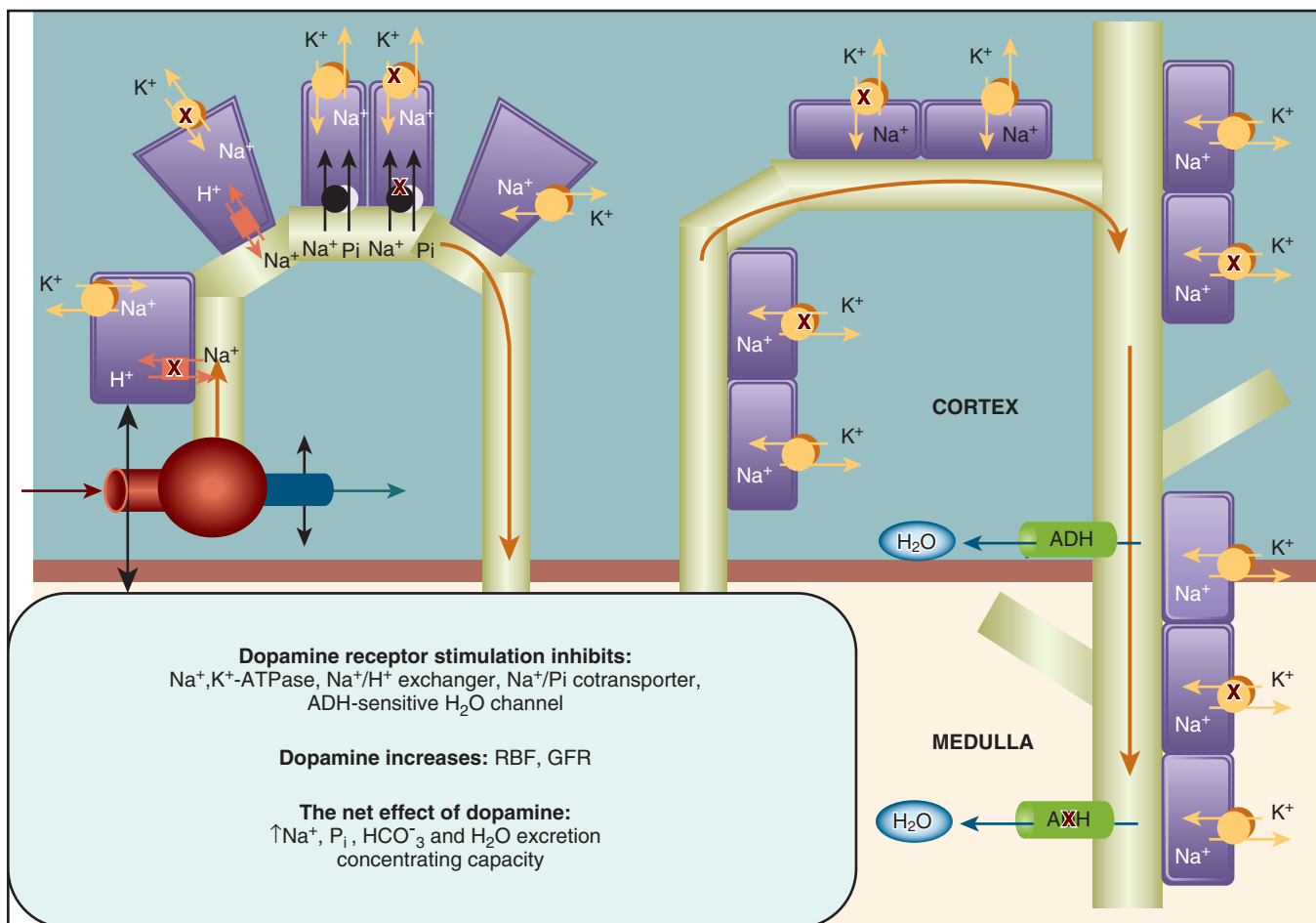
As discussed earlier, because of the weak relationship between blood pressure and systemic blood flow in very preterm neonates during the immediate postnatal period, an increase in blood pressure does not necessarily guarantee that tissue perfusion has improved along with the blood pressure (Kluckow and Evans, 1996; Lopez et al., 1997; Kluckow and Evans, 2000a; Pladys et al., 1999; Kluckow and Seri, 2012). Although a recent study using laser Doppler measurements showed no evidence of peripheral vasoconstriction even with dopamine dosages greater than 10 $\mu\text{g/kg}$ per minute (Ishiguro et al., 2012), other studies have demonstrated impairment in systemic blood flow in a subset of patients despite improvements in blood pressure with higher doses of dopamine (Osborn et al., 2002). Therefore if there is evidence of peripheral vasoconstriction, especially in VLBW neonates during the first postnatal day, high-dose dopamine treatment should be attempted only if systemic blood flow can be monitored by functional echocardiography, NIRS, and/or other advanced perfusion monitoring techniques. However, in clinical practice these measures of blood flow monitoring, with the possible exception of NIRS, are not routinely available, and the neonatologist must rely on monitoring blood pressure and the indirect measures of cardiovascular function. If evidence of vasoconstriction is present with higher doses of dopamine (or epinephrine), the neonatologist should consider accepting blood pressures in the lower range for gestational age and postnatal age and decrease the dose of vasopressor-inotrope to levels where significant α -adrenoreceptor stimulation is less likely (Seri, 1995; Kluckow and Seri, 2012). In these cases and depending on the pathophysiology of the cardiovascular compromise, addition of dobutamine or milrinone should be considered, and the blood pressure should be maintained in the low-normal range. As systemic hypotension has been linked to poor long-term neurodevelopmental outcome in the VLBW neonatal patient population (Miall-Allen et al., 1987; Bada et al., 1990), careful consideration of the lowest acceptable perfusion pressure in the given patient is warranted. A combination of dobutamine or milrinone and low-dose dopamine may achieve the most important goals of treatment by maintaining blood pressure and systemic blood flow in acceptable ranges if monitoring of both cardiovascular parameters is possible. In most of these patients, physiologic glucocorticoid and mineralocorticoid replacement with hydrocortisone is likely to be effective, although the potential side effects of early hydrocortisone exposure in preterm neonates should be kept in mind (see later discussion) (Seri et al., 2001; Seri and Noori, 2005; Cole, 2008; Kluckow and Seri, 2012; Biniwale et al., 2013). In summary, as maintenance of appropriate perfusion pressure is necessary to ensure appropriate tissue oxygen delivery (systemic and organ blood flow) and because both hypotension and low systemic blood flow have been associated with impaired neurodevelopmental outcome, the primary goal of management of the hypotensive very preterm neonate should be the correction of both measures of cardiovascular function.

In hypotensive term and preterm neonates beyond the immediate postnatal period, where vasodilatory shock is the more likely presentation, dopamine (or epinephrine) administration in doses tailored to the cardiovascular response is warranted and appears to be beneficial (DiSessa et al., 1981; Martinez et al., 1992; Pellicer et al., 2005; Seri and Noori, 2005) unless evidence of primary myocardial dysfunction is present (Seri, 1995, 2001).

Epithelial and Neuroendocrine Effects

Independently of the aforementioned cardiovascular effects, dopamine exerts direct renal (Seri, 1995) and endocrine (Seri, 1995) actions in the newborn. Via its actions on renal blood flow and glomerular filtration rate as well as through its direct effects on sodium, phosphorus, and water transport processes and Na^+ , K^+ -ATPase activity in renal tubules, dopamine increases sodium, phosphorus, and free water excretion (Fig. 51.12) and may increase the hypoxic threshold of renal tubular cells during episodes of hypoperfusion and hypoxemia (Seri, 1995). Via its renal vascular and epithelial actions, dopamine also potentiates the diuretic effects of furosemide (Tulassay and Seri, 1986) and theophylline (Bell et al., 1998). Although dopamine has the theoretical potential to attenuate renal side effects of indomethacin, the data in the literature are contradictory (Seri, 1995). Differences in the level of maturity, disease severity, ductal shunting, intravascular volume

status, and indomethacin dose may be responsible for the conflicting results. Among its endocrine actions, the dopamine-induced decreases in plasma prolactin, thyrotropin, and growth hormone levels (Seri, 1995) may be of clinical importance. The decrease in plasma prolactin level may attenuate the preterm infant's propensity to edema formation but may also have a modulating effect on immune function (Seri, 1995). The inhibition of thyrotropin release necessitates the postponement of routine neonatal thyroid screening until after dopamine administration has been discontinued (Seri, 1995; Filippi et al., 2007). Dopamine, compared with dobutamine, decreases serum thyroid-stimulating hormone and thyroxine levels by approximately 70% to 80% and 50%, respectively (Filippi et al., 2007). As dobutamine does not stimulate dopaminergic receptors, its administration is devoid of neuroendocrine effects. The potential impact on long-term neurodevelopmental outcome and immunologic function of the drug-induced alterations in neuroendocrine function is not known in the preterm and term neonate.



• **Fig. 51.12** Effects of Dopamine Along the Nephron. Dopamine increases renal blood flow (RBF) by selective renal vasodilation and by increasing mesangial cell surface area and glomerular hydrostatic pressure via the preferential dilation of the afferent arteriole (black arrows). These changes result in increased single-nephron glomerular filtration rate (GFR). Administration of dopamine to normotensive preterm neonates results in increased sodium, phosphorus, and free water excretion (Seri, 1993, 1995). These effects are primarily caused by the drug-induced inhibition of Na^+ , K^+ -ATPase, Na^+ / H^+ exchanger, and the Na^+ /inorganic phosphate (Pi) cotransporter in the proximal tubule as well as inhibition of antidiuretic hormone (ADH)-induced phosphorylation of water channels in the collecting duct (Seri, 1995). The proposed resultant increase in bicarbonate excretion has not been documented in preterm infants. X in red represents inhibition of enzyme or transporter function. (Courtesy Kelly L, Seri I. Renal developmental physiology: relevance to clinical care. *NeoReviews*. 2008;9:e150–e161.)

Epinephrine, Norepinephrine, and Other Cardiovascular Agents and Hormones

Epinephrine

In an RCT, low to medium doses of epinephrine, when titrated to optimal hemodynamic response, were shown to increase blood pressure and CBF in hypotensive VLBW neonates (see earlier discussion) (Pellicer et al., 2005). Because of epinephrine's significant effect on glycogenolysis, its administration is associated with an increase in serum lactate levels independently of the drug's cardiovascular actions (Valverde et al., 2006). This effect should be kept in mind when one is following serum lactate levels to assess the changes in cardiovascular status due to epinephrine administration. Therefore the epinephrine-induced improvement in perfusion cannot be ascertained by the following of serum lactate levels alone, because these levels will likely increase independently of drug-induced improvement in the cardiovascular status.

It is not known whether there is a difference in the cardiovascular response and/or side effects of sympathomimetic amines with the combined use of epinephrine and dopamine compared with the use of increasing dosages of dopamine beyond 20 µg/kg per minute with or without dobutamine. In preliminary publications, no detrimental vasoconstrictive effects have been reported with the use of either high doses of epinephrine with or without dopamine or norepinephrine in sick neonates and children (Derleth, 1997; Campbell and Byrne, 1998; Seri and Evans, 1998). These findings are best explained by the downregulation of cardiovascular adrenergic receptors and thus the decreased cardiovascular sensitivity of critically ill preterm infants to catecholamines, necessitating escalation of sympathomimetic support. However, one must be extremely careful when escalating treatment with these potent vasopressors–inotropes to decrease the risk of unwarranted severe vasoconstriction and compromised tissue perfusion. As referred to earlier, with the appropriate use of low-dose hydrocortisone in patients with evidence of relative adrenal insufficiency (also see later), high-dose vasopressor support is rarely required.

Norepinephrine

Norepinephrine is the drug of choice for the treatment of septic (vasodilatory) shock in adult patients and is being increasingly used in children with septic shock (Lampin et al., 2012; Dellinger et al., 2013). However, because of a number of developmentally regulated factors, the pharmacokinetics and pharmacodynamics of norepinephrine are different in neonates. Unfortunately, few data are available on the effectiveness and safety of norepinephrine in the neonatal population (Oualha et al., 2014). Until very recently, findings on the cardiovascular effects of norepinephrine in neonates were published only in abstract form (Derleth, 1997). However, a more recent observational study in late preterm and term neonates with persistent pulmonary hypertension of the newborn (PPHN) treated with inhaled nitric oxide (iNO) who also had signs of circulatory failure found that medium doses of norepinephrine improved systemic and pulmonary cardiovascular function by decreasing the pulmonary artery to aortic pressure gradient and improving cardiac performance (Tourneux et al., 2008a). Another observational study found improvement in blood pressure, urine output, and lactic acidosis in term infants with refractory hypotension unresponsive to conventional treatment (Tourneux et al., 2008b). Although these findings are encouraging, the routine use of norepinephrine in the treatment of neonates with PPHN and cardiovascular compromise requires further confirmation.

Milrinone

There are few data available on the cardiovascular effects of milrinone, a phosphodiesterase 3 inhibitor in the neonatal patient population (Samiee-Zafarghandy et al., 2015). It has been widely used to reduce afterload in neonates with congenital heart disease with low cardiac output syndrome after surgery (Hoffman et al., 2003). With regard to its use in term infants without congenital heart disease, milrinone administration has been shown in a small case series to improve the oxygenation index without compromising systemic blood pressure when given to term neonates with PPHN treated with iNO during the first 2 days postnatally (McNamara et al., 2006). Another small retrospective study found improvement in indices of cardiac function about 24 hours after initiation of milrinone treatment in late preterm and term infants with pulmonary hypertension treated with iNO (James et al., 2015). The preferential effect of milrinone on the pulmonary circulation in neonates with PPHN treated with iNO may be due to the upregulation of phosphodiesterase 3 in pulmonary vessels in response to iNO administration (Chen et al., 2009). It is unclear if milrinone has a positive inotropic effect in neonates (Barrington and Dempsey, 2006). A retrospective study of 1446 neonates exposed to milrinone found that 42% of the neonates had adverse effects attributed to the drug administration (Samiee-Zafarghandy et al., 2015). Arterial hypotension prompting vasopressor–inotrope administration and thrombocytopenia were the most frequently noted adverse effects.

A randomized placebo-controlled blinded clinical trial investigated whether the use of milrinone would minimize or prevent the suspected increase in systemic vascular resistance and the associated systemic hypoperfusion in VLBW infants during the first postnatal day (Paradis et al., 2009). However, there was no reduction in the incidence of low SVC flow used as a surrogate of systemic blood flow in patients treated with milrinone compared with the placebo group. These findings may be explained, at least in part, by the little-appreciated complexity of the pathophysiology of cardiovascular compromise in the very preterm neonate after delivery (Wu et al., 2016). They further suggest that systemic and cerebral hypoperfusion and reperfusion in this patient population may not solely occur because of the inability of the immature myocardium to pump against the sudden postnatal increase in systemic vascular resistance (Noori et al., 2009, 2014a).

Vasopressin

The primary cardiovascular effect of vasopressin in the systemic circulation is vasoconstriction. However, in the pulmonary circulation, low doses of vasopressin have been shown to induce vasodilation in adults. In neonates with pulmonary hypertension, including patients with congenital diaphragmatic hernia, several case series have also reported improvement in oxygenation and decrease in pulmonary pressure following low-dose vasopressin administration (Radicioni et al., 2012; Acker et al., 2014; Mohamed et al., 2014). However, as expression of vasopressin receptors is developmentally regulated, it is unclear if vasopressin truly induces selective pulmonary vasodilation in the neonatal population (Enomoto et al., 2014).

Its systemic pharmacodynamic profile makes vasopressin a good candidate for treatment of circulatory failure when the underlying pathophysiology is primarily that of vasodilation such as in septic shock without myocardial dysfunction or systemic inflammatory response following cardiac surgery. However, there are only case series reporting improvement in systemic hemodynamics in preterm and term infants with refractory hypotension or septic shock (Meyer

et al., 2006a, 2006b; Bidegain et al., 2010; Ikegami et al., 2010). A recent randomized pilot study compared vasopressin with dopamine as the first line of treatment in hypotensive preterm infants (Rios and Kaiser, 2015). It found that vasopressin resulted in similar improvement in blood pressure and urine output compared with dopamine. In addition, the vasopressin group had less tachycardia. Yet, more data on the safety and efficacy of vasopressin are needed before it can be introduced in the routine clinical care of critically ill neonates with vasodilatory shock (Shivanna et al., 2013).

Steroid Administration

Steroid Administration as Primary or Rescue Treatment

A small RCT showed that low-dose hydrocortisone improves blood pressure but is less effective than dopamine in hypotensive preterm infants (Bouchier and Weston, 1997). Given the concerns about adverse long-term effects of steroids (primarily dexamethasone) and inadequate data on their efficacy, steroids are not recommended as the first-line treatment of hypotensive infants (Ibrahim et al., 2011).

For rescue treatment, there is now overwhelming evidence provided by descriptive studies (Helbock et al., 1993; Ng et al., 2001; Seri et al., 2001; Heckmann and Pohlandt, 2002; Noori et al., 2006a) and confirmed by randomized blinded prospective trials (Bouchier and Weston, 1997; Gaissmaier and Pohlandt, 1999; Watterberg et al., 1999; Efrid et al., 2005; Ng et al., 2006) that brief steroid treatment stabilizes the cardiovascular status and decreases the need for vasopressor–inotropic support in the critically ill preterm and term newborn with vasopressor-resistant hypotension. Because there is overwhelming evidence that early and/or medium-to-high cumulative doses of dexamethasone have detrimental effects on the developing brain (O’Shea et al., 1999; Shinwell et al., 2000; Halliday et al., 2003; Baud, 2004; Yeh et al., 2004; Hitzert et al., 2012), and because in addition to glucocorticoids, mineralocorticoids also have significant effects on the cardiovascular system (Wehling et al., 1995; Wehling, 1997), even low-dose dexamethasone administration to treat vasopressor-resistant hypotension has fallen by the wayside in recent years. This section therefore addresses only the actions, side effects, recommended doses, and remaining clinically relevant concerns of hydrocortisone administration to treat vasopressor-resistant hypotension in the critically ill neonate. It is of note that the available evidence for the effectiveness of low-dose hydrocortisone to increase blood pressure and decrease vasopressor–inotrope requirement in the preterm neonate is so strong that it would take 74 and 188 future studies, respectively, demonstrating no effect of hydrocortisone on blood pressure increase and on the decrease in vasopressor requirement to eliminate the statistical power of the present findings (Higgins et al., 2010).

Rationale for Hydrocortisone Treatment

Because in most patients and after some time cardiovascular stability will be achieved by the use of vasopressors–inotropes and/or inotropes alone, it is important to understand the rationale for hydrocortisone administration, especially because the drug has its own side effects (see later discussion).

First, it is widely accepted although not proven that the sooner one normalizes blood pressure and systemic perfusion, especially in the VLBW neonate during the immediate postnatal period, the better the outcome. However, because normal blood pressure in the first 24 hours may not guarantee normal cerebral perfusion (see earlier discussion) and because an association but not causation

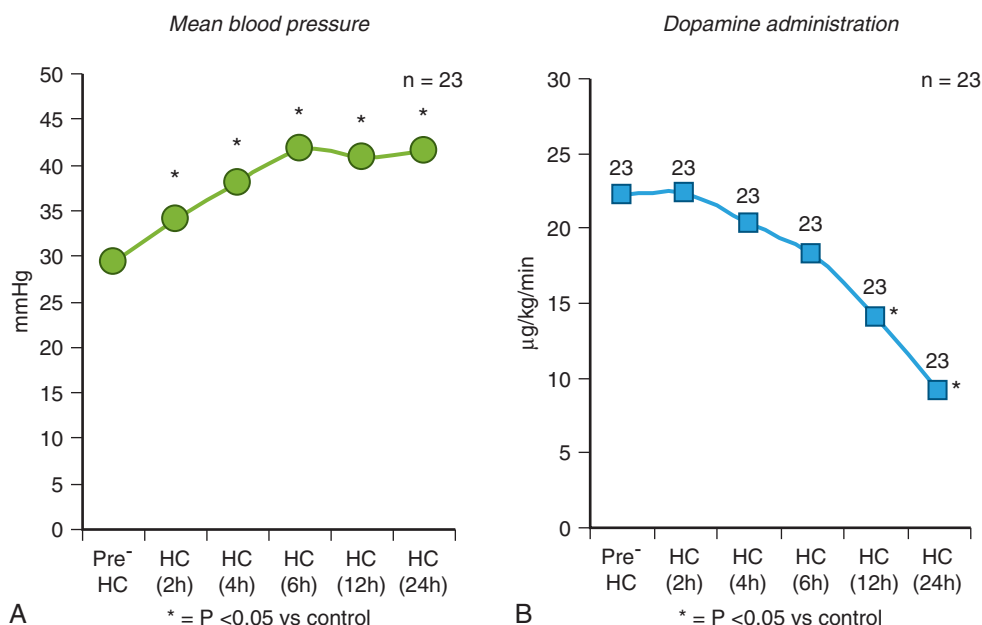
has been documented between hypotension and adverse outcomes, stabilization of the cardiovascular status with little blood pressure fluctuation remains an intuitively desirable goal but one without much direct evidence to support it.

Second, and more importantly, hydrocortisone specifically addresses the underlying cause of the cardiovascular instability in most critically ill neonates and thus is the logical choice of treatment. Indeed, developmental endocrinology and cardiovascular physiology findings as well as recent clinical data support a role for hydrocortisone use in critically ill hypotensive neonates, especially in VLBW neonates (Biniwale et al., 2013). In critical illness, desensitization of the cardiovascular system to catecholamines occurs through the downregulation of cardiovascular adrenergic receptors and second messenger systems (Hausdorff et al., 1990). This process is attenuated or prevented by the regulatory actions of glucocorticoids on the expression of cardiovascular adrenergic receptors and second messenger systems (Hausdorff et al., 1990). In addition, the direct increase in myocardial and vascular smooth muscle cell contractility induced by mineralocorticoids also plays an effective role (Wehling et al., 1995; Wehling, 1997). Furthermore, corticosteroids contribute to the maintenance of capillary integrity, inhibit catecholamine metabolism and reuptake of norepinephrine into sympathetic nerve endings, increase the expression of angiotensin type 2 receptors in the myocardium, and inhibit prostacyclin production and the induction of inducible nitric oxide synthase. Each of these actions of corticosteroids aids in maintaining the sensitivity of the cardiovascular system to catecholamines in response to acute stress or critical illness.

Third, because of the role of glucocorticoids in the physiologic regulation of adrenergic receptor expression (Hausdorff et al., 1990), the emergence of vasopressor resistance in itself may indicate a state of relative adrenal insufficiency. The findings of several recent studies indicate that critically ill preterm and term infants are likely to develop relative adrenal insufficiency because of their immature adrenal function (Watterberg and Scott, 1995; Watterberg, 2002; Ng et al., 2004) and hypothalamopituitary axis (Fernandez et al., 2008; Fernandez and Watterberg, 2009), respectively. It is important to emphasize that, on the basis of these findings, it appears that in the VLBW neonate adrenal unresponsiveness to endogenous or exogenous adrenocorticotrophic hormone (Watterberg, 2002; Ng et al., 2004) and in the late preterm and term neonate unresponsiveness of the hypothalamopituitary axis to stress (Fernandez et al., 2008; Fernandez and Watterberg, 2009) are the primary causes for the development of relative adrenal insufficiency. Thus these patients have limited capacity to mount sufficient adaptive increases in endogenous steroid production to prevent the development of cardiovascular adrenergic receptor downregulation and desensitization of the cardiovascular system to catecholamines during their illness. Therefore in critically ill preterm and term infants with vasopressor-resistant hypotension, steroid administration also serves as hormone substitution therapy.

Clinical Applications of Hydrocortisone

As mentioned earlier, use of hydrocortisone as primary treatment in hypotensive preterm infants is not recommended. On the other hand, although long-term effects need to be further studied (see later), there is justification for the use of hydrocortisone as a secondary rescue treatment. Available evidence indicates that hydrocortisone in preterm and term infants with vasopressor-resistant hypotension increases blood pressure within 2 hours of the initiation of treatment (nongenomic effects) and decreases vasopressor requirement within 8 to 12 hours of the first dose of the drug (genomic effects)



• **Fig. 51.13** Effect of Hydrocortisone on Mean Blood Pressure and the Dose of Dopamine During the First 24 Hours of Hydrocortisone Treatment in 23 Preterm Neonates With Vasopressor-Resistant Shock. (A) Mean blood pressure and (B) mean dopamine requirement during the 12 hours before and the first 24 hours after the first dose of hydrocortisone (HC). Before HC administration, blood pressure remained low (A), despite significantly increased dopamine doses (B; asterisk $P < .05$ vs the baseline [0 hour]). However, mean blood pressure increased significantly by 2 hours after the first dose of HC (A; asterisk $P < .05$ vs the baseline [0 hour]) and continued to rise until 6 hours of HC therapy, remaining stable thereafter (A; asterisk $P < .05$ vs the baseline [0 hour]; $P < .05$ vs HC therapy [2 hours]). In addition, the dose of dopamine significantly decreased by 12 and 24 hours of HC therapy (B; asterisk $P < .05$ vs the baseline [0 hour]). (Courtesy Seri I, Tan R, Evans J. Cardiovascular effects of hydrocortisone in preterm neonates with pressor resistant hypotension. *Pediatrics*. 2001;107:1070–1074.)

(Fig. 51.13) (Seri et al., 2001; Ng et al., 2006; Noori et al., 2006a). A study in preterm infants without a PDA documented that the hydrocortisone-induced improvement in blood pressure is associated with improvements in all aspects of cardiovascular function, including stroke volume and tissue perfusion (Fig. 51.14) (Noori et al., 2006b). It is important to note that because of the decreased clearance of hydrocortisone, lower and less frequent dosing are sufficient to induce the desired hemodynamic effects in preterm and term infants (Noori et al., 2006b; Vezina et al., 2014; Waterberg, 2016).

Short-Term Side Effects

With regard to the side effects of early or late low-dose hydrocortisone administration, the potential occurrence of short-term and long-term sequelae is of great interest. For the short-term side effects, it has been repeatedly documented that coexposure of preterm neonates to indomethacin and hydrocortisone during the first postnatal week significantly increases the risk of spontaneous gastrointestinal (mostly ileal) perforations (Waterberg et al., 2004; Peltoniemi et al., 2005). This serious untoward effect curtails the use of hydrocortisone in the VLBW neonate during the first postnatal week, the very patient population in whom vasopressor-resistant hypotension and relative adrenal insufficiency are most prevalent during the immediate postnatal period. The preliminary findings of one of these studies might suggest that VLBW neonates with low baseline serum cortisol levels may be at less risk of developing ileal perforations when treated with low-dose hydrocortisone and a cyclooxygenase inhibitor during the first few postnatal days (Peltoniemi et al., 2005). However, this observation

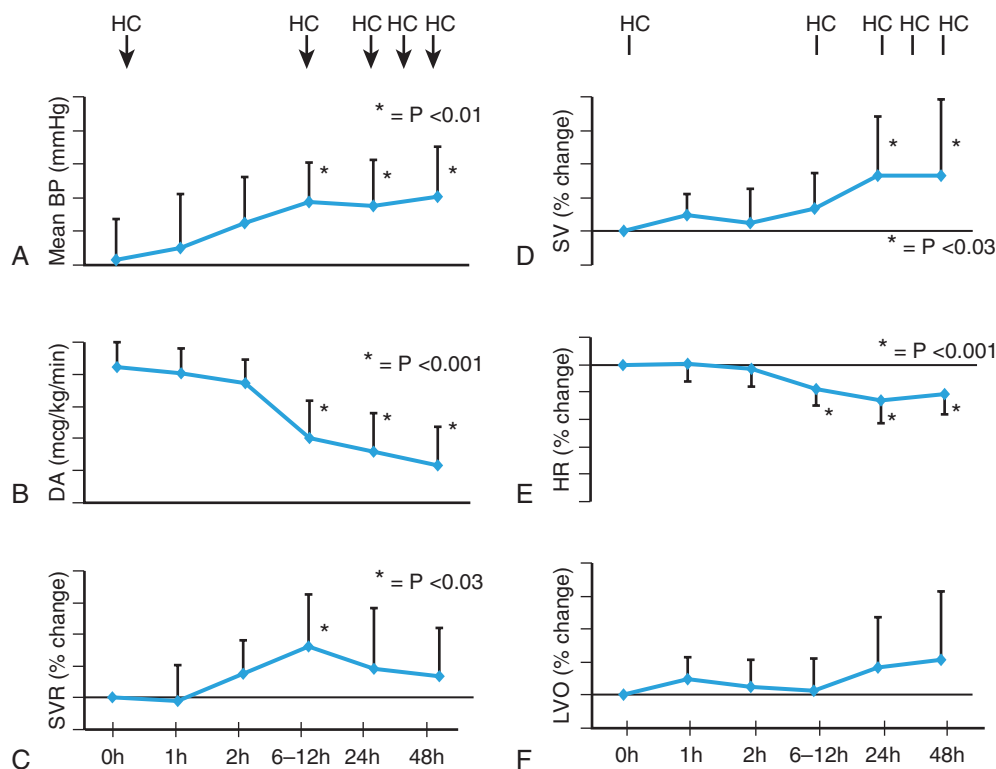
needs to be confirmed before it can be used to predict the likelihood of spontaneous intestinal perforation based on the basal serum cortisol level alone. For the other short-term side effects of hydrocortisone, one earlier study found an increase in the incidence of systemic fungal infections (Botas et al., 1995). However, none of the other studies have documented a significant increase in bacterial or fungal infections in neonates. Finally, hydrocortisone administration–associated transient hyperglycemia and hypertension have been reported in a few cases in the literature.

Long-Term Side Effects

Regarding the potential long-term side effects, the most pressing question is whether low to moderate doses of hydrocortisone interfere with neurodevelopment, especially in preterm neonates.

A placebo-controlled trial showed that early, low-dose hydrocortisone treatment for the prevention of bronchopulmonary dysplasia used in the first 10 days postnatally was not associated with an increased incidence of cerebral palsy (Waterberg et al., 2004) and might have been associated with evidence of improvement in some measures of neurodevelopmental outcome at 18 to 22 months' corrected age when compared with controls (Waterberg et al., 2007).

With regard to the use of higher cumulative doses of hydrocortisone for the treatment of evolving bronchopulmonary dysplasia outside the immediate transitional period, the results of a study examining structural and functional brain development at an age of 8 years suggest that hydrocortisone, used for the treatment of bronchopulmonary dysplasia in ventilator-dependent preterm



• **Fig. 51.14** Effect of Hydrocortisone on Systemic Hemodynamics and Dopamine Requirement During the First 48 Hours of Hydrocortisone Treatment in 15 Preterm Neonates With Vasopressor-Resistant Shock. Changes in mean blood pressure (BP; A) and dopamine dosage (DA; B) and percentage changes relative to the baseline (0 hours) in systemic vascular resistance (SVR; C), stroke volume (SV; D), heart rate (HR; E), and left ventricular output (LVO; F) at 1 hour, 2 hours, 6 to 12 hours, 24 hours, and 48 hours after the first dose of hydrocortisone (HC). Arrows indicate the approximate timing of hydrocortisone doses. Significant *P* values for pairwise comparisons versus the baseline (0 hour) with adjustment for multiple comparisons (Bonferroni) are shown. See the text for details. (Courtesy Noori S, Friedlich P, Wong P, et al. Hemodynamic changes following low-dose hydrocortisone administration in vasopressor-treated neonates. *Pediatrics*. 2006;118:1456–1466.)

neonates at a median age of 18 days and at cumulative doses exceeding 50 mg, does not interfere with brain development (Lodygensky et al., 2005). In addition, case-control studies have shown no difference in cerebral and cerebellar tissue volumes on MRI at term equivalent in preterm infants treated with hydrocortisone for bronchopulmonary dysplasia compared with the controls (Benders et al., 2009; Kersbergen et al., 2013). A small RCT of hydrocortisone with a cumulative dose of 17 mg/kg started moderately early (at 10–21 days) also showed no difference in regional brain volume at term equivalent compared with the controls (Parikh et al., 2013). However, one small RCT found a trend for a higher rate of neurodevelopmental impairment in the hydrocortisone group compared with the placebo group (61% vs 39%) at school age (Peltoniemi et al., 2016).

Although overall hydrocortisone appears to be safer than dexamethasone, the lack of power of the RCTs and the retrospective nature of the observational or retrospective studies warrant caution before the use of early or late and low-dose to medium-dose hydrocortisone can be declared safe in preterm neonates, at least from a neurodevelopmental standpoint.

Supportive Measures

Maintenance of a normal intravascular volume, arterial pH, and serum ionized calcium concentration is necessary for the optimum

cardiovascular response to catecholamines. A previous study found that metabolic acidosis (pH < 7.25) may compromise myocardial function in the preterm infant (Fanconi et al., 1993), and so it has been recommended that the arterial pH be maintained in or above this range in cases of acidosis with a significant metabolic component. On the other hand, respiratory acidosis has been shown to have no to minimal effect on cardiac function, myocardial contractility, and systemic vascular resistance in hemodynamically stable, preterm infants during the transitional period (Noori et al., 2013). However, and as discussed earlier, respiratory acidosis has significant effects on cerebral hemodynamics, and its cardiovascular effects in neonates receiving cardiovascular support are not known (see *Respiratory Support and Hemodynamics*). Therefore more data are needed to define the optimal pH and acid-base status in preterm and term infants receiving respiratory support. Administration of sodium bicarbonate (Fanconi et al., 1993) or, in neonates with severe combined respiratory and metabolic acidosis, the administration of tromethamine rapidly improves arterial pH. However, the efficacy and potential short-term and long-term adverse effects of such supportive treatment measures have not been studied in the neonatal patient population, and there is indeed very little evidence that bicarbonate administration for management of metabolic acidosis caused by tissue hypoperfusion is beneficial (Aschner and Poland, 2008). Finally, although positive pressure ventilation may raise pleural pressure and has the potential to

reduce venous return, it may also reduce left ventricular afterload by reducing transmural pressure and decreasing or eliminating the work of breathing. Decreasing or eliminating the work of breathing will also decrease or eliminate the concomitant cardiac output diverted to respiratory muscles. Therefore the net hemodynamic effect of positive pressure ventilation is improved systemic oxygen delivery independent of any potential improvement in pulmonary gas exchange. On the other hand, excessive positive pressure and lung hyperexpansion are associated with decreases in pulmonary and systemic blood flow.

In summary, sustained stabilization of the cardiovascular status with provision of appropriate blood pressure, cardiac output, and tissue perfusion and oxygenation remains a difficult task in most of the critically ill hypotensive neonates. Treatment of these patients requires the ability to continuously monitor the most important measures of cardiovascular function (blood pressure, systemic blood flow, and tissue oxygenation), a thorough understanding of the pathogenesis and pathophysiology of neonatal shock, and a thorough understanding of the mechanisms of action, pharmacodynamics, and potential side effects of sympathomimetic amines and other medications used in the management of neonatal shock.

Suggested Readings

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Persistent Pulmonary Hypertension

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KEY POINTS

- Persistent pulmonary hypertension of the newborn (PPHN) can occur with parenchymal disease, with lung hypoplasia, or without associated lung disease (*idiopathic*) and has an incidence of 0.2% in term infants and up to 2% in preterm infants.
- Maternal factors such as diabetes, high body mass index, smoking, use of selective serotonin receptor inhibitors or nonsteroidal antiinflammatory drugs, and cesarean delivery increase the risk of PPHN. Postnatal factors include perinatal asphyxia, hyperoxia, hypoxia, infection, and lung inflammation.
- Medical management of PPHN requires careful optimization of right and left ventricular function. Lung recruitment strategies should be applied in patients with parenchymal lung disease.
- Inhaled nitric oxide improves oxygenation and reduces the need for extracorporeal membrane oxygenation support in term and near-term infants with PPHN.
- Extracorporeal membrane oxygenation support is indicated for term and near-term neonates with severe pulmonary hypertension and/or hypoxemia that is refractory to inhaled nitric oxide therapy and optimization of respiratory and cardiac function.
- Chronic pulmonary hypertension occurs in a subset of infants with congenital diaphragmatic hernia and bronchopulmonary dysplasia and increases morbidity and mortality.
- Infants who survive moderate to severe PPHN are at a high risk of neurodevelopmental impairment and should undergo neuroimaging and neurodevelopmental follow-up.

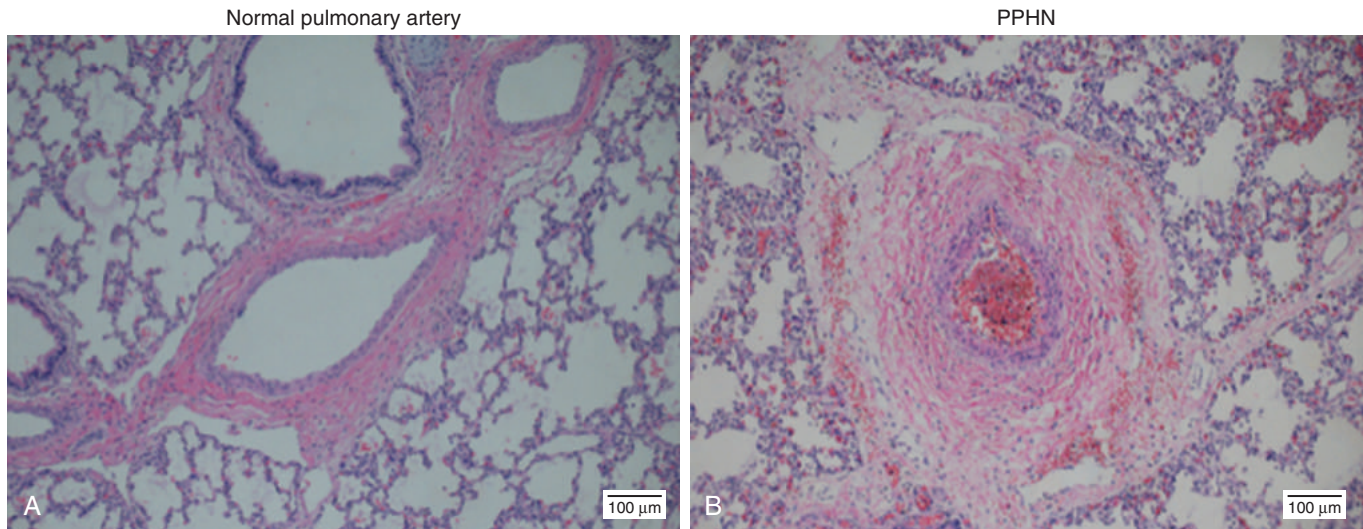
Neonatal respiratory failure affects 2% of all live births and contributes to more than one-third of all neonatal mortality (Angus et al., 2001). Persistent pulmonary hypertension of the newborn (PPHN) is a frequent complication of neonatal respiratory disease and is defined as the failure to achieve or sustain the normal decrease in pulmonary vascular resistance (PVR) at birth. PPHN can produce severe respiratory distress and hypoxemia because of severe pulmonary vascular disease remodeling (Fig. 52.1) in term and near-term infants. Death and morbidity are common (Kinsella and Abman, 1995; Walsh-Sukys et al., 2000), including chronic lung disease and neurodevelopmental sequelae. While PPHN can be idiopathic, it is more commonly associated with a wide array of lung diseases, including meconium aspiration syndrome, perinatal asphyxia, congenital diaphragmatic hernia (CDH), pneumonia, and respiratory distress

syndrome. PPHN can also occur in premature infants with early respiratory distress, particularly after prolonged rupture of membranes and pulmonary hypoplasia (Aikio et al., 2012; de Waal and Kluckow, 2015). Chronic pulmonary hypertension is associated with lung diseases such as bronchopulmonary dysplasia (BPD) and CDH and is a common complication of congenital heart disease. This chapter will review the pathophysiology of PPHN, clinical treatment of newborns with severe disease, and outcome data.

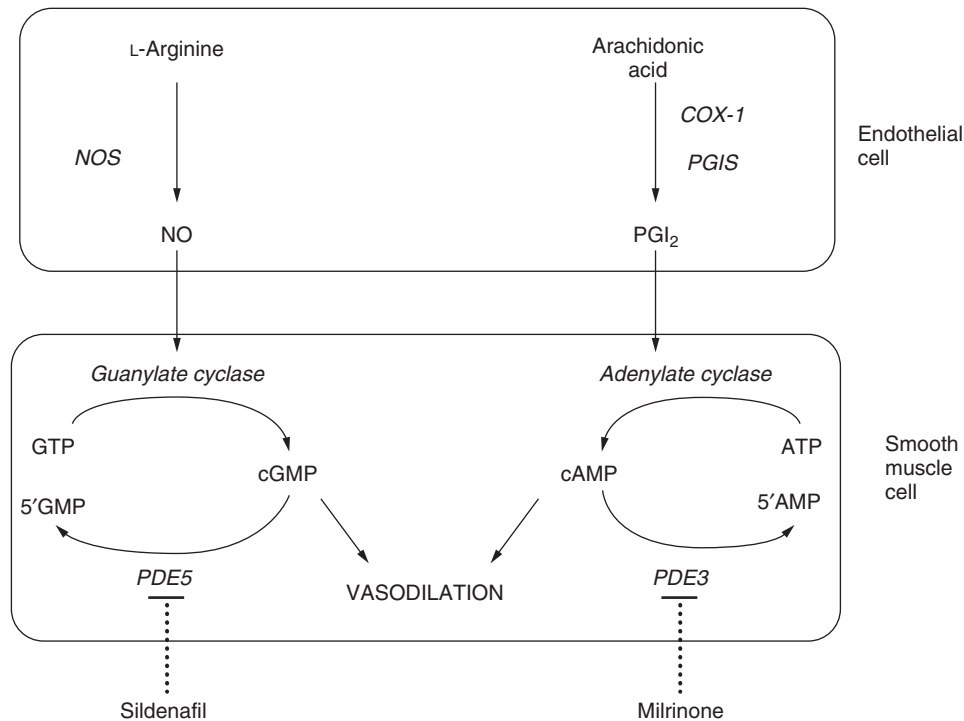
Normal Fetal Pulmonary Vascular Development and Transition

During fetal life, placental vascular resistance is low and PVR is high. This state of physiologic pulmonary hypertension is needed to maintain the patterns of blood flow that support gas exchange by the placenta. In the human fetus, only 10%–20% of the combined ventricular output is directed to the pulmonary vascular bed, and most of the right ventricular output crosses the ductus arteriosus to the descending aorta. During late gestation there is a marked increase in cross-sectional area of the pulmonary vascular bed. During this same timeframe, pulmonary vessels become more sensitive to vasoconstrictive mediators, such as endothelin and hypoxia, resulting in active pulmonary vasoconstriction and a net increase in PVR. The dilatory response to increased oxygen also emerges in late gestation. Pulmonary vasoconstriction is also promoted by low basal production of vasodilators such as prostacyclin and nitric oxide (NO) and low activity of pulmonary arterial soluble guanylate cyclase.

At birth, a rapid and dramatic decrease in PVR redirects half of the combined ventricular output to the lung and increases pulmonary blood flow by 8-fold to 10-fold. Increased pulmonary blood flow increases pulmonary venous return and left atrial pressure, promoting functional closure of the one-way valve of the foramen ovale. Clamping of the umbilical cord removes the low-resistance placental circulation, increasing systemic vascular resistance. The largest drop in PVR and pulmonary arterial pressure occurs shortly after birth, although both will continue to drop during the first few months of life until the low levels that are typical of the adult circulation are reached. As PVR falls below systemic levels, blood flow through the patent ductus arteriosus reverses. During the first several hours of life the ductus arteriosus functionally closes, largely in response to the increased oxygen tension of the newborn. This



• **Fig. 52.1** Histologic appearance of a pulmonary vessel from an infant with fatal persistent pulmonary hypertension of the newborn (PPHN) illustrating the dramatic remodeling that can be associated with severe PPHN.

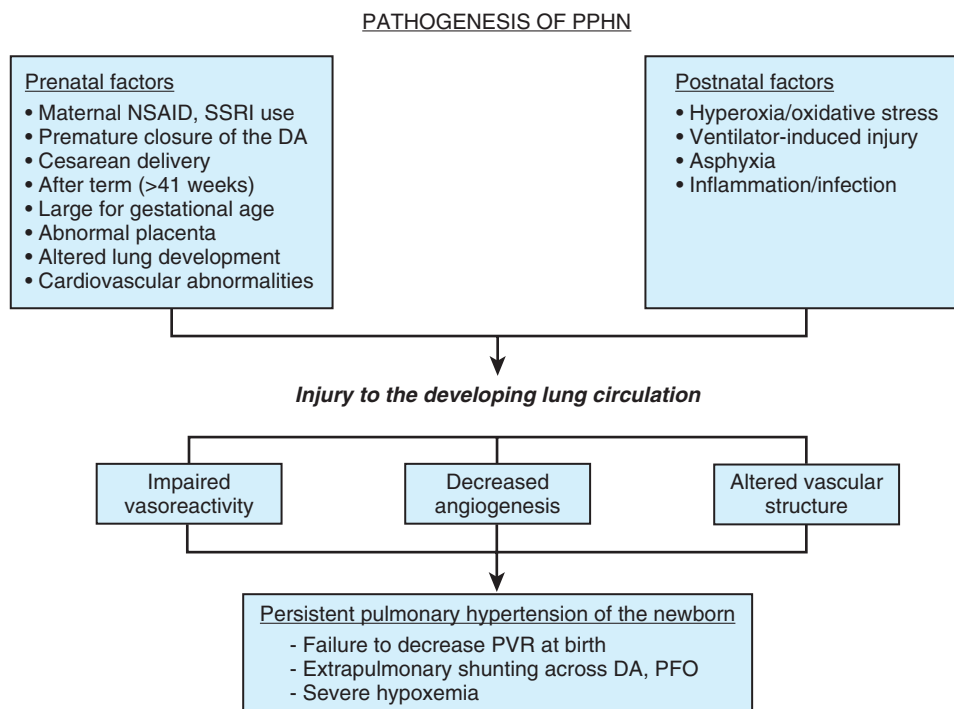


• **Fig. 52.2** Nitric oxide (NO) and prostacyclin (PGI₂) signaling pathways that regulate pulmonary vascular tone in the developing lung. ATP, Adenosine triphosphate; cAMP, cyclic AMP; cGMP, cyclic GMP; COX-1, cyclooxygenase 1; GTP, guanosine triphosphate; NOS, nitric oxide synthase; PDE3, phosphodiesterase 3; PDE5, phosphodiesterase 5; PGIS, prostacyclin synthase.

effectively separates the pulmonary and systemic circulations and establishes the normal postnatal circulatory pattern.

These transitional changes in the pulmonary vasculature are initiated by ventilation of the lung and an increase in oxygen tension and are mediated by several vasoactive compounds. The fetus prepares for the transition late in gestation by increasing pulmonary vascular expression of NO synthases and soluble guanylate cyclase (Fig. 52.2). Pulmonary endothelial NO production increases markedly at the time of birth, partly as a response to

increased oxygen tension and shear stress. NO exerts its action through soluble guanylate cyclase, an enzyme whose level peaks in late gestation (Bloch et al., 1997) and increases the levels of cyclic guanosine monophosphate (cGMP), a central mediator responsible for vascular relaxation. Phosphodiesterase 5 (PDE5) catalyzes the breakdown of cGMP and, similarly to soluble guanylate cyclase, exhibits peak expression and activity in the immediate newborn period (Hanson et al., 1998; Sanchez et al., 1998). The arachidonic acid–prostacyclin pathway also plays a significant role



• **Fig. 52.3** Pathogenesis of persistent pulmonary hypertension of the newborn (PPHN). DA, Ductus arteriosus; NSAID, nonsteroidal antiinflammatory drug; PFO, patent foramen ovale; PVR, pulmonary vascular resistance; SSRI, selective serotonin receptor inhibitor.

in the transition at birth (see Fig. 52.1). The enzyme cyclooxygenase acts on arachidonic acid to produce prostaglandin endoperoxides. Prostaglandins activate adenylate cyclase to increase cyclic adenosine monophosphate (cAMP) concentrations in vascular smooth muscle cells, which, similarly to increases in cGMP concentrations, leads to vasorelaxation. Phosphodiesterase 3A catalyzes the breakdown of cAMP.

Several events can disrupt the perinatal transition and contribute to the pathogenesis of PPHN (Fig. 52.3). Birth by elective cesarean delivery delays the decrease in pulmonary arterial pressure and increases the risk of PPHN (Makihara et al., 1993), and delivery before 39 weeks' gestation likely amplifies this effect. When compared with matched controls, infants with PPHN are more likely to have been born by cesarean delivery, and elevated risk of PPHN is also associated with maternal diabetes, asthma, and high body mass index (Hernandez-Diaz et al., 2007; Wilson et al., 2011). Perinatal asphyxia modifies the perinatal adaptation through a number of mechanisms that delay the fall in PVR and increase the risk of PPHN (Lapointe and Barrington, 2011), such as fetal hypoxemia, ischemia, acidosis, meconium aspiration, and ventricular dysfunction. Acute asphyxia is associated with reversible pulmonary vasoconstriction, but chronic in utero asphyxia can also induce vascular remodeling (Murphy et al., 1984). The adoption of therapeutic hypothermia for management of perinatal asphyxia raised many questions about its capacity to increase the risk of PPHN in asphyxiated infants. Deep levels of hypothermia (to 30°C–32°C) increased mean pulmonary arterial pressure in neonatal lambs (Toubas et al., 1978) and have been associated with an increased use of extracorporeal membrane oxygenation (ECMO) and inhaled nitric oxide (iNO) use in human studies. Furthermore, hypothermia shifts the oxygen dissociation curve to the left and decreases PaO₂ at a given peripheral oxygen saturation (SpO₂). However, pooled analysis of randomized trials of standard

hypothermia (33.5°C) has not shown an increased incidence of PPHN (25% vs 20%) in treated infants (Thoresen, 2011).

Pathophysiology of Persistent Pulmonary Hypertension of the Newborn

Failure of the normal cardiopulmonary transition after birth results in PPHN. The first reports of PPHN described term newborns with profound hypoxemia who lacked radiographic evidence of parenchymal lung disease and echocardiographic evidence of structural cardiac disease (Gersony et al., 1969; Levin et al., 1976). In these patients, hypoxemia was caused by marked elevations of PVR leading to right-to-left extrapulmonary shunting of blood across the patent ductus arteriosus or patent foramen ovale. Because of the persistence of high PVR and blood flow through these “fetal shunts,” the term *persistent fetal circulation* was originally used to describe these findings.

Consequently, it was recognized that these physiologic patterns can complicate the clinical course of term or preterm neonates with diverse causes of hypoxemic respiratory failure. As a result, the term *persistent pulmonary hypertension of the newborn* now denotes a syndrome characterized by common pathophysiologic features, including sustained elevation of PVR and hypoxemia due to right-to-left extrapulmonary shunting of blood flow across the ductus arteriosus or foramen ovale. These physiologic findings may be found in association with a wide range of cardiopulmonary disorders, such as meconium aspiration, sepsis, pneumonia, asphyxia, CDH, and respiratory distress syndrome. The term is often used interchangeably with *hypoxemic respiratory failure* although the term *persistent pulmonary hypertension of the newborn* should be reserved to describe neonates in whom extrapulmonary shunting contributes to hypoxemia and impaired cardiopulmonary function.

Most studies suggest an overall incidence for PPHN of 1.9 in 1000 live births or an estimated 7400 cases per year in the United States, although these rates differ widely from hospital to hospital (Walsh-Sukys et al., 2000; Huybrechts et al., 2015).

The 2013 Nice classification of pulmonary hypertension was expanded to capture the range of pediatric and neonatal disease (Ivy et al., 2013). Because of its specific anatomic and physiologic characteristics, PPHN was assigned a separate subcategory in group 1 (pulmonary arterial hypertension). In group 3 pulmonary hypertension (pulmonary hypertension due to lung disease or hypoxia), developmental lung diseases are specifically listed because of the important role that abnormal lung vascular growth plays in the pathogenesis of impaired lung development and pulmonary hypertension. CDH, BPD, and several other developmental lung disorders, such as surfactant protein deficiencies and alveolar capillary dysplasia, are now understood to be important causes of pulmonary hypertension in infants.

PPHN is commonly described according to its primary cause. Idiopathic PPHN is the least common, occurring in about 10% of cases. It refers to the absence of parenchymal lung disease to explain elevated pulmonary arterial pressure and implies intrauterine pulmonary vascular remodeling. However, PPHN is usually associated with other acute respiratory conditions, such as meconium aspiration syndrome, respiratory distress syndrome, or pneumonia, and is referred to as *secondary PPHN*. The hypoplastic vasculature, as seen in CDH, is associated with underdevelopment of the pulmonary vasculature and parenchyma and often leads to chronic pulmonary vascular disease. In many cases it can be more difficult to separate chronic intrauterine remodeling from acute pulmonary vasoconstriction due to parenchymal lung disease. For example, neonates with meconium aspiration often have clinical evidence of parenchymal disease and altered vasoreactivity, but excessive muscularization from intrauterine vascular remodeling is often found at autopsy.

Based on the significant remodeling found in lethal cases of PPHN, intrauterine events were presumed to affect pulmonary vascular growth, reactivity, and structure (Gegge and Reid, 1984; see Fig. 52.1). Pulmonary vascular development can be disrupted by environmental, placental, toxic, or other influences (see Fig. 52.3). Case-control surveillance studies indicate that maternal risk factors of black or Asian maternal race, elevated body mass index ($>27 \text{ kg/m}^2$), diabetes, and asthma predict a higher risk of PPHN. Neonatal risk factors include male sex, large for gestational age infants, birth by cesarean delivery, and delivery before 37 weeks' gestation or after 41 weeks' gestation (Hernandez-Diaz et al., 2007).

Recent animal and epidemiologic studies also suggest that maternal exposures can alter fetal pulmonary vascular development and function. There are strong associations between PPHN and maternal smoking, and two classes of maternal medications, nonsteroidal antiinflammatory drugs and selective serotonin receptor inhibitors, have also been implicated. Exposure to nonsteroidal antiinflammatory drugs such as aspirin or ibuprofen during the third trimester can cause constriction of the fetal ductus arteriosus, which in turn can trigger pulmonary vascular remodeling and PPHN. Based on the findings of a recent epidemiologic study, the relationship is complex and may be dependent on the specific agent and the timing of exposure (Van Marter et al., 2013), although aspirin use during late pregnancy remains a consistent risk factor for PPHN. Use of selective serotonin receptor inhibitors during the last half of pregnancy has been associated with an increased incidence of PPHN in several population studies, although the severity of PPHN has not been well described, and other studies

have not found this association (Kieler et al., 2012; Grigoriadis et al., 2014; Huybrechts et al., 2015). Maternal depression is a risk factor for adverse pregnancy outcomes, so maternal physical and psychologic well-being remain the primary factors guiding antidepressant therapy during pregnancy and the postpartum period.

Unlike pulmonary hypertension in adults and older children, few genetic risk factors for PPHN have been identified. Children with Down syndrome (trisomy 21) commonly develop pulmonary hypertension in association with structural heart defects but also have a 10-fold increased risk of idiopathic PPHN. In a Dutch cohort, PPHN was documented in 5.2% of Down syndrome infants (Weijerman et al., 2010), and other studies have shown that Down syndrome infants are overrepresented in neonates requiring ECMO support (Southgate et al., 2001). Polymorphisms of genes for bone morphogenetic protein receptor or other transforming growth factor β receptors, other critical growth factors, or vasoactive enzymes (e.g., NO synthase, phosphodiesterase) have not been shown to increase the risk of neonatal PPHN. In one rigorous genotype analysis of 88 neonates with documented PPHN (Byers et al., 2012), there was a significant association with genetic variants for cortisol signaling (*CRHR1* and *CRHBP*), as well as increased levels of 17-hydroxyprogesterone. These data are supported by animal data indicating that prenatal or postnatal steroid therapy normalizes NO synthase function and improves pulmonary vascular function in experimental PPHN (Chandrasekar et al., 2008; Perezet et al., 2012).

Disruptions of the NO-cGMP, prostacyclin-cAMP, and endothelin signaling pathways play an important role in the vascular abnormalities associated with PPHN. The NO-cGMP pathway has been a topic of particularly intense investigation in the last two decades. Decreased expression and activity of endothelial nitric oxide synthase (eNOS) have been documented in lamb models of chronic intrauterine pulmonary hypertension (Shaul et al., 1997; Villamor et al., 1997), and decreased eNOS expression was found in umbilical venous endothelial cell cultures from human infants with meconium staining who developed PPHN (Villaneuva et al., 1998). These important findings were rapidly followed by clinical testing of iNO, leading to its adoption as the primary vasodilator therapy for PPHN. However, numerous other signaling abnormalities limit the effect of endogenous or exogenous NO. In PPHN, expression and activity of soluble guanylate cyclase are decreased, and cGMP phosphodiesterase activity is increased, leading to lower cGMP levels and limitation of NO-induced vasodilation. Oxidant stress associated with vascular dysfunction and/or exposure to hyperoxia can oxidize and reduce soluble guanylate cyclase activity and increase cGMP phosphodiesterase activity, accentuating these vascular abnormalities.

Prostacyclin is important in the normal pulmonary vascular transition, although less is known about abnormal prostacyclin-cAMP signaling in PPHN. Data from animal models suggest reductions in prostacyclin synthesis and downstream adenylate cyclase responses, analogous to the abnormalities reported for NO-cGMP signaling (Shaul et al., 1991; Lakshminrusimha et al., 2009a). In addition, production of thromboxane, a vasoconstrictor arachidonic acid metabolite, plays a role in pulmonary hypertension produced by chronic hypoxia (Fike et al., 2005). Circulating levels of the potent vasoconstrictor endothelin 1 (ET-1) are elevated in lambs and newborns with PPHN (Rosenberg et al., 1993; Christou et al., 1997). Endothelin effects are mediated through two receptors: ET_A receptors on smooth muscle cells that mediate vasoconstriction and ET_B receptors on endothelial cells that mediate vasodilation, and the balance of endothelin receptors is shifted to the vasoconstrictor (ET_A) pathways in PPHN (Ivy et al., 1998b). Endothelin may

affect vascular tone by increasing production of vasoconstrictor reactive oxygen species such as superoxide and hydrogen peroxide (Wedgwood et al., 2001) and by decreasing expression and activity of peroxisome proliferator-activated receptor γ , which maintains the vasodilatory balance in the fetal lung.

Other Causes of Neonatal Pulmonary Hypertension

Congenital Diaphragmatic Hernia

CDH affects approximately 1 in 2500–3000 pregnancies when prenatal diagnosis is factored in and represents approximately 8% of all major congenital anomalies. CDH includes abnormal diaphragm development, herniation of abdominal viscera into the chest, and a variable degree of lung hypoplasia. Herniation occurs most often in the posterolateral segments of the diaphragm, and 80% of the defects occur on the left side. Severe CDH develops early in the course of lung development and involves an arrest in the normal patterns of airway branching in both lungs, resulting in reduced lung volume and impaired alveolarization. Abnormal distal vascular and air space growth is likely because of the loss of stretch-induced stimulation of lung development from impaired fetal breathing movements due to disruption of the diaphragm. The pulmonary vascular findings include vascular remodeling superimposed on hypoplasia or pruning of the pulmonary vascular bed, producing increased vascular tone and altered vasoreactivity after birth (Pierro and Thebaud, 2014).

After birth, PVR often remains at suprasystemic levels, causing acute pulmonary hypertension, extrapulmonary right-to-left shunting across the foramen ovale and ductus arteriosus, and profound hypoxemia. High PVR in the newborn with CDH is related to multiple factors, including the small cross-sectional area of pulmonary arteries, structural vascular remodeling, and vasoconstriction with altered reactivity. The mediators of altered pulmonary vascular reactivity in CDH remain under investigation, with substantial evidence pointing to disruptions in NO–cGMP and ET-1 signaling (Keller et al., 2010). The level of vascular endothelial growth factor, a key stimulator of angiogenesis, is increased in the lungs of infants with CDH who died; this has been interpreted as a compensatory mechanism for reduced eNOS expression and NO production (Pierro and Thebaud, 2014). In some infants, chronic pulmonary hypertension will persist for months or years, including structural and functional abnormalities of pulmonary circulation in the ipsilateral and contralateral lung. Cardiac catheterization of infants with prolonged pulmonary hypertension has revealed numerous vascular abnormalities, including left pulmonary artery hypoplasia or stenosis, pulmonary vein stenosis, and delayed venous return (Kinsella et al., 2005).

In addition to pulmonary vascular disease, it is important to appreciate that structural and functional abnormalities of the left ventricle create a relative left ventricular hypoplasia that impairs filling and creates pulmonary venous hypertension. Often, newborns with severe CDH have severe pulmonary hypertension with right-to-left shunting at the ductus arteriosus but have left-to-right shunting at the atrial septum because of high left atrial pressure from left ventricular dysfunction (Gien and Kinsella, 2016). This circulatory pattern will diminish the clinical response to iNO during the first few days after birth and produce significant pulmonary and systemic hemodynamic instability. Some infants may have exceptionally severe left ventricular dysfunction that leads to dependence on the right ventricle for systemic perfusion; this subset

of patients may benefit from a management approach that maintains patency of the ductus arteriosus, reduces left ventricular afterload, and delays the use of iNO until left ventricular performance improves (McNamara et al., 2013; Abman et al., 2015). In addition to its role in the acute setting, left ventricular dysfunction can contribute to the severity of pulmonary hypertension during the late and chronic courses in infants with CDH.

Alveolar Capillary Dysplasia

Unlike pediatric and adult pulmonary hypertension, few genetic factors have been identified for PPHN. A notable exception is alveolar capillary dysplasia with misalignment of lung vessels, a rare but universally lethal cause of pulmonary hypertension in the newborn (Bishop et al., 2011). Shortly after birth, affected infants typically exhibit cyanosis, minimal parenchymal disease, and respiratory distress refractory to all known therapies, including extracorporeal support, although later presentations (at several weeks or months of age) have been recognized. More than 50% of infants exhibit other anomalies, most commonly affecting the genitourinary, cardiovascular, and gastrointestinal systems. The diagnosis is established by direct examination of lung tissue by lung biopsy or autopsy. Characteristic findings include simplification of lung architecture, widened and poorly cellular septa with a paucity of capillaries, and strikingly muscularized small arterioles. “Misaligned pulmonary veins” have been described within the same adventitial sheath as pulmonary arteries; these were recently identified to be congested bronchial veins that represent intrapulmonary vascular anastomoses creating right-to-left intrapulmonary shunts and profound refractory hypoxemia (Galambos et al., 2014). Approximately 10% of reported alveolar capillary dysplasia cases have a familial association, and mutations or deletions in the transcription factor forkhead box F1 gene (*FOXF1*) or deletions upstream of *FOXF1* are identified in 40% of infants with alveolar capillary dysplasia (Stankiewicz et al., 2009). A murine model of forkhead box F1 deficiency has been studied, which demonstrates the importance of the forkhead box F1 in embryonic development of the pulmonary vasculature (Kalinichenko et al., 2001).

Pulmonary Hypertension in Premature Infants

Fetal physiologic studies in lambs and humans have shown that the pulmonary vascular response to oxygen does not develop until the latter part of gestation (Morin et al., 1988; Rasanen et al., 1998). However, pulmonary vasoconstriction and reactivity can contribute to high PVR in early preterm lambs, as demonstrated by an increase in PVR with NO synthase inhibition and marked responsiveness to iNO (Kinsella et al., 1994). Birth at 23–26 weeks’ gestational age is associated with high rates of PPHN (Aikio et al., 2012), particularly when associated with oligohydramnios, and high rates of clinical use of iNO have been reported for these gestational ages in regional databases (Handley et al., 2016). Furthermore, echocardiographic findings suggestive of pulmonary hypertension, such as ventricular septal wall flattening and right ventricular dilation, were found in approximately 40% of extremely low birth weight babies at 7 days of age and predicted BPD and late pulmonary hypertension (Mourani et al., 2015).

BPD has emerged as an important cause of chronic pulmonary hypertension, with an incidence of 16%–25% in infants with BPD (Mourani and Abman, 2015). Severe or prolonged pulmonary hypertension increases the risk of late morbidity and death to nearly 50% (Khemani et al., 2007). The pathogenesis of pulmonary

hypertension associated with BPD involves complex interactions between prenatal factors, such as growth restriction, preeclampsia, oligohydramnios, or fetal inflammation, and postnatal injury due to ventilator-induced lung injury, hyperoxia, hemodynamic stress, and infection. The result is impaired angiogenesis, abnormal vascular signaling, and vascular pruning, resulting in a reduction in the alveolar–capillary surface area. Identifying pulmonary hypertension in infants with BPD requires a high index of suspicion and systematic longitudinal evaluation. Echocardiography remains the most practical screening tool and should be considered in infants who continue to have cyanotic spells or require oxygen supplementation at 36 weeks' corrected gestational age. In infants with evidence of significant pulmonary hypertension by echocardiogram, cardiac catheterization may provide a more accurate quantification of the severity of disease, as well as allow vasoreactivity testing (Mourani et al., 2008).

Clinical Evaluation of Persistent Pulmonary Hypertension of the Newborn

PPHN typically presents as respiratory distress and cyanosis within hours of birth and may be associated with a variety of lung and/or cardiac disorders (Box 52.1). Clinically, PPHN is most often recognized in the term or near-term neonate but should also be considered in premature neonates who have cyanosis out of proportion to their parenchymal lung disease (Kinsella et al., 2016). While PPHN is often associated with signs of perinatal distress, such as asphyxia, low Apgar scores, or meconium staining, idiopathic PPHN can present without signs of acute perinatal distress.

PPHN is characterized by hypoxemia that is poorly responsive to supplemental oxygen. An important goal for the initial clinical evaluation is to rule out cyanotic cardiac disease and to determine whether a hypoxemic infant has PPHN-type physiology. Because a patent foramen ovale and ductus arteriosus are normally present early in life, elevated PVR in the newborn will produce extrapulmonary shunting of blood through these fetal channels, leading to severe and potentially unresponsive hypoxemia. In the presence of right-to-left shunting across the patent ductus arteriosus, “differential cyanosis” is often present, which is difficult to observe by physical examination but may be detected by a gradient in PaO₂ and/or oxygen saturation between the right radial artery versus descending aorta sites. Considering the left subclavian artery may have either a preductal or a postductal origin from the aorta, the oximeter probe should be applied to a foot for postductal pulse oximetry monitoring. Saturation differences greater than 5%–10% are generally a significant sign of PPHN, but it is important to remember that a similar pattern of postductal desaturation may be observed in ductus-dependent cardiac diseases, including hypoplastic left-sided heart syndrome, coarctation of the aorta, or interrupted aortic arch.

In many infants, intrapulmonary shunt or ventilation–perfusion mismatch resulting from parenchymal lung disease is the predominant abnormality rather than shunting of blood flow across the patent ductus arteriosus and patent foramen ovale. In this setting, hypoxemia is the result of pulmonary arterial blood perfusing the nonaerated lung regions. Although PVR is often elevated in hypoxemic newborns without PPHN, high PVR does not contribute significantly to hypoxemia in these cases. Rapid improvement in response to supplemental oxygen or increased mean airway pressure suggests ventilation–perfusion mismatch due to primary lung disease, although this may not be obvious with severe parenchymal

• BOX 52.1 Disorders Associated With Neonatal Pulmonary Hypertension

Pulmonary

Meconium aspiration syndrome
Respiratory distress syndrome (term and preterm newborns)
Lung hypoplasia—primary
Congenital diaphragmatic hernia
Pneumonia/sepsis
Idiopathic pulmonary arterial hypertension
Transient tachypnea of the newborn
Developmental and genetic lung diseases, including:

- Alveolar capillary dysplasia (e.g., *FOXF1*)
- Genetic lung disease associated with surfactant proteins, ABCA3, TTF-1, and other proteins
- Pulmonary interstitial glycogenosis
- Congenital lobar emphysema (rare association)
- Cystic adenomatoid malformation (rare association)

Cardiovascular

Myocardial dysfunction (asphyxia; infection; stress)

Structural cardiac diseases:

- Mitral stenosis, cor triatriatum
- Endocardial fibroelastosis
- Pompe disease
- Aortic atresia, coarctation of the aorta, interrupted aortic arch
- Transposition of the great vessels
- Ebstein anomaly, tricuspid atresia

Pulmonary atresia

Hepatic arteriovenous malformations

Cerebral arteriovenous malformations

Pulmonary venous abnormalities:

- Total anomalous pulmonary venous return
- Pulmonary vein stenosis (isolated)
- Pulmonary capillary hemangiomatosis
- Pulmonary veno-occlusive disease

Associations With Other Conditions

Neuromuscular disease

Metabolic disease

Polycythemia

Thrombocytopenia

Maternal drug use or smoking

ABCA3, Member A3 of ATP binding cassette family; TTF-1, thyroid transcription factor 1.

lung disease. Furthermore, most infants with PPHN have at least a transient improvement in oxygenation in response to interventions such as high inspired oxygen concentration therapy and/or mechanical ventilation. Therefore these clinical findings can only suggest and not confirm the diagnosis. At present, there is no single biochemical marker that has emerged with sufficient sensitivity and specificity for the diagnosis and management of PPHN.

Radiographic findings are variable, depending on the primary disease associated with PPHN. Classically, the lung on a chest X-ray in idiopathic PPHN is oligemic, normally or slightly hyperinflated, and lacks parenchymal infiltrates. In general, the degree of hypoxemia is disproportionate to the severity of radiographic evidence of lung disease. Laboratory findings may include hypoglycemia, hypocalcemia, polycythemia, and thrombocytopenia.

Echocardiography is the gold standard to confirm the diagnosis and is an important tool for monitoring the response to therapy. The initial echocardiographic evaluation rules out structural heart disease causing hypoxemia or ductal shunting (e.g., coarctation of the aorta or total anomalous pulmonary venous return), determines

the predominant direction of shunting at the patent foramen ovale and patent ductus arteriosus, and assesses ventricular function. The diagnosis of PPHN is made with certainty if bidirectional or predominantly right-to-left shunting across the foramen ovale or ductus arteriosus is observed, although other signs such as flattening or left deviation of the intraventricular velocity, tricuspid regurgitant velocity, and increased right ventricular dilation also suggest the diagnosis. The echocardiogram is also critical for the evaluation of right and left ventricular function. In some infants, predominant right-to-left shunting at the ductus arteriosus associated with left-to-right shunt at the foramen ovale is observed, indicating a significant contribution of left ventricular dysfunction to the underlying pathophysiology. When severe left ventricular dysfunction accompanies pulmonary hypertension, pulmonary vasodilation alone may be ineffective in improving oxygenation and must be accompanied by targeted therapies to increase cardiac performance and decrease left ventricular afterload. Thus careful echocardiographic assessment provides invaluable information about the underlying pathophysiology and will help guide the course of treatment.

General Management

The American Heart Association and the American Thoracic Society recently developed guidelines for management of pediatric pulmonary hypertension, including PPHN (Abman et al., 2015; Box 52.2). The general management principles for PPHN include maintenance of normal temperature (except for those undergoing therapeutic hypothermia), electrolytes (particularly calcium), glucose, hemoglobin, and intravascular volume. Mechanical ventilation is usually required to optimize lung volumes, although few guidelines exist to standardize ventilator management. Some advocate a “gentle ventilation” approach, with avoidance of paralysis and blood gas goals consisting of PaO_2 of 50–70 mmHg and PaCO_2 of 40–60 mmHg. This strategy has never been rigorously tested (Wung et al., 1985) but may well be the optimal strategy for infants still in the early phases of disease. When significant parenchymal lung disease is present, lung recruitment strategies, such as high-frequency ventilation, improve lung expansion and amplify the response to iNO (Kinsella et al., 1997; Kinsella and Abman, 2000). However, care should be taken to avoid settings that may cause lung overdistension, which can lead to inflammatory changes, pulmonary edema, and decreased lung compliance, all of which can exacerbate pulmonary hypertension.

Use of surfactant should be considered only for infants with parenchymal lung disease. Single-center trials have shown that surfactant improves oxygenation, reduces air leak, and reduces the need for ECMO in infants with meconium aspiration (Findlay et al., 1996). A multicenter trial showed benefit in infants with parenchymal lung diseases such as meconium aspiration syndrome and sepsis (Lotze et al., 1998). This trial also demonstrated the greatest benefit for infants with earlier or milder disease (oxygenation index 15–22) but no benefit in the subset of newborns with idiopathic PPHN. A recent systematic review and metaanalysis of surfactant therapy for neonates with meconium aspiration syndrome showed that those who received bolus surfactant needed ECMO less often than controls. However, the limited number of studies and neonates enrolled led the authors to conclude that more studies are urgently needed to evaluate the efficacy and cost-effectiveness of this therapy (Natarajan et al., 2016). Registry studies indicate that surfactant therapy does not benefit the infant with CDH (Van Meurs, 2004).

• BOX 52.2 American Heart Association and American Thoracic Society Management Guidelines for Persistent Pulmonary Hypertension of the Newborn

1. Inhaled nitric oxide therapy is indicated to reduce the need for extracorporeal membrane oxygenation (ECMO) support in term and near-term infants with persistent pulmonary hypertension of the newborn (PPHN) or hypoxemic respiratory failure who have an oxygenation index that exceeds 25 (class I, level A).
2. Lung recruitment strategies can improve the efficacy of inhaled nitric oxide therapy and should be applied in patients with PPHN associated with parenchymal lung disease (class 1, level B).
3. ECMO support is indicated for term and near-term neonates with severe pulmonary hypertension and/or hypoxemia that is refractory to inhaled nitric oxide therapy and optimization of respiratory and cardiac function (class I, level A).
4. Evaluation for disorders of lung development, such as alveolar capillary dysplasia and genetic surfactant protein diseases, is reasonable for infants with severe PPHN whose condition fails to improve after vasodilator, lung recruitment, and/or ECMO therapy (class IIa, level B).
5. Sildenafil use is a reasonable adjunctive therapy for infants with PPHN who are refractory to inhaled nitric oxide therapy, especially with an oxygenation index that exceeds 25 (class IIa, level B).
6. Inhaled prostacyclin analogues may be considered as adjunctive therapy for infants with PPHN who are refractory to inhaled nitric oxide therapy and have an oxygenation index that exceeds 25 (class IIb, level B).
7. Intravenous administration of milrinone is reasonable therapy for infants with PPHN and signs of left ventricular dysfunction (class IIa, level B).
8. Inhaled nitric oxide can be beneficial for preterm infants with severe hypoxemia that is primarily due to PPHN physiology rather than parenchymal lung disease (class IIa, level B).
9. Inhaled nitric oxide therapy and other pulmonary arterial hypertension–targeted drug therapies should be used cautiously in individuals with congenital diaphragmatic hernia, especially in those with confirmed or suspected left ventricular dysfunction (class IIa, level B).

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Acidosis is a pulmonary vasoconstrictor, particularly when combined with hypoxia (Rudolph and Yuan, 1966), and pH should be maintained above 7.30. However, alkalosis (pH >7.45) is not recommended. The use of alkalosis was frequent before the approval of iNO therapy, based on studies that found transient increases in PaO_2 after acute hyperventilation (Drummond et al., 1981). However, the pulmonary vascular response to alkalosis is transient, and prolonged alkalosis may paradoxically worsen pulmonary vascular tone, reactivity, and permeability edema (Laffey et al., 2000). Furthermore, alkalosis produces cerebral vasoconstriction, reducing cerebral blood flow and oxygen delivery to the brain, and may be associated with worse neurodevelopmental outcomes (Ferrara et al., 1984). Similarly, there is no evidence to suggest that the use of sodium bicarbonate infusions to induce alkalosis provides any short-term or long-term benefit (Walsh-Sukys et al., 2000).

Systemic hemodynamics should be optimized, with use of volume to provide sufficient preload and cardiostimulant therapy to enhance cardiac output and systemic O_2 transport. Systemic hypotension may worsen right-to-left shunting, impair oxygen delivery, and worsen gas exchange in patients with parenchymal lung disease. In addition, high right ventricular systolic pressure reduces systolic

flow through the right coronary artery, and low systemic blood pressure leads to a decline in right coronary artery diastolic flow, so correction of both hemodynamic abnormalities is key to avoiding right ventricular ischemia (Giesinger and McNamara, 2016). However, the goal is more complex than simply increasing blood pressure, and careful attention should be paid to right and left ventricular function and the choice of medication. Front-line agents typically include dopamine (5–20 µg/kg per minute) and milrinone

(0.2–0.99 µg/kg per minute). Milrinone, which inhibits cAMP phosphodiesterase 3 (PDE3) activity in cardiomyocytes and pulmonary and systemic arterial smooth muscle, is viewed as an “inodilator” that may be especially useful in the context of left ventricular dysfunction and pulmonary venous hypertension (James et al., 2015). Recent anecdotal evidence supports the use of arginine vasopressin for severe PPHN (Table 52.1), based on its ability to raise systemic pressure via V_1 receptors, and to reduce

TABLE 52.1

Pharmacologic Management of Persistent Pulmonary Hypertension of the Newborn

| Drug | Dose | Mechanism of Action | Adverse Reactions/ Safety Monitoring | Indications |
|----------------------|--|---|--|---|
| Inhaled nitric oxide | 5–20 ppm | Activates soluble guanylate cyclase in vascular smooth muscle cells Selective pulmonary vasodilator Low doses (<10 ppm) enhance ventilation–perfusion matching with parenchymal lung disease (“microselective effects”) | Rebound PH with rapid discontinuation of inhaled nitric oxide therapy Methemoglobinemia with higher doses | Hypoxemic respiratory failure with PPHN |
| Sildenafil (Revatio) | Intravenous: loading dose: 0.4 mg/kg in 3 h Maintenance dose 1.6 mg/kg per day | Selective PDE5 inhibitor that increases vascular cGMP levels and pulmonary vascular relaxation | Hypotension (particularly with rapid loading dose) Hypoxemia | PPHN refractory to inhaled nitric oxide therapy and other conventional therapies |
| Milrinone | Intravenous: loading dose: 0.75 µg/kg per minute for 3 h (for infants with hypotension or severe illness, may choose to not use loading dose) Maintenance dose: 0.2–0.33 µg/kg per minute. Increasing by 0.33 µg/kg per minute to a maximum of 0.99 µg/kg per minute | PDE3 inhibitor that increases cAMP levels Improves cardiac performance through reduced systemic afterload, enhanced contractility, and pulmonary vasodilation | Hypotension, thrombocytopenia, arrhythmias | Left ventricular dysfunction, poor cardiac output |
| Prostacyclin | Flolan: Inhaled: 20–100 ng/kg per minute. Start at 2 ng/kg per minute and increase to 20 ng/kg per minute within 3 h. Continuous intravenous infusion: starting dose: 1–2 ng/kg per minute, incremental increases as tolerated, monitor newborn for tachyphylaxis Remodulin: Continuous infusion: 1.25 ng/kg per minute to start, titrate up slowly to 10 ng/kg per minute Can be delivered subcutaneously in the same dosing range | Activates adenylate cyclase in vascular smooth muscle cells Pulmonary vasodilator | Ventilation–perfusion mismatch may complicate use in the setting of lung disease. Nonselective vasodilator, can cause systemic hypotension Risk of rebound PH with sudden withdrawal Flushing, diarrhea | PPHN refractory to iNO therapy and other conventional therapies |
| Bosentan | Enteral: 1–2 mg/kg every 12 h | Dual ET_A and ET_B receptor inhibitor | Hepatotoxicity, edema, anemia, teratogen Monitor monthly LFT results. | PPHN refractory to iNO and other conventional therapies Generally used for chronic PH (CDH, BPD) |
| Vasopressin | 0.0003–0.004 units/kg per minute | Predominantly used for enhancement of systemic hemodynamics in severe PPHN May have pulmonary vasodilator effects via activation of eNOS | Hypotension, arrhythmias Hyponatremia: increases cAMP level in distal tubules, leading to decreased urine volume | |

cAMP, Cyclic AMP; BPD, bronchopulmonary dysplasia; CDH, congenital diaphragmatic hernia; cGMP, cyclic AMP; eNOS, endothelial nitric oxide synthase; iNO, inhaled nitric oxide; LFT, liver function test; PDE3, phosphodiesterase 3; PDE5, phosphodiesterase 5; PH, pulmonary hypertension; PPHN, persistent pulmonary hypertension of the newborn.

pulmonary arterial pressure via activation of NO synthase (Mohamed et al., 2014).

Infants who fail to respond to medical treatment, as evidenced by failure to sustain improvement in oxygenation with good hemodynamic function, may require treatment with ECMO (UK Collaborative ECMO Trial Group, 1996). ECMO is the only proven lifesaving rescue modality for severe PPHN, although it is also costly, labor-intensive, and associated with potential adverse effects, such as intracranial hemorrhage and ligation of the right common carotid artery. The oxygenation index (calculated as $[\text{mean airway pressure} \times \text{FiO}_2 \times 100]/\text{PaO}_2$) is used to gauge the severity of disease, with an oxygenation index greater than 40 used as an indication for transfer to an ECMO center. Even with all available therapies, the mortality for PPHN remains between 5% and 10%.

Oxygen

Supplemental oxygen is a mainstay of PPHN therapy to maintain oxygen delivery to the brain and other tissues and for its pulmonary vasodilator properties. Alveolar hypoxia and hypoxemia should be avoided, as both are pulmonary vasoconstrictors and contribute to the pathophysiology of PPHN. On the other hand, the degree of hyperoxic ventilation needed for pulmonary vasodilation remains under investigation. The use of oxygen concentrations greater than 60% has not been shown to provide any additional benefit in pulmonary vasodilation. On the other hand, hyperoxic ventilation increases oxidant stress and lung injury and may paradoxically impair the vasodilator response to iNO. Therefore the oxygen concentration and PaO_2 should be titrated to maximize pulmonary vasodilation. Studies in newborn lambs suggest that the lowest PVR can be maintained with a preductal SpO_2 in the 91%–97% range with preductal PaO_2 between 60 and 80 mmHg (Lakshminrusimha et al., 2007, 2009b). Overall, oxygen should be used like any other drug in the therapeutic context, considering its potential benefits and side effects.

Inhaled Nitric Oxide

The primary goal of PPHN therapy is selective pulmonary vasodilation. Inhaled NO (iNO) is FDA approved as a specific pulmonary vasodilator therapy for PPHN, based on extensive safety and efficacy data from large placebo-controlled trials (Abman et al., 2015). It is well suited for the treatment of PPHN. It is a rapid and potent vasodilator, and because NO is a small gas molecule, it can be delivered as inhalation therapy to air spaces approximating the pulmonary vascular bed. Inhaled NO immediately improves oxygenation and decreases the need for ECMO support in newborns with PPHN and an oxygenation index greater than 25. The appropriate starting dose is 20 ppm; higher doses in nonresponders did not improve immediate responses or outcomes (Neonatal Inhaled Nitric Oxide Study Group, 1997), and treatment with high NO doses (80 ppm) increases the risk of methemoglobinemia (Davidson et al., 1998). Similarly, to term infants, preterm infants with early PPHN, particularly after prolonged rupture of membranes or oligohydramnios, will show marked improvement in oxygenation after treatment with iNO (Kinsella et al., 2016). Recent reports indicate that iNO use ranges from 4% to 8% in extremely preterm infants (Handley et al., 2016).

The optimal window for introduction of iNO therapy remains uncertain. The initial randomized trials studied term and near-term infants with severe PPHN and an oxygenation index of 25–40 (Neonatal Inhaled Nitric Oxide Study Group, 1997; Clark et al.,

2000). A subsequent large trial tested earlier use of iNO in infants with moderate respiratory failure (median oxygenation index of approximately 20) but did not report reductions in ECMO use/death relative to controls (16.7% vs 19.5%; Konduri et al., 2004, 2007). Once an infant has stabilized, weaning can generally be accomplished in 4 to 5 days. Prolonged need for iNO therapy without resolution of disease should lead to a more extensive evaluation to determine whether other underlying anatomic lung or cardiovascular disease is present, such as pulmonary venous stenosis, alveolar capillary dysplasia, and severe lung hypoplasia.

Up to 40% of infants will not respond or sustain a response to iNO. The reasons for an inadequate response are diverse and require the clinician to carefully analyze the relative roles of parenchymal lung disease, pulmonary vascular disease, and cardiac dysfunction for each infant. For instance, if severe air space disease is associated with PPHN, strategies such as high-frequency ventilation that optimize lung expansion are likely to be effective, and the two therapies used together are more effective than either used individually (Kinsella et al., 1997). Considering iNO is usually delivered with high concentrations of oxygen, these therapies could interact and lead to enhanced production of reactive oxygen and reactive nitrogen metabolites, both of which may contribute to vasoconstriction and/or inadequate responses to iNO.

Other Therapeutic Agents

Phosphodiesterase Inhibitors

Increased activity of PDE5 has been consistently reported in experimental models of PPHN (Hanson et al., 1998; Farrow et al., 2010). More recently, in vitro and in vivo studies have shown that even brief periods of hyperoxia independently increase the activity of PDE5, by a mechanism that appears to involve reactive oxygen species (Farrow et al., 2008, 2010, 2012). This finding could partially explain why some infants do not respond favorably to iNO.

Sildenafil is the best studied of the available phosphodiesterase inhibitors and can be administered by the enteral and intravenous routes. A small randomized trial found that enteral administration of sildenafil improved oxygenation and survival in term and late preterm neonates with PPHN (Baquero et al., 2006). In a subsequent dose-finding study, intravenous administration of sildenafil improved oxygenation in neonates with and without concurrent iNO treatment (Steinhorn et al., 2009), suggesting that sildenafil may be a useful adjunct in patients with partial or poorly sustained responses to iNO. Theoretically, sildenafil may be particularly effective in neonates with PPHN following prolonged hyperoxic ventilation, considering PDE5 expression and activity are increased following ventilation with high concentrations of oxygen and exposure to reactive oxygen species. Sildenafil is generally well tolerated, although hypotension can occur, particularly if a loading dose is given too rapidly (Steinhorn et al., 2009). The availability of sildenafil as an enteral preparation also makes it feasible for long-term therapy for infants with chronic pulmonary hypertension associated with CDH (Keller et al., 2006) and BPD (Mourani et al., 2009), although this aspect of treatment needs more study and long-term follow-up. Other phosphodiesterase inhibitors include tadalafil and vardenafil, but their use in PPHN has not yet been reported.

Prostanoids

Prostacyclin (epoprostenol) was approved by the FDA in 1995 for the treatment of adult pulmonary arterial hypertension. While

intravenously administered prostacyclin remains a mainstay of therapy for pulmonary hypertension in adults, rapid dosage escalation is often necessary for acute disease and can produce systemic hypotension. Its utility in the neonatal intensive care unit setting is also limited by the need for a dedicated central venous catheter, the potential to worsen ventilation–perfusion matching, and other systemic side effects including pain.

Aerosolized prostacyclin has been widely adopted in adult critical care units for the treatment of pulmonary hypertension (Walmrath et al., 1996). While fewer reports describe its use in infants, case series indicate that continuous inhaled prostacyclin therapy (50–100 ng/kg per minute) is well tolerated and improves oxygenation in infants with severe PPHN and inadequate response to iNO (Kelly et al., 2002; Porta and Steinhorn, 2012). The risks include airway irritation from the alkaline solution needed to maintain drug stability, rebound pulmonary hypertension if use of the drug is abruptly discontinued, and inconsistent drug delivery due to drug loss into the circuit. New, stabler preparations are emerging that are specifically designed for intermittent nebulization, such as iloprost or treprostinil. Treprostinil is particularly promising because it is also suitable for systemic administration, including by the subcutaneous route (Ferdman et al., 2014).

Inhibition of PDE3, which metabolizes cAMP, might also enhance cAMP signaling in PPHN (Chen et al., 2009). Milrinone, a PDE3 inhibitor, has been shown to decrease pulmonary arterial pressure and resistance and to act additively with iNO in animal studies (Thelitz et al., 2004). A report indicates that the addition of intravenous milrinone therapy for neonates with severe PPHN and poor iNO responsiveness was associated with improvements in oxygenation without hemodynamic status being compromised (McNamara et al., 2006).

Endothelin Receptor Antagonists

ET-1 is a potent vasoconstrictor synthesized by vascular endothelial cells that acts through two receptors, ET_A and ET_B. The ET_A receptor plays a critical role in vasoconstriction, and selective blockade of the ET_A receptor causes fetal pulmonary vasodilation (Ivy et al., 1994). *ET-1* gene expression and ET-1 levels are increased in the lungs and pulmonary arterial endothelial cells in the fetal

lamb model of PPHN (Ivy et al., 1998a; Gien et al., 2013). Long-term intrauterine ET_A receptor blockade following ductal ligation decreases pulmonary arterial pressure and distal muscularization of small pulmonary arteries in utero, decreases right ventricular hypertrophy, and increases the fall in PVR at delivery in newborn lambs with PPHN (Ivy et al., 1997). Thus it is likely that ET-1, acting through the ET_A receptor, contributes to the pathogenesis and pathophysiology of PPHN. Use of bosentan, a nonspecific ET-1 receptor blocker, is an established therapy for pulmonary hypertension in adults. Two recent trials show that bosentan is well tolerated in neonates with PPHN. One single-center trial reported that bosentan led to substantial improvements in oxygenation in an iNO-naïve population of PPHN infants (Mohamed and Ismail, 2012), but the FUTURE-4 multicenter trial found that bosentan as an adjunctive therapy with iNO therapy did not improve PPHN outcomes, reduce the time in receipt of iNO therapy, or reduce the time to extubation, possibly in part due to inconsistent intestinal absorption (Steinhorn et al., 2016).

Outcomes

Early reports began to establish the high risk of death and abnormal neurodevelopmental outcomes for survivors of PPHN (Bifano and Pfannenstiel, 1988). At least one report showed that oxygenation (measured by the alveolar–arterial oxygen difference) correlated well with survival but not with long-term outcome (Hageman et al., 1988). Some reports further indicated that low Apgar scores and prolonged hypoxemia were predictive of adverse neurodevelopmental outcomes (Bifano and Pfannenstiel, 1988; Marron et al., 1992).

The long-term outcome data for the largest iNO trials are summarized in Table 52.2, which also provides the most comprehensive outcome data for PPHN infants treated with and without iNO. Overall, these results highlight that neurodevelopmental impairment is high in this population (14%–30%) and is not reduced by iNO therapy (Neonatal Inhaled Nitric Oxide Study Group, 2000; Lipkin et al., 2002; Clark et al., 2003; Konduri et al., 2007). In each of these trials, a trend toward higher motor impairment was observed in the iNO group, and in the early iNO trial reported by Konduri et al. (2007), the difference in the Bayley

TABLE
52.2

Neurodevelopmental Outcomes for the Major Inhaled Nitric Oxide Clinical Trials

| Study | Number of Participants | Initial OI | Age at Follow-Up (Months) | NDI (%) | | BAYLEY MENTAL DEVELOPMENTAL INDEX | | BAYLEY PSYCHOMOTOR DEVELOPMENTAL INDEX | | CEREBRAL PALSY (%) | |
|--|------------------------|------------|---------------------------|---------|-----|-----------------------------------|----------------|--|----------------|--------------------|------|
| | | | | Control | iNO | Control | iNO | Control | iNO | Control | iNO |
| Neonatal Inhaled Nitric Oxide Study Group (2000) | 235 | 44 | 18–24 | 30 | 35 | 87 | 85 | 93.6 | 85.7 | 10.3 | 11.8 |
| Clark et al. (2003) | 248 | 39 | 12 | 14 | 19 | 95 | 95 | 85 | 92 | 1.4 | 4 |
| Davidson (2002) ^a | 155 | 24.7 | 12 | 20 | 18 | — ^b | — ^b | — ^c | — ^b | 6 | 8 |
| Konduri et al. (2007) | 299 | 19.2 | 18–24 | 25 | 28 | 86.1 | 83.3 | 98 | 89 | 6.3 | 8.2 |

^aThe reference reporting the outcomes for the Davidson trial is entered as Lipkin 2002.

^bSeventy-one percent of the control group and 69% of the inhaled nitric oxide (iNO) group were reported as normal (mental developmental index ≥85).

^cEighty-two percent of the control group and 76% of the iNO group were reported as normal (psychomotor developmental index ≥85).

NDI, Neurodevelopmental impairment; OI, oxygenation index.

psychomotor developmental index was statistically significant. The Neonatal Inhaled Nitric Oxide Study Group (2000) reported that the rate of disability was not affected by the need for ECMO, leading the authors to speculate that the timing of brain injury was most likely before ECMO.

Neurodevelopmental impairment is high even for those infants with moderate PPHN (oxygenation index of 15–25). The overall rates of neurodevelopmental impairment were similar to those of other trials that enrolled patients with more advanced disease, and early iNO use did not translate into improved outcomes (Konduri et al., 2007). The proportion of infants with medical problems, such as the need for home medications, oxygen, and gastrostomy feedings, also tended to be higher in the early iNO group, although these differences were not statistically significant.

ECMO is the only PPHN therapy that has been proven to be lifesaving, but it remains a technically complex and invasive therapy with many potential complications. More than 27,000 newborns have been treated with ECMO for respiratory failure, with a cumulative overall survival to discharge of 75% (Paden et al., 2014). Mild to major neurodevelopmental impairment is described in 15%–25% of neonatal ECMO survivors (van Heijst et al., 2014).

Summary

Persistent pulmonary hypertension complicates the course of up to 10% of neonates with respiratory failure. It is associated with a diverse set of cardiopulmonary conditions, and its pathophysiologic mechanisms are characterized by vascular dysfunction, injury, and remodeling that occur before and after birth. In the last 20 years, experimental work on the basic mechanisms of vascular regulation of the developing pulmonary circulation has improved the range of therapeutic approaches for neonates with PPHN. In particular, iNO has proven to be a selective and effective pulmonary vasodilator

However, determining the role of ECMO versus other disease factors in the neurodevelopmental outcomes for ECMO survivors is difficult. From the pre-iNO era, the UK Collaborative Randomized Trial of ECMO provides the most direct comparison of outcomes after ECMO versus conventional treatment and included neurodevelopmental assessments at ages 1, 4, and 7 years. At age 1 year, a significant survival benefit (68% vs 41%) was observed in the ECMO-treated group versus the conventionally treated group (UK Collaborative ECMO Trial Group, 1998), and survivors demonstrated similar rates of impairment or disability. At the 4-year follow-up, there continued to be a higher proportion of survivors without disability in the ECMO-treated group versus the conventionally treated group (50% vs 37%) (Bennett et al., 2001), although the rates of disability were similar at age 7 years (55% vs 50%) (McNally et al., 2006). The high rate of neurodevelopmental impairment in both groups suggests that the underlying disease process, rather than the treatment, is the major influence on neurodevelopmental outcome. Several more recent studies also indicate that ECMO survivors may be at risk of academic problems (Ijsselstijn and van Heijst, 2014), so these children will benefit from detailed assessment as they reach school age.

for infants with PPHN, although successful clinical management requires meticulous care for all aspects of the associated lung and cardiac disease. Current research is focused on developing a better understanding of cellular responses in the remodeled vasculature and will likely elucidate additional signaling pathways and lead to new therapeutic strategies. More work is needed to further reduce mortality and improve neurodevelopmental outcomes of sick newborns with pulmonary hypertension, especially in patients with lung hypoplasia and advanced structural vascular disease.

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53

Fetal and Neonatal Echocardiography

MARGARET M. VERNON AND MARK B. LEWIN

KEY POINTS

- Echocardiography is used to assess cardiac anatomy and physiology.
- Appropriate interpretation requires an understanding of its limitations.

*E*chocardiography is the application of ultrasound to the evaluation of the cardiovascular system. Over the past several decades echocardiography has established itself as the gold standard for the diagnosis of congenital heart disease (CHD) and acquired heart disease. Instrumentation has become more sophisticated, and utilization has moved from an anatomic evaluation to include an assessment of myocardial function; an understanding of the basic principles and limitations of ultrasound is increasingly valuable to clinicians. This chapter reviews the basic principles of echocardiography and their applications in the prenatal and newborn settings.

Application of Ultrasound to Cardiac Imaging

Physics of Ultrasound

Ultrasound is the use of ultra-high-frequency sound waves (those too high in frequency to be heard by the human ear) to create an image. Echocardiography is the application of ultrasound to create an image of the heart. In both, energy is generated by the stimulation of piezoelectric crystals housed in a small handheld device called a transducer. When the transducer is placed on the chest or abdomen, ultrasound waves are transmitted into the tissue. The majority of these waves are absorbed; however, a small subset are reflected (echoed) back to the transducer. These reflected waves are processed, and the resultant image is displayed on the screen. Soft tissue (such as the liver) and fluid (such as blood and amniotic fluid) are excellent media for ultrasound transmission and provide optimal windows for detailed cardiac imaging (Weyman, 1994). Ultrasound scatter, which negatively impacts image clarity, is greatest at the interfaces between biologic tissues of widely disparate densities. Hence, bone and air, when adjacent to soft tissues such as the heart, create poor acoustic windows for ultrasound transmission.

Ultrasound generally refers to sound waves of frequencies greater than 20,000 Hz (cycles per second), with the range of frequencies used for diagnostic assessment of biologic tissue between 2 and 12 megahertz (MHz) (megahertz = 1,000,000 Hz). Low-frequency waves penetrate tissue better than high-frequency ultrasound waves; however, higher-frequency waves provide for greater spatial resolution of fine structures. This principle is dictated by a fundamental law of physics that defines the relationship between ultrasound frequency and wavelength:

$$\text{velocity} = \text{frequency} \times \text{wavelength}$$

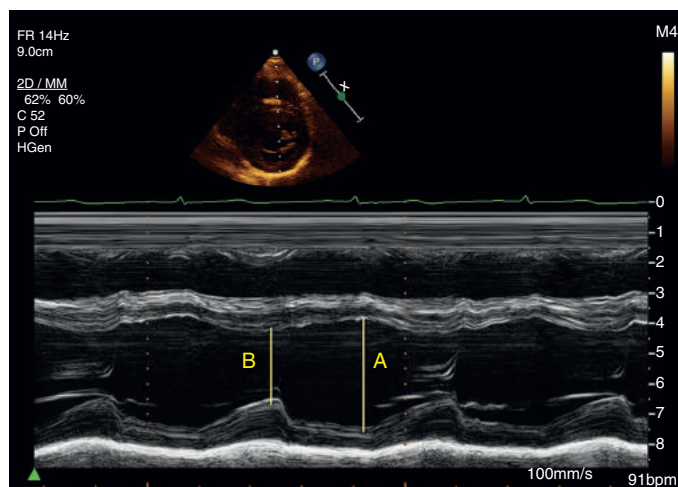
An ultrasound wavelength distance is the physical limit beyond which two structures cannot be distinguished. Hence, the shorter the wavelength (i.e., the higher the frequency), the greater the ability to distinguish the two points from each other.

Let us look at the following example: two structures are 0.5 mm apart. The velocity of sound in biologic tissue is a constant, at approximately 1540 m/s. Applying a frequency of 2 MHz of ultrasound energy will result in a wavelength of 0.77 mm, whereas applying a frequency of 8 MHz of ultrasound energy will result in a wavelength of 0.19 mm. Hence, to the operator using the 2 MHz transducer, the two structures will not be distinguishable from one another and will appear as one (in that the distance between them is less than the ultrasound wavelength), whereas the operator using the 8 MHz transducer will be able to differentiate between the structures with ease. Accordingly, higher frequencies, at a minimum 5 MHz and more typically 8–12 MHz, are used in the neonatal setting because penetration is less important, and the objective is to maximize fine structure resolution.

Basic Imaging Components

M-Mode

The earliest form of echocardiographic imaging, M-mode echocardiography, displays the visualized cardiac structures along a single plane of insonation. The displayed image shows the structures as they change over the cardiac cycle. Today, M-mode echocardiography is primarily used for measurement of ventricular wall thickness, cavity dimensions, and in the calculation of the ventricular *shortening fraction*—an estimate of ventricular function. By angling the plane of insonation through the short axis of the left ventricle at the level of the tips of the papillary muscles, one can obtain an M-mode display of the change in ventricular cavity dimension over time (Fig. 53.1). The ratio of the difference between left



• **Fig. 53.1** M-Mode Tracing of the Left-Ventricular Dimensions Over Time Obtained in the Short-Axis View. The electrocardiographic tracing helps identify the timing of the cardiac cycle as systolic or diastolic. Measurement A demonstrates the left ventricle end-diastolic dimension and measurement B, the left ventricle end-systolic dimension. The shortening fraction is calculated at 35%.

ventricle end-diastolic dimension (LVEDD) and left ventricle end-systolic dimension (LVESD) to the left ventricle end-diastolic dimension is the shortening fraction ($(LVEDD - LVESD)/LVEDD \times 100 = \%SF$). The normal range is 28% to 38%, which correlates with a ventricular volumetric ejection fraction of 55%–65%.

When interpreting a shortening fraction, it is important to note the dimension of the left ventricle. For example, an infant with a ventricular volume load caused by a large patent ductus arteriosus (PDA) may have a normal shortening fraction; however, hemodynamic significance is suggested by the presence of a dilated left ventricle when normalized for body surface area.

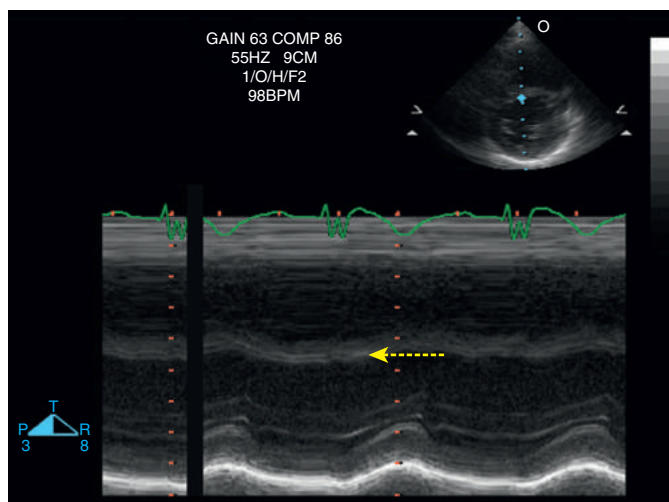
The shortening fraction (%SF) also can be helpful in investigating the cause of hypotension in neonates. In the setting of hypotension caused by myocardial dysfunction, the infant will have a diminished %SF frequently accompanied by a dilated left ventricle. Alternatively, when hypotension is secondary to systemic vasodilatation, such as in sepsis, the neonate may have a normal or hyperdynamic %SF, often exceeding 40%. Finally, volume-depleted infants will exhibit a normal %SF with a small left-ventricular cavity size.

Although the %SF is easily calculated and commonly used, it has a number of limitations. It provides for only a single-plane assessment of ventricular contraction and is invalid in conditions in which there is abnormal interventricular septal wall position or motion, including right-ventricular hypertension, right-ventricular enlargement, or a right-bundle branch block (Fig. 53.2).

Two-Dimensional Imaging

The development of phased-array transducers has allowed for sector rather than linear scanning and the display of two-dimensional (2D) images. Today, high-resolution 2D echocardiography is the most commonly applied modality of echocardiography. Detailed, reliable, and reproducible images of cardiac structures can be obtained, and, when images from multiple planes are mentally combined, precise cardiac diagnoses can be made.

Imaging typically follows a standard protocol. Cardiac and abdominal situs are assessed, a complete evaluation of the anatomic structure of the heart and proximal vasculature from multiple



• **Fig. 53.2** M-Mode Tracing of the Left-Ventricular Dimensions Over Time Obtained in the Short-Axis View. The electrocardiographic tracing helps identify the timing of the cardiac cycle as systolic or diastolic. The interventricular septum moves paradoxically (arrow) because of elevated right-ventricular pressure. Therefore a shortening fraction cannot be calculated accurately.

planes is made, and a determination is made as to the functional (systolic and diastolic) properties of the myocardium.

For an accurate determination to be made as to the dimensions of chambers, valves, and vessels, comparison with a normative dataset is required. The most common methodology involves normalizing measurements to body surface area. A number of datasets have been published and are available for comparison. In order to achieve consistency, each pediatric echocardiography laboratory must decide which of these datasets they will use. Various normative (Z-score) calculators are also available online (<http://www.parameterz.com> and <http://zscore.chboston.org/>) with references and descriptors included for ease of use. The identification of a pathologically large or small structure is especially troublesome when the patient is extremely premature. Comparative data for such infants are scant and are often based on data collected from a very small number of “normal” patients. Determination of dimensions (e.g., left ventricle, aortic valve, or pulmonary artery) in such premature patients is therefore subject to a fair bit of interpretation; in these cases, consultation with the pediatric cardiologist is imperative in order to make appropriate management decisions.

2D imaging also allows for an alternate means of measuring the end-diastolic and end-systolic dimensions, thus providing another means of estimating ventricular systolic contractility.

Doppler

Application of the Doppler principle allows for determination of flow direction and velocity of moving objects. As blood and myocardial tissue both are in motion throughout the cardiac cycle, either can be assessed by Doppler echocardiography. Doppler imaging can be performed in a number of ways. In pulsed-wave Doppler echocardiography, single ultrasound crystals alternately fire pulses of energy and then “listen” for reflected signal return. This mode allows for determination of spatial signal position but is limited in its ability to accurately measure blood traveling at higher velocities. In continuous-wave Doppler echocardiography, half of the transducer crystals continuously transmit, while the

other half continuously receive, and all received signals are displayed as a composite. This mode allows for unambiguous assessment of increased velocities but limits the ability to pinpoint the precise location at which the velocity is obtained. In combination, pulsed-wave and continuous-wave Doppler modes provide a complete picture of blood flow direction and velocity.

Color Doppler echocardiography uses pulsed-wave principles to create a 2D sector display of all velocities within a given region of interest. A sector within a 2D image is identified, and pixels of color are displayed overlying the area of interrogation. Each color pixel reflects the direction of motion; the shade of color reflects the velocity. By convention, “warm” colors such as red and orange designate direction of flow toward the transducer, and “cold” colors such as blue and white designate flow away from the transducer (Fig. 53.3). When flow velocities exceed the characteristics of the transducer, the smooth, laminar color pattern changes to a speckled color pattern, suggesting a region of more turbulent flow. Whether this represents a pathologic velocity change requires pulsed Doppler interrogation.

Doppler techniques are also applied to motion of the myocardium (i.e., tissue Doppler), yielding information helpful in ascertaining parameters of myocardial function. Although tissue signals move at much lower velocities than blood signals, determination of Doppler-derived tissue velocities can aid in understanding complex states of systolic and diastolic dysfunction. Distinct patterns of normal and abnormal motion of various myocardial segments have been described (Mertens and Friedberg, 2009).

Extremely premature infants are at risk for bronchopulmonary dysplasia (BPD), also referred to as chronic lung disease of pre-

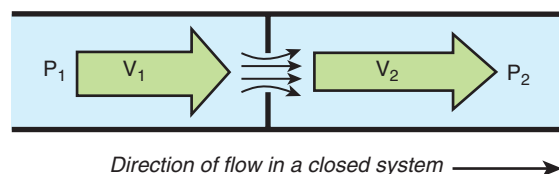
maturity (see Chapter 48). Primarily in those born at less than 26 weeks' gestation, pulmonary vascular and alveolar growth are disrupted, which can result in abnormal gas exchange and vaso-reactivity. The resultant pulmonary vascular remodeling can be accompanied by a persistent elevation of pulmonary vascular resistance (PVR) and consequent pulmonary hypertension, which in turn can lead to right-ventricular dysfunction and failure. Transthoracic echocardiography, and the comparison of serial studies, is a key clinical tool for monitoring pulmonary hypertension and optimizing treatment plans for those with, or at risk for, chronic lung disease. Right-ventricular pressure (and pulmonary-arterial pressure) can be estimated using the tricuspid regurgitation jet velocity as well as qualitative evidence of pulmonary hypertension (flattening of the interventricular septum and right-ventricular hypertrophy). Recently, novel echocardiographic parameters have been introduced, including the myocardial performance index (Tei index), a marker of ventricular dysfunction calculated by the sum of the isovolumetric contraction and relaxation times divided by the ejection time. This index is elevated in primary pulmonary hypertension and may be elevated in those with BPD. Tissue Doppler measurements have been found to correlate with BPD severity, including the ratio of the early Doppler inflow velocity to the early myocardial tissue Doppler velocity (E/E'). Furthermore, cardiovascular abnormalities are found in many of those with BPD, including large aortopulmonary collaterals, pulmonary vein stenosis, and persistent ductal patency. Right-ventricular function is an important determinant of prognosis in pulmonary hypertension. Noninvasive assessment of the right-ventricular function is often limited by complex geometry and poor endocardial definition. The degree of tricuspid annular displacement (tricuspid annular plane systolic excursion [TAPSE]) is a useful echo-derived measure of right-ventricular function with prognostic significance in pulmonary hypertension, which has recently been validated in the neonatal and pediatric population. TAPSE has been found to be a feasible and reproducible marker of right-ventricular systolic function in infants with pulmonary hypertension and correlates with the severity of pulmonary hypertension (Zakaria et al., 2015).

Data derived from Doppler echocardiography are used to provide physiologic information. Using the principles of flow hydraulics across a tubular system with discrete narrowing, velocity data can be translated into pressure data via modification of the Bernoulli equation (Fig. 53.4).

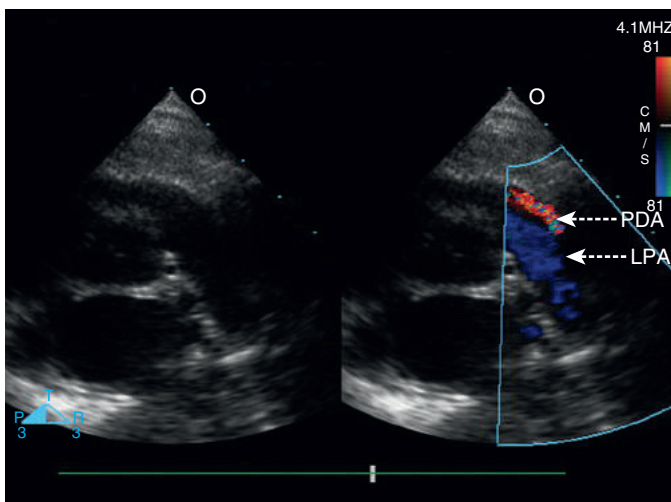
Normal velocities in the heart are rarely greater than 2 m/s and typically less than 1 m/s. These low velocities proximal to an area of narrowing (V_1) can be ignored and the Bernoulli equation simplified to:

$$\text{pressure change} = 4 \times V_2$$

$$\text{Bernoulli Equation} \\ P_1 - P_2 = 4 \times (V_2 - V_1)^2$$



• **Fig. 53.4** The Bernoulli Equation of Flow Dynamics. The Bernoulli equation of flow dynamics describes the relationship between pressure differences and velocity differences across an area of narrowing in a closed fluid system. P_1 , Proximal pressure; P_2 , distal pressure; V_1 , proximal velocity; V_2 , distal velocity.



• **Fig. 53.3** Side-by-Side Two-Dimensional and Color Doppler Echocardiographic Images of a Ductus Arteriosus. Flow in the patent ductus arteriosus (PDA) is red, indicating flow toward the transducer (in this case left-to-right) and is directed toward the main pulmonary artery (MPA) from the aorta. The PDA color signal is speckled, indicating a more turbulent region of flow. High velocity in the PDA is the normal state, indicating appropriately elevated pressures in the aorta as compared to the lower pressures in the pulmonary artery. The flow in the left pulmonary artery (LPA) is blue, indicating flow away from the transducer. The LPA color flow signal is laminar (smooth), indicating a lower velocity of flow. Turbulent flow at this portion of the LPA in a neonate is most commonly associated with physiologic branch pulmonary artery stenosis, a transient phenomenon associated with neonatal transitional physiology.

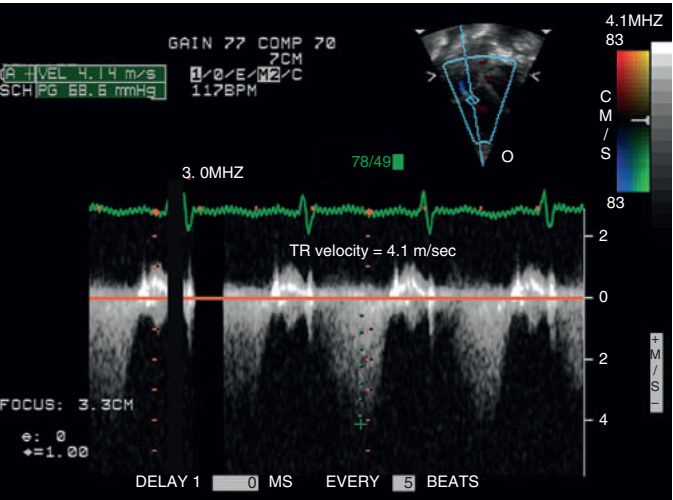
This principle can be applied in a variety of clinical settings. For example, in the presence of tricuspid regurgitation (TR), the peak right ventricle pressure (which will equal the pulmonary artery pressure in the absence of pulmonary outflow obstruction) can be estimated by measuring the peak velocity of the regurgitant jet. A TR peak velocity of 2 m/s suggests a pressure difference between the right atrium and the right ventricle of 16 mmHg ($4 \times [2]^2$). We express this as a right-ventricular pressure of 16 mmHg + right-atrial (or central venous) pressure. In infants and children, right-atrial pressure is estimated to be between 3 and 10 mmHg, with 5 mmHg typically chosen as an estimated guess. Hence, in the preceding example, the patient would have a right ventricle pressure of 21 mmHg, which is within normal limits. Alternatively, an infant with severe chronic lung disease may have a TR peak jet of 4.1 m/s (Fig. 53.5). This would translate to a pressure estimate of 64 mmHg ($4 \times [4.1]^2$); 68 mmHg added to the estimated right-atrial pressure of 5 mmHg gives a right ventricle pressure of 73 mmHg, which suggests pulmonary hypertension. The Bernoulli equation is also commonly used to assess pressure gradients across stenotic valves or vessels: for example, aortic valve stenosis or coarctation of the aorta. Using the maximum velocity, we derive the “peak instantaneous” gradient across the site of obstruction. When interpreting a Doppler-derived velocity, it is essential to recognize that the most precise estimates are obtained when the direction of blood flow is parallel to the ultrasound beam. Small changes in the angle of interrogation can introduce large errors in the calculated velocity; therefore the sonographer angles the ultrasound beam until the highest-pitched audio signal is detected, and assessment is attempted from several different echocardiographic windows in order to obtain the highest velocity. Superimposing

color Doppler imaging with pulsed-wave or continuous-wave Doppler makes it possible to visually align the Doppler beam with the flow jet.

Neonatal Echocardiography: Goals of Imaging

As previously mentioned, echocardiography is the most common imaging modality used in the assessment of the neonatal heart. Echocardiography is widely available and noninvasive. Furthermore, the information obtained is real-time, reproducible, and acquired at the bedside. These properties allow for serial assessment and comparison of data points. Echocardiography is established as the gold standard for the diagnosis of CHD, and obtained images frequently support the clinical assessment in the management of a variety of neonatal conditions. The indications for the performance of an echocardiogram in the nursery or newborn intensive care unit include the assessment of the neonate with a presumed syndromic or genetic condition at high risk for cardiac disease (e.g., trisomy 21), the infant with a noncardiac lesion where there is an increased risk of associated CHD (e.g., omphalocele), and the neonate with a condition that may adversely affect hemodynamics (e.g., arteriovenous malformations). These are summarized in Table 53.1.

Serial echocardiographic evaluations are often necessary in order to care for the neonate. This is particularly the case when changes are occurring in the patient’s clinical condition. Left-atrial and left-ventricular chamber dimensions can be followed as PVR drops in order to determine the left-to-right shunt impact in the face of



• **Fig. 53.5** Example of a Doppler Signal. Time is on the x-axis, and velocity is in meters/second on the y-axis. The Doppler signal is obtained by positioning a marker just proximal to the tricuspid valve annulus in order to sample the tricuspid regurgitant (TR) jet flow. In this example the TR peak velocity measures 4.1 m/sec. Using the modified Bernoulli equation, the predicted right-ventricular pressure is $4 \times (4.1)^2 = 68$ mmHg + right-atrial pressure. This predicts elevated right-ventricular pressure (normal is typically in the range of 20 mmHg). In the absence of obstruction to flow through the pulmonary artery, the right-ventricular pressure is equivalent to pulmonary artery pressure. We can therefore quantify pulmonary artery pressure based on the velocity of the TR jet.

TABLE 53.1 Indications for Echocardiographic Evaluation in the Neonate

| Conditions Associated With Congenital Heart Defects | |
|--|--|
| Syndromes | Nonsyndromes |
| Trisomies 13, 18, 21 22q11 microdeletion syndromes: <ul style="list-style-type: none"> • DiGeorge • Velocardiofacial VACTERL association CHARGE association Holt–Oram syndrome Goldenhar syndrome De Lange syndrome Turner syndrome Noonan syndrome Williams syndrome Infantile Marfan syndrome | Congenital diaphragmatic hernia Gastroschisis Omphalocele Tracheoesophageal fistula |
| Conditions Associated With Impact on Cardiovascular System | |
| Congenital diaphragmatic hernia Chronic lung disease Gestational diabetes mellitus Sacroccygeal teratoma Twin–twin transfusion syndrome Arteriovenous malformation Perinatal asphyxia Severe anemia | |

a PDA or ventricular septal defect (VSD); the notation of left-atrial or left-ventricular dilation in these scenarios suggests the presence of pulmonary overcirculation. The interventricular thickness can be serially measured as can the Doppler pattern and peak velocity across the left-ventricular outflow tract obstruction in the infant of a diabetic mother to assess for baseline hemodynamic impact and improvement over time. The neonatal cardiopulmonary system is a highly fluid system, subject to perturbation from numerous outside influences and thus requires close monitoring in the labile child. Echocardiography is but one tool in the armamentarium of the neonatologist and cardiologist to assist with this monitoring.

Fetal-to-Neonatal Transitional Physiology: Echocardiographic Assessment

During fetal life, the placenta serves as the organ of gas exchange. This fundamental difference between the fetal and neonatal circulations is behind the complex physiologic transition that occurs following birth and clamping of the umbilical cord. With the initiation of respiration and expansion of the lungs, oxygen is brought to the alveoli. This results in a dramatic fall in PVR, with a resultant increase in pulmonary blood flow. Simultaneously, the umbilical cord is clamped, resulting in a sudden increase in systemic vascular resistance (SVR). The combination of this sudden increase in SVR and decrease in PVR leads to a reversal of flow through the ductus arteriosus. In utero, blood flow through the ductus arteriosus was from right-to-left (pulmonary artery to descending aorta) while after birth, the flow direction reverses and now becomes left-to-right (from descending aorta to pulmonary artery). Finally, because of the marked increase in pulmonary blood flow, venous return to the left atrium increases and left-atrial pressure exceeds right-atrial pressure, leading to flap closure of the foramen ovale.

When clinical findings such as tachypnea, tachycardia, or persistent cyanosis suggest an abnormality in transitional physiology, echocardiography is a very useful, noninvasive diagnostic tool. While anything that increases PVR or delays its fall, such as acidosis, hypoxemia, polycythemia, lung disease, or immaturity, may impair the normal neonatal transition, it is the uniqueness of the fetal circulation that allows many complex lesions to be tolerated silently in utero only to unmask themselves during the transitional period.

Suspected Congenital Heart Disease

Alterations from the normal fetal to neonatal transition are most frequently associated with persistent patency of the ductus arteriosus and/or persistently elevated PVR, and the confirmation of the absence of structural heart disease can be extremely helpful to the team caring for an ill neonate, particularly one with a recognized extra-cardiac anomaly. That said, in those in whom CHD is confirmed, high-quality 2D images combined with color and pulsed-wave and continuous-wave Doppler are frequently sufficient for development of a comprehensive management strategy, including the need for prostaglandin therapy and catheter-based or surgical-based interventions (Tworetzky et al., 1999).

Assessment of the Ductus Arteriosus

Before the routine use of echocardiography, a hemodynamically significant PDA was diagnosed clinically by the presence of a murmur, hyperdynamic precordium, bounding and palmar pulses,

increased ventilatory support, and radiographic evidence of cardiomegaly with increased pulmonary vascular markings. In the present era, a complete transthoracic echocardiogram is recommended to not only confirm the presence of a PDA but also to ensure the absence of structural heart defects. Most commonly, treatment is not considered unless there is clinical as well as echocardiographic evidence of hemodynamic significance (Condo et al., 2012). The echocardiographic assessment of a PDA includes an assessment of its size and direction of flow across it. Size is frequently assessed as a ratio of the diameter of the duct to the left pulmonary artery, with a value greater than 1 suggesting a large ductus (Ramos et al., 2010). A large ductus is also suggested by a percentage of retrograde to antegrade flow in the descending aorta of greater than 50% and the presence of left-ventricular enlargement (Sehgal and McNamara, 2009). Additionally, pulsed-wave and continuous-wave Doppler can be used to establish flow velocity and predict the pressure difference between the aorta and pulmonary artery. Finally, the determination of aortic arch sidedness and branching pattern is made should surgical ligation be required (Murdison et al., 1990). The most common finding is that of a left-aortic arch with a left-sided ductus; however, a right-aortic arch with a right-sided ductus can occur and alters the surgical approach to ductal ligation.

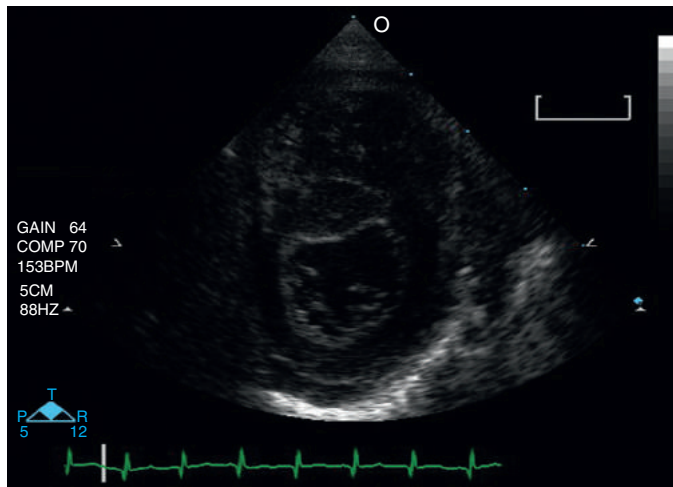
Evaluation of Persistent Pulmonary Hypertension

Persistent pulmonary hypertension of the newborn (PPHN), previously referred to as persistent fetal circulation, results when there is a failure of the normal neonatal transition with persistent severe vasoconstriction of the pulmonary vasculature resulting in the maintenance of elevated pulmonary arterial pressure and right-ventricular pressure. Blood continues to bypass the lungs, shunting across the foramen ovale and through the ductus arteriosus and resulting in systemic desaturation. Risk factors for PPHN include meconium aspiration, neonatal asphyxia, infection, and the presence of a congenital anomaly associated with underdevelopment of the lungs such as a diaphragmatic hernia.

In infants with PPHN, transthoracic echocardiography confirms the absence of structural heart disease (specifically, total anomalous pulmonary venous return with obstruction), demonstrates atrial-level and ductal-level shunting, and provides an estimate of pulmonary arterial pressure by measuring the velocity of the tricuspid regurgitation jet, if present. The Doppler pattern in the ductus arteriosus establishes flow direction as well as velocity. From flow velocity a pressure gradient (or lack thereof) between the aorta and pulmonary artery can be estimated by applying the Bernoulli equation (Musewe et al., 1990). The appearance of the Doppler pattern sampled in the branch pulmonary arteries may also provide information about the PVR and response to therapy.

In addition, echocardiography may be used to assess ventricular function in an infant with PPHN (Fig. 53.6). Neonates with PPHN typically have right-ventricular dysfunction (Valdes-Cruz et al., 1981). Although the etiology of cardiac dysfunction in PPHN is unclear, prenatal constriction of the ductus arteriosus has been suggested as one possible mechanism (Morin, 1989).

Extracorporeal membrane oxygenation (ECMO) is frequently used in infants with severe PPHN whose hypoxemia does not respond to mechanical ventilatory support and pulmonary vasodilators such as supplemental oxygen and nitric oxide. Echocardiography can be used to document improvement and identify the etiology of poor circuit flow.

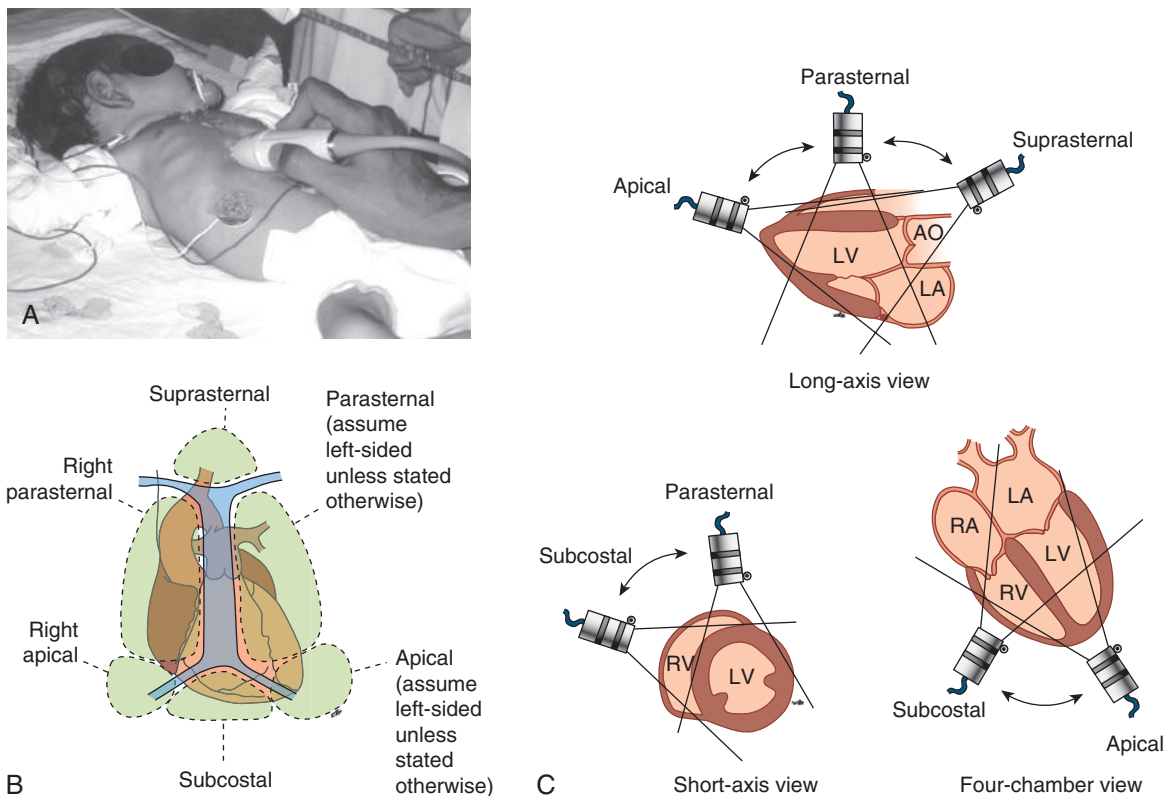


• **Fig. 53.6** Short Axis Imaging of the Ventricles. Normally the left-ventricular pressure exceeds the right, resulting in bowing of the ventricular septum into the right-ventricular cavity. In persistent pulmonary hypertension of the newborn, the right-ventricular pressure (RVp) may be equivalent or even exceed the left-ventricular pressure (LVP), altering the ventricular septal position, as seen in this image where the septum bows abnormally into the left ventricle. This septal position suggests the RVp exceeds the LVP.

Neonatal Imaging Techniques

A systematic and standardized approach is key to performing a comprehensive neonatal echocardiogram. Follow-up studies may be curtailed and limited in scope; however, an initial evaluation should include a detailed assessment of intracardiac and great vessel anatomy using 2D, Doppler, color Doppler, and M-mode imaging. Both still frame and cine clips are obtained. Short cine clips capture both structural motion through the cardiac cycle as well as tomographic cuts of the heart as the transducer is fanned through it. These acquired 2D imaging “sweeps” allow for the “mental reconstruction” of the three-dimensional heart and any complex perturbations from normal. Standard imaging locations are used to create a complete picture of the heart and great vessels (Fig. 53.7). These include images obtained mid-chest (parasternal imaging plane), four-chamber views (apical imaging), and abdominal views through the liver and diaphragm (subcostal imaging) and between the clavicles (suprasternal notch views). Subcostal imaging is unique to neonates and pediatric patients. The liver acts to enhance acoustic transmission, and the proximity of the transducer to the heart optimizes image resolution.

Although echocardiography is a noninvasive diagnostic test, monitoring should be performed during the performance of an echocardiogram, particularly in small, premature infants, as a



• **Fig. 53.7** (A) Demonstration of the position of the ultrasound transducer using the subcostal approach in a neonate. (B) By moving the transducer to multiple windows surrounding the heart, echocardiographic two-dimensional tomographic imaging provides for a three-dimensional look at the heart via “mental reconstruction” once each of the corresponding windows has been examined. (C) Long-axis, short-axis, and four-chamber views of the heart contribute to visualization of the overall detail of complex cardiac anatomy. AO, Aorta; LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle. (B and C, Courtesy American Society of Echocardiography.)

complete study routinely takes 30–60 minutes and if CHD is identified can last significantly longer. Application of cold ultrasound gel and environmental exposure may cause temperature instability. Applying pressure on the abdomen during subcostal imaging or extending the neck during suprasternal imaging may cause destabilization of the patient because of either cardiac or pulmonary compromise. Even in neonates, occasionally sedation is required to obtain a complete study.

Limitations of Neonatal Echocardiography

Although echocardiography is an extremely useful tool that has revolutionized the diagnostic evaluation of the neonatal cardiovascular system, it has a number of limitations:

- **Discrepancy between “echo-derived” and “catheter-derived” gradients.** Doppler echocardiography measures the peak instantaneous pressure difference at any point in time during flow across a stenotic valve. In the case of a stenotic aortic valve, this may occur during the upstroke of systole, not at the peak of systole. Catheter-based measures of pressure are taken at the peak of systole; hence, echo-derived pressure gradients do not equal, and usually exceed, catheter-derived measures. This discrepancy does not invalidate the measures of either modality but rather highlights the point that they are measures of gradients occurring during two different points in systole. Historically, clinical decision making has been based on catheter-derived information; therefore echocardiographically derived data should be interpreted in this light. Other factors that may have an impact on differences in echocardiographic versus catheter-based gradients are level of sedation, hydration status, medications (inotropes), and ventilatory strategy.
- **Inadequate visualization of structures.** Lung disease can cause acoustic impedance, limiting the ability to visualize structures. Air within the thorax caused by air trapping, pneumothorax, or pneumopericardium can result in a dramatic deterioration in image clarity.
- **Inadequate Doppler signal for velocity measurement.** Small blood volumes traveling at low velocity may be difficult to identify with echocardiography. Examples are coronary arteries in the very small, premature infant and pulmonary vein flow in patients with severe lung disease in whom pulmonary blood flow may be limited. Identification of pulmonary venous anatomy and flow in infants treated with ECMO also may be difficult as a result of reduced pulmonary circulation. Transesophageal echocardiography (TEE) may be indicated in these patients because of the enhanced imaging resolution of this modality.

Fetal Echocardiography: Goals of Imaging

CHD, which refers mainly to anatomic malformations of the heart, constitutes one of the most common congenital anomalies. The reported incidence rate per 100 live-born infants is nearly 1 by the end of the first year of life (Bjornard et al., 2013). An interest in the prenatal detection of major congenital malformations, including CHD, has blossomed over the past two to three decades as technologic advances have improved image quality such that a complete anatomic assessment, including the screening views of the fetal heart, can be obtained in nearly all women by 18 weeks' gestation and in many late in the first trimester. Furthermore, prenatal detection rates have improved as obstetric societies have adopted guidelines for the comprehensive anatomic assessment of the mid-trimester fetus, including the fetal heart. The

TABLE 53.2

Indications for Fetal Echocardiography

| Maternal Indications | Fetal Indications |
|--|---|
| Family history of CHD, including prior child or pregnancy with CHD | Abnormal obstetric screening ultrasound |
| Metabolic disorders (e.g., diabetes) | Extra-cardiac abnormality |
| Exposure to teratogens | Chromosomal abnormality |
| Exposure to prostaglandin synthetase inhibitors (ibuprofen) | Arrhythmia |
| Infection (rubella, Coxsackie, parvovirus B19) | Hydrops |
| Autoimmune diagnosis (e.g., Sjögren syndrome, SLE) | Increased first-trimester nuchal translucency |
| Familial inherited disorder (Marfan, Noonan syndromes) | Multiple gestation and suspected twin-twin transfusion syndrome |
| In vitro fertilization | |

CHD, Congenital heart disease; SLE, systemic lupus erythematosus.

four-chambered view of the heart was included in the guidelines released in 2004 by the American Institute of Ultrasound in Medicine in conjunction with the American College of Radiology, American College of Obstetricians and Gynecologists, and the Society of Radiologists in Ultrasound; however, it was not until 2013 that the outflow tracts were identified as essential components (American Institute of Ultrasound in Medicine Practice Guideline, 2013).

Similar to neonatal echocardiography, fetal echocardiography is the mainstay of imaging the fetal cardiovascular system. Fetal echocardiography refers to a comprehensive cardiovascular evaluation in excess of the limited screening views obtained as part of the mid-trimester anatomic scan. A fetal echocardiogram is recommended in those with identified maternal or fetal risk factors (Table 53.2). However, the majority of CHD is identified in fetuses with no known risk factors. Despite this tiered approach, CHD continues to be one of the most frequently missed malformations, with reported rates consistently less than predicted.

The ultimate goal of fetal echocardiography is the accurate definition of any anatomic abnormality of the heart during the prenatal time period. Once a diagnosis is established, the secondary goal of fetal echocardiography, parental counseling, can begin. Complete parental counseling includes not only a discussion of the abnormality identified but also the increased risk for extra-cardiac anomalies and chromosomal abnormalities (if applicable), management strategy, and the long-term outcomes of those with an identified lesion. Prenatal management plans need to be discussed, including local laws regarding termination of a pregnancy. Finally, perinatal management plans can be created to smooth the transition to the postnatal time period. In the fetus identified with ductal-dependent CHD, prenatal detection has been shown to improve outcome by reducing postnatal acidosis, morbidity, and mortality (Tworetzky et al., 2001; Verheijen et al., 2001).

Fetal Imaging Techniques

Cardiac formation is complete by 8 weeks after conception, and ventricular contraction can be detected by ultrasound imaging at that time. The optimal timing for performance of a comprehensive transabdominal fetal echocardiogram is 18–20 weeks'

gestation; however, as resolution has improved, earlier diagnosis and reassurance may be possible late in the first trimester (Smrcek et al., 2006).

Similar to a pediatric transthoracic echocardiogram, a fetal echocardiogram involves assessing cardiac anatomy in a series of standard imaging planes. The components of a comprehensive evaluation are listed in Table 53.3, although not all may be visualized in every fetus at every examination. In addition, some lesions can evolve in utero. Initial imaging establishes fetal position in utero (vertex, breech, or transverse). Once fetal left and right are established, the fetal abdominal situs is confirmed as well as the position of the heart within the thorax. Although uncommon as a whole, CHD and abnormalities of laterality (heterotaxy syndrome) are commonly found together.

Further imaging follows in a less predictable and frequently nonsequential order, accepting the inability to position the fetus in a standard position as well as frequent movement throughout the examination. The cardiovascular system is evaluated, beginning below the diaphragm in the abdomen and ending at the thoracic

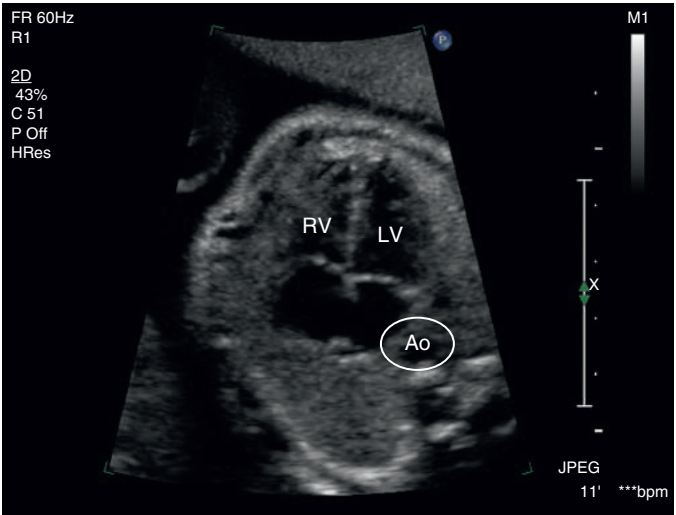
inlet as a series of imaging planes. The four-chamber view (Fig. 53.8) is the most important in a comprehensive examination of the fetal heart. In this view, cardiac position, size, rate and rhythm, and qualitative contractility can all be assessed. Additionally many congenital heart defects can be detected (Fig. 53.9). After a four-chamber view is obtained, attention turns to visualization of the left- and right-ventricular outflow tracts and an assessment of ventriculoarterial concordance. Establishing the origin of the main pulmonary artery from the right ventricle and the aorta from the left ventricle is mandatory. Finally, systemic and pulmonary venous return are evaluated.

Structures are evaluated in orthogonal imaging planes in order to form a composite picture of the heart, including any identified malformation. In addition to obtaining clear pictures of each structure, flow is evaluated with color Doppler and pulsed Doppler.

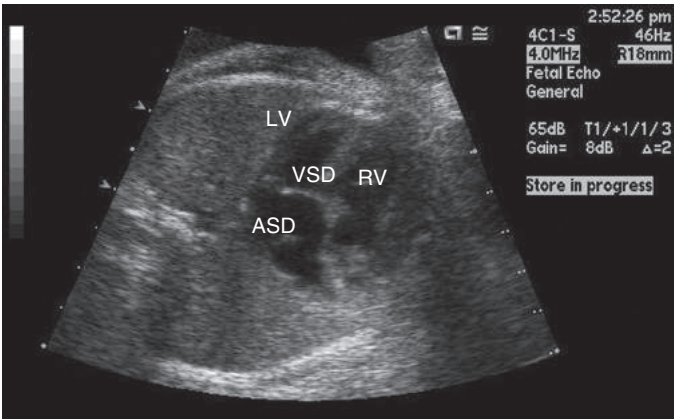
TABLE 53.3 Components of the Fetal Echocardiogram

| | |
|--------------------------------|---|
| Overview | Fetal number and position Stomach position and abdominal situs Cardiac position |
| Biometric examination | Cardiothoracic ratio Biparietal diameter and head circumference Femur length Abdominal circumference |
| Cardiac imaging | Four-color view LVOT RVOT Great arteries Three-vessel view Bicaval view Ductal arch Aortic arch |
| Doppler examination | Inferior and superior vena cava Pulmonary veins Ductus venosus Foramen ovale Atrioventricular valves Semilunar valves Ductus arteriosus Transverse aortic arch Umbilical artery Umbilical vein |
| Measurement data | Atrioventricular valve diameter Semilunar valve diameter Main pulmonary artery Ascending aorta Branch pulmonary arteries Transverse aortic arch Ventricular length Ventricular short-axis dimensions |
| Examination of rate and rhythm | M-mode study of atrial and ventricular wall motion Doppler examination of atrial and ventricular flow patterns |

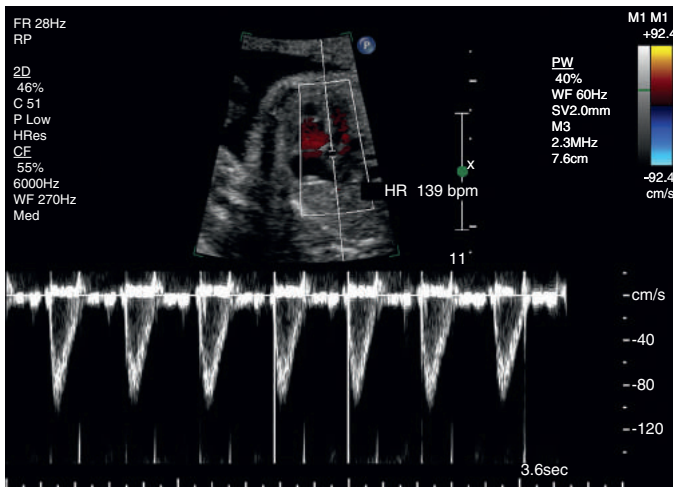
LVOT, Left-ventricular outflow tract; RVOT, right-ventricular outflow tract.



• **Fig. 53.8** Four-Chamber View of a Normal Fetal Heart. All four chambers are visible, with relative symmetry in size between the ventricles and atria. The right ventricle is identified by the presence of the moderator band and increased myocardial trabeculations in comparison with the left ventricle. The descending aorta is seen directly behind the left atrium.



• **Fig. 53.9** Four-Chamber View of the Fetal Heart With Complex Congenital Heart Disease. There is a large ventricular septal defect as well as a large primum atrial septal defect. There is size discrepancy between the two ventricular chambers. This fetus was diagnosed with an unbalanced complete atrioventricular canal defect.



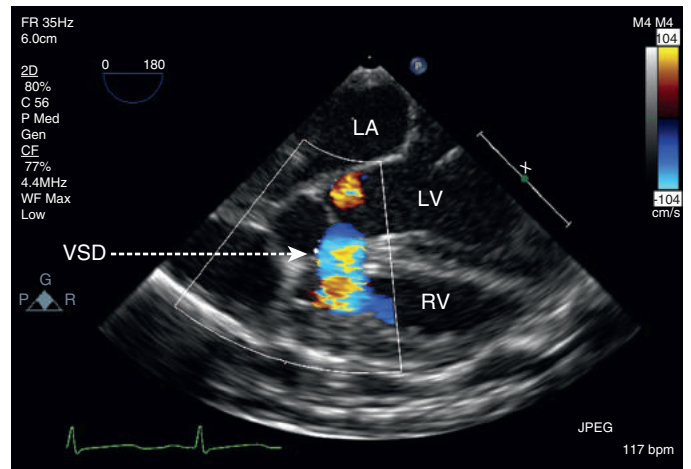
• **Fig. 53.10** The Fetal Heart Rate Is Documented Using Aortic Valve Doppler. The time between successive beats is measured and a heart rate per minute calculated.

Valve regurgitation and/or stenosis can be detected and valve leaflet motion observed. From 2D images, structures can be measured and compared with established norms for varying gestation ages. Once a comprehensive assessment of the cardiac anatomy is complete, the heart rate is documented (Fig. 53.10). The rate and rhythm of the fetal heart are evaluated by mechanical surrogate events, specifically the movement of atria and ventricles or blood flow across valves.

Limitations of Fetal Echocardiography

Many of the same limitations associated with pediatric echocardiography apply to fetal echocardiographic imaging, including inadequate Doppler assessment for velocity measurement and limited visualization of cardiac structures. In addition, there are a number of limitations unique to fetal echocardiography, including:

- *In utero physiology.* Fetal circulation precludes exclusion of persistency of the ductus arteriosus postnatally. In addition, it is difficult or impossible to exclude a secundum atrial septal defect (ASD) because of the fetal requirement for a patent foramen ovale. Preferential right-to-left flow across the PDA can make assessment for coarctation of the aorta very challenging unless there is severe arch hypoplasia.
- *Imaging limitations.* While there are some advantages to fetal imaging in that an unlimited number of planes can be utilized, potential disadvantages include evolving calcification of bony structures, early gestational age when cardiac structures are at the lower limits of resolution, and noncardiac anomalies that may complicate understanding of fetal cardiac anatomy and/or physiology (examples include diaphragmatic hernia and congenital cystic adenomatoid malformation or congenital pulmonary airway malformation).
- *Maternal body habitus.* Obesity prevalence in the United States is epidemic. This has resulted in an ever-increasing population of overweight pregnant women. Obesity complicates fetal cardiac imaging in that increasing distance and mass between the fetal heart and echo transducer reduces imaging resolution.
- *Evolution with time.* Prediction of the degree of postnatal cyanosis caused by pulmonary outflow obstruction (e.g., tetralogy of



• **Fig. 53.11** Color Doppler Image Obtained During Transesophageal Imaging in the Operating Room Preceding Surgical Closure of a Moderate-Sized Membranous Ventricular Septal Defect. The color signal is blue, indicating flow away from the transducer, in this case left-to-right. LA, Left atrium; LV, left ventricle; RV, right ventricle; VSD, ventricular septal defect.

Fallot) can be challenging given the normal elevation in downstream pulmonary artery pressure in the fetus. In addition, restriction at the atrial septum (e.g., hypoplastic left heart syndrome and dextro-transposition of the great arteries) and obstruction in the setting of total anomalous pulmonary venous return can prove difficult to predict. Fetal echo specialists have developed tools and techniques (e.g., maternal hyperoxygenation testing) that have resulted in improvement in our ability to predict early postnatal morbidity.

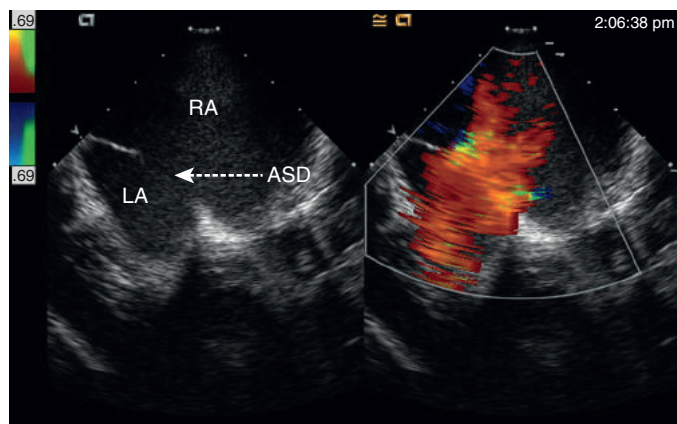
Other Cardiovascular Imaging Modalities

Transesophageal Echocardiography

Technologic advancements in miniaturization led to the development of tiny transducer housings with small footprints such that a transthoracic echocardiogram can be performed in even the most premature of infants. Similar technology allowed for the development of neonatal TEE, in which a transducer can be introduced into the esophagus, and technology is available that expands the size range for TEE such that premature infants weighing just over 1 kg can be safely imaged. TEE is helpful in elucidating structures in the posterior mediastinum, such as pulmonary veins, and in overcoming acoustic impediments at the surface such as air and bone (Randolph et al., 2002). Most importantly, TEE has allowed for imaging to take place during interventional procedures in the operating room or catheterization laboratory (Fig. 53.11). This eliminates the need to place a transducer directly on the chest or abdomen, thereby avoiding potential contamination of the operative field. Interventional procedures can be guided by TEE imaging, yielding immediate feedback to the proceduralist that has been well validated as a means of improving overall outcomes.

Intracardiac Echocardiography

Recently, ultrasound transducer technology has been incorporated into the tips of catheters, allowing for intracardiac imaging (Hijazi

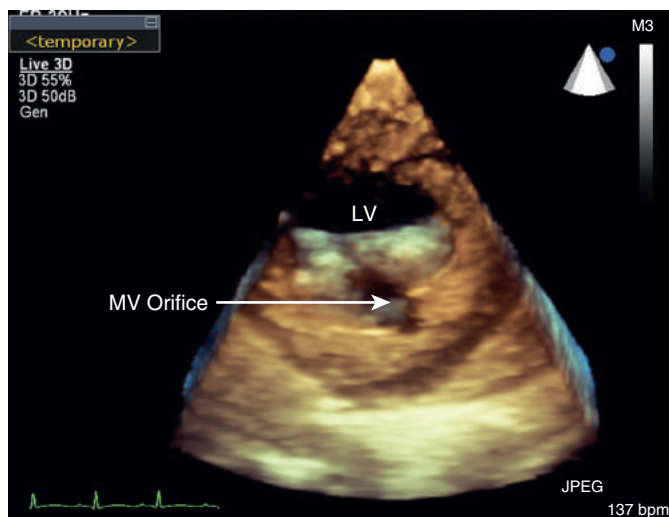


• **Fig. 53.12** Side-by-Side Two-Dimensional and Color Doppler Imaging of a Large Secundum Atrial Septal Defect Obtained by Intracardiac Echocardiographic Imaging. The color signal is red and laminar, indicating low-velocity, left-to-right flow through the atrial septal defect. The right atrium is dilated in comparison with the left atrium because of the volume load on the right heart caused by this shunt lesion. ASD, Atrial septal defect; LA, left atrium; RA, right atrium.

et al., 2009). This is primarily of use in the cardiac catheterization laboratory during the percutaneous device closure of ASDs (Fig. 53.12). Although these catheters provide an additional imaging modality, the size of these catheters (8 French) precludes their use in the femoral veins of younger children and neonates.

Three-Dimensional Echocardiography

Integration of multiplanar image data into a real-time three-dimensional (3D) display has been a goal of echocardiography. The earliest methods required acquisition of data that then required offline 3D image rendering. Over the past several years systems have been made available that are capable of displaying “live” 3D echocardiographic images (Fig. 53.13). This has resulted in our ability to interpret data as they are being obtained, making possible real-time bedside feedback. 3D echocardiography allows for enhanced identification of spatial relationships and structural malformations (Marx and Su, 2007). 3D echocardiographic technology has been miniaturized such that it is now available in an adult-sized TEE transducer. This allows for the benefits of TEE



• **Fig. 53.13** Three-Dimensional Echocardiographic Image of a Pathologic Mitral Valve. Using this imaging technique, a view of the mitral valve position within the left ventricular cavity is obtained that allows for a more thorough understanding of the nature of the anomaly. In this case, the mitral valve is seen in a cross-sectional, short-axis view peering into the left-ventricular cavity. The mitral orifice is hypoplastic, resulting in obstruction to inflow. LV, Left ventricle; MV, mitral valve.

(improved image resolution and ease of image acquisition during procedures) to be combined with the enhanced data available from 3D imaging. In the foreseeable future, 3D TEE should be available in small transducers amenable to imaging the newborn and small child. 3D echocardiography will continue to evolve and promises to become an important modality as technologic advances continue.

Computed Tomography and Magnetic Resonance Imaging

While echocardiography remains the gold standard for the diagnosis of CHD, magnetic resonance imaging and computed tomography are increasingly serving as adjunctive imaging modalities, especially in cases where there are concerns regarding extra-cardiac vasculature (e.g., pulmonary artery branch pathology and aortic arch anomalies) (Sigal-Cinqualbre et al., 2011).

Summary

The echocardiographic assessment has become inseparable from effective management of the critically ill neonate. The ability to provide noninvasive real-time, bedside cardiac evaluation not only aids in the identification of congenital cardiac lesions but also, just as importantly, facilitates a thorough understanding of the current physiologic state. Pharmacologic, ventilatory, and surgical decision making are by necessity based on clarity surrounding how

these management strategies affect, and are affected by, cardiac status. In addition, the understanding of prenatal cardiac issues (e.g., anatomic anomalies, rhythm disturbances, myocardial function) can only be performed via fetal echocardiographic imaging. Neonatal outcomes are therefore dependent on both accurate fetal and postnatal echocardiographic diagnosis and the application of these data to overall multidisciplinary newborn care.

Suggested Readings

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Patent Ductus Arteriosus in the Preterm Infant

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KEY POINTS

- Clinical signs of a symptomatic patent ductus arteriosus (PDA) usually appear later than echocardiographic signs and are related to the degree of left-to-right ductal shunting, with associated blood flow changes to the lungs, brain, kidneys, and intestines.
- Factors known to play a prominent role in regulation of ductal patency involve those that promote constriction (oxygen, endothelin, calcium channels, catecholamines, and Rho kinase) and those that oppose it (intraluminal pressure, prostaglandins, nitric oxide, carbon monoxide, potassium channels, and cyclic adenosine monophosphate and guanosine monophosphate).
- Closure of the ductus arteriosus occurs in two phases: (1) “functional” closure of the lumen by smooth muscle constriction, within hours after birth; (2) “anatomic” occlusion of the lumen over the next several days, due to neointimal thickening and loss of smooth muscle cells from the inner muscle media.
- The most important mechanism that prevents the preterm ductus from constricting after birth is its increased sensitivity to the vasodilating effects of prostaglandin E_2 . As a result, inhibitors of prostaglandin production (e.g., indomethacin, ibuprofen, acetaminophen) are usually effective in promoting ductus closure in preterm infants.
- Early surgical ligation, while eliminating the detrimental effects of a PDA on lung development, may create its own set of problems for the preterm infant that counteract those benefits (e.g., postligation hypotension, bronchopulmonary dysplasia, vocal cord paralysis, neurodevelopmental abnormalities).
- While there may be general consensus on the efficacy of indomethacin for treatment of a PDA, questions about proper dosage, treatment duration, optimal timing, and treatment criteria remain controversial.
- Although a moderate-to-large PDA increases pulmonary blood flow and edema and decreases systemic blood pressure, it is not clear which is preferable: (1) to close the PDA (surgically or pharmacologically) or (2) to deal with the pulmonary edema and hypotension through other means, while awaiting spontaneous ductal closure.
- Further investigation is needed to determine which preterm infants are most likely to benefit from ductal closure and which might be best left untreated.

The ductus arteriosus represents a persistence of the terminal portion of the sixth branchial arch. During fetal life, the ductus arteriosus serves to divert blood away from the fluid-filled lungs toward the descending aorta and placenta. After

birth, constriction of the ductus arteriosus and obliteration of its lumen separate the pulmonary and systemic circulations. In full-term infants, obliteration of the ductus arteriosus takes place through a process of vasoconstriction and anatomic remodeling. In the preterm, the ductus arteriosus frequently fails to close. The clinical consequences of a patent ductus arteriosus (PDA) are related to the degree of left-to-right PDA shunt with its associated change in blood flow to the lungs, kidneys, and intestine.

Diagnosis

Phase contrast magnetic resonance imaging appears to offer the most accurate measurements of ductal shunt volume, and the effects of a PDA on left ventricular and systemic blood flow volumes (Broadhouse et al., 2014). Unfortunately these measurements are difficult to obtain in extremely immature, sick preterm infants. As a result, two-dimensional echocardiography and color Doppler flow mapping have been used as the gold standards for assessing the magnitude and direction of PDA shunting. Ductus diameter ≥ 1.5 mm (or $>50\%$ of the diameter of the left pulmonary artery), left atrial-to-aortic root (LA/Ao) ratio ≥ 1.5 , reversal of forward blood flow in the descending aorta during diastole, and end diastolic flow velocity in the left pulmonary artery ≥ 0.20 m/s are signs consistent with a moderate-to-large PDA shunt (Table 54.1) (El Hajjar et al., 2005). Unfortunately, the inter-observer repeatability of all echocardiographic parameters is relatively poor (Schwarz et al., 2016).

Clinical signs of a PDA (systolic murmur, hyperdynamic precordial impulse, full pulses, widened pulse pressure and/or worsening respiratory status) usually appear later than echocardiographic signs and are less sensitive in determining the degree of left-to-right shunt. Certain signs such as continuous murmur or hyperactive left ventricular impulse are relatively specific for a PDA but lack sensitivity; conversely, worsening respiratory status, although a sensitive indicator, is relatively nonspecific for a PDA. Tachycardia is not a useful or reliable indicator of a PDA in preterm infants. Infants with large left-to-right shunts may have evidence of cardiomegaly and increased pulmonary arterial markings on their chest X-rays; however, in general, the chest X-ray and electrocardiogram are not useful in diagnosing a PDA. Although elevated plasma concentrations of Brain Natriuretic Peptide (BNP) and N-terminal pro-BNP (NTpBNP) have been found to correlate with the presence of a moderate sized left-to-right PDA shunt,

TABLE 54.1 Rates of Spontaneous Ductus Arteriosus Closure Among Neonates Who Survive to 72 Hours

| Gestation | Closed on Day 4 % | Closed on Day 7 % | Closed at Discharge % |
|-------------|-------------------|-------------------|-----------------------|
| Full-Term | 100 | 100 | 100 |
| ≥30 Weeks | 90 | 98 | 98 |
| 28–29 Weeks | 45 | 67 | 94 |
| 26–27 Weeks | 16 | 32 | 78 |
| 24–25 Weeks | 4 | 13 | 54 |

changes in BNP and N-terminal pro-BNP (NTpBNP) concentrations have poor sensitivity and specificity in predicting increases or decreases in PDA shunt magnitude and cannot be used to replace echocardiography in the management of PDA shunts (Chen et al., 2010). Although there has been little consensus in the past about what constitutes a clinically important PDA, recent studies have shown that although moderate and large PDA shunts are associated with significant neonatal morbidities, small PDA shunts have similar outcomes as no PDA shunts (Sellmer et al., 2013; Schena et al., 2015). Whereas the magnitude of PDA shunt plays a significant role in creating neonatal morbidity, equally important factors are the duration of exposure to the shunt and the infant's ability to compensate for the shunt.

Incidence

Functional closure of the ductus occurs in almost 50% of full-term infants by 24 hours, in 90% by 48 hours, and in nearly 100% by 72 hours after birth (Reller et al., 1993). The rate of ductus closure is delayed in preterm infants; however, essentially all preterm infants who are of 30 weeks' gestation or more (including those with respiratory distress syndrome) will close their ductus by the fourth day after birth. Infants born at less than 30 weeks' gestation have a 65% incidence of persistent ductus patency beyond day 4. Even among the most immature infants (≤27 weeks' gestation), spontaneous closure can occur during the neonatal period. However, when it does occur, it usually occurs late during the neonatal intensive care unit (NICU) hospitalization (average age is 61 ± 37 days) (Reller et al., 1993; Koch et al., 2006; Nemerofsky et al., 2008; Herrman et al., 2009; Rolland et al., 2015). Among preterm infants discharged from the hospital with a persistent PDA, 86% will achieve PDA closure by 1 year of age. The remainder will require continued observation or coil closure (Herrman et al., 2009) (Table 54.1).

Factors like surfactant administration, infection, being small for gestational age, and excessive fluid administration increase the likelihood of developing a symptomatic PDA (Cotton et al., 1981; Alpan et al., 1995; Gonzalez et al., 1996; Bell et al., 2001; del Moral et al., 2007; Rakza et al., 2007). However, non-white infants and infants who receive prenatal glucocorticoids have a reduced risk of PDA (Clyman et al., 1981; Furzan et al., 1985; Cotton et al., 1991; Chorne et al., 2007; Durrmeyer et al., 2010; Waleh et al., 2015).

Regulation of Ductus Arteriosus Patency

In the full-term infant, closure of the ductus arteriosus occurs in two phases: (1) "functional" closure of the lumen within the first

hours after birth by smooth muscle constriction and (2) "anatomic" occlusion of the lumen over the next several days because of extensive neointimal thickening and loss of smooth muscle cells from the inner muscle media.

Balance Between Vasoconstriction and Vasorelaxation

Ductus arteriosus patency is determined by the balance between dilating and constricting forces. The factors known to play a prominent role in ductus arteriosus regulation involve those that promote constriction (oxygen, endothelin, calcium channels, catecholamines, and Rho kinase) and those that oppose it (intraluminal pressure, prostaglandins [PGs], nitric oxide [NO], carbon monoxide, potassium channels, and cyclic adenosine monophosphate [cAMP] and cyclic guanosine monophosphate [cGMP]). The relative importance of each of these factors depends on the intrauterine and extrauterine environment, the degree of ductus maturation, and the genetic background and species being studied.

In Utero Regulation

The fetal ductus normally has a high level of intrinsic tone (Kajino et al., 2001). The intrinsic tone is due to mechanisms that both depend on and are independent of extracellular calcium (Kajino et al., 2001). The contractile proteins (smooth muscle myosin, calponin, and caldesmon) are more differentiated in the ductus than they are in the adjacent aorta and pulmonary artery (Sakurai et al., 1996). In addition, the fetal ductus arteriosus is more sensitive to the contractile effects of calcium than are the aorta and pulmonary artery (Crichton et al., 1997). This may be due in part to increased Rho kinase activity in the ductus (Clyman et al., 2007). Endothelin-1 also appears to play a role in producing the elevated basal tone of the fetal ductus arteriosus (Cocceani et al., 1999).

The relative importance of the factors that oppose ductus arteriosus constriction in utero is more clearly defined than the factors that promote constriction. The elevated vascular pressure within the ductus lumen (due to the constricted pulmonary vascular bed) plays an important role in opposing ductus constriction (Clyman et al., 1989). The fetal ductus also produces several vasodilators that maintain ductus patency. Vasodilator PGs appear to be the dominant vasodilators that oppose ductus constriction in the later part of gestation. Inhibitors of PG synthesis constrict the fetal ductus both in vitro and in vivo. Prostaglandin E₂ (PGE₂) is the most potent PG produced by the ductus (Clyman et al., 1978; Cocceani et al., 1978). Ductus smooth muscle is extraordinarily sensitive to the vasodilating effects of PGE₂. PGE₂ produces ductus relaxation by interacting with PGE receptors EP₂, EP₃, and EP₄. In the ductus, all three of the EP receptors participate in vasodilation by activating adenylate cyclase (Bouayad et al., 2001). The increased cAMP concentrations inhibit the sensitivity of the contractile proteins to calcium. Inhibitors of phosphodiesterase (the enzyme that degrades cAMP) relax the ductus in utero (Toyoshima et al., 2006). Low phosphodiesterase levels in the fetal ductus account for its increased sensitivity to PGE₂ (Liu et al., 2008).

Both isoforms of the enzyme responsible for synthesizing PGs (cyclooxygenase [COX]-1 and COX-2) are expressed in the fetal ductus. In the fetal mouse, COX-2 appears to be the COX isoform responsible for producing the PGs that regulate the ductus (Loftin et al., 2001) whereas in the fetal sheep both COX-1 and COX-2 play a role in ductus patency (Takahashi et al., 2000). In addition to the PGs that are made within the ductus, the fetal ductus is

also under the influence of circulating concentrations of PGE₂. Circulating concentrations of PGE₂ appear to be of placental origin and are high in the fetus because of the reduced pulmonary clearance of circulating PGs resulting from the low fetal pulmonary blood flow (Clyman et al., 1981).

NO, formed mainly by endothelial nitric oxide synthase (eNOS), is made by the fetal ductus arteriosus and appears to play an important role in maintaining ductus patency in rodent fetuses early in gestation (Momma et al., 1999). PGE₂ and NO appear to be preferentially coupled for reciprocal compensation since cyclooxygenase inhibition upregulates NO (Sodini et al., 2008). Although NO is also made in the ductus of larger species, its importance in maintaining ductus patency under normal in utero conditions has not been conclusively demonstrated (see later in the chapter for the role of NO in fetuses exposed to indomethacin tocolysis and in premature newborns).

Carbon monoxide relaxes the ductus arteriosus. Under physiologic conditions the amount of carbon monoxide made by the ductus does not seem to affect ductus tone; however, in circumstances where its synthesis is upregulated, e.g., endotoxemia, it may exert a relaxing influence on the ductus (Cocceani et al., 1997). Hydrogen sulfide has also been identified as another endogenous factor that inhibits fetal ductus tone (Baragatti et al., 2013).

Although short term, pharmacologic inhibition of PG synthesis and signaling produces ductus constriction in utero (Momma et al., 2005); chronic inhibition of PG synthesis and signaling produces the opposite effect: a persistent patent ductus in utero and a persistent patent ductus after birth (Nguyen et al., 1997; Loftin et al., 2001). It is now clear that, in addition to its role in maintaining fetal ductus patency, PGE₂ is essential for the induction of pathways necessary for postnatal closure. Blocking PGE₂ activity by disrupting the PG EP₄ receptor decreases the expression of several genes that control postnatal oxygen-induced ductus constriction (see “in utero regulation”). Specifically, PGE₂ increases the expression of calcium L (CaL)- and potassium (K⁺)-channel genes (*CaLalpha1c*, *CaLbeta2*, *Kir6.1*, and *Kv1.5*), which regulate calcium entry, without affecting genes that regulate calcium sensitization (Rho kinase-associated genes). Chronic inhibition of PG synthesis decreases the expression of CaL- and K⁺-channel genes. Phosphodiesterase expression, which decreases the ductus’ sensitivity to cAMP- or cGMP-dependent vasodilators, is also decreased by chronic COX inhibition (Reese et al., 2009). Inhibition of PG signaling may also contribute to delayed closure by inhibiting hyaluronic acid production and intimal cushion formation in the ductus (Yokoyama et al., 2006). Intimal cushions play an important role in permanent ductus closure after birth (see “Anatomic Closure-Histologic Changes”). PGE₂, acting through its EP₄ receptor, has been shown to affect intimal thickening, elastogenesis, and contraction-related genes through pathways that involve cAMP, cAMP-protein kinase A, exchange protein activated by cAMP, phospholipase C, and Wnt/β-catenin (Yokoyama, 2015).

It is interesting to note that pharmacologic inhibition of PG synthesis in human pregnancy is also associated with an increased incidence of PDA after birth (Norton et al., 1993). However, this appears to be due to the ability of indomethacin to produce ductus constriction in utero. In utero constriction produces ischemic hypoxia, increased NO production, and smooth muscle cell death within the ductus wall (see “Relationship Between Vasoconstriction and Anatomic Closure”). These factors prevent the ductus from constricting after birth and make it resistant to the constrictive effects of postnatal indomethacin (Clyman et al., 2001; Goldbarg et al., 2002) (Box 54.1).

• BOX 54.1 Pathways Affected by In Utero Chronic Inhibition of Prostaglandin Production and Signaling That Contribute to Persistent Ductus Arteriosus Patency After Birth

Pathways Involved With Smooth Muscle Contraction

- Decreased expression of CaL-channel and K⁺-channel genes (*CaLalpha1c*, *CaLbeta2*, *Kir6.1*, and *Kv1.5*) that facilitate calcium entry into the cytoplasm during oxygen-induced constriction
- Increased nitric oxide production
- Decreased phosphodiesterase expression. Phosphodiesterases decrease the ductus’ sensitivity to cAMP or cGMP-dependent vasodilators
- Decreased expression of myocardin, an important transcriptional coactivator that regulates production of smooth muscle cell contractile proteins
- Decreased Wnt/β-catenin activation of myocardin

Pathways Involved With Neointimal Mound Formation

- Decreased hyaluronic acid production, which is important in neointimal mound formation
- Decreased hyaluronic acid dependent and Epac (exchange protein activated by cAMP) dependent smooth muscle migration into the neointimal mounds
- Decreased elastic fiber fragmentation and increased elastic fiber formation by ductus smooth muscle cells

Additional Pathways Involved When Ductus Arteriosus Depends on Vasa Vasorum to Supply Oxygen to the Outer Vessel Wall (i.e., Fetuses Older Than 28 Weeks’ Gestation)

- Collapse of vasa vasorum in the muscle media
- Ischemic hypoxia of the smooth muscle cells in the muscle media
- Increased nitric oxide production
- Smooth muscle cell death

cAMP, Cyclic adenosine monophosphate; CaL, calcium L channel; cGMP, cyclic guanosine monophosphate; K⁺, potassium ion.

Postnatal Regulation

There are several events that promote ductus constriction in the full-term newborn following delivery: (1) an increase in arterial PO₂, (2) a decrease in blood pressure within the ductus lumen (because of the postnatal decrease in pulmonary vascular resistance), (3) a decrease in circulating PGE₂ (because of the loss of placental PG production and the increase in PG removal by the lung), and (4) a decrease in the number of PGE₂ receptors in the ductus wall. Although the newborn ductus continues to be sensitive to the vasodilating effects of NO, it loses its ability to respond to PGE₂ (Abrams et al., 1995). All of these factors promote ductus constriction after birth.

The postnatal increase in arterial PO₂ plays an important role in ductus constriction. Oxygen-induced constriction occurs in the presence of inhibitors of PG, NO, and endothelin signaling. This suggests that these vasoactive substances are not essential for normoxic constriction. In most species, oxygen appears to constrict the ductus arteriosus through both mechanisms that involve smooth muscle depolarization and mechanisms that are independent of membrane depolarization (Roulet et al., 1981). Oxygen depolarizes the ductus smooth muscle cells by inhibiting K⁺ channels (Michelakis et al., 2000). Following the depolarization of the membrane, calcium enters the ductus smooth muscle through L-type (Clyman et al., 2007) and T-type (Yokoyama et al., 2006) voltage-dependent

calcium channels. Several oxygen-sensitive K^+ channels have been found in the fetal ductus (including Kv1.5 and Kv2.1). These vary with species and gestational age and may account for the differing sensitivity of the ductus to oxygen (Hayama et al., 2006; Wu et al., 2007). Oxygen also appears to have a direct effect on the CaL-channels themselves (Thebaud et al., 2008) and on the store-operated calcium channels (Hong et al., 2006). In addition, oxygen may increase smooth muscle sensitivity to calcium by activating Rho kinase-mediated pathways (Hong et al., 2006).

Unique oxygen sensors within the ductus wall have been identified; however, their relative importance has not been agreed upon due to species variations in addition to multiple interacting downstream pathways. In some species, a mitochondrial oxygen sensor within the ductus smooth muscle cells regulates K^+ and calcium channels, as well as Rho kinase activity through the production of reactive oxygen molecules such as hydrogen peroxide (H_2O_2). Elevated PO_2 causes mitochondrial fission, which increases H_2O_2 production (Hong et al., 2013; Dunham-Snary et al., 2016). In contrast, in other species H_2O_2 acts as a vasodilator (Clyman et al., 1989) or as both a vasodilator and a vasoconstrictor (depending on its concentration) (Van der Sterren et al., 2014).

The oxygen-induced constriction may also involve a cytochrome P450 hemoprotein (Baragatti et al., 2011; Ciofini et al., 2013). This is an intriguing observation since preterm infants have an increased incidence of PDA when treated with cytochrome P450 inhibitors (Cotton et al., 2013). Oxygen also activates the epidermal growth factor receptor (EGFR) in ductus smooth muscle cells, and EGFR inhibition attenuates oxygen-induced constriction. The EGFR vasoconstriction appears to be mediated through tyrosine kinases and phosphatases (Hong et al., 2014).

Oxygen also increases the formation of isoprostanes that can modulate the postnatal constriction (Chen et al., 2012), as well as the potent vasoconstrictor, endothelin-1 (Cocceani et al., 1989). The role of endothelin-1 in postnatal ductus closure is still unclear due to the marked species variation in its contribution to the oxygen-induced ductus constriction. Endothelin receptor stimulation accounts for 44% of the oxygen-induced constriction in the rat but only 13% in the rabbit. In the human ductus, inhibition of endothelin production does not inhibit oxygen-induced constriction (Fineman et al., 1998; Cocceani et al., 1999; Michelakis et al., 2000; Shen et al., 2002). The postnatal increase in PaO_2 also has profound modulatory effects on other vasoactive systems (Smith, 1998). Elevated oxygen tensions can increase the ductus' contractile response to neural mediators (Ikeda et al., 1973) and decrease the formation of vasodilator PGs (Kajino et al., 2001). Although the contractile effects of oxygen play an important role in postnatal ductus constriction, they may not be essential for postnatal ductus closure. For example, mice lacking the endothelin A receptor have diminished oxygen-induced ductus constriction; however, their ductus closes normally after birth (Cocceani et al., 1999).

Developmental Regulation

In contrast with the full-term ductus, the premature ductus is less likely to constrict after birth (Box 54.2). This is due to several mechanisms. The intrinsic tone of the extremely immature ductus (<70% of gestation) is decreased compared with the ductus at term (Kajino et al., 2001). This may be due to the presence of immature smooth muscle myosin isoforms, with a weaker contractile capacity (Colbert et al., 1996; Sakurai et al., 1996), and decreased Rho kinase expression and activity (Kajimoto et al., 2007). Calcium entry through L-type calcium channels appears to be impaired in the immature ductus (especially under hypoxic conditions)

• BOX 54.2 Factors Affecting the Constriction of the Ductus Arteriosus After Birth: Preterm Compared With Full-Term Ductus Arteriosus

Constriction of the ductus arteriosus after birth—preterm compared with full-term ductus arteriosus:

- Immature smooth muscle isoforms and decreased intrinsic tone
- Decreased calcium entry through calcium L-channels
- Decreased Rho kinase activity
- Increased potassium K_{Ca} channels (which are not regulated by oxygen)
- Decreased potassium K_v channels (which are regulated by oxygen)
- Decreased endothelin production
- Decreased pulmonary clearance of circulating prostaglandin E2
- Increased number of postnatal prostaglandin receptors (EP4)
- Increased EP4 coupling with adenylyl cyclase and increased cAMP production
- Decreased cAMP degradation by phosphodiesterases

cAMP, Cyclic adenosine monophosphate; K_{Ca} , calcium-activated potassium channel; K_v , oxygen-sensitive potassium ion channel.

(Clyman et al., 2007; Thebaud et al., 2008). The potassium channels that promote ductus relaxation also change during gestation (switching from K_{Ca} channels (which are not regulated by oxygen tension) to K_v channels (which can be inhibited by increased oxygen concentrations). Reduced expression and function of the putative oxygen-sensing K_v channels appear to contribute to ductus patency in the preterm rabbit, sheep, baboon, mouse, and chicken (Thebaud et al., 2004; Cogolludo et al., 2009; Waleh et al., 2009). In contrast, a decrease in K_v channel expression occurs with advancing gestation in the rat, which suggests that in that species ductus arteriosus closure may occur by eliminating K_v channels (Wu et al., 2007).

Premature infants have elevated circulating concentrations of PGE2, which may play a significant role in maintaining ductus patency during the first days after birth. This is due to the decreased ability of the premature lung to clear circulating PGE2 (Clyman et al., 1981). In addition, during episodes of bacteremia and necrotizing enterocolitis (NEC), circulating concentrations of PGE2 reach the pharmacologic range and are often associated with reopening of the ductus arteriosus (Gonzalez et al., 1996).

The most important mechanism that prevents the preterm ductus from constricting after birth is its increased sensitivity to the vasodilating effects of PGE2. The increased sensitivity to PGE2 is due to both increased cAMP production (from enhanced receptor coupling with adenylyl cyclase) and decreased cAMP degradation by phosphodiesterase (Waleh et al., 2004; Liu et al., 2008). As a result, inhibitors of PG production (e.g., indomethacin, ibuprofen, mefenamic acid, and acetaminophen) are usually effective agents in promoting ductus closure in the premature infant.

The factors responsible for the changes that occur with advancing gestation are currently unknown. Prenatal administration of vitamin A has been shown to increase both the intracellular calcium response and the contractile response of the preterm ductus to oxygen (Wu et al., 2001). However, vitamin A administration does not improve the rate of ductus closure in preterm infants (Ravishankar et al., 2003). During normal fetal development there are increases in the circulating concentrations of thyroid hormones and cortisol with advancing gestation. Thyroid hormones may play a role in ductus arteriosus maturation since full-term infants with congenital

hypothyroidism have an increased incidence of PDA that appears to respond to thyroid hormone replacement therapy (Guarnieri et al., 2008). Similarly, a lower occurrence of PDA has been found in thyroid hormone-treated preterm infants (Osborn et al., 2007). Elevated cortisol concentrations in the fetus also foster ductus maturation by decreasing the sensitivity of the ductus to the vasodilating effects of PGE₂ (Clyman et al., 1981). Prenatal administration of glucocorticoids significantly reduces the incidence of PDA in premature humans and animals (Thibeault et al., 1978; Clyman et al., 1981). Postnatal glucocorticoid administration also reduces the incidence of PDA (Vermont Oxford Network Steroid Study Group, 2001). However, postnatal glucocorticoid treatment also increases the incidence of several other neonatal morbidities (Watterberg et al., 2004).

Although PGs play a dominant role in maintaining ductus patency in the preterm ductus, they also contribute to persistent ductus patency in infants born at term. Indomethacin can produce a substantial degree of ductus constriction even among term infants (Takami et al., 2007) as well as in infants born with congenital cardiac anomalies (Takami et al., 2013).

Genetic Regulation

Both species and genetic background play a significant role in determining the relative importance of ductus regulatory pathways. There is a marked species difference among several of these pathways; for example, ductus patency is critically dependent on vasodilator PGs in most species; however, notable exceptions exist in the guinea pig, chicken, and emu ductus, where locally derived PGs do not appear to play a role in its patency (Bodach et al., 1980; Agren et al., 2007; Dzialowski et al., 2008).

Recent studies using genome-wide transcriptome analysis to examine differences in gene expression between the ductus and the aorta (as well as the effects of oxygen and birth on ductus gene expression) (Costa et al., 2006; Liu et al., 2013; Shelton et al., 2014) have found that the expression of genes controlling matrix molecules (such as fibronectin and lysyl oxidase), actin–myosin interactions, potassium and calcium ion signaling, and genes involved with PG, endothelin, and angiotensin II signaling are increased in the ductus. Mutations in several of these ductus-dominant genes (examined in either mouse knock-out models or human genetic syndromes) are associated with delayed ductus closure or a persistent PDA in term neonates (Huang et al., 1998; Segi et al., 1998; Cocceani et al., 1999; Bergwerff et al., 2000; Morano et al., 2000; Loftin et al., 2001; Zhao et al., 2001; Mani et al., 2002; Trivedi et al., 2006; Zhu et al., 2006; Chang et al., 2007; Huang et al., 2008; Ko et al., 2008; Newbern et al., 2008; Anderson et al., 2009; Choudhary et al., 2009; Chang et al., 2010; Feng et al., 2010; Peng et al., 2010; Sinibaldi et al., 2010; Banka et al., 2011; Fallahi et al., 2011; Shen et al., 2011; Zhang et al., 2011; Zhao et al., 2011; Gruzdev et al., 2012; Paterick et al., 2013; Cooper et al., 2014; Roulez et al., 2014).

Genetic or familial factors also may play a role in persistent ductus patency in preterm infants (Lavoie et al., 2008; Bhandari et al., 2009). Several single nucleotide polymorphisms (SNPs) have recently been associated with preterm PDA: angiotensin receptor-alpha gene (T/C) Pvu II polymorphism (ATR) type 1 (Treszl et al., 2003), interferon (IFN) γ (Bokodi et al., 2007), estrogen receptor- α PvuII (Derzbach et al., 2005), Transcription Factor AP-2 Beta (TFAP2B), prostacyclin synthase (PGI) synthase, and TNF receptor-Associated Factor 1 (TRAF1) (Dagle et al., 2009). These associations will need to be replicated in other studies. There is a growing body of evidence to suggest that SNPs in TFAP2B may be responsible

for some of the PDAs that occur in preterm infants. TFAP2B is uniquely expressed in ductus smooth muscle; it regulates other genes that are important in ductus smooth muscle development; mutations in TFAP2B produce PDA in mice and humans (Zhao et al., 2001); and TFAP2B polymorphisms are associated with preterm PDAs (especially those that are unresponsive to indomethacin) (Dagle et al., 2009).

Anatomic Closure—Histologic Changes

In the full-term newborn there are progressive intimal thickening and fragmentation of the internal elastic lamina after delivery. As the intima increases in size, it ultimately forms mounds that occlude the already constricted lumen. The increase in intimal thickening is due (1) to migration of smooth muscle cells from the muscle media into the intima and (2) to proliferation of luminal endothelial cells. The process of intimal cushion formation starts with the accumulation of hyaluron (HA) below the luminal endothelial cells. This is accompanied by the loss of laminin and collagen IV from the basement membrane of the endothelial cells and their subsequent separation from the internal elastic lamina. The hygroscopic properties of HA cause an influx of water and widening of the subendothelial space; this creates an environment well-suited for cell migration (Boudreau et al., 1991). The endothelial and smooth muscle cells of the ductus arteriosus differ from those of the adjacent vessels in their ability to form neointimal cushions. Isolated endothelial cells of the ductus arteriosus have an increased rate of HA accumulation compared with those of the aorta or pulmonary artery; this increase appears to be due to transforming growth factor β (Boudreau et al., 1992), which is markedly increased in the ductus after birth. PGs, acting through the EP₄ receptor, also appear to play a critical role in HA production in the ductus (Yokoyama et al., 2006). Fibronectin and chondroitin sulfate also play an important role in facilitating ductus smooth muscle cell migration (Boudreau et al., 1991).

Intimal cushion formation in the ductus is also associated with striking alterations in elastin fiber assembly. In the aorta, well-developed elastic laminae in the muscle media provide elasticity and prevent the vascular wall from collapsing. In contrast, smooth muscle cells in the ductus muscle media are surrounded by thin and fragmented elastin fibers that do not prevent it from collapsing and closing when it vasoconstricts. The disruption of normal elastin fiber assembly in the ductus does not appear to be due to increased elastase activity or decreased tropoelastin production. Rather, it appears to be due to developmental mechanisms that reduce insolubilization of elastin and prevent formation of intact elastic laminae. PGE₂ and its receptor EP₄ appear to play a critical role in inhibiting thick elastin fiber formation in the ductus. Lysyl oxidase, which catalyzes elastin cross-linking, is degraded when the EP₄ receptor is stimulated. Interestingly, the ductus of the EP₄ knock-out mouse has an increased elastic phenotype that is similar to the aorta (Yokoyama et al., 2014).

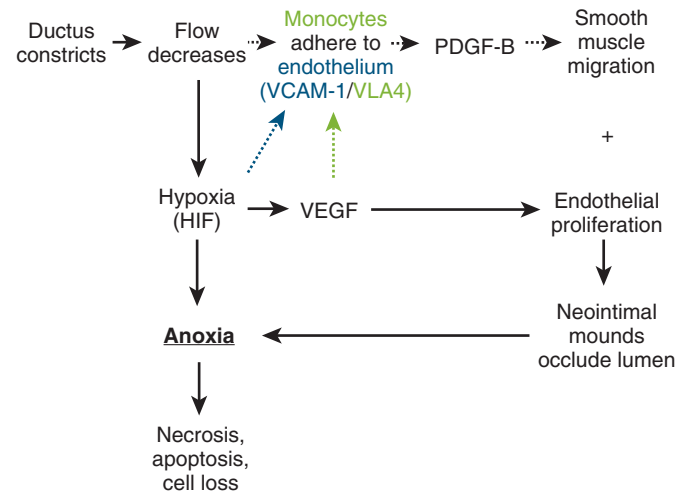
The exact relationship between impaired elastin assembly and smooth muscle migration into the neointima is still open for speculation. Impaired assembly of thick elastic laminae might facilitate smooth muscle cell migration by removing a physical barrier. In addition, truncated tropoelastin may act as a chemoattractant for smooth muscle cells (Mecham et al., 1984). Conversely, in some genetic forms of PDA, the elastic laminae of the ductus appear abnormally well developed and similar to those in the aorta; when this occurs, intimal cushions fail to develop (de Reeder et al., 1990; Hsieh et al., 2014; Yokoyama et al., 2014).

Relationship Between Vasoconstriction and Anatomic Closure

In full-term animals, loss of responsiveness to PGE₂ shortly after birth prevents the ductus arteriosus from reopening once it has constricted (Clyman et al., 1983; Abrams et al., 1995). This is due, in part, to decreased synthesis of PGE₂ receptors in the ductus after birth. Both the loss of vasodilator regulation and the anatomic events that lead to permanent closure appear to be controlled by the degree of ductus smooth muscle constriction. Experimental models that alter the ability of the ductus to constrict at term also prevent the normal histologic changes that occur after birth (Clyman et al., 1989; Nguyen et al., 1997; Loftin et al., 2001). Constriction produces ischemic hypoxia of the vessel wall (Clyman et al., 1999).

Because of the thickness of the term ductus, intramural vasa vasorum are needed to provide oxygen and nutrients to the outer half of its wall. These collapsible intramural vasa vasorum provide the ductus with a unique mechanism for controlling the maximal diffusion distance for oxygen and nutrients across its wall. Ductus constriction obliterates vasa vasorum flow to the outer muscle media, which turns the entire thickness of the muscle media into a virtual avascular zone (Kajino et al., 2002). The profound ischemic hypoxia that follows the constriction inhibits local production of PGE₂ and NO, induces local production of hypoxia inducible factors such as hypoxia inducible factor (HIF)1 α and vascular endothelial growth factor (VEGF), and produces smooth muscle apoptosis in the ductus wall. VEGF plays a critical role in the migration of the ductus smooth muscle cells into the neointima and in the proliferation of intramural vasa vasorum (Clyman et al., 2002). After postnatal ductus constriction, several genes known to be essential for vascular remodeling (*HIF1 α* , *VCAM-1*, *E-selectin*, *interleukin [IL]-8*, *MCSF-1*, *CD154*, *IFN γ* , *IL-6*, and *tumor necrosis factor [TNF]- α*) are increased in the ductus wall. Circulating mononuclear cells are attracted to the ductus, and, once adherent to the ductus wall, they become activated monocytes/macrophages and produce platelet derived growth factor (PDGF), which stimulates smooth muscle migration and proliferation within the neointima. The inflammatory response that follows postnatal ductus constriction appears to be necessary for neointimal remodeling since the extent of remodeling is determined by the degree of mononuclear cell adhesion (Waleh et al., 2005) (Fig. 54.1).

In preterm infants, the ductus frequently remains open for many days after birth. Even when it does constrict, the premature ductus frequently fails to develop profound hypoxia and anatomic remodeling. The preterm infant requires a greater degree of ductal constriction than the term infant to develop a comparable degree of hypoxia. In contrast with the term ductus, the thin-walled preterm ductus does not depend on intramural vasa vasorum to provide oxygen and nutrients to its wall. Before 26 weeks' gestation, intramural vasa vasorum are absent from the ductus wall. The absence of intramural vasa vasorum leaves the preterm ductus without a mechanism to rapidly increase the diffusion distance across its wall during postnatal constriction. As long as any degree of luminal patency exists, the thin-walled preterm ductus fails to become profoundly hypoxic and fails to undergo anatomic remodeling after birth. As a result, the preterm ductus requires complete cessation of luminal flow before it can develop the same degree of hypoxia as found at term. Once the preterm ductus develops the same degree of hypoxic ischemia as the term ductus, most of the anatomic changes seen at term will occur (Seidner et al., 2001). However, if the premature ductus does not develop the degree of



• **Fig. 54.1** Ductus Remodeling: Role of Constriction, Hypoxia, and Mononuclear Cell Adhesion in Neointima Formation and Smooth Muscle Cell Death. Postnatal constriction produces hypoxia in the ductus wall. The hypoxic smooth muscle and endothelial cells increase their expression of vascular endothelial growth factor (VEGF) and VCAM-1, respectively. VEGF is required for endothelial cell proliferation. VEGF also attracts circulating mononuclear cells (expressing VLA-4) to the endothelial cell surface. Under very low flow conditions, the weakly adherent VLA-4⁺ mononuclear cells attach to VCAM-1 on the endothelial cell surface and release platelet derived growth factor and matrix metalloproteinase 9 (which promote smooth muscle migration into the neointima). Tight constriction and loss of luminal flow are essential for VEGF expression, mononuclear cell adhesion, neointimal formation, and luminal occlusion. *HIF*, hypoxia inducible factor; *MMP-9*, matrix metalloproteinase-9; *PDGF-B*, platelet derived growth factor B; *VCAM-1*, vascular cell adhesion molecule-1; *VEGF*, vascular endothelial growth factor; *VLA-4*, integrin very late antigen-4.

ischemic hypoxia needed to induce cell death and anatomic remodeling, it will continue to be responsive to vasodilators and continue to be susceptible to vessel reopening.

Although inhibitors of PG production are very effective in closing the ductus when given on postnatal day 1, they become less effective by the end of the first postnatal week. A number of factors conspire to make the postnatal preterm ductus increasingly resistant to indomethacin-induced and ibuprofen-induced closure. In contrast to the term newborn ductus, where all of the PGE₂ EP receptors are downregulated after birth, the preterm newborn ductus increases its synthesis of the dominant PGE₂ receptor, EP₄, and continues to respond to PGE₂ after birth. In addition to its persistent responsiveness to PGE₂, the premature ductus synthesizes increased amounts of other vasodilators (e.g., NO, TNF- α , and IL-6) after birth in response to the postnatal ductus wall hypoxia (Waleh et al., 2005). As a result, a change occurs in the relative balance of the vasodilators that maintain ductus patency after birth. Ductus patency becomes less dependent on PG generation and more dependent on other vasodilators after the first few days following birth. In addition, adenosine triphosphate concentrations are significantly altered in the preterm PDA. The decreased adenosine triphosphate concentrations prevent the immature neonatal ductus from contracting as vigorously as the fetal ductus (Levin et al., 2005). As a result of these postnatal changes, the effectiveness of indomethacin and ibuprofen wanes with increasing postnatal age (Clyman, 1996). In premature animals and humans, the combined use of an NO synthase inhibitor and indomethacin produces a much greater degree of ductus constriction than

indomethacin alone. It follows that drugs that interfere with NO synthesis could become a useful adjunct, especially in situations where indomethacin has been found to be ineffective (Seidner et al., 2001; Keller et al., 2005).

Hemodynamic and Pulmonary Alterations

The pathophysiologic features of a PDA depend both on the magnitude of the left-to-right shunt and on the cardiac and pulmonary responses to the shunt. There are important differences between immature and mature infants in the heart's ability to handle a volume load. The immature fetal ventricles have less cardiac sympathetic innervations, are less distensible than at term, and generate less force per gram of myocardium (Romero et al., 1979). The relative lack of left-ventricular distensibility in immature infants is more a function of the ventricle's tissue constituents than of poor muscle function. As a result, left-ventricular distention secondary to a large left-to-right PDA shunt may produce a higher left-ventricular end-diastolic pressure at smaller ventricular volumes. The increase in left-ventricular pressure increases pulmonary venous pressure and causes pulmonary congestion.

Studies in preterm animal and human newborns (Clyman et al., 1987; Shimada et al., 1994) have shown that, despite these limitations, preterm newborns are able to increase left-ventricular output and maintain their "effective" systemic blood flow, even with left-to-right PDA shunts equal to 50% of left-ventricular output. With shunts greater than 50% of left-ventricular output, "effective" systemic blood flow falls, despite a continued increase in left-ventricular output. The increase in left-ventricular output associated with a PDA is accomplished not by an increase in heart rate but by an increase in stroke volume. Stroke volume increases primarily as a result of the simultaneous decrease in afterload resistance on the heart and the increase in left-ventricular preload. Despite the ability of the left ventricle to increase its output in the face of a left-to-right ductus shunt, blood flow distribution is significantly rearranged. Blood flow to the gastrointestinal (GI) tract and kidneys is decreased due to a combination of decreased perfusion pressure and localized vasoconstriction. Mesenteric blood flow is decreased in both fasting and fed states in the presence of a PDA (McCurnin, Clyman, 2008). Significant decreases in organ blood flow occur before there are signs of left-ventricular compromise (Shimada et al., 1994) and may contribute to the decreased glomerular filtration rate (Clyman, 1996) that has been observed with ductus patency. Cerebral blood flow and oxygenation are also compromised in the presence of a moderate PDA left-to-right shunt because of the preterm newborn's limited ability to autoregulate its cerebral vascular bed (Lemmers et al., 2010).

The decreased ability of the preterm infant to maintain active pulmonary vasoconstriction may be responsible in part for early development of a "large" left-to-right PDA shunt. Randomized controlled trials have shown that the presence of a PDA increases the incidence of early hemorrhagic pulmonary edema/pulmonary hemorrhage (Al Faleh et al., 2008; Aranda et al., 2009; Kluckow et al., 2014). Therapeutic maneuvers or prenatal conditions that lead to a rapid postnatal drop in pulmonary vascular resistance, such as surfactant replacement or intrauterine growth retardation, can exacerbate the amount of left-to-right shunt and lead to pulmonary hemorrhage (Alpan et al., 1995; Rakza et al., 2007). Although phototherapy has been associated with persistence of a PDA, randomized controlled trials (RCTs) have not found that chest shielding alters the incidence or severity of PDA (Bhola et al., 2015).

In premature animals, a wide open PDA increases the hydraulic pressures in the pulmonary vasculature on both the arterial and venous sides of the capillary bed. In addition, the increase in pulmonary blood flow and immature precapillary arterial tone shift the distribution of intravascular hydraulic pressures toward downstream capillary fluid filtration sites (Perez Fontan et al., 1987). This, in turn, increases the rate of fluid transudation into the pulmonary interstitium (Alpan et al., 1991). Any increase in microvascular perfusion pressure in premature infants with respiratory distress syndrome may increase interstitial and alveolar lung fluid because of their low plasma oncotic pressures and increased capillary permeability. Leakage of plasma proteins into the alveolar space inhibits surfactant function, increases surface tension, and decreases compliance of the immature air sacs (Ikegami et al., 1983), which are already compromised by surfactant deficiency. The increased fraction of inspired oxygen and mean airway pressures required to overcome these early changes in compliance may play a role in the development of chronic lung disease. Depending on the gestational age and the species examined, changes in pulmonary mechanics may occur as early as 1 day after birth or not before several days of exposure to a PDA left-to-right shunt (Perez Fontan et al., 1987; McCurnin et al., 2008).

Although preterm animals with a PDA have increased fluid and protein clearance into the lung interstitium (which is due to an increase in pulmonary microvascular filtration pressure), a simultaneous increase in lung lymph flow appears to eliminate the excess fluid and protein from the lung (Alpan et al., 1991). This compensatory increase in lung lymph acts as an "edema safety factor," inhibiting fluid accumulation in the lungs. As a result, there is no net increase in water or protein accumulation in the lung, and there is no change in pulmonary mechanics (Perez Fontan et al., 1987; Alpan et al., 1989; Clyman, 1996). This delicate balance between the PDA-induced fluid filtration and lymphatic reabsorption is consistent with the observation, made in human infants, that closure of the ductus arteriosus within the first 24 hours of birth has no effect on the course of the newborn's hyaline membrane disease. However, after several days of mechanical ventilation, there is a decrease in pulmonary capillary surface area (Mokres et al., 2010), which increases both the pulmonary microvascular pressure and rate of hydraulic fluid filtration. As a result, it is not uncommon for infants with a persistent PDA to develop pulmonary edema and alterations in pulmonary mechanics at 6 to 10 days after birth. In these infants, improvement in lung compliance occurs following closure of the PDA (Clyman 1996; Szymankiewicz et al., 2004).

Preterm baboon newborns, delivered at 67% term gestation and mechanically ventilated for 2 weeks, have been used to examine the effects of a PDA on pulmonary mechanics (McCurnin et al., 2008). Preterm baboons were either treated with a cyclooxygenase inhibitor to close the PDA or allowed to have a persistent PDA. Exposure to a persistent PDA for 2 weeks did not appear to alter surfactant secretion, pulmonary epithelial protein permeability, or presence of surfactant inhibitory proteins in the alveoli, nor did the presence of a PDA alter the expression of proinflammatory or tissue remodeling genes. In contrast with full-term baboons, which mobilize lung fluid rapidly after birth, preterm newborns (with either an open and closed ductus) mobilize lung fluid much more slowly. As a result, a persistent PDA led to a small but significant increase in lung water at 2 weeks after delivery. In addition, closure of the PDA with indomethacin or ibuprofen produced increased expression of alveolar epithelial sodium channels, which facilitate fluid removal from the alveolar compartment (McCurnin et al.,

2008). This finding may account for the decreased incidence of significant pulmonary edema and hemorrhage in infants treated with prophylactic indomethacin after birth (Al Faleh et al., 2008; Aranda et al., 2009; Kluckow et al., 2014).

Pharmacologic closure of the PDA was associated with improved lung development in the preterm baboons. In contrast to the animals with an open ductus, where an arrest in alveolar development (the hallmark of the “new” bronchopulmonary dysplasia [BPD]) was noticeable by 2 weeks after birth, pharmacologic closure of the PDA led to improved alveolarization (McCurnin et al., 2008). Whether the improvement in alveolarization was due to the closure of the ductus or the pharmacologic agents (indomethacin/ibuprofen) used to close it is unknown at this time.

The same improvements in pulmonary mechanics and alveolar surface area have not been observed after surgical ligation of the PDA. Early surgical ligation increases the expression of genes involved with pulmonary inflammation and decreases the expression of genes associated with pulmonary epithelial sodium channels (which are critical for alveolar water clearance) (Waleh et al., 2011). These changes may contribute to the lack of improvement in pulmonary mechanics after PDA ligation. In addition, early surgical ligation impedes lung growth (Chang et al., 2008). These findings raise the possibility that early ductus ligation, while eliminating the detrimental effects of a PDA on lung development, may create its own set of problems that counteract many of the benefits derived from ductus closure (Clyman et al., 2009).

Not all of the changes associated with a PDA are necessarily detrimental to the immature infant with respiratory distress syndrome. Persistence of the left-to-right shunt maintains an elevated PaO_2 in the presence of atelectasis. This phenomenon is due to recirculation of oxygenated arterial blood through lungs that are not fully expanded (Clyman et al., 1987). Closing the PDA can lead to decreases in systemic arterial oxygen content despite the absence of any alterations in pulmonary mechanics.

Treatment

Treatment Options for Closing a PDA

In full-term infants, transcatheter PDA ligation is the treatment of choice for closing a PDA. However, its use in the premature population is just beginning to be explored (Zahn et al., 2015).

Open surgical ligation produces definitive ductus arteriosus closure; however, it is associated with several important morbidities: thoracotomy and exposure to surgical anesthesia, pneumothorax, chylothorax, scoliosis, and infection. In addition, following PDA ligation, between 20% and 60% of extremely low birth weight (ELBW) infants will develop unilateral vocal cord paralysis (which increases the requirements for tube feedings and respiratory support and persists well beyond the neonatal period) (Nichols et al., 2014; Strychowsky et al., 2014). Profound hypotension during the postoperative period is another complication that affects 30%–50% of infants with birthweights of 1000 g and lower. The incidence of profound postoperative hypotension is inversely related to the infant's corrected age and appears to be due to impaired adrenal stimulation, low cortisol release, and decreased vascular tone (Clyman et al., 2014; Noori et al., 2015). Early surgical ligation also contributes to the development of BPD (Chorne et al., 2007; Clyman et al., 2009). Neurodevelopmental abnormalities are also more frequent following early PDA ligations. If surgical expertise is not readily available, neonatal transport to another facility may be required. Therefore avoiding or delaying ligation appears to be

beneficial since many of the morbidities associated with ligation (postligation hypotension [Teixeira et al., 2008], vocal cord paralysis [Smith et al., 2009], BPD [Clyman et al., 2009]) and abnormal neurocognitive development (Wickremasinghe et al., 2012) appear to be reduced when ligation is delayed (Sung et al., 2014).

Inhibition of PG synthesis with nonselective inhibitors of COX-1 and COX-2 (indomethacin and ibuprofen) appears to be an effective alternative to surgical ligation. However, both have been associated with several potential adverse effects in the newborn. Indomethacin produces significant reductions in renal (Pezzati et al., 1999), mesenteric (Van Bel et al., 1990), and cerebral blood flow (Van Bel et al., 1989). Indomethacin also reduces cerebral oxygenation (Patel et al., 2000). Alterations in creatinine clearance and oliguria (which are minimally responsive to dopamine or furosemide therapy) are common problems. Furosemide, when used in combination with indomethacin, actually increases the incidence of acute renal failure (Brion et al., 2001). Renal function returns to normal after the initial doses of indomethacin or after drug discontinuation (Seyberth et al., 1983). There are some reports that COX inhibitors may affect glomerular development in neonatal animals; however, these findings have not been consistently observed (Bueters et al., 2013; Kent et al., 2014; Bueters et al., 2015). The action of indomethacin on these organ systems may not be due entirely to its inhibition of PG synthesis (Chemtob et al., 1991; Malcolm et al., 1993; Speziale et al., 1999). Indomethacin also has effects on lipoxygenase activity and histamine and endothelin release, although the relevance of these effects to any neonatal morbidities is still unknown.

Although indomethacin produces significant physiologic alterations and has been associated with several morbidities, none of the controlled randomized trials that have examined the relationship between indomethacin and neonatal morbidity have found an increase in the incidence of NEC, GI perforation, retinopathy of prematurity (ROP) chronic lung disease, or cerebral white matter injury following indomethacin treatment (Fowlie et al., 2003). Indomethacin, by itself, has not been shown to increase the incidence of GI perforations; however, the combination of indomethacin and postnatal steroids, administered simultaneously, does increase the incidence of GI perforations/NEC (Watterberg et al., 2004; Peltoniemi et al., 2005).

The cerebral vasoconstrictive effects of indomethacin are frequently cited as a concern for neonatologists (Edwards et al., 1990); however, a Cochrane systematic review found that indomethacin prophylaxis is more likely to decrease rather than increase the incidence of periventricular leukomalacia (Fowlie et al., 2003). There is no evidence that prophylactic indomethacin has any adverse effect on neurodevelopmental outcome at 18 months (Schmidt et al., 2001); in fact, there is evidence that it may have long-term benefits at 4.5 and 8 years (Vohr et al., 2003; Ment et al., 2004).

While there may be general consensus on the efficacy of indomethacin for treatment of a PDA, questions about proper dosage, treatment duration, and optimal timing of treatment remain quite controversial. The plasma clearance of indomethacin depends on postnatal age. Therefore a dosing regimen recommended for infants at the end of the first week (when the half-life of the drug is 21 hours) (Yaffe et al., 1980; Yeh et al., 1989) may lead to elevated and prolonged plasma concentrations when used in infants on day 1 (when the half-life is 71 hours). Conversely, a single loading dose of indomethacin (0.2 mg/kg), without subsequent maintenance doses, can be effective in closing a PDA when administered within the 24 hours following delivery (Krueger et al., 1987).

Many variations in dosage regimens have been evaluated (Gork et al., 2008). A prolonged low-dose course of indomethacin (0.1 mg/kg every 24 hours for 5–7 days) may increase the rate of permanent closure, especially in infants who still have residual ductus flow after completing the standard short course (2–3 doses over 24 hours), and may decrease the impairment of renal function from indomethacin (Hammerman et al., 1990). This dosage regimen still needs further evaluation since a higher NEC rate was observed in infants receiving prolonged maintenance indomethacin (Herrera et al., 2007). Although some have suggested that the dose of indomethacin be increased when conventional dosing fails to produce ductus closure (Sperandio et al., 2005), an RCT examining this approach found that the rate of ductus closure was not substantially improved despite a nearly threefold increase in serum indomethacin concentrations. More worrisome was the fact that the higher indomethacin concentrations produced significant increases in the incidence of moderate/severe ROP and late renal dysfunction (Jegatheesan et al., 2008).

The postnatal age at which indomethacin is administered plays an important role in determining its effectiveness. With advancing postnatal age, dilator PGs play less of a role in maintaining ductus patency (see Relationship Between Vasoconstriction and Anatomic Closure) (Cotton et al., 1991; Chorne et al., 2007). Even when indomethacin concentrations have been maintained in the “desired” range, the drug’s ability to produce complete ductus closure declines during the first days after birth. Despite its decreased effectiveness, it may still be advisable to attempt PDA closure by indomethacin before surgical ligation even in cases of advanced postnatal age (Sterniste et al., 1998).

Recurrence of a symptomatic PDA can occur after initial successful treatment. The rate of reopening, which is greatest among the most immature infants, appears to be related to the timing and completeness of ductus closure after the first treatment course (Narayanan et al., 2000). Permanent anatomic closure requires tight constriction of the ductus lumen and the development of ductus wall hypoxia (see Relationship Between Vasoconstriction and Anatomic Closure). Unfortunately, there are limitations in the ability of Doppler ultrasound to detect complete luminal closure. Even when there is no evidence of ductus patency on the Doppler/echocardiogram, a significant number of preterm infants will still have a tiny patent ductus lumen. In addition, the thinner the ductus wall, the less the likelihood that profound hypoxia and anatomic remodeling will occur. Both increasing degrees of immaturity and increasing degrees of growth restriction increase the treatment failure rate (Madeleneau et al., 2015). Twenty percent of PDAs in neonates delivered before 26 weeks reopen in spite of echocardiographic evidence of closure; in contrast, only 9% of those in neonates delivered between 26 and 27 weeks will reopen if the ductus is found to be closed on echocardiography. Early treatment produces a tighter degree of ductus constriction and as a result produces higher rates of ductus wall hypoxia and permanent closure (Narayanan et al., 2000). Recurrence of a PDA can also occur after bacterial infections (Gonzalez et al., 1996). Bacterial endotoxin increases NO production, but not PG production, in the ductus wall (Kajimura et al., 2016). This may explain why indomethacin is less effective in closing the PDA after infection.

Ibuprofen, another nonselective cyclooxygenase inhibitor, appears to be as effective as indomethacin in producing PDA closure (at least in infants with a mean gestational age of 28 weeks) (Van Overmeire et al., 2000). Compared with indomethacin, ibuprofen treatment results in reduced rates of NEC and transient renal

insufficiency (Ohlsson et al., 2015). Orogastic administration of ibuprofen appears to be as effective as intravenous administration (Ohlsson et al., 2015). In contrast with indomethacin, ibuprofen does not appear to affect mesenteric blood flow and has less of an effect on renal perfusion, oliguria, and cerebral blood flow (Malcolm et al., 1993; Mosca et al., 1997; Pezzati et al., 1999; Speziale et al., 1999; Patel et al., 2000). Animal studies suggest that ibuprofen may have some cytoprotective effects in the intestinal tract (Grosfeld et al., 1983). Unfortunately, most of the studies that have compared ibuprofen with indomethacin have not included ELBW infants (≤ 25 weeks’ gestation), which is the group that is most resistant to pharmacologic ductus closure and where the optimal age-appropriate dosing schedule for ibuprofen is still under consideration (Hirt et al., 2008). Some of the higher ibuprofen dose options are concerning because of the effects of ibuprofen on total and free serum bilirubin concentrations (Zecca et al., 2009). Ibuprofen does not have the same effects as indomethacin on cerebral autoregulation. As a result, prophylactic ibuprofen does not appear to have the same intracranial hemorrhage (ICH) sparing effect that is seen with indomethacin (see Indomethacin and Intracranial Hemorrhage (ICH)). Oral ibuprofen has been reported to have a high incidence of adverse GI events when administered prophylactically (Kanmaz et al., 2013).

Paracetamol (acetaminophen) may be an effective agent for inducing PDA closure. Although its mechanism of action has not been fully elucidated, the vasoconstrictive effect of acetaminophen appears to be mediated through inhibition of the peroxidase moiety of PGH2 synthetase (Allegraert et al., 2013). Three RCTs found oral paracetamol to have comparable effectiveness in closing the PDA with oral ibuprofen or intravenous indomethacin (Dang et al., 2013; Oncel et al., 2014; Dash et al., 2015). These results should be interpreted with caution since only a limited number of relatively mature neonates (mean gestational age greater than 28 weeks) have been treated in the paracetamol RCTs so far. In addition, since paracetamol use in pregnancy has been associated with an increased risk of infantile autism with hyperkinetic symptoms (Liew et al., 2014), long-term follow-up studies will be needed before paracetamol can be fully adopted as an alternative to indomethacin and ibuprofen.

Indomethacin and Intracranial Hemorrhage

Previous studies have shown that indomethacin can decrease the incidence of ICH in preterm infants and experimental animals (this has not been the case with ibuprofen or paracetamol). The effects of indomethacin on ICH do not appear to be due to its effects on ductus patency (Ment et al., 1988). Indomethacin decreases cerebral blood flow, decreases reactive postasphyxial cerebral hyperemia, and accelerates maturation of the germinal matrix microvasculature (Ment et al., 1992; Ballabh, 2014). Since most ICHs occur within the first 3 days of birth, one would expect to see beneficial effects only when indomethacin is given in a *prophylactic* strategy (anytime during the first 18 hours after birth [Mirza et al., 2013]). When prophylactic indomethacin is given to infants with normal echoencephalograms, there is a significant reduction of all grades (I–IV), as well as the most severe grades (III, IV) of ICH. Fortunately, when indomethacin is given to infants with a preexisting intraventricular hemorrhage (IVH) there does not appear to be an increased risk of parenchymal extension of the hemorrhage (Ment et al., 1994). However, it should be noted that infants who are coagulopathic or have low platelet counts (below $100 \times 10^9/L$) at the time COX inhibitors are

administered are at increased risk for intracerebral bleeding (Brunner et al., 2013).

PDA and Neonatal Morbidity: To Close or Not to Close

Clear evidence is lacking for or against many of the approaches to PDA treatment. Although a prolonged, persistent moderate-to-large left-to-right shunt through a PDA shortens the life span of animals and humans (Campbell 1968; Loftin et al., 2001), there has been a growing debate in recent years about whether or not the PDA needs to be closed during the neonatal period (Bose et al., 2006; Benitz et al., 2016). Preterm infants have a high rate of spontaneous PDA closure during the first 2 years. Therefore early treatment runs the risk of exposing infants to drugs or procedures they might not need. Although a moderate-to-large PDA increases pulmonary blood flow and edema and decreases systemic blood pressure, it is not clear which is preferable: (1) to close the PDA (either surgically or pharmacologically) or (2) to deal with the pulmonary edema and hypotension through other means while awaiting spontaneous ductus closure. For example, dopamine can decrease PDA shunting by increasing pulmonary vascular resistance (Bouissou et al., 2008); fluid restriction can minimize PDA symptoms (Bell et al., 2001) but fails to reduce the PDA shunt (and also is associated with reduced systemic blood flow [De Buyst et al., 2012]); and modest increases in end-expiratory pressure can improve pulmonary edema and compliance but have only a modest effect on the left-to-right PDA shunt (Fajardo et al., 2014).

Published RCTs provide only a limited amount of information to help guide current PDA treatment choices. Most of our information about the risks and benefits of indomethacin treatment comes from studies that compared two different forms of indomethacin treatment: prophylactic treatment (i.e., starting indomethacin within 12 hours of birth, before the onset of symptoms) versus symptomatic treatment (i.e., delaying treatment until PDA-related symptoms appear, which usually occur by 2–8 days after birth). Unfortunately, the trials were not designed to address the question of whether to close the PDA or to leave it alone. Prophylactic treatment (compared with waiting until the end of the first postnatal week before deciding to treat the PDA) did not decrease the incidence of important morbidities such as BPD, NEC, or neurodevelopmental impairment and resulted in overtreatment of 40%–50% of the infants that would have had spontaneous ductus closure by day 7. On the other hand, prophylactic treatment decreased a number of short-term morbidities: (1) severe early pulmonary hemorrhages, (2) severe grades of IVH, (3) the risk of developing a symptomatic PDA, (4) dopamine-dependent hypotension, (5) the need for higher levels of ventilator support, and (6) the need for surgical PDA ligation (Schmidt et al., 2001; Fowlie et al., 2003; Koch et al., 2006; Al Faleh et al., 2008; Clyman et al., 2008; Kluckow et al., 2014).

Little information exists about the consequences of even longer exposure to a persistent, symptomatic, moderate-to-large PDA shunt. Only two small RCTs, performed more than 30 years ago, specifically examined the role of a persistent untreated symptomatic PDA on neonatal morbidity (Cotton et al., 1978; Kaapa et al., 1983). Both studies found that a persistent PDA prolonged the need for respiratory support and increased pulmonary morbidity. Whether these findings are still applicable in the setting of modern neonatal respiratory care, with “gentle ventilation” and acceptance of elevated arterial partial pressure of carbon dioxide, needs to be reexamined. It is interesting that even the authors of the original

studies speculated that the PDA might not be source of the respiratory morbidity they observed. They attributed the cause of the respiratory morbidity to the “extensive use of mechanical ventilation,” used to control the PDA-induced pulmonary edema (Cotton et al., 1978).

The role of a PDA in the development of spontaneous intestinal perforation or NEC is even more controversial. Retrospective population-based observational studies have reported opposing results. There are no RCTs in the medical literature that specifically address this issue nor is there information about the advisability of continuing or stopping enteral feeding in the presence of a PDA or during its treatment. Although the presence of a PDA and indomethacin treatment are both known to decrease postprandial mesenteric blood flow, several large population-based studies (Kelleher et al., 2014; Louis et al., 2016) and a recent RCT (Clyman et al., 2013) found that infants receiving small “trophic” enteral feeds during indomethacin treatment attain full enteral feedings (without any adverse GI outcomes) at a faster rate than those kept nil per os during treatment.

In the United States, 95% of neonatologists believe that a moderate-to-large PDA should be treated if it persists in ELBW infants who continue to require mechanical ventilation or vasopressor support; in contrast, less than 30% use indomethacin prophylactically (Jhaveri et al., 2009). The number of neonatologists that treat a persistent PDA when it occurs in infants who do not require mechanical ventilation or vasopressor support varies significantly by geographic region. Marked differences in neonatologists’ willingness to feed infants in the presence of a PDA appear to account for the large geographic variations in indomethacin use and rates of PDA ligation. It is interesting to note that at one time, 70% of US neonatologists believed that enteral feedings needed to be stopped in the presence of a PDA whereas, at the same time, non-US neonatologists had exactly the opposite opinion: 70% believed that enteral feedings should continue in the presence of a PDA (Jhaveri et al., 2009).

A Personalized Approach

A moderate-to-large left-to-right PDA shunt can lead to dopamine-dependent systemic hypotension and increased ventilator needs. The University of California, San Francisco (UCSF) NICU’s approach has been to use pharmacologic treatment early in the newborn period since early pharmacotherapy has only been linked to transient renal dysfunction, and early treatment is more likely to result in successful ductus closure. In certain settings (where ICH, pulmonary hemorrhage, and PDA ligations are frequent occurrences), indomethacin prophylaxis may even be a preferred alternative. On the other hand, ductus ligation, while eliminating one potential cause for neonatal morbidity, may introduce its own set of problems. Further investigations will be needed to determine which infants are most likely to benefit from surgical ligation and which infants might best be left untreated when pharmacologic approaches are no longer an option.

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55

Congenital Heart Disease

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KEY POINTS

- Congenital heart disease (CHD) is the most common birth heart defect encountered in the clinical setting, affecting 1% of live births.
- Surgical outcomes for all forms of CHD continue to improve. Early detection through fetal echocardiography, physical examination, and pulse oximetry screening allows for improved neonatal management and decreased short-term and long-term morbidity.
- The etiology of CHD remains elusive in many cases. Advances in cellular biology and genetic testing will continue to improve our understanding of its origins.
- An in-depth understanding of neonatal cardiac anatomy and physiology is necessary for proper management of infants with CHD.

Congenital heart disease (CHD) is the most common birth defect encountered in the clinical setting. The prevalence of all forms of congenital heart lesions is approximately 1% of live births, although estimates in the newborn are likely compromised by underdiagnosis of some lesions such as bicuspid aortic valve and overdiagnosis of normal structures that are in transition, including the foramen ovale and ductus arteriosus. The presentation of the newborn with CHD can range from being asymptomatic to complete cardiovascular collapse. Most commonly, neonates with CHD present with a murmur, cyanosis, and/or congestive heart failure (CHF). Lesions that result in each of these clinical findings will be considered separately although considerable overlap exists among the three groups.

General Considerations

Fetal-to-Postnatal Transition

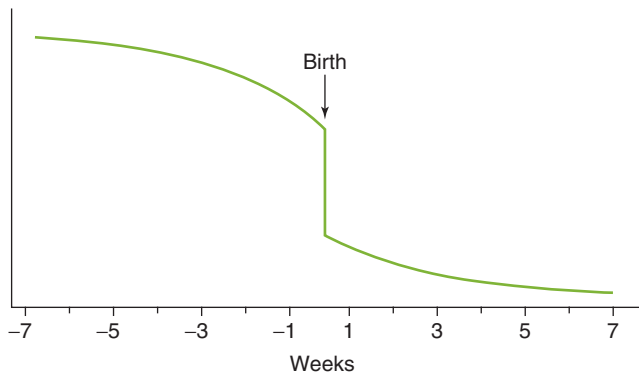
The hemodynamic state of the fetus is much different from the newborn. In the fetus, a relatively low systemic vascular resistance exists because of the presence of the placenta, and the pulmonary vasculature maintains a high resistance. Central shunts exist that provide alternate routes on the venous side (ductus venosus), within the heart (foramen ovale), and on the arterial side of the circulation (ductus arteriosus).

The ductus venosus predominantly collects oxygen-rich and nutrient-rich blood from the placenta via the umbilical vein and delivers this blood directly to the right atrium, largely bypassing the hepatic and portal venous systems. Based on studies in fetal

sheep, less than one-half of the umbilical venous return enters the left lobe of the liver and reaches the ductus venosus near its insertion into the inferior vena cava (IVC), returning as relatively nutrient-rich blood (Edelstone et al., 1978). The lateral position of the IVC within the right atrium results in streaming of this nutrient-rich blood across the foramen ovale and into the left atrium. The most desaturated blood to return to the right atrium comes from the coronary sinus, which combines with the venous return from the superior vena cava (SVC) and is directed across the tricuspid valve into the right ventricle.

In the fetus, the presence of the ductus arteriosus, which is nonrestrictive, results in both ventricles being subjected to a comparable afterload. Compared with the postnatal heart, this results in an increase in right ventricular workload and some restriction to filling of the right ventricle. The nutrient-rich blood deriving from the umbilical vein, which has crossed the foramen ovale to enter the left side of the heart, predominantly supplies the heart and brain. Output from the right ventricle supplies the lungs (less than 8% of the combined cardiac output) and flows right-to-left through the ductus arteriosus to supply the remainder of the body.

At birth, several important transitions take place that allow the fetus to adapt to extrauterine life. First, the gradual decline in pulmonary vascular resistance (PVR) that was occurring during the last trimester of pregnancy undergoes an abrupt drop with the first breath taken by the newborn (Fig. 55.1). This decline in PVR results in a more than 20-fold increase in pulmonary blood flow and reversal of flow (left-to-right) in the ductus arteriosus before its closure (Teitel, 1988). Second, the central shunts present in the fetus undergo closure such that blood flows in series through the body. The ductus venosus closes largely because of lack of flow following separation of the placenta, although some contractile elements may be present in the vessel wall (Adeagbo et al., 2004). The foramen ovale becomes occluded as the flap of the septum primum abuts the septum secundum following the increased pulmonary blood flow that increases filling of the left atrium. Small residual left-to-right shunts at the foramen ovale may persist although will generally decrease with time (see “Atrial Septal Defects” later in the chapter). Closure of the ductus arteriosus is mediated by a variety of pathways, although patency of the ductus can usually be maintained by exogenous prostaglandin administration. The third important transition at birth is an increase in the combined ventricle output as the metabolic demands of the body increase at birth.



• **Fig. 55.1** Change in Pulmonary Vascular Resistance. A gradual decline in pulmonary vascular resistance is seen during the latter part of gestation followed by an abrupt decline at birth. A gradual decline occurs postnatally over the next 6–8 weeks.

The dramatic hemodynamic changes that occur at birth continue to evolve over the next few months. There is a continued decline in PVR during the first 6–8 weeks after birth. In addition, the right ventricle remodels to a thinner and more compliant ventricle. Probe patency of the foramen ovale may persist for years, although in most individuals the septa become adherent.

Nomenclature

The complete description of any heart requires more than a description of the presence or absence of a specific congenital heart defect. Each heart has a specific set of structures and connections that may be normal or abnormal. While the terminology used for cardiac lesions is relatively consistent among pediatric cardiologists, different nomenclatures have been developed to completely define the cardiac anatomy and various systems have evolved that are based on surgical approaches, embryologic origins, or spatial relationships, which has hampered communication between individuals and institutions. A common method of describing cardiac anatomy would be a benefit but seems unlikely to be agreed upon in the near future. While the brief summary of nomenclature that is given here is based on the segmental approach of [Anderson et al. \(1984\)](#), the embryologic approach of [Van Praagh \(1972\)](#) is equally valid and used by several institutions.

The segmental approach to describing cardiac anatomy includes:

1. Cardiac position
2. Visceral sidedness
3. Systemic and pulmonary venous connections
4. Atrial sidedness and their connections
5. Atrioventricular (AV) valves
6. Ventricle sidedness
7. Ventriculoarterial connections
8. Great vessel number and position

The description of cardiac position in the chest can be separated into where the heart is located and the direction in which the apex of the heart is pointed. Normally, the heart is in the left chest with the apex pointed to the left. Dextro- (right) or meso- (midline) position of the heart can occur with decreased right lung volume, severe scoliosis, or an elevated left diaphragm. Typically, the position of the heart in the chest is determined by a chest X-ray. The normal leftward-pointing apex of the heart (levocardia) can vary to mesocardia (in various heterotaxy syndromes) or dextrocardia (in situs inversus). The orientation of the apex of the heart is usually defined by echocardiography.

Visceral sidedness is often defined separately for the abdominal organs, the cardiac structures, and the lungs, although they frequently follow one another. Sidedness is referred to solitus (normal), inversus (mirror image), or ambiguus (isomerism or indeterminate). In the latter situation, effort is made to define whether the organs that appear on both sides are right-sided (liver, right atrium, and trilobed lung) or left-sided (stomach/spleen, left atrium, bilobed lung) structures since this can have prognostic and therapeutic importance. For instance, patients with bilateral right-sidedness typically lack a spleen and require lifelong prophylactic antibiotics for encapsulated organisms and have malrotation of the intestine.

Venous connections of the superior and inferior venous systems must also be delineated. The usual connection of the SVC to the right atrium may also be accompanied by a persistent left SVC to the coronary sinus, with or without a bridging brachiocephalic vein. The IVC is derived from various embryologic vessels and can have its suprahepatic segment interrupted, in which case the normal connection of the IVC to the right atrium does not occur. In this case, lower extremity blood flow is routed to the SVC through the azygous or hemiazygous systems. Various pulmonary venous connections are described below.

Atria can be solitus with the morphologic right atrium on the right (normal), inversus, mirror image, common, or, rarely, indeterminate. The right atrium is typically identified by its venous connections (in particular, the coronary sinus), the presence of the crista terminalis, the large sail-shaped appendage, and the coarse pectinate muscles of the free wall. The left atrium is characterized by its smooth walls and narrow, finger-shaped appendage. Atrial morphology can typically be discerned by echocardiography, although angiography may also aid in their distinction. When the morphologic right atrium connects to the morphologic right ventricle (and similarly on the left), the connection is *concordant*. A *discordant* connection occurs when the morphologic right atrium connects to the morphologic left ventricle as in corrected transposition of the great arteries (TGA). When both atria connect to one ventricle (as in double inlet left ventricle) or a single ventricle, the type of connection is referred to as *univentricular*. An *ambiguous* connection occurs in cases of atrial isomerism.

The AV valves usually travel with their ventricle. As such, the tricuspid valve, when present, connects to the morphologic right ventricle, and the mitral valve connects to the morphologic left ventricle. The tricuspid valve has three leaflets and is distinguished from the mitral valve by the septal attachments of its papillary muscles and the slight inferior position of the septal leaflet of the tricuspid valve relative to the anterior leaflet of the mitral valve. When the AV valves fail to undergo septation, a common AV valve is found as in children with a complete AV septal defect. The position of the AV valves and their chordal attachments are used to define whether the valves are *malaligned* or *straddling*. A malaligned AV valve is not completely positioned over its respective ventricle, which is sometimes referred to as *overriding*. If the chordal attachments of an AV valve cross the septum and connect to the other ventricle, an AV valve is referred to as *straddling*.

The morphology of the ventricles, the associated AV valve, and the outflow portion of the ventricle can generally be used to identify the right and left ventricles. The right ventricle, besides being associated with the tricuspid valve, is more heavily trabeculated at its apex and anterior free wall than the left ventricle. In addition, an infundibular or conus ring exists in the right ventricle that separates the tricuspid and semilunar valves. The left ventricle, besides being more smooth walled with finer trabeculations at its apex than the right ventricle, demonstrates fibrous continuity

between the mitral and semilunar valves. When the ventricular morphology is uncertain, the ventricles are said to be *indeterminate*. A *common ventricle* is defined by virtual absence of the interventricular septum.

The great vessels are largely defined by their branching pattern. The pulmonary artery bifurcates shortly after exiting the heart into the right and left pulmonary arteries that undergo subsequent branching to supply the segments of the lung. The right pulmonary artery is positioned anterior to the right upper bronchus while the left pulmonary artery is posterior to the left upper bronchus. The pulmonary arteries typically follow the situs of the lungs such that mirror image pulmonary artery branching is seen in situs inversus, bilateral branch pulmonary arteries anterior to the upper bronchus are seen in right isomerism, and branch pulmonary arteries posterior to the upper bronchus are seen in left isomerism. The aorta is normally left sided, traveling to the left of the main bronchus, and gives rise to the three arch vessels. A right aortic arch passes to the right of the main bronchus before crossing back to the left side of the spine in the thorax and gives rise to mirror image arch vessels such that the first arch vessel, the brachiocephalic artery, bifurcates to give rise to the right subclavian and right carotid. While the aorta is typically posterior and rightward to the main pulmonary artery, the relative position of the vessels can vary greatly. Most commonly, in D-TGA, the aorta is anterior and rightward to the main pulmonary artery. In situations where only a single semilunar valve is present, a truncus arteriosus (or common truncal artery) is found that gives rise to both the aorta and pulmonary artery.

The ventriculoarterial connections are said to be *concordant* when the right ventricle connects to the pulmonary artery and the left ventricle gives rise to the aorta. The ventriculoarterial connection is *discordant* when the opposite occurs. The ventriculoarterial connection can also be *double*, *single*, or *common*. If both great arteries arise from one ventricle, a double outlet occurs. The definition of a double outlet connection is somewhat controversial. For example, in the case of double outlet right ventricle (DORV) with normally related great vessels, some clinicians have proposed basing the definition on whether greater than 50% of the aorta overrides the right ventricle, while others define the double outlet on whether a subaortic conus exists that results in mitral–aortic discontinuity. From a patient management perspective, both situations are relevant for either placement of the ventricular septal defect (VSD) patch or the potential for development of subaortic obstruction, respectively. A single outlet occurs when severe pulmonary hypoplasia occurs such that no main pulmonary artery segment is present. A common outlet occurs in truncus arteriosus.

Clinical Evaluation of the Newborn

Even with the technologic advances in prenatal and postnatal echocardiography and genetic testing, a careful history and physical examination are needed in every newborn with suspected CHD. Birth history, including complications during pregnancy, labor, and delivery, is important to document. Often the child with cyanosis because of structural heart disease has an unremarkable birth history. A difficult labor or delivery may point toward noncardiac causes of cyanosis such as persistent fetal circulation, infection, or pneumothorax. For the child with poor systemic perfusion, a history of premature rupture of membranes or maternal fever may suggest sepsis as a cause for the diminished cardiac function. Hematologic abnormalities that may cause cardiovascular dysfunction in the neonate, such as polycythemia or anemia, may

be suggested by a history of placental abruption or twin–twin transfusion.

Family history is critical to review with the biologic parents. There is a genetic basis for a growing number of congenital heart defects (see later). A sibling with CHD more than doubles the likelihood of future children having CHD (Nora and Nora, 1988). A history of CHD in either of the parents also increases the chance of developing a congenital heart lesion (Nora and Nora, 1987; Whittemore et al., 1994).

Physical examination of the newborn should initially include a general assessment looking for dysmorphic features and the degree of distress of the infant. The child with cardiac obstructive physiology may have shallow, rapid respirations with intercostal and suprasternal retractions. Cyanosis may or may not be seen depending on the degree of hemoglobin desaturation (roughly 5 g of hemoglobin must be desaturated to be clinically evident). Vital signs, including four extremity blood pressures, should be determined along with preductal and postductal oxygen saturation measurement. Palpation of the precordium may identify an overactive or displaced cardiac impulse or the sensation of a thrill caused by turbulent flow. Palpation of the abdomen for a liver edge or spleen tip can often provide an indication of volume overload or neonatal infection. Assessment of femoral and upper extremity pulses is essential. Simultaneous palpation of the right branchial and right femoral pulses allows assessment of comparable timing and intensity of the pulsations. Perfusion and capillary refill of the extremities are also important to determine.

Auscultation is often challenging in the sick neonate. However, characterizing the presence, timing, intensity, position, and radiation of murmurs that are present may provide a clue to the underlying diagnosis. In the tachypneic and tachycardic child, it is critical to listen over the head and liver for a continuous murmur that may indicate an arteriovenous malformation (AVF). The presence of a click or gallop over the precordium may indicate valvar disease or cardiac failure. Assessment of the second heart sound is particularly important. It has been suggested that the presence of physiologic splitting of the second heart sound nearly always suggests a structurally normal heart (El-Segaier et al., 2007).

Signs of CHF in the newborn may be subtle and include resting tachypnea (with no periodic variation), sinus tachycardia, and enlarged liver. Tachypnea may be accompanied by nasal flaring and intercostal and subcostal retractions, particularly in situations where elevated pulmonary venous pressures are present. Grunting respirations are a particularly concerning sign in a newborn and often accompany severe heart failure and decreased systemic perfusion.

Because many infants with CHD are asymptomatic in the newborn period and have a normal physical examination and because routine prenatal ultrasound does not detect all defects, it has been recommended that pulse oximetry screening should be added to the routine newborn screening panel (Mahle et al., 2012). The screen specifically targets critical congenital heart defects or those that usually require an intervention in the first month of life and can lead to death or significant morbidity if not diagnosed in a timely manner. Since this recommendation, there have been several studies focusing on the feasibility, implementation, and impact of the screen (Garg et al., 2013).

Laboratory Assessment of the Neonate

As mentioned previously, initial laboratory assessment should include measurement of preductal (right hand) and postductal (foot) oxygen

TABLE 55.1 Age-Dependent Standards

| Age Group | QRS axis | PRI | QRSD | QV6 (mm) | RV1 (mm) | SV1 (mm) | RV6 (mm) | SV6 (mm) | SV1 + RV6 |
|-----------|----------|-----------|-------------|----------|----------|----------|----------|----------|-----------|
| <1 day | 59–163 | 0.08–0.16 | 0.031–0.075 | 2 | 5–26 | 0–23 | 0–11 | 0–9.5 | 28 |
| 1–2 days | 64–161 | 0.08–0.14 | 0.032–0.066 | 2.5 | 5–27 | 0–21 | 0–12 | 0–9.5 | 29 |
| 3–6 days | 77–163 | 0.07–0.14 | 0.031–0.068 | 3 | 3–24 | 0–17 | 0.5–12 | 0–10 | 24.5 |
| 1–3 wks | 65–161 | 0.07–0.14 | 0.036–0.08 | 3 | 3–21 | 0–11 | 2.5–16.5 | 0–10 | 21 |
| 1–2 mo | 31–113 | 0.07–0.13 | 0.033–0.076 | 3 | 3–18 | 0–12 | 5–21.5 | 0–6.5 | 29 |
| 3–5 mo | 7–104 | 0.07–0.15 | 0.032–0.08 | 3 | 3–20 | 0–17 | 6.5–22.5 | 0–10 | 32 |
| 6–11 mo | 6–99 | 0.07–0.16 | 0.034–0.076 | 3 | 1.5–20 | 0.5–18 | 6–22.5 | 0–7 | 32 |
| 1–2 yr | 7–101 | 0.08–0.15 | 0.038–0.076 | 3 | 2.5–17 | 0.5–21 | 6–22.5 | 0–6.5 | 39 |
| 3–4 yr | 6–104 | 0.09–0.16 | 0.041–0.072 | 3.5 | 1–18 | 0.2–21 | 8–24.5 | 0–5 | 42 |
| 5–7 yr | 11–143 | 0.09–0.16 | 0.042–0.079 | 4.5 | 0.5–14 | 0.3–24 | 8.5–26.5 | 0–4 | 47 |
| 8–11 yr | 9–114 | 0.09–0.17 | 0.041–0.085 | 3 | 0–12 | 0.3–25 | 9–25.5 | 0–4 | 45.5 |
| 12–15 yr | 11–130 | 0.09–0.18 | 0.044–0.087 | 3 | 0–10 | 0.3–21 | 6.5–23 | 0–4 | 41 |
| Adult | –30–90 | 0.12–0.2 | 0.05–0.1 | | 0–10 | 0.5–23 | 4–23 | 0–4 | 35 |

saturations. Values less than 93% are considered abnormal. The *oxygen challenge test* is performed by increasing the inspired oxygen concentration to 100% for at least 5 minutes. Oxygen saturation values that increase into the normal range may be useful to distinguish an admixture cyanotic heart lesion from lung disease, although this test does not discriminate perfectly. History and physical examination should be included and guide the need for further evaluation. A decrease in postductal oxygen saturations compared with preductal values suggests right-to-left shunting because of an increase in PVR. The unusual situation in which the preductal saturation reading is less than the postductal reading occurs when TGA is combined with pulmonary hypertension and a patent ductus arteriosus (PDA).

An electrocardiogram (ECG) should be obtained in the initial evaluation of the newborn with suspected CHD, although, in the absence of an arrhythmia, it rarely provides a specific diagnosis. The neonatal ECG demonstrates prominent rightward forces and may have an upright T wave in the right precordial leads in the first few days after birth and thus may not be diagnostic of right ventricular hypertrophy. Age-dependent standards are available and should be referred to when evaluating the ECG (Table 55.1). Certain lesions may have distinctive findings on ECG such as extreme right axis deviation and Q waves in leads I and aVL (complete AV canal), preexcitation and right atrial enlargement (Ebstein anomaly), and Q waves in leads V1–V3 (corrected TGA).

A chest X-ray should also be obtained in every newborn that is evaluated for CHD. The chest X-ray may help to determine if lung disease is contributing to cyanosis. The heart size, shape, and border contours should be evaluated on the chest X-ray along with pulmonary vascular markings. A prominent thymic shadow in the newborn may make identification of classic chest X-ray findings difficult (such as the “boot-shaped” heart in tetralogy of Fallot (TOF), the “egg on a string” in transposition, and the “snowman” appearance in supracardiac total anomalous pulmonary venous return (TAPVR) although the massively increased heart size typically

found with Ebstein anomaly will not be missed. An absent thymic shadow may suggest DiGeorge syndrome (22q11 deletion), although genetic testing is still required. Increases in pulmonary vascular markings typically found in left-to-right shunt lesions may not be immediately apparent in the newborn because of the relatively high PVR and may take days or weeks to become apparent. Often, decreased pulmonary vascular markings in lesions with diminished pulmonary blood flow, such as tricuspid or pulmonary atresia, will be apparent in the newborn period. Presence of a PDA, however, will improve pulmonary blood flow in these lesions.

Echocardiography is truly the mainstay in the diagnosis of CHD in the neonate (described in detail in Chapter 53). While we encourage complete cardiovascular evaluation and consultation with a pediatric cardiologist in all children with suspected CHD, more programs are performing echocardiograms in neonates without the direct evaluation of the newborn by a pediatric cardiologist. In these instances, it is critical to have the echocardiographic examination reviewed by a pediatric cardiologist with some knowledge of the clinical condition of the child. Further, the pediatric cardiologist reading the echocardiogram should discuss the findings directly with the caregivers in order for the team to define relevant therapy and follow-up.

Genetics and Congenital Heart Disease

Our understanding of the genetic basis of CHD is progressing at a rapid pace. The early identification of the association of specific syndromes with CHD suggested that it would be possible to identify genes relevant to abnormal cardiac morphogenesis. With the completion of the Human Genome Project and the availability of large scale sequencing techniques, genetic causes for CHD continue to become more highly refined. For example, copy number variants, a term used to reflect the quantity of genes expressed when large segments of DNA are amplified or deleted, contribute to the syndromic CHD found in DiGeorge syndrome where a 3 megabase

disruption occurs at 22q11. A variety of copy number variants contributing to both syndromic and nonsyndromic CHD disease have been identified (Fahed et al., 2013).

The list of point mutations that cause a particular CHD continues to grow as individuals and families are studied using next-generation sequencing. Considerable postprocessing of the sequence data is needed to exclude common polymorphisms in the sequence. Syndromic CHD found in Alagille syndrome, Noonan syndrome, and Holt–Oram syndrome has been linked to point mutations. The list of nonsyndromic point mutations causing isolated CHD continues to grow (Allen et al., 2008; Fahed et al., 2013).

Despite these advances in the understanding of the genetic basis of CHD, the genetic basis of more than half of children with CHD is not known. It is likely that altered interactions with noncoding regions of the genome impact the degree to which genes that regulate cardiac development are expressed. There may also be gene–environment interactions, yet to be defined, which impact cardiac development.

A child with suspected CHD should be carefully evaluated for dysmorphic features that may indicate an associated syndrome. The converse is also true in that if a newborn is suspected to have a syndrome, careful cardiac evaluation, including an echocardiogram, should be considered.

Screening chromosomes are an important initial step in the genetic evaluation of the newborn with a presumed syndrome. Well-described associations of congenital cardiac lesions with chromosome abnormalities include trisomy 21 with complete AV septal defect, Turner syndrome (45 XO) with coarctation of the aorta, William syndrome (7q11 deletion) with valvar and supravalvar pulmonic stenosis, and DiGeorge or velocraniofacial syndrome (22q11 deletion) with conotruncal anomalies. These syndromes, and others, make karyotype analysis with fluorescent in situ hybridization (FISH) analysis an important initial step in the work-up of infants with these lesions.

Genetic testing should be performed in all newborns with suspected syndromes whether or not CHD is present. FISH studies for 22q11 deletions should be performed in all infants with conotruncal anomalies such as TOF, truncus arteriosus, and interrupted aortic arch. Other FISH studies can be specifically ordered at most institutions although the maximum number of fluorescent probes is typically four or five due to detection limitations of the range of wavelengths available. Genome-wide chromosome microarray screening is now also available that can detect deletions and duplications across the genome.

Genetic testing is not only useful to help provide prognostic information for a specific patient but also to provide counseling for the family. Referral to a consulting genetics faculty should be considered whenever a karyotype, FISH study, or genome-wide chromosome microarray is found to be abnormal. Consideration of services available to the infant, screening of additional family members, and risk to future pregnancies will be reviewed during the genetics consultation that will impact on the overall care of the patient.

Heart Transplantation

Surgical palliation for congenital heart lesions allows most structural abnormalities in the newborn period to be corrected or palliated. In instances where the abnormality is primarily myocardial or surgical repair has not sufficiently corrected the child's hemodynamics, cardiac transplantation may be the only therapeutic option.

The first heart transplant in an infant was reported in 1968 (Kantrowitz et al., 1968). Since then, understanding of transplant immunology and medical management has made heart transplant in infants and children an important option for inoperable patients or those with end-stage cardiac disease. In a recent scientific statement from the American Heart Association (Canter et al., 2007), the indications for pediatric heart transplant were defined (adapted from Canter et al., 2007):

1. Need for ongoing intravenous inotropic or mechanical circulatory support
2. Complex CHD not amenable to conventional surgical repair or palliation or for which the surgical procedure carries a higher risk of mortality than transplantation
3. Progressive deterioration of ventricular function or functional status despite optimal medical care
4. Malignant arrhythmia or survival of cardiac arrest unresponsive to medical therapy, catheter ablation, or an automatic implantable defibrillator
5. Progressive pulmonary hypertension that could preclude future transplantation
6. Growth failure secondary to severe CHF unresponsive to conventional medical therapy
7. Unacceptably poor quality of life

There are a variety of lesions in the neonate for which cardiac transplantation has been used as primary palliation. These have included hypoplastic left heart, pulmonary atresia with intact ventricular septum and presence of coronary sinusoids, complex heterotaxy or unbalanced complete AV canal with poor common AV valve function, and single ventricle hearts where the dominant semilunar valve is severely insufficient. When considering transplantation in these patients, there is a balance between the success of palliative surgery and the availability of organs for transplantation. For example, over the past decade, survival of patients undergoing surgical palliation of hypoplastic left heart syndrome (HLHS) has continued to improve (Alsoufi et al., 2007a; Gordon et al., 2008). With the availability of infant donors increasing only slightly over this period of time (Table 55.2), the balance for treatment of these newborns has shifted toward surgical palliation with the Norwood or hybrid Norwood procedures.

Patients with the other lesions noted in this section continue to have relatively poor surgical outcomes, suggesting that palliation with heart transplantation may be the best approach. Given the limited availability of organs, however, it should be recognized that some of these infants will not survive to transplant (Kirklin et al., 2006). The long-term survival of infants who undergo heart transplantation is quite good. The 10-year survival in infants continues to improve, with current survival rates of 66% (Boucek et al., 2007). It is interesting that there appears to be a survival advantage in children who receive their transplant prior to 1 month of age versus those between 1 and 12 months (del Rio, 2000). While the mechanism of the improved survival in the younger neonates is not known,

TABLE 55.2 Number of Available Donors for Infant Heart Transplant by Year in the United States

| Year | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 |
|--------|------|------|------|------|------|------|------|------|------|------|
| Donors | 89 | 87 | 100 | 116 | 105 | 110 | 106 | 117 | 120 | 126 |

Data from the Organ Procurement and Transplant Network Database (<http://optn.transplant.hrsa.gov>)

it may reflect an immunologic window where graft rejection and transplant coronary artery disease are limited.

Ventricular Assist Devices

Over the past several years, mechanical circulatory support devices have increasingly been developed for the pediatric population to serve as a bridge for postoperative cardiac recovery or heart transplantation. While the availability of devices for neonates lags far behind older children and adults, options are now available.

The mechanical circulatory support device approved for use in children is the Berlin Heart EXCOR pediatric ventricular assist device (VAD). This is a pneumatically driven device that comes in five sizes, with the smallest of the devices delivering a volume of 10 mL per stroke. One or two devices can be implanted to support one or both ventricles, respectively. Studies have looked at survival and complications in infants in the 3–5 kg range and a corrected gestational age of at least 37 weeks of age, in comparison with older children (Almond et al., 2013). Early mortality was increased in association with lower body weight. In the 33 infants included in the study who were less than 5 kg, 64% died following VAD implantation.

Given the challenges of the use of current VADs in infants, other devices continue to be developed (Baldwin et al., 2011; Vanderpluym et al., 2014) and are either in the experimental stage or available for compassionate use. The development of these smaller devices has been supported, in part, by the Pediatric Circulatory Support Program of the National Heart, Lung, and Blood Institute. Several devices are in various stage of testing (Baldwin et al., 2011), some of which are small enough in size for use in infants.

Murmurs in the Newborn—Congenital Cardiac Lesions

Patent Ductus Arteriosus and Aortopulmonary Window

As discussed earlier, virtually every child is born with a PDA that typically closes within the first week of life. Prematurity is a risk factor for a persistent PDA and is covered in Chapter 54. Persistent PDA in term infants occurs more commonly in females and may have a genetic component in some patients, as suggested by an animal model of inbred poodles (Knight et al., 1973) and linkage to chromosome 12 (Mani et al., 2002).

The pathophysiology of a PDA largely depends on the degree of shunting from the aorta to the pulmonary artery, which is determined by the inner diameter and length of the PDA and the relative pulmonary and systemic resistances. If the PDA diameter is small, the ductus itself will provide the primary site of resistance to flow, and the shunt will be small. In the case of a larger PDA, low PVR may allow for a significant shunt that places the patient at risk for developing heart failure and, eventually, pulmonary vascular occlusive disease. The low resistance pathway through the lungs provides a route for diastolic run-off from the aorta that can result in decreased coronary perfusion pressure. The diastolic steal by the pulmonary artery can lead to myocardial ischemia.

Clinically, patients with a small shunt will be asymptomatic. With a larger PDA shunt, the progressive decline in PVR postnatally will cause an increase in left-to-right shunt flow with signs of increasing heart failure. The murmur in a child with a PDA is generally continuous and has been described as a “machinery”

murmur in the left infraclavicular region. The character of the murmur, however, varies greatly, although the continuous nature is generally present once the PVR has declined. Examination of the patient with a PDA will also include a wide pulse pressure because of decreased diastolic pressure and bounding pulses.

The diagnosis of PDA, when suspected on examination, can nearly always be confirmed by echocardiography. Even in the face of high PVR with limited left-to-right shunting, differences in the pulse waveforms between the aorta and pulmonary artery will allow left-to-right and/or right-to-left shunting to be observed by color Doppler imaging. Echocardiography can also provide information regarding the degree of left-to-right shunting, as the dimensions of the left atrium and left ventricle are increased and the left atrium-to-aorta ratio is increased. In addition, retrograde flow will be seen in the proximal descending aorta when a large shunt is present. Cardiac catheterization is rarely needed unless coil closure of the PDA is considered.

The prognosis for small PDAs is quite good, and debate exists as to whether closure of “silent” PDAs, incidentally identified by echocardiography, should be performed (Giroud and Jacobs, 2007). With the most recent recommendations from the American Heart Association suggesting that PDAs do not require subacute bacterial endocarditis prophylaxis (Wilson et al., 2007), there is little need to close these small vessels.

Because of the long-term concerns of pulmonary overcirculation and the development of pulmonary vascular occlusive disease, closure of PDAs by 1 year of age is recommended when a significant left-to-right shunt is present. Surgical ligation and division can readily be performed through a lateral thoracotomy. However, coil or device closure in the catheterization lab has a lower morbidity and success rates equal to surgery (Arora, 2005). As a result, catheter closure of PDAs has become the preferred method in nonpremature infants. Success of device closure depends in part on the weight of the infant and size and shape of the PDA. With the current array of devices available, closure can be safely performed in most infants weighing more than 4 kg and can be considered in some infants weighing between 2.5 and 4 kg (Brunetti et al., 2010; Dimas et al., 2010).

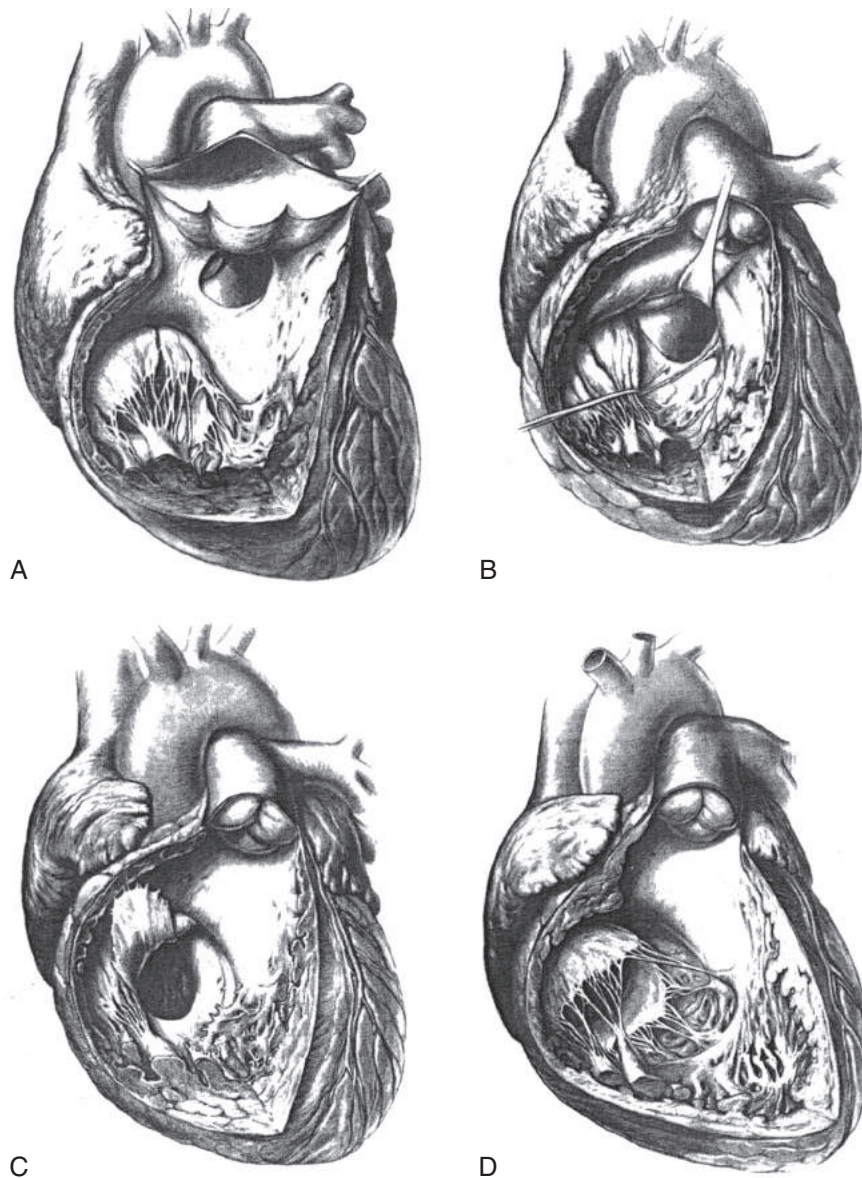
Aortopulmonary (AP) window occurs when there is direct communication between the aorta and main pulmonary artery and is a rare lesion that can readily be confused with PDA on both physical and laboratory evaluation. Nearly half of patients with an AP window will have an associated cardiac anomaly. These lesions nearly always result in a large degree of left-to-right shunting. Patients will show signs of CHF on examination and have physical and laboratory findings similar to those for a large PDA. Generally, all AP windows should be closed when they are identified. Closure of an AP window must be done surgically, generally with patch closure from the pulmonary artery side being the preferred technique.

Ventricular Septal Defect

VSDs are the most common type of CHD (excluding bicuspid aortic valve). Generally, VSDs are classified into four types (Fig. 55.2):

1. Perimembranous
2. Muscular
3. Inlet
4. Outlet

The perimembranous VSD is the most common of the four types and has variably been referred to as membranous, paramembranous, or infracristal. From the right ventricular side of the heart, these defects lie under the septal leaflet of the tricuspid valve below the



• **Fig. 55.2** Anatomic Varieties of Ventricular Septal Defect. (A) Subpulmonary defect. (B) Membranous defect. (C) Inlet (atrioventricular septal defect type) defect. (D) Apical muscular defects. (From Mavroudis C, Backer CL. *Pediatric Cardiac Surgery*. St. Louis, MO: Mosby Year Book; 1994.)

crista supraventricularis and posterior to the papillary muscle of the conus. Muscular VSDs can occur in isolation or as multiples (“Swiss cheese septum”) and, as the name implies, can occur anywhere in the muscular septum. Apical muscular VSDs are the most common and are sometimes difficult to accurately size by echocardiography because of the heavy trabeculations at the apex of the right ventricle. Inlet VSDs are located posterior and inferior to perimembranous defects, and, although the nomenclature is controversial, this is the location of the defect in patients with complete atrioventricular septal defects. The location of the VSD in patients with outlet defects is above the crista supraventricularis and typically undermines the right aortic valve leaflet. A variety of synonyms have been used for outlet VSDs including subarterial, subaortic, supracristal, and conal VSD.

The clinical importance of any VSD is dependent upon the size of the defect and the relative pulmonary-to-systemic vascular resistance, which together determine the degree of left-to-right shunting. An

additional consideration with outlet VSDs is the degree to which the right coronary cusp of the aortic valve prolapses into the defect and results in aortic insufficiency. Defects whose cross-sectional area is equal to, or greater than, the cross-sectional area of the aortic valve will not restrict flow leaving the left ventricle and entering the right ventricle. In this case, the degree of shunting will be determined by the relative resistance to flow in the pulmonary and systemic vascular beds. The normal postnatal decline in PVR will result in a progressive increase in left-to-right shunting and signs of CHF. A small percentage of children do not have the usual postnatal decline in PVR and may never develop signs of pulmonary overcirculation and heart failure despite the presence of a large VSD. This is an indication for early surgical closure of the defect.

When the VSD is small relative to the aortic valve, the defect itself will be the primary point of resistance to shunt flow. In this case, changes in PVR will have little impact on the degree of left-to-right shunting.

An important associated lesion that is critical to rule out is coarctation of the aorta. The coarctation results in a fixed elevated systemic vascular resistance that can result in important left-to-right shunting even in the presence of a small VSD. In this case, medical therapy is often unable to control CHF symptoms, and surgery is needed.

The volume of shunted blood is most accurately quantitated at cardiac catheterization based on the step-up in oxygen saturation from the right atrium (mixed venous) to the pulmonary artery and is represented as the ratio of pulmonary-to-systemic blood flow ($Q_p : Q_s$). Generally, a $Q_p : Q_s$ less than 1.5 is considered below the threshold for surgery, while a $Q_p : Q_s$ greater than 2.0 is an indication for surgery. The $Q_p : Q_s$ can also be estimated by echocardiography and by magnetic resonance imaging (MRI).

The examination of the patient with a VSD depends on the magnitude of the shunt. Small defects that provide considerable restriction to flow often have the loudest murmur. The rapid drop in PVR immediately after birth often allows VSD murmurs to be heard in the newborn nursery, although the full extent of the murmur, and perhaps a thrill at the lower left sternal border, may not be appreciated for several weeks. The murmur may have a more ejection quality in the newborn nursery in the face of high PVR and somewhat elevated right ventricular pressure. The more typical holosystolic murmur will be more apparent as the PVR falls.

With large VSDs, little or no murmur may be heard depending on the PVR. With low PVR, signs of heart failure will likely be present including tachypnea with nasal flaring and retractions, tachycardia, diaphoresis, poor feeding, and diminished weight gain. A systolic murmur at the lower left sternal border (caused by flow across the VSD) or upper left sternal border (caused by increased flow across the right ventricular outflow tract [RVOT]) may be heard along with a diastolic inflow rumble (an absence of silence) at the apex. If PVR is high, the pulmonic component of the second heart sound may be increased although difficult to appreciate. Occasionally, large defects may allow transient right-to-left shunting to occur, particularly when the infant is crying.

In patients with an outlet VSD, the holosystolic murmur is often present, but the murmur is located higher on the left sternal border. Care should be taken to listen for the diastolic decrescendo of aortic insufficiency at the mid-left sternal border or at the apex.

The laboratory evaluation of the infant with a suspected VSD should include an ECG, chest X-ray, and echocardiogram. In infants, the ECG may not be distinctive unless an inlet VSD is present. Obtaining a chest X-ray, even in the neonate, is important in order to assess heart size and pulmonary vascular markings. It can also be an important tool in the follow-up of newborns with VSDs when used to assess progression in left-to-right shunting as the PVR declines. Echocardiography is the gold standard for characterizing the location and size of VSDs. Associated lesions, such as coarctation of the aorta, can also be readily assessed by echocardiography. Doppler studies can estimate the degree of restriction by calculating the pressure drop at the defect (see Chapter 53). M-mode measurements can be used to determine left ventricular dimensions, which will be increased when a significant left-to-right shunt is present. As mentioned above, cardiac catheterization can accurately quantitate the degree of shunting but is rarely needed in the initial assessment of the newborn with VSD.

Up to 80% of small, muscular VSDs and 30%–50% of perimembranous defects will close spontaneously. It is uncommon for these defects to increase in size although the degree of left-to-right shunting can increase as PVR drops. Occasionally, inlet VSDs will undergo closure secondary to chordal attachments of the AV valves,

but closure is generally present at birth if it is going to occur. Outlet VSDs virtually never close and have the associated risk of progressive aortic insufficiency because of prolapse of the right coronary cusp into the defect.

The short-term consideration in following patients with VSDs is the management of CHF if the left-to-right shunt is excessive. Medications that are used include furosemide (typically 1 mg/kg per dose b.i.d [twice daily]), digoxin (5 mcg/kg per dose b.i.d), and afterload reduction with enalapril (initial dose of 0.05 mg/kg per dose b.i.d, up to 0.2 mg/kg per dose b.i.d, when monitoring blood pressure). The primary goal in controlling CHF is to allow the newborn to grow adequately and hopefully allow progressive closure of the VSDs. Weight gain is a useful and objective measure to follow.

The long-term goal of therapy or intervention is to prevent the development of irreversible pulmonary vascular occlusive disease. Shunts with a $Q_p : Q_s$ greater than 2.0 are at long-term risk of developing Eisenmenger disease, and closure of the defect is warranted beyond 2–4 years of age when further decline in defect size is unlikely.

If medical therapy fails to control heart failure and the infant exhibits failure to thrive, surgical closure of the defect in the first 6 months of life is required. Surgical closure is also indicated in the 6–12-month-old child with a large VSD who has not demonstrated signs and symptoms of CHF due to lack of decline in PVR. In these infants, irreversible changes in the pulmonary vasculature may occur if the pressure load is not taken off the lungs. As mentioned previously, defects with a $Q_p : Q_s$ greater than 2.0 and outlet defects also require closure. The use of pulmonary artery banding is falling out of favor for palliation of patients with VSDs unless the patient's clinical condition makes complete repair untenable. There is growing use of hybrid procedures where the surgeon and interventional cardiologist work together to close defects in small children (Contrafouris et al., 2009). This combined approach has successfully been applied to the closure of muscular VSDs in infants that are positioned in a location that is difficult for the surgeon to visualize from a right atrial approach. Additional indications for the hybrid approach to VSD closure will likely be determined in the coming years.

Atrial Septal Defects

Although atrial septal defects (ASDs) rarely produce a murmur in the neonatal period, the systolic murmur they generate later in life makes them appropriate for this section. As discussed earlier, atrial level shunting through the foramen ovale in utero allows the nutrient-rich placental blood to gain access to the left ventricle and ascending aorta. In the immediate postnatal period, careful echocardiography examination of the interatrial septum will usually identify residual left-to-right or bidirectional shunting through the foramen ovale. Measurement of the size of the shunt provides an indication of whether there will be a persistent septal defect. An opening less than 6 mm in a term infant will most likely close and is referred to as a patent foramen ovale (PFO) to distinguish it from a true ASD. ASDs that represent congenital lesions are classified as:

1. Secundum
2. Primum
3. Sinus venosus
4. Coronary sinus

Secundum defects are the most common and, when present in the neonate, allow for left-to-right or bidirectional shunting. The

direction of the shunt, and the reason an ASD murmur is typically not heard in the neonate, is that right ventricular compliance remains low until the RV remodels postnatally. The reduced RV compliance increases right atrial pressure and limits the amount of left-to-right atrial level shunting. Secundum ASDs are associated with Holt–Oram syndrome (abnormal radii, first degree AV block, and ASD). Primum ASDs are in the spectrum of atrioventricular septal defects and will be considered in the next section. Sinus venosus defects occur when the wall separating the upper or lower right pulmonary vein is deficient so that pulmonary venous return from either or both veins spills into the right atrium. This results in a left-to-right shunt. This variant of anomalous pulmonary venous return will be considered in greater detail further in the chapter. Coronary sinus defects result from an “unroofing” of the coronary sinus so that the coronary sinus enters at the left atrium–right atrium junction where the septum is deficient. Often, this lesion is associated with a persistent left SVC that enters into the coronary sinus.

In later childhood, when the compliance of the right ventricle increases and left-to-right shunting through an ASD increases, the classic physical findings of fixed splitting of the second heart sound. A systolic ejection murmur at the left upper sternal border, and a diastolic right ventricular inflow murmur at the lower left sternal border become apparent in lesions with a $Q_p : Q_s$ more than 2.0. ECG will often demonstrate an rsR' pattern in the right precordial leads with evidence of right ventricular hypertrophy. Cardiomegaly with a prominent main pulmonary artery segment and increased vascular markings will be seen on chest X-ray. Echocardiogram remains the gold standard for identifying and sizing the defects.

It is uncommon for infants and children with an isolated ASD to develop heart failure. Typically, intervention for a significant ASD is performed to prevent the long-term sequelae of pulmonary vascular occlusive disease. As such, closure is rarely performed before the age of 3 or 4 years. Device closure of secundum ASDs is now routinely performed in the catheterization lab when children are greater than 15 kg, although surgical closure is still performed when lesions are large or the rim of tissue between the ASD and aorta is deficient.

Atrioventricular Septal Defects

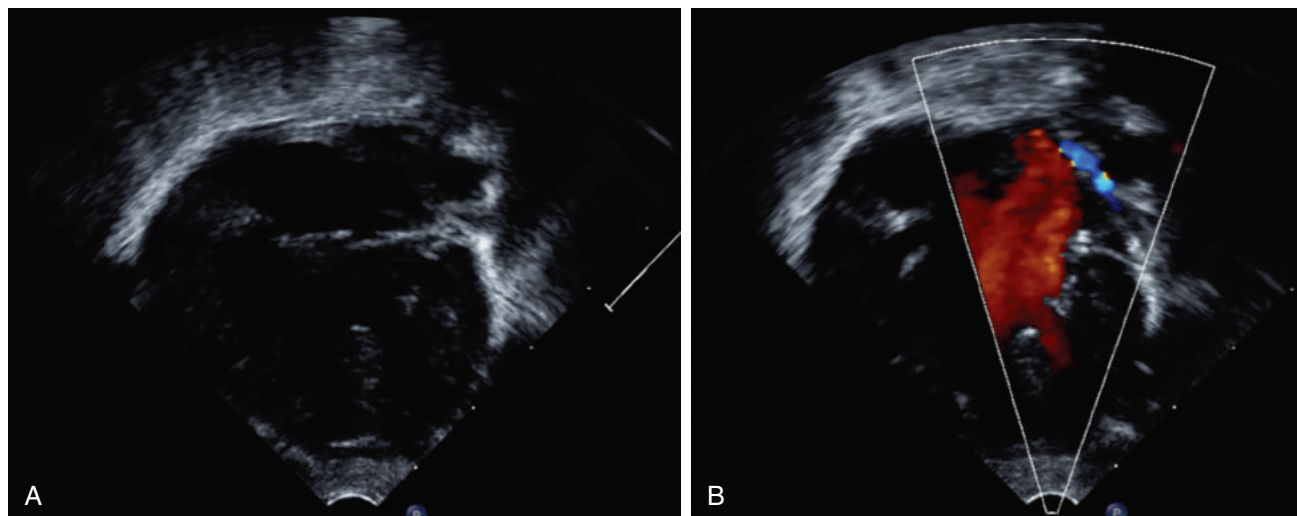
A variety of terms have been used to describe AV septal defects including AV canal defect, endocardial cushion defect, and common AV orifice. In addition, there is a spectrum of AV septal defects:

1. *Complete*—common AV valve is present along with a significant primum ASD and inlet VSD.
2. *Intermediate*—common AV valve annulus although separate AV valves along with a primum ASD and inlet VSD.
3. *Transitional*—completely separate AV valves along with a primum ASD and inlet VSD.
4. *Partial*—separate AV valves with a primum ASD and no VSD.

These four groups have some therapeutic relevance with regard to the degree of AV valve abnormality that must be considered at surgery, although each has a cleft between the anterior bridging leaflet and the lateral leaflet of the left-sided (mitral) valve that must be addressed. In infants with a complete AV septal defect, it is important to assess whether the right and left ventricles are *balanced*, that is, equally developed so that a two-ventricle repair is possible, and the degree of AV insufficiency, which has prognostic importance in the surgical outcome. AV septal defects are common in children with Down syndrome.

In the immediate postnatal period, variations in PVR that occur, particularly in infants with Down syndrome, can result in a right-to-left shunt through a nonrestrictive primum ASD and/or inlet VSD and result in systemic desaturation. Many infants with complete AV septal defects will develop heart failure in the first 2 months of life as PVR falls. A small percentage of infants will not have a decline in PVR, making the lack of heart failure a troubling sign and an indication for early surgical repair.

While diagnosis largely rests on the echocardiogram, AV septal defects can be suspected on ECG with the presence of a superior frontal QRS axis (ranging from -30 to -120 degrees), evidence of right ventricular hypertrophy, and Q waves in leads I and aVL. Echocardiogram is used to define the type of AV septal defect and identify associated anomalies (Fig. 55.3). As mentioned above, critical assessment of relative ventricular sizes and the degree of AV valve insufficiency is necessary. Interrogation of the RVOT is



• **Fig. 55.3** Echocardiographic View of Complete Atrioventricular Septal Defect. (A) Four chamber view showing the common atrioventricular valve that separates the atria and ventricles, which in this patient are well balanced. The large primum atrial septal defect and inlet ventricular septal defect are seen. (B) With color Doppler, inflow across the atrioventricular valve is seen.

also needed due to the associated pulmonic stenosis or TOF that can occur with complete AV septal defects.

Correction of complete AV septal defects is performed surgically at 4–6 months of age. While awaiting surgery, care must be taken not to treat minor desaturation episodes with excessive oxygen since oxygen-induced lowering of PVR can rapidly worsen heart failure and lead to further desaturation, a spiral that can be difficult to reverse. Furosemide, digoxin, and afterload reduction are often needed to control heart failure, and some cardiologists will start these medications in the immediate postnatal period because of the high likelihood of infants developing CHF. Complete repair (septation atria, ventricles, and AV valves) is preferred. It is very rare that palliative banding of the pulmonary artery is needed to control heart failure symptoms. As mentioned above, early repair should be considered in the infant who does not have a decline in PVR and the development of heart failure, because of the concern of early development of irreversible increases in PVR, particularly in non-Down syndrome complete AV septal defect patients.

Peripheral Pulmonic Stenosis

Peripheral pulmonic stenosis (PPS) can range widely in its severity. In many instances, mild narrowing of the branch pulmonary arteries occurs in the neonate and produces a murmur that is heard widely throughout the chest. The murmur caused by this mild PPS is considered by some to be an “innocent murmur” of infancy and usually resolves by 2–4 months of age. This lesion likely reflects mild hypoplasia of the branch pulmonary arteries because of decreased in utero pulmonary blood flow and the postnatal transition where these vessels must accommodate the entire cardiac output following ductal closure.

More severe PPS is seen in cases of congenital rubella syndrome or in association with Williams or Noonan syndrome. Branch pulmonary artery stenoses can also occur with TOF. In these cases, multiple levels of obstruction may exist that require catheterization or surgical intervention.

In isolated or mild PPS, minimal right ventricular pressure overload occurs. With increasing stenosis, right ventricular pressure overload will result in right ventricular hypertrophy and, in later stages, right atrial enlargement on ECG. Echocardiogram can interrogate the proximal pulmonary arteries, but more-distal lesions require other imaging modalities such as MRI or cardiac catheterization.

Intervention to treat severe branch stenoses should be considered when right ventricular pressure is greater than 75% of systemic pressure or any clinical or laboratory evidence of right ventricular dysfunction is present. Surgical management is possible for proximal areas of stenosis, although the treatment preferred by most clinicians is balloon dilation or expandable stent placement in the cardiac catheterization laboratory. Repeated interventions may be needed to enlarge vessels as the patient grows or to dilate other areas of stenosis that develop.

Pulmonic Stenosis

Isolated pulmonary valve stenosis is a common cause of a systolic ejection murmur in the neonatal period. While cases typically occur sporadically, more than 50% of infants with Noonan syndrome will have pulmonic stenosis, and thus the pulmonary valve should be evaluated in all these children.

The degree of pulmonic stenosis determines the pathophysiology of the disease process. As the stenosis of the valve worsens, right

ventricular pressure increases along with the degree of right ventricular wall stress. Right ventricular hypertrophy will develop if the stenosis is left untreated. In severe or critical pulmonic stenosis (discussed later in the section), heart failure can develop in the neonate accompanied by cyanosis due to right-to-left shunting at the atrial level.

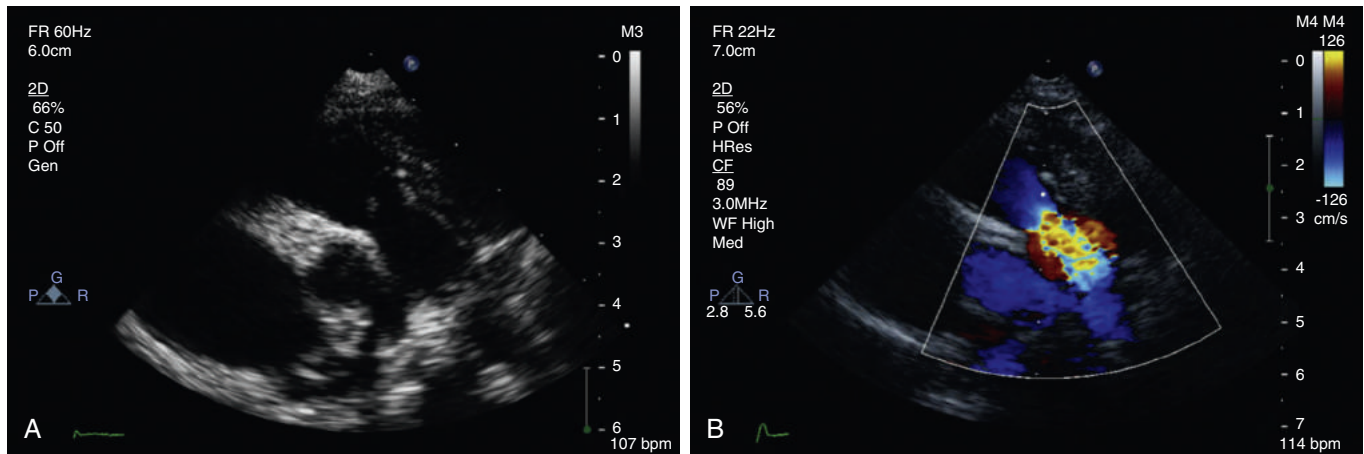
The degree of stenosis is generally classified based on the pressure drop across the pulmonic valve with mild stenosis defined as a gradient less than 30 mmHg, moderate stenosis as a gradient of 30–60 mmHg, and severe stenosis as higher than 60 mmHg. While these definitions were initially based on cardiac catheterization, current follow-up of patients with pulmonic stenosis utilizes echocardiogram, which overestimates the gradient compared with direct hemodynamic measurement obtained from cardiac catheterization. Thus some use an *echo gradient* of more than 40 mmHg as mild pulmonic stenosis.

Consideration of the valve gradient is critical because of the prognostic significance of the value. In older children, mild stenosis rarely progresses (Nugent et al., 1977). In infants, however, follow-up of patients with echo gradients over 40 mmHg found that 29% developed progressive valve stenosis, with half of those showing an increase in the first 6 months of life (Rowland et al., 1997). Neonates with moderate valve stenosis may face an even greater likelihood of developing progressive stenosis, although limited data exist.

A systolic ejection murmur of pulmonic stenosis can be heard in the neonatal period at the upper left sternal border. Typically, although the fast heart rate in the neonate may make it difficult to appreciate, a systolic ejection click just after the first heart sound (S1) can be heard in most of these infants and is an important feature to distinguish pulmonic stenosis from other lesions. Much like PPS (see earlier), the murmur of pulmonic stenosis radiates throughout the lung fields. As the gradient across the valve worsens, a thrill may be palpable at the upper left sternal border. As the degree of stenosis progresses further and becomes severe, the murmur and click will diminish and may even be absent as right ventricular dysfunction worsens. Of note is that while progressive pulmonic stenosis may be able to be estimated on the basis of the murmur, the clinical condition of the infant may not change appreciably until the degree of stenosis becomes severe.

The findings of laboratory studies in infants with pulmonic stenosis will vary depending on the degree of stenosis. ECG will demonstrate right ventricular hypertrophy in most patients with moderate stenosis, although the study may be normal when mild stenosis is present. Chest X-ray is often normal unless poststenotic dilation of the main pulmonary artery has developed. Echocardiography will be diagnostic. The valve will have varying degrees of dysplasia that is characterized by thickened, poorly mobile leaflets that dome during systole. Turbulence distal to the valve will be seen by color Doppler imaging (Fig. 55.4), while pulse wave Doppler is used to determine the degree of stenosis. As discussed previously, Doppler echo generally gives a value of stenosis that is 20%–30% higher than the peak-to-peak pressure gradient measured as cardiac catheterization.

Treatment of isolated pulmonic stenosis, even in the neonate, can readily be performed by balloon valvotomy in the cardiac catheterization laboratory. The exception may be in infants with Noonan syndrome where the degree of valvar dysplasia may prevent an adequate result, although most will initially attempt a balloon valvotomy before progressing to surgery. Valvotomy should be considered in infants with more than mild stenosis. Mild or moderate pulmonary insufficiency, should it develop after balloon



• **Fig. 55.4** Pulmonic Stenosis. (A) Short-axis view of pulmonic stenosis with a thickened-appearing pulmonic valve. (B) Using color Doppler imaging, turbulence in the main pulmonary artery is seen above the pulmonic valve.

valvotomy, is usually well tolerated. Recurrent stenosis and more significant pulmonic insufficiency are found more often when valvotomy is needed in the neonatal period (Garty et al., 2005).

Aortic Stenosis

Similar to stenosis of the pulmonic valve, aortic valve stenosis can produce a murmur in the neonatal period. However, aortic stenosis is more likely to be progressive than pulmonic stenosis. Levels of obstruction of the left ventricular outflow tract can also occur at the subvalve and supravalue levels. Subvalvar stenosis is rarely diagnosed in the neonatal period but often progresses later in life as a fibromuscular ridge beneath the aortic valve. Supravalar stenosis can present in the newborn period and is often associated with Williams syndrome.

As with pulmonic stenosis, the pathophysiology and physical findings associated with aortic valve stenosis depend on the degree of obstruction. Certainly the most common aortic valve abnormality addressed in the neonatal period is critical aortic stenosis associated with decreased left ventricular function. This medical emergent situation is addressed in detail later. Mild (aortic valve gradient <30 mmHg) and moderate (valve gradient of 30–60 mmHg) aortic valve stenosis is not commonly encountered in the neonatal period. When it is, these milder forms of stenosis are generally associated with a bicuspid aortic valve and tend to progress with age. The rate of change of progression throughout infancy and childhood is quite variable and necessitates frequent follow-up of these patients.

The murmur in the newborn with aortic stenosis may be difficult to localize to the upper right sternal border as in older children. A thrill is usually palpable at the suprasternal notch with even mild aortic valve stenosis, with a precordial thrill felt as the degree of stenosis increases. Usually a click from the stenotic aortic valve is heard at the apex or lower left sternal border.

Electrocardiography may be of limited benefit in the newborn with mild or even moderate aortic valve stenosis, although some degree of left ventricular hypertrophy may be seen. Chest X-ray is of limited benefit in following these patients, with the focus of the diagnostic assessment being the echocardiogram. On echocardiogram, the aortic valve can be characterized. Fused leaflets that are thickened and domed are seen. Pulsed Doppler is used to determine the gradient across the valve while color Doppler imaging can define the presence of aortic insufficiency.

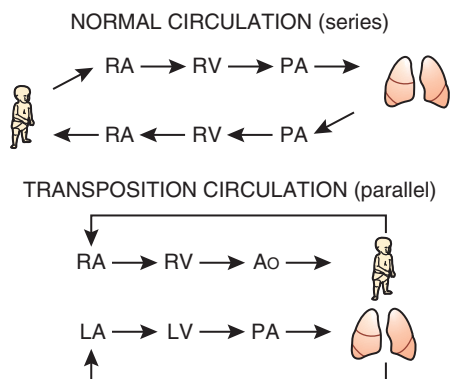
Initial management of moderate aortic valve stenosis is balloon valvotomy in the cardiac catheterization laboratory. The balloon is sized in an effort to limit aortic insufficiency, which is much less well tolerated than insufficiency of the pulmonic valve. If significant aortic valve insufficiency is present or develops, surgical repair or valve replacement is needed. In neonates and infants, the Ross procedure is usually the procedure of choice (Elkins et al., 1994). In this procedure, the pulmonary valve is removed intact from the patient and sewn into the aortic position, and a homograft is placed in the pulmonic position. Interestingly, the pulmonary autograft generally demonstrates good growth and excellent function in the aortic position. While the child will outgrow the pulmonary homograft, replacing it and managing progressive pulmonic stenosis are much easier than addressing the aortic valve.

Cyanosis in the Newborn

Congenital heart defects that present with cyanosis do so because of a right-to-left shunt. The shunt results in mixing of the systemic and pulmonary venous returns that can occur between the atria, ventricles, or great vessels. Defects presenting predominantly with cyanosis can be further subclassified by the amount of associated pulmonary blood flow. If there is no restriction to pulmonary blood flow, cardiac output to the lungs will increase as the normal postnatal drop in PVR occurs. Clinically, this results in tachypnea, poor feeding, hepatomegaly, and pulmonary edema. Much of the neonatal management is aimed at balancing the ratio of systemic to pulmonary blood flow. Defects with restriction to pulmonary blood flow typically present with cyanosis without associated symptoms of CHF. If the restriction is severe, pulmonary blood flow may be dependent upon a left-to-right shunt through the ductus arteriosus. These patients typically have some degree of right ventricular hypertension. If the ductus closes profound cyanosis results. Lesions presenting primarily with cyanosis in the newborn period are discussed later.

Transposition of the Great Arteries

TGA accounts for 5% of all cases of cyanotic CHD and is the most common cyanotic cardiac defect in the newborn. There is a strong male predominance (60%–70%) but no other clear association between TGA and maternal conditions, teratogen exposure, or



• **Fig. 55.5** Series and Parallel Circulations. The series circulation in the normal newborn is compared with the parallel circulation in transposition of the great arteries (TGA). In TGA, survival after birth is dependent on mixing at either the atrial, ventricular, or great vessel level. Ao, Aorta; LA, left atrium; LV, left ventricle; PA, pulmonary artery; RA, right atrium; RV, right ventricle. (From Wernovsky G: *Transposition of the great arteries*. In: Allen HD, Gutgesell HP, Clark EB, Driscoll DJ, eds. *Moss and Adams' Heart Disease in Infants, Children, and Adolescents: Including the Fetus and Young Adult*, 6th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:1027–1084.)

genetic abnormalities. TGA is typically an isolated defect, with less than 10% of cases associated with extracardiac malformations.

In the most common form of TGA (D-TGA, complete transposition, or simple transposition), the aorta arises from the right ventricle anteriorly and slightly rightward of the pulmonary artery, which arises from the left ventricle. Desaturated blood returns to the right ventricle and is recirculated to the body via the aorta, while oxygenated blood returns to the left ventricle and is recirculated to the lungs. The end result is separate, parallel circulations (Fig. 55.5). Survival is dependent upon communication between the two circulations, typically in the form of bidirectional shunting at the PFO and PDA. With absent or small communications between the circulations, severe systemic acidosis and hypoxia develop after birth resulting in death.

Cyanosis is apparent within the first few hours of life and is not responsive to oxygen. Cardiac examination is typically normal with the exception of a loud single S₂. Because of the arrangement of the great vessels, the louder closure of the anterior aortic valve obscures closure of the more posterior pulmonary valve. Chest X-ray may be normal or reveal a narrow mediastinum with slight predominance of the right ventricle resulting in an “egg on a string” appearance. Pulmonary vascular markings are normal to slightly increased. ECG is typically normal but may reveal right ventricular hypertrophy. Echocardiogram reveals the transposed great vessels and is used to determine the size of the PFO and PDA and define any associated cardiac defects.

Initial management of infants with TGA is aimed at maintaining communication between the systemic and pulmonary circulations. Prostaglandin E₁ (PGE₁) is started to maintain patency of the ductus arteriosus (dose of 0.03–0.1 mcg/kg per min). In patients with a restrictive PFO, or with persistent hypoxia and acidosis despite a PDA, a balloon atrial septostomy is performed within the first 24 to 48 hours of life to encourage mixing at the atrial level. In the setting of adequate mixing, pulmonary overcirculation may develop as PVR drops. Surgical treatment is the arterial switch procedure (Fig. 55.6), typically performed within the first week after birth (Castaneda et al., 1989; Prifti et al., 2002).

While TGA is usually an isolated lesion, 40%–45% of patients have an associated VSD, 10% have a VSD with left ventricular outflow tract obstruction, and 5% have left ventricular outflow tract obstruction alone. In addition, there is a wide variety in coronary artery anatomy in patients with TGA. The clinical presentation of patients with TGA and VSD depends upon the size of the VSD. Those with a restrictive VSD present like patients with TGA and intact ventricular septum. Those with a large VSD may not demonstrate any symptoms initially. As PVR drops, symptoms of pulmonary overcirculation develop. Patients with restricted pulmonary blood flow (TGA with VSD and pulmonic stenosis) appear clinically similar to patients with TOF. The timing and type of surgical repair in patients with complex TGA are variable and beyond the scope of this chapter.

Double Outlet Right Ventricle

DORV is a relatively rare, diverse group of lesions characterized by the origination of both great vessels from the right ventricle and by the persistence of the subaortic and subpulmonary conus. There are multiple anatomic variations of this lesion that are commonly classified by the relationship of the great vessels to one another and/or by the location of the VSD to the great vessels (Fig. 55.7) (Anderson et al., 1983; Stellin et al., 1991; Mahle et al., 2008). With this in mind, there are four types of DORV, each with a slightly different neonatal presentation and surgical management.

DORV of the tetralogy type typically has normally related great vessels with a subaortic VSD. Because the subaortic conus is pulled anteriorly, the subpulmonary conus is typically smaller, resulting in variable obstruction to pulmonary blood flow. The clinical presentation and surgical management are similar to those of TOF.

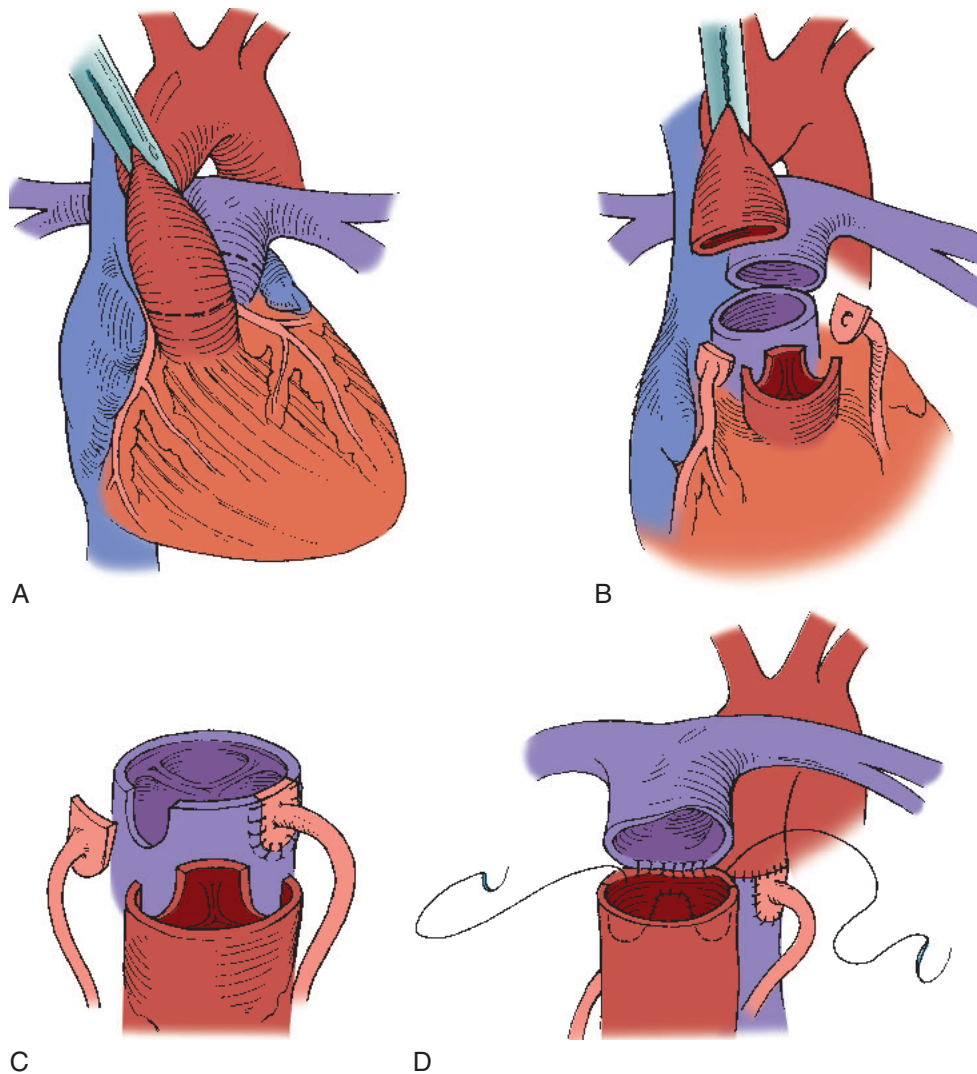
DORV of the transposition type typically has malposed great vessels with the aorta located either anterior to or rightward of the pulmonary artery. The VSD is subpulmonary and directs flow into the pulmonary artery, resulting in transposition-like physiology. This lesion is frequently associated with variable obstruction to aortic blood flow and coarctation of the aorta. Surgical repair consists of baffling the VSD to the pulmonary valve and performing an arterial switch. In the setting of coronary artery anomalies that prevent an arterial switch, the left ventricle is baffled to both the aortic and pulmonary valves, and a right-ventricle-to-pulmonary-artery conduit is placed. Arch obstruction is corrected if needed.

In DORV with a “doubly committed” VSD, the VSD is below both great vessels. The physiology and clinical presentation are variable, depending upon the size and orientation of the VSD and the degree of outflow tract obstruction. Surgical repair is directed at closing the interventricular communication without obstructing either ventricular outflow.

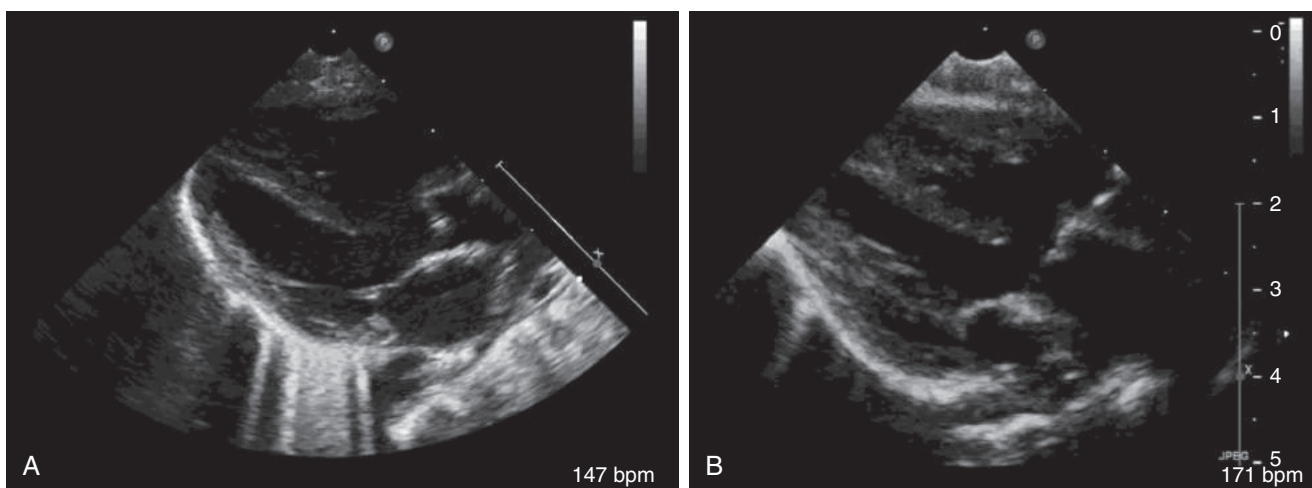
The least common variety of DORV is that in which the VSD is remote from both great vessels. Because of the distance between the VSD and the great vessels, a two-ventricle repair is difficult and often impossible. Pulmonary banding is frequently performed in the newborn period to limit pulmonary blood flow, delaying attempts at a biventricular repair or single ventricle palliation until several months of age. Recently, there has been increasing interest in biventricular repair for this lesion through creative intracardiac baffles (Lacour-Gayet, 2002).

Truncus Arteriosus

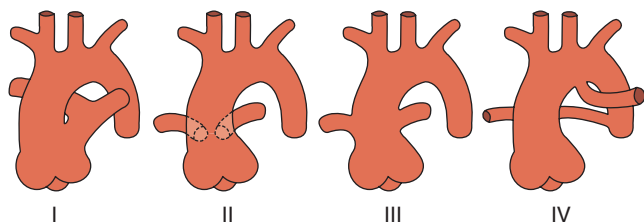
In truncus arteriosus, a single arterial trunk gives rise to the pulmonary, systemic, and coronary circulation. The arterial trunk



• **Fig. 55.6** Arterial Switch Operation for Dextro-Transposition of the Great Arteries. (A) The *dashed lines* depict the planned location of transection of the great vessels. (B) The coronary arteries are removed with surrounding aortic wall as "buttons." (C) The coronary buttons are translocated to the posterior neoaortic root. (D) The Lecompte maneuver brings both pulmonary arteries anterior to the neo-aorta. The aortic suture line is completed, incorporating the coronary buttons. The coronary donor sites are filled in with patches of autologous pericardium, and the pulmonary anastomosis is completed. (From Wernovsky G, Jonas RA. Transposition of the great arteries. In: Chang AC, Hanley FL, Wernovsky G, Wessel DL, eds. *Pediatric Cardiac Intensive Care*. Baltimore, MD: Williams & Wilkins; 1998:289–301.)



• **Fig. 55.7** Echocardiographic Views of Double Outlet Right Ventricle. (A) The aorta is committed to the right ventricle, and the ventricular septal defect is below the aortic valve. (B) The great vessels are transposed in this patient with the pulmonary artery overriding the ventricular septal defect.



• **Fig. 55.8** Types of Truncus Arteriosus. The classification of truncus arteriosus is determined by the origin of the pulmonary arteries (see text for details).

typically overrides a VSD. Truncus is classified by the origin of the pulmonary arteries (Fig. 55.8). In type I, a small pulmonary artery arises from the arterial trunk and bifurcates in the right and left branch pulmonary arteries. The right and left pulmonary arteries can also arise from separate ostia that are either close to one another (type II) or some distance apart (type III) (Collett and Edwards, 1949). Type IV is similar to TOF with pulmonary atresia. The truncal valve has between one and six cusps. It is occasionally insufficient but rarely stenotic. Truncus usually occurs as an isolated cardiovascular defect although has been associated with microdeletion of chromosome 22q11 (McElhinney et al., 2003).

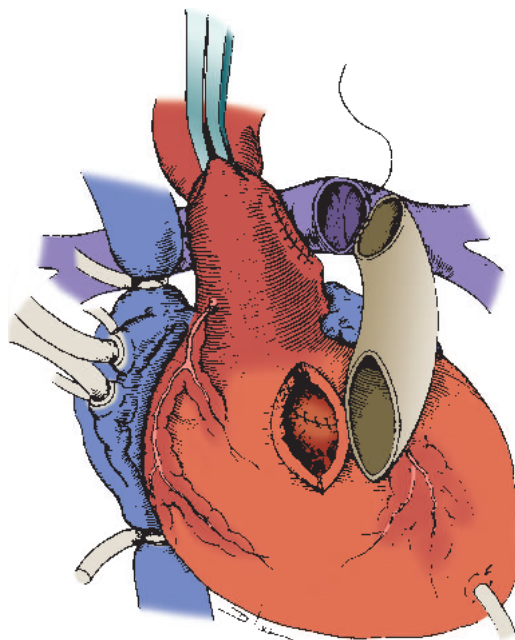
As the PVR drops over the first several weeks after birth, increased shunting from the arterial trunk to the pulmonary arteries occurs, and pulmonary overcirculation develops. If left untreated, symptoms of heart failure develop. Long-term pulmonary vascular obstructive disease occurs.

The clinical presentation of truncus changes with the PVR. Initially, infants are minimally cyanotic and do not appear to be in distress. As pulmonary overcirculation occurs, symptoms of heart failure develop. Cardiac examination reveals a hyperdynamic precordium and a loud single S₂. As PVR drops, the pulses become bounding, and the pulse pressure widens because of run-off into the pulmonary arteries. A low-pitched diastolic inflow rumble may be heard at the apex. In addition, a systolic ejection murmur may be present and a medium- to high-pitched diastolic murmur may be heard from truncal valve insufficiency. Chest X-ray typically reveals cardiomegaly with pulmonary vascular marking that increases over the first few weeks of life. ECG demonstrates right, left, or biventricular hypertrophy. Echocardiogram is used to demonstrate the anatomy and evaluate for associated cardiac defects such as right aortic arch, coronary artery anomalies, interrupted aortic arch, and secundum ASDs.

Initially, heart failure symptoms are managed with medications. Surgical repair is typically performed within the first 2 months of life because of the difficulty in controlling heart failure. The usual repair involves closure of the VSD so that the arterial trunk arises from the left ventricle. A right-ventricle-to-pulmonary-artery conduit is then placed (Fig. 55.9). The right-ventricle-to-pulmonary-artery conduit needs to be replaced several times over the course of the patient's lifetime.

Total Anomalous Pulmonary Venous Return

In TAPVR, the pulmonary veins return to a systemic vein or directly to the right atrium, rather than to the left atrium. Both systemic and pulmonary venous return pass through the right atrium, right ventricle, and pulmonary artery, creating pulmonary overcirculation and right heart volume overload. An ASD is present in all cases. The right-atrium-to-left-atrium shunt supplies the

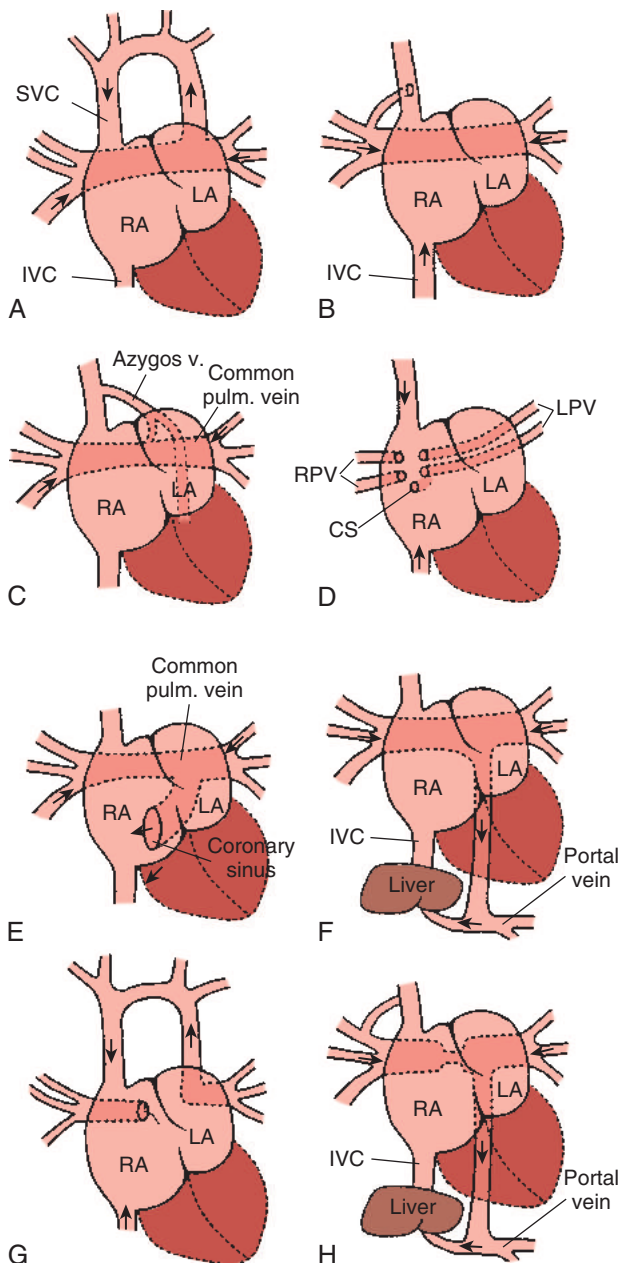


• **Fig. 55.9** Anatomy and Repair of Truncus Arteriosus. After the branch pulmonary arteries are removed from the common trunk (and the resulting aortic defect is closed), a ventriculotomy is performed. The ventricular septal defect is closed through this incision, and a conduit is placed from the right ventricle to the pulmonary arteries. (From Behrendt DM, Dick M. Truncus repair with a valveless conduit in neonates. *J Thorac Cardiovasc Surg.* 1995;110:1148–1150.)

systemic cardiac output. If the ASD is restrictive, then systemic output will be limited.

Several common patterns of anomalous pulmonary venous return are seen (Fig. 55.10) (Edwards and Helmholtz, 1956). Most commonly, the anomalous veins drain in a supracardiac fashion, entering the left innominate vein or SVC via a vertical vein and draining into the right atrium via the SVC. Cardiac drainage occurs when the anomalous veins drain into the right atrium via the coronary sinus. Alternatively, a descending vein may pass through the diaphragmatic hiatus and enter the hepatic or portal venous system resulting in infracardiac drainage. Rarely, a mixed pattern of drainage may exist, or the anomalous veins may drain directly into the right atrium. Drainage directly into the right atrium occurs almost exclusively in patients with heterotaxy.

The clinical presentation of TAPVR varies, depending upon the degree of obstruction to pulmonary venous flow. Patients with unobstructed pulmonary venous flow will have minimal symptoms in the newborn period, and cyanosis is usually not apparent. Symptoms of right heart failure develop over time because of the progressive right heart volume overload. Physical examination may reveal a right ventricular heave, fixed split S₂, and a soft systolic ejection murmur in the pulmonic area. Chest X-ray may demonstrate cardiomegaly due to an enlarged right atrium, right ventricle, and main pulmonary artery. Pulmonary vascular markings increase over time. ECG reveals peaked P waves and right ventricular hypertrophy. Patients with moderate restriction to pulmonary blood flow are typically cyanotic in the newborn period. There is usually adequate mixing of venous return to allow for oxygen delivery to tissues. These patients may, however, benefit from supplemental oxygen, mechanical ventilation, and sedation to decrease oxygen consumption. The presentation of patients with obstructed



• **Fig. 55.10** Anatomic Varieties of Total Anomalous Pulmonary Venous Return. (A–C) Supracardiac. (D, E) Cardiac. (F) Infracardiac. (G, H) Mixed. See text for details. CS, Coronary sinus; IVC, inferior vena cava; LA, left atrium; LPV, left pulmonary vein; Pulm., pulmonary; RA, right atrium; RPV, right pulmonary vein; SVC, superior vena cava; V, vein. (From Eimbcke F, Enriquez G, Gomez O, Zilleruelo R. Total anomalous pulmonary venous connection. In: Moller JH, Hoffman JIE, eds. *Pediatric Cardiovascular Medicine*. Philadelphia, PA: Churchill Livingstone; 2000:409–420.)

pulmonary blood flow will be discussed later in this chapter under obstructive lesions.

Treatment of TAPVR is surgical. In most cases, a pulmonary venous confluence is seen near the left atrium. Cardiac catheterization is used to define pulmonary venous anatomy if the pulmonary veins are not well seen by echocardiography. During surgery, the pulmonary venous confluence is anastomosed to the left atrium, the ASD is closed, and the connections to systemic veins are ligated. In the absence of pulmonary venous obstruction and in the setting

of uncomplicated anatomy, surgical mortality is low and long-term results are good. Late pulmonary venous obstruction occurs in approximately 20% of cases, with relatively poor surgical and interventional cardiac catheterization results.

Tetralogy of Fallot

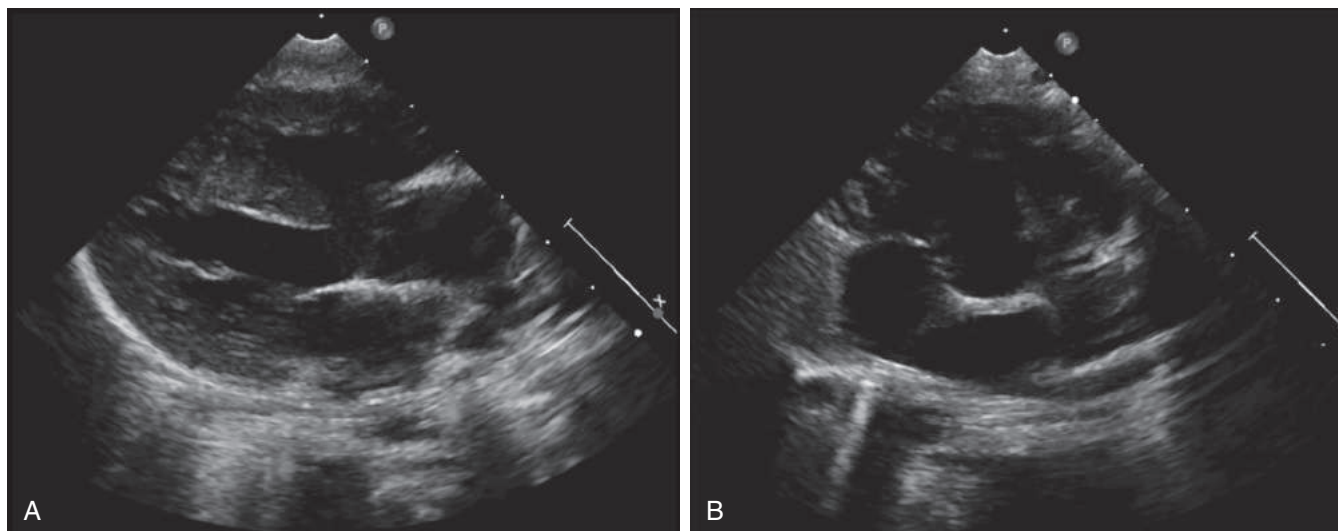
TOF consists of a VSD, overriding aorta, RVOT obstruction, and right ventricular hypertrophy (Fig. 55.11). The severity and location of the RVOT obstruction are variable. Infundibular hypertrophy, a small, frequently bicuspid pulmonary valve, and small main pulmonary artery can jointly or independently create obstruction at the subvalve, valve, or supravalve level, respectively. Complete obstruction of the RVOT (pulmonary atresia) with a VSD is an extreme form of TOF. TOF is associated with a right aortic arch in 20% of the cases and aberrant coronary arteries in 5%–10% of cases, both of which can affect surgical planning. The etiology of TOF is heterogeneous. Maternal diabetes, phenylketonuria, and retinoic acid exposure have been associated with TOF. In addition, genetic syndromes including 22q11 deletion, Alagille syndrome, and VACTERL/VATER association have been associated with TOF.

The pathophysiology and clinical presentation of TOF are directly tied to the severity of the RVOT obstruction. With mild RVOT obstruction, the predominant shunt through the VSD is left to right. Cyanosis is minimal, and symptoms of heart failure are the typical presenting symptoms. The main and branch pulmonary arteries are usually of normal size. With moderate RVOT obstruction, the shunt through the VSD is right to left, but the prograde flow through the RVOT is adequate, resulting in mild cyanosis. The main and branch pulmonary arteries may have areas of focal stenosis or be diffusely small. With severe RVOT obstruction, there is minimal or no prograde flow across the RVOT. Pulmonary blood flow is thus dependent upon left-to-right shunt through the PDA. Patients are severely cyanotic. PDA closure leads to cardiovascular collapse. The main and branch pulmonary arteries may be confluent and small. With pulmonary atresia (pulmonary atresia VSD or tetralogy pulmonary atresia), the pulmonary vascular bed is variable. The pulmonary arteries can be confluent, normal-sized, and fed entirely by the ductus arteriosus; confluent and small with a small ductus; or virtually absent with pulmonary blood flow supplied by multiple AP collateral vessels.

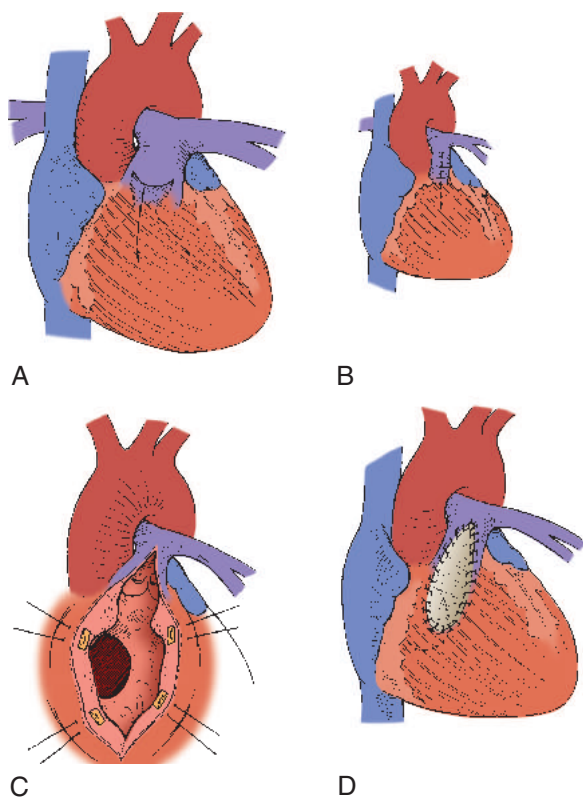
Initial management depends upon the amount of pulmonary blood flow. Patients without significant symptoms or hypoxemia undergo a complete repair in the first 6 months after birth. Complete repair consists of VSD closure and relief of RVOT obstruction through a combination of infundibular muscle resection, pulmonary valvotomy, or RVOT patch that can extend out to the branch pulmonary arteries as needed (Fig. 55.12). In patients with significant cyanosis or ductal-dependent circulation, management options include palliation with a systemic-to-pulmonary artery shunt in the newborn period (modified Blalock–Taussig [BT] shunt) and complete repair at a later date or a complete anatomic correction in the newborn period. The recent trend has been toward earlier complete correction in symptomatic infants (Reddy et al., 1995; Ooi et al., 2006; Tamesberger et al., 2008).

Tetralogy of Fallot Absent Pulmonary Valve

TOF with absent pulmonary valve (APV), or APV syndrome, is a spectrum of disorders with a rudimentary pulmonary valve. The resultant pulmonary valve stenosis and insufficiency cause dilation



• **Fig. 55.11** Echocardiographic View of Tetralogy of Fallot. (A) Some of the anatomic features of tetralogy of Fallot are readily demonstrated on the long-axis echocardiographic view including the hypertrophy of the right ventricle, the ventricular septal defect (VSD), and the aorta overriding the VSD. B. The short-axis view on echocardiogram shows the infundibular or right ventricular outflow tract stenosis.



• **Fig. 55.12** Repair of Tetralogy of Fallot. (A) Dashed line depicts a non-transannular incision, used when the pulmonary valve and annulus are of adequate size. (B) A transannular incision (dashed line) is used when there is annular hypoplasia. (C) An example of transventricular exposure of the ventricular septal defect. Alternatively, the ventricular septal defect may be closed via a right atriotomy through the tricuspid valve. (D) External view of patch closure of the transannular incision. (From Spray TL, Wernovsky G. Right ventricular outflow tract obstruction. In: Chang AC, Hanley FH, Wernovsky G, Wessel DL, eds. *Pediatric Cardiac Intensive Care*. Baltimore, MD: Williams & Wilkins; 1998:257–270.)

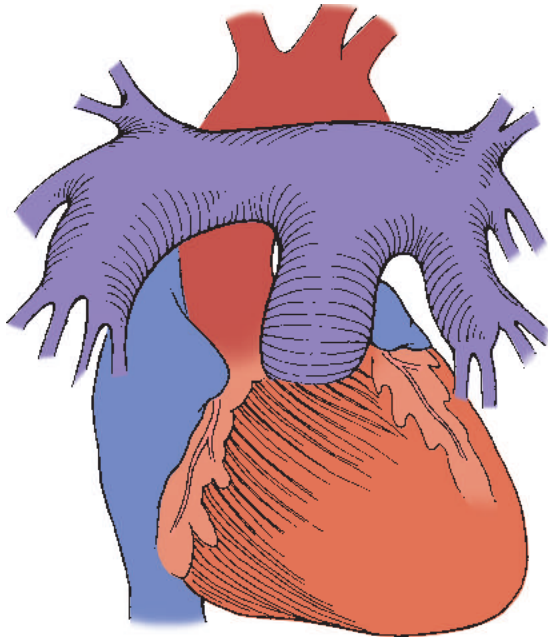
of the right ventricle and main and branch pulmonary arteries that is apparent in utero (Fig. 55.13). The vast majority of cases also have a malalignment VSD. Presumably secondary to airway compression by the dilated pulmonary vasculature, this syndrome is commonly associated with airway anomalies. There is a high prevalence of 22q11 deletion among patients with APV.

Physical examination reveals a characteristic “to and fro” murmur of pulmonary stenosis and insufficiency. A prominent right ventricular heave and hepatomegaly are present secondary to the right ventricular volume overload. There is a wide range of symptoms, depending upon the degree of right ventricular volume overload and degree of airway disease. At one end of the spectrum is an asymptomatic neonate with mild cyanosis and the gradual development of heart failure symptoms during the normal postnatal decline in PVR. At the other end of the spectrum is a critically ill infant with severe cyanosis and respiratory failure caused by the combination of right-to-left shunt through the VSD and underlying airway disease. Mechanical ventilation is necessary and occasionally unsuccessful secondary to airway compression by the dilated pulmonary vasculature. Urgent surgery is required in these infants.

Surgery consists of VSD closure, pulmonary artery plication, relief of RVOT obstruction, and insertion of a valve or homograft in the RVOT position. There is significant variation in surgical technique between centers (Alsoufi et al., 2007b; Tissot et al., 2007). The surgical mortality and short-term and long-term outcomes are related primarily to the severity of pulmonary artery dilation and airway compression. More severely affected infants have a higher surgical mortality and need for prolonged postoperative ventilation. Those with less significant pulmonary artery dilation have low postoperative mortality and long-term outcomes similar to those with TOF.

Pulmonary Atresia With Intact Ventricular Septum

In pulmonary atresia with intact ventricular septum, the pulmonary valve leaflets are fused or fail to form. Without egress from the

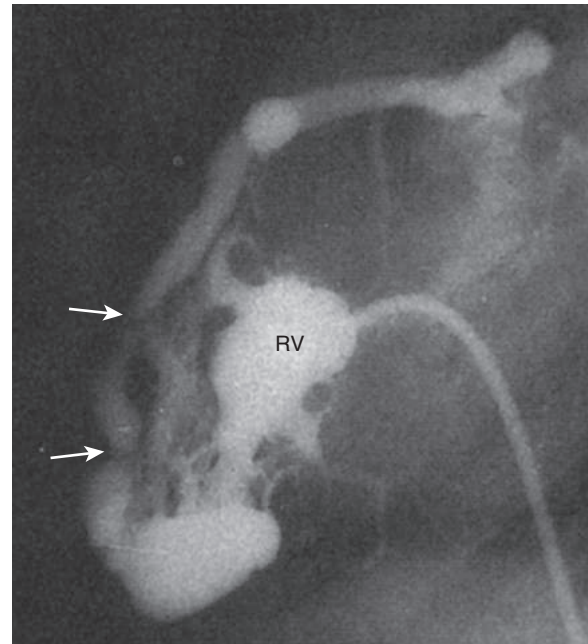


• **Fig. 55.13** Tetralogy of Fallot With Absent Pulmonary Valve. Severe dilatation of the main and branch pulmonary arteries is seen and is frequently associated with bronchial compression and large and small airway disease. The intracardiac anatomy is usually similar to that in standard tetralogy of Fallot. (From Spray TL, Wernovsky G. Right ventricular outflow tract obstruction. In: Chang AC, Hanley FH, Wernovsky G, Wessel DL, eds. *Pediatric Cardiac Intensive Care*. Baltimore, MD: Williams & Wilkins; 1998:257–270.)

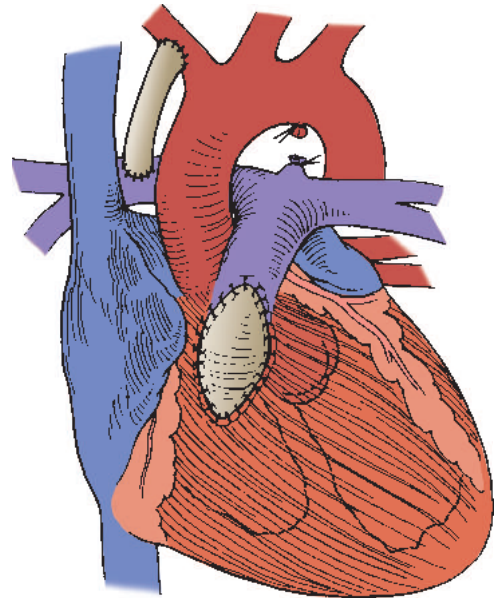
right ventricle, systemic venous return passes through the PFO and mixes with pulmonary venous return. Pulmonary blood flow is dependent upon the PDA, with the left ventricle providing cardiac output to both the systemic and pulmonary circulations. Tricuspid valve and right ventricular size vary, ranging from nearly normal-sized structures to nearly atretic tricuspid valve and diminutive right ventricle chamber. The latter patients may have sinusoidal channels in the right ventricle myocardium that communicate with the coronary circulation. The high right ventricular pressure results in retrograde perfusion of the coronary arteries, resulting in right ventricle-dependent coronary circulation (Fig. 55.14).

Pulmonary atresia with intact ventricular septum presents with cyanosis. Cardiac examination reveals a single loud second heart sound. A murmur is typically not present, other than from the PDA. ECG typically reveals a relative lack of right-sided forces with a QRS axis between 0 and 90 degrees. The P wave is peaked from right atrial enlargement. Pulmonary vascular markings on chest X-ray are typically decreased. Echocardiography confirms the diagnosis and can be used to look for coronary sinusoids. Cardiac catheterization is performed in patients where coronary sinusoids are known or suspected to completely define the extent and connections of the sinusoids.

Initially, PGE₁ is used to maintain ductal patency until a more permanent source of pulmonary blood flow is provided. In patients with right ventricle-dependent coronary circulation, the outcome of surgical repair is often poor. A systemic-to-pulmonary shunt followed by staged single ventricle palliation may be tried, but proceeding directly to heart transplantation may be appropriate. In patients without right ventricular coronary-dependent circulation, an egress from the right ventricle to pulmonary arteries is created by surgical valvotomy or RVOT augmentation or valve perforation,

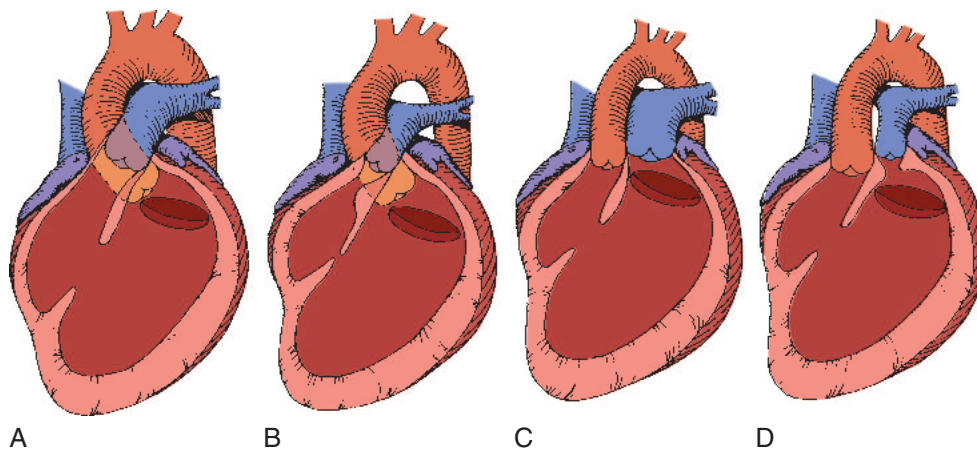


• **Fig. 55.14** Lateral Angiogram From a Newborn With Pulmonary Atresia and Intact Ventricular Septum. Following injection of contrast through the hypertrophied and diminutive right ventricle, there is retrograde filling of tortuous coronary arteries through sinusoidal connections to the right ventricle (arrows). RV, Right ventricle.



• **Fig. 55.15** Newborn Surgical Palliation for Pulmonary Atresia With Intact Ventricular Septum and Normal Coronary Arteries. A right ventricular outflow tract patch is placed, the ductus is ligated and divided, and a modified Blalock-Taussig shunt is placed. (From Wernovsky G, Hanley FL. Pulmonary atresia with intact ventricular septum. In: Chang AC, Hanley FL, Wernovsky G, Wessel DL, eds. *Pediatric Cardiac Intensive Care*. Baltimore, MD: Williams & Wilkins; 1998:265–270.)

and valvuloplasty is performed in the catheterization lab (Fig. 55.15) (Justo et al., 1997). At times, additional pulmonary blood flow is provided by a modified BT or other systemic-to-pulmonary shunt. The postoperative course can be complicated by low cardiac output or a circular shunt.



• **Fig. 55.16** Anatomic Variants in Tricuspid Atresia. (A) Normally related great vessels with a large ventricular septal defect (VSD) and normal-sized pulmonary arteries (PAs). (B) Normally related great vessels with a small VSD and PAs. (C) Transposed great arteries (left ventricle aligned with the PA, right ventricle with the aorta) with a relatively small VSD and aorta. Many patients with this variant have coarctation as well (see text). (D) Transposed great vessels with a VSD, subpulmonary obstruction, and small PAs. (From Fyler DC. Tricuspid atresia. In: Fyler DC, ed. *Nadas' Pediatric Cardiology*. Philadelphia, PA: Hanley & Belfus; 1992:659–667.)

Tricuspid Atresia

In tricuspid atresia, there is no outlet from the right atrium to the right ventricle. Systemic venous return passes from the right atrium, through a PFO or ASD, to the left ventricle. More than 90% of patients with tricuspid atresia have an associated VSD, allowing blood to pass from the left ventricle to the pulmonary arteries (Fig. 55.16). The size of the VSD determines the amount of pulmonary blood flow. Patients with a small or absent VSD have a very hypoplastic right ventricle and pulmonary artery. The majority of pulmonary blood flow passes through the PDA. If a large VSD is present, pulmonary blood flow is prograde through the pulmonary valve, and the ductus is not necessary. A variant of tricuspid atresia is associated with TGA. Pulmonary blood flow is derived from the left ventricle, and systemic blood flow must pass through the VSD. There may be an associated coarctation or hypoplastic aortic arch in these patients.

Clinically, cyanosis is present at birth, the extent of which is dependent upon the degree of restriction to pulmonary blood flow. A holosystolic murmur consistent with a VSD and a prominent left ventricular impulse are present on exam. ECG is nearly diagnostic and reveals left axis deviation and left ventricular hypertrophy. Chest X-ray reveals variable pulmonary vascular markings, depending on the size of the VSD and relationship of the great vessels. Echocardiogram demonstrates a fibromuscular plate in place of the tricuspid valve and a variably small right ventricle and pulmonary valve. The relationship of the great vessels can also be determined by echocardiogram. The degree of obstruction at the VSD or across the RVOT can be evaluated.

PGE₁ is used initially to maintain a PDA. If there is restriction to flow at the atrial level, a balloon septostomy is performed. Further management depends on the amount of pulmonary blood flow and the relationship of the great vessels. Patients with ductal-dependent pulmonary blood flow have a systemic-to-pulmonary artery shunt placed (modified BT shunt). Those with adequate pulmonary blood flow undergo a SVC-to-pulmonary-artery anastomosis (bidirectional Glenn) at approximately 6 months of age, with completion of the Fontan procedure around 3 years of

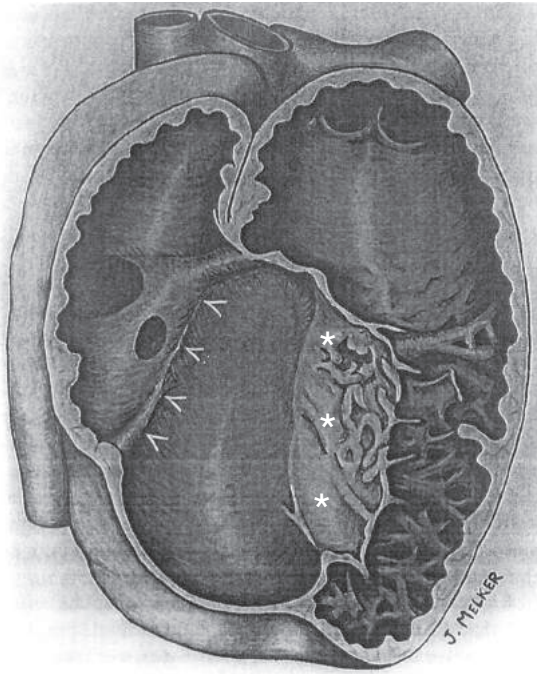
age. Patients with transposition, or more complex anatomy, undergo more extensive palliative procedures initially but continue down the pathway to Fontan palliation.

Ebstein Anomaly of the Tricuspid Valve

Ebstein anomaly is a rare form of CHD with variable presentation. Dysplasia of the tricuspid valve, with downward displacement of the septal and posterior leaflets, is the defining feature (Fig. 55.17). The anterior leaflet, although normally positioned in the valve annulus, frequently has abnormal chordal attachments and is large and redundant. The tricuspid valve abnormality can be accompanied by tricuspid regurgitation, right atrial dilation, abnormal right ventricular myocardium, and an increased risk of Wolf–Parkinson–White syndrome. In addition, functional and true pulmonary atresia can occur with severe Ebstein malformation. With displacement of the tricuspid valve, a significant portion of the right ventricle becomes atrialized, making it an ineffective pumping chamber. In this setting it can be difficult to differentiate functional pulmonary atresia from true pulmonary atresia. Severe Ebstein anomaly of the tricuspid valve is associated with heart failure in utero (Tongsong et al., 2005).

The clinical presentation is variable and depends upon the degree of displacement of the tricuspid valve and severity of RVOT obstruction. In many patients, symptoms are mild and do not present until later in life. In more severe disease, cyanosis results when a right-to-left shunt occurs at the atrial level, secondary to the tricuspid regurgitation and elevated right atrial pressures. Cardiac examination reveals a holosystolic murmur at the lower left sternal border with associated gallop and clicks. Neonates with severe Ebstein present with marked cyanosis, cardiomegaly, and ductal-dependent pulmonary blood flow. Chest X-ray is characteristic in severe cases with severe cardiomegaly evident at birth (Fig. 55.18). Death can occur because of the significant heart failure and hypoxemia. Clinical improvement may occur as the PVR drops, improving the right ventricle's ability to contribute to pulmonary blood flow.

Initially, management of the severely cyanotic infant is aimed at promoting pulmonary blood flow. PGE₁ is used to maintain



• **Fig. 55.17** Inferior Displacement of Tricuspid Valve Leaflet Into the Right Ventricular Cavity. The *white arrowheads* indicate the normally positioned tricuspid valve annulus. The asterisks (*) indicate varying degrees of inferior or apical displacement of the septal leaflet of the tricuspid valve. The area between the true annulus and the displaced valve leaflet is considered "atrialized." (From Epstein ML. Congenital stenosis and insufficiency of the tricuspid valve. In: Allen HD, Gutgesell HP, Clark EB, Driscoll DJ, eds. *Moss and Adams' Heart Disease in Infants, Children and Adolescents: Including the Fetus and Young Adult*. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:810–819.)



• **Fig. 55.18** Chest Radiograph in a 1-Day-Old Newborn With Ebstein Anomaly of the Tricuspid Valve. Note the massive cardiomegaly and relative pulmonary oligemia and hypoplasia.

ductal patency. Supplemental oxygen, inhaled nitric oxide, and mild respiratory alkalosis can have marginal success in improving pulmonary blood flow by lowering PVR. After several days, attempts are made at weaning the PGE₁. If unsuccessful, surgical options are considered. Options include tricuspid valve repair or replacement, 1½ ventricle repair, or other palliative procedures such as right ventricular exclusion with a fenestrated patch and placement of a modified BT shunt (Reemtsen et al., 2007). While surgical outcomes have improved, the need for neonatal repair for symptomatic disease remains a risk factor for death (McElhinney et al., 2005; Sarris et al., 2006).

Lesions That Present Primarily With Heart Failure

Hypoplastic Left Heart Syndrome

HLHS is an anatomically heterogeneous lesion characterized by varying degrees of underdevelopment of the left ventricle, hypoplasia or atresia of the aortic and mitral valves, and hypoplasia of the aortic arch. While accounting for only 1.4%–3.8% of all congenital heart defects, HLHS accounts for 23% of cardiac deaths in the first week of life and 15% of cardiac deaths in the first month after birth (Samaneck, 2000). HLHS is likely multifactorial in cause. There is a slight male predominance. While there is no clear genetic cause, familial clustering of various left heart obstructive lesions has been noted.

It is theorized that the growth of developing vascular structures is dependent upon flow. HLHS likely results from in utero obstruction of left ventricular inflow or outflow. The fetal left ventricle is predominantly filled with blood that passes through the foramen ovale. Restriction to flow or reversal of flow through the foramen ovale could then result in decreased flow to the left heart and its underdevelopment. Similarly, several studies have documented the progression of severe aortic stenosis to HLHS in utero (Danford and Cronican, 1992; Hornberger et al., 1995). The progressive left ventricular hypertrophy, dilation, and fibrosis associated with severe aortic stenosis can lead to decreased ventricular compliance, elevated left atrial pressures, and reversal of flow through the foramen ovale in utero. Prenatal cardiac intervention is still in its infancy, and much continues to be learned about fetal intervention, but successes with in utero balloon dilation of the aortic valve suggest that the progression to HLHS can be altered (Makikallio et al., 2006; McElhinney et al., 2010).

Because of the underdevelopment of the left heart structures, pulmonary venous return must exit the left atrium through the foramen ovale. Pulmonary venous blood then mixes with systemic venous return in the right atrium and enters the right ventricle. Right ventricular output then passes either into the pulmonary circulation or through the ductus arteriosus into the systemic circulation; that is, the pulmonary and systemic circulations are in parallel. The ratio of systemic to pulmonary blood flow is determined by the relative resistance of the vascular beds. As the normal postnatal drop in PVR occurs, pulmonary flow increases at the expense of systemic flow. Thus the management of HLHS is dependent upon an adequate egress from the left atrium and a balancing of the resistances of the pulmonary and systemic vascular beds.

Most cases of HLHS are now diagnosed prenatally when an abnormal four chamber view of the heart is noted on a screening obstetric ultrasound. Ideally, delivery should occur at a tertiary care center. In the absence of prenatal diagnosis, postnatal

presentation is somewhat variable and dependent upon ductal patency and the degree of restriction to flow at the atrial septum. The infant with an unrestrictive atrial septum and PDA is largely asymptomatic at birth and may be missed in the newborn period. Cyanosis is minimal and pulmonary overcirculation is mild while PVR is high. Cardiac exam is relatively unremarkable. The second heart sound is single and loud. A third heart sound becomes apparent as heart failure develops. As PVR drops and ductal closure occurs, feeding difficulties and respiratory distress become apparent with rapid progression to cardiovascular collapse. Physical examination following ductal restriction is significant for lethargy, pallor, and diminished or absent pulses. Chest X-ray typically reveals a relatively normal-sized heart and pulmonary edema. ECG is nonspecific but may reveal relative lack of left-sided forces.

The patient with a restrictive atrial septum presents with tachypnea and profound cyanosis shortly after birth. The elevated left atrial and pulmonary venous pressures result in pulmonary venous congestion that is apparent on chest X-ray.

The preoperative management of patients with HLHS is directed at balancing the ratio of pulmonary-to-systemic blood flow ($Q_p : Q_s$) to allow for sufficient oxygenation of blood while maintaining adequate systemic cardiac output. Prostaglandins should be started immediately postnatally to ensure ductal patency. Echocardiography is utilized to confirm cardiac anatomy and determine the degree of restriction to flow through the foramen ovale. If the atrial level shunt is restrictive with profound cyanosis and metabolic acidosis, a balloon atrial septostomy, surgical septectomy, or emergent stage I palliation should be performed (see later in the section). If the restriction was present in utero, pathologic fibrosis and arterialization of the pulmonary veins and medial hypertrophy of the pulmonary arterioles occur. Even following atrial septostomy, lung disease can persist and PVR can remain high. Oxygen may be needed to maintain saturations in an appropriate range. This subset of patients has a high mortality rate.

A small group of patients will have adequately balanced pulmonary and systemic blood flow at the time of presentation. A small degree of restriction to flow through the foramen ovale may be associated with slight cyanosis but has the beneficial effect of restricting pulmonary blood flow. In the absence of acidosis or end-organ dysfunction, this state is generally tolerated until stage I palliation is performed.

An unrestrictive ASD allows increased pulmonary blood flow as the PVR falls. Because of the parallel arrangement of the circulations, increasing pulmonary flow decreases systemic flow. In this setting, there is increasing oxygen saturation associated with inadequate oxygen delivery to the tissues. Worsening metabolic acidosis and end-organ dysfunction ensue. The ratio of pulmonary-to-systemic blood flow is balanced by manipulating the resistances of the pulmonary and systemic vascular beds. The success of these therapies is monitored by measurement of oxygen saturation, mixed venous saturation, lactate, and arterial blood gases.

PVR can be manipulated through mechanical ventilation and alteration in the amount of oxygen delivered. Mechanical ventilation with high positive end-expiratory pressure can limit pulmonary blood flow. PVR can also be increased by adding carbon dioxide or nitrogen to the ventilator circuit to reduce the fraction of inspired oxygen (FiO_2) to 0.17 (Riordan et al., 1996; Shime et al., 2000). Both CO_2 and nitrogen have been shown to decrease the $Q_p : Q_s$ and decrease oxygen saturation. Only hypercarbia has been shown to improve systemic oxygen delivery (Tabbutt et al., 2001). The goal of this therapy is to maintain normal lactate systemic oxygen saturation at 75%–85% and mixed venous oxygen saturation

approximately 25 percentage points lower than systemic saturations. Mechanical ventilation is typically required when manipulating inspired gas. Mechanical ventilation alone has been associated with increased infection risk, more labile preoperative hemodynamics, and increased mortality. Both elective mechanical ventilation and alteration of the inspired gas mixture may be less in favor in the current era (Johnson et al., 2008).

Vasoactive medications can be used to alter systemic vascular resistance and improve ventricular function. Use of these medications is determined by clinical presentation and echocardiographic findings. Milrinone can be used to provide some afterload reduction, if tolerated by blood pressure. The inotropic effects of milrinone are also an advantage if ventricular function is poor. In addition to decreasing pulmonary blood flow, afterload reduction has the added benefit of decreasing tricuspid valve regurgitation if it is present. Milrinone also dilates the pulmonary vascular bed, so care should be taken when it is used. While counterintuitive, when faced with an unoperated patient with high oxygen saturation and low peripheral blood pressure, the gentle addition of milrinone may improve blood pressure simply by increasing systemic blood flow (Johnson et al., 2008).

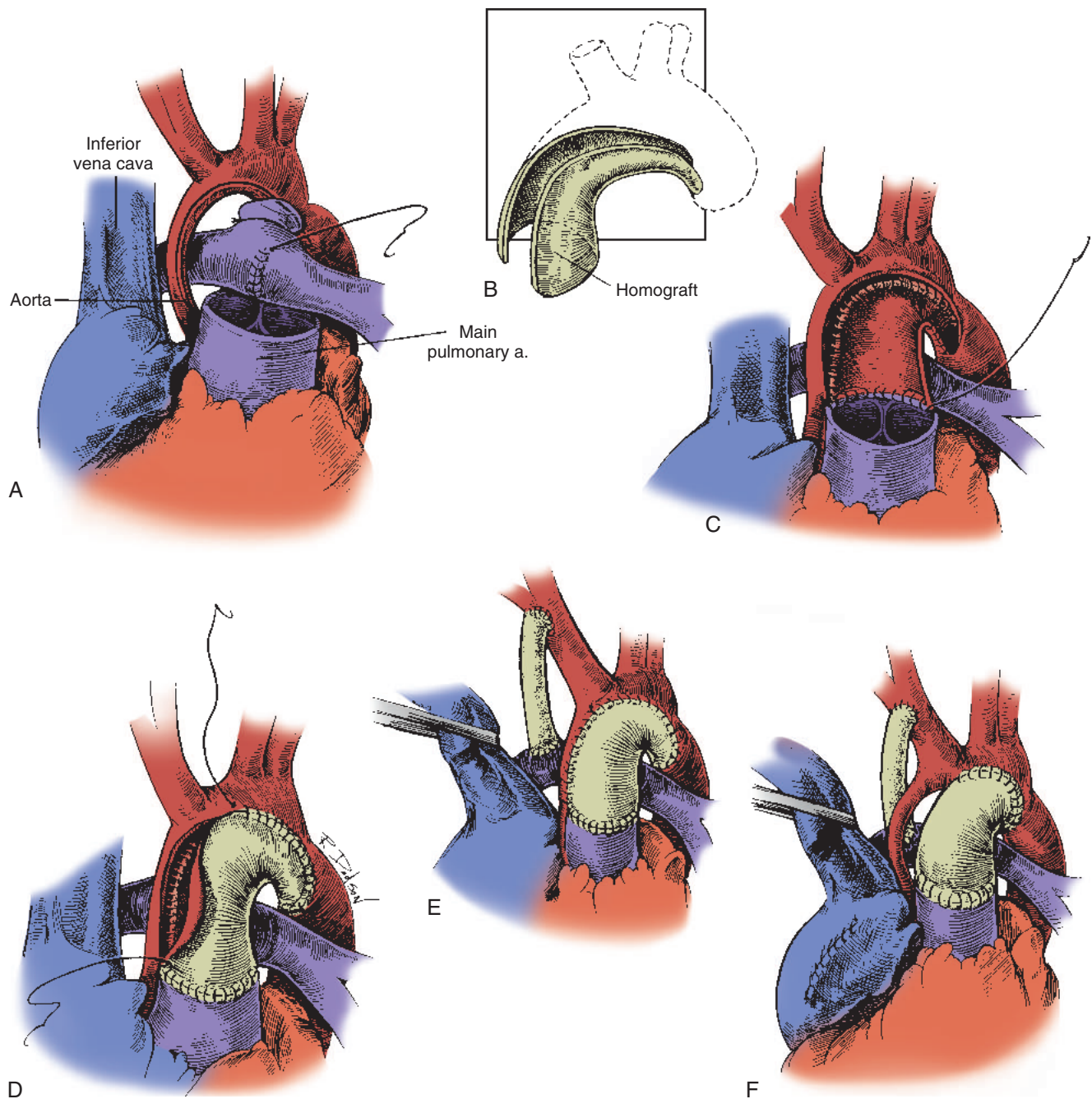
The immediate goal of surgical palliation is to provide stable unrestricted systemic and coronary blood flow and reliably restricted pulmonary blood flow. There are several strategies for stage I palliation. Traditionally, aortic arch reconstruction was performed using pulmonary artery tissue, and pulmonary blood flow was supplied using a modified BT shunt (Norwood procedure, Fig. 55.19). A recent modification of a right ventricle-to-pulmonary artery conduit has been used to supply pulmonary blood flow (Sano modification, Fig. 55.20). The Sano modification has the presumed benefit of providing pulsatile flow to the pulmonary arteries without AP diastolic run-off and coronary steal. The downside of this procedure is the need for a ventriculotomy. A hybrid procedure that combines stent placement in the ductus arteriosus by the cardiologist and pulmonary artery banding by the surgeon is an approach being taken by a number of institutions and provides a relatively noninvasive stage I palliation for HLHS (Caldarone et al., 2007). Each of these procedures has pros and cons and advocates and detractors (Malec et al., 2003; Ghanayem et al., 2006; Caldarone et al., 2007). Longer-term prospective studies are needed to determine the optimal approach to stage I palliation. In the low-risk patient, survival following the first stage nears 80%–90%.

The remaining palliative surgeries occur outside the newborn period. Stage II palliation unloads the right ventricle and begins to separate the pulmonary and systemic circulations. The superior cavopulmonary anastomosis (bidirectional Glenn) is usually performed between 4 and 6 months of age. During this procedure, the conduit providing pulmonary flow is removed, and the SVC is anastomosed to the pulmonary artery.

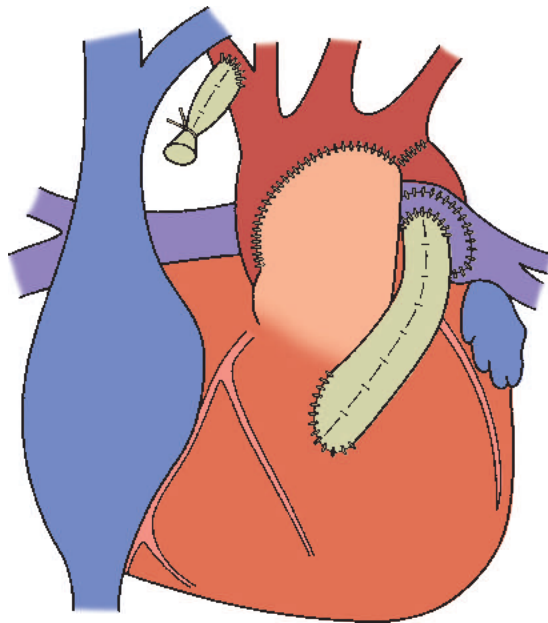
Stage III palliation completely separates the pulmonary and systemic circulations. An inferior cavopulmonary anastomosis (Fontan completion) is performed by one of several techniques.

Obstructed Total Anomalous Pulmonary Venous Return

Obstructed TAPVR represents one of the few remaining neonatal surgical emergencies in pediatric cardiology. While obstruction can occur with any type of TAPVR, it is most common in infra-diaphragmatic TAPVR. Physiologically, obstruction to pulmonary venous flow results in pulmonary venous hypertension that is transmitted to the pulmonary capillary bed, resulting in pulmonary



• **Fig. 55.19** Stage I Palliation for Hypoplastic Left Heart Syndrome: Classic Norwood Procedure. (A) The main pulmonary artery (a) is transected and the distal end oversewn. The ductus arteriosus is ligated, and an incision is made from the proximal ascending aorta around the aortic arch to the level of the ductus. (B) A pulmonary homograft is utilized to create a patch to reconstruct the neo-aorta. (C, D) This homograft patch is used to connect the proximal main pulmonary artery and pulmonary (neo-aortic) valve to the ascending aorta and transverse arch. (E) A modified Blalock-Taussig shunt is placed from the base of the innominate artery to the right pulmonary artery. (F) An alternate technique utilizing a circumferential tube graft from the proximal main pulmonary artery to the distal transverse aortic arch. *Not shown:* Atrial septectomy is performed to provide unobstructed egress from the pulmonary veins to the right ventricle. (From Castañeda AR, Jonas RA, Mayer JE Jr, Hanley FL. *Cardiac Surgery of the Neonate and Infant*. Philadelphia, PA: WB Saunders; 1994.)



• **Fig. 55.20** Right Ventricle–Pulmonary Artery, or Sano, Modification of Stage I Reconstruction. The arch reconstruction is similar to that shown in Fig. 55.19. The Blalock–Taussig shunt is replaced with a Gore-Tex tube inserted from the right ventricle to the main pulmonary artery. (From Sano S, Ishino K, Kawada K, et al. Right ventricle–pulmonary artery shunt in first-stage palliation of hypoplastic left heart syndrome. *J Thorac Cardiovasc Surg.* 2003;126:504–510.)

edema. Pulmonary blood flow is severely limited. Newborns thus present with profound cyanosis and respiratory distress that are not responsive to medical management. Prostaglandins may help minimize the obstruction by maintaining patency of the ductus venosus, but patency of the ductus arteriosus does not improve the clinical picture as the limitation to pulmonary blood flow is not due to insufficient antegrade flow but rather obstructed outflow. Chest X-ray reveals a normal cardiac silhouette with pulmonary venous congestion that may be interpreted as interstitial pneumonia (Fig. 55.21). Echocardiography can be challenging in these patients. The pulmonary veins can be small due to limited flow, making them difficult to detect by two-dimensional imaging and color Doppler. Surgical treatment is the same as for unobstructed TAPVR. The postoperative course is frequently marked by PVR lability, right ventricular hypertension, and low cardiac output syndrome. The risk of mortality, need for prolonged ventilation, and incidence of late postpresence of postoperative obstruction all increase when obstruction is present preoperatively (Frommelt et al., 2010).

Cor Triatriatum

Embryologically, the pulmonary veins enter a common pulmonary vein that initially has no connection to the left atrium (Neill, 1956). During normal development, the common pulmonary vein becomes incorporated into the left atrium, resulting in the usual pattern of two right and two left pulmonary veins entering the left atrium. Abnormal incorporation of the common pulmonary vein can result in cor triatriatum, a condition in which the common pulmonary vein joins the left atrium through a single opening. If the opening is small and restrictive, the clinical presentation is similar to that of obstructed TAPVR. If the opening is nonrestrictive, no symptoms are present. Surgical resection of the membrane that separates the left atrium and common pulmonary vein is an effective



• **Fig. 55.21** Chest X-Ray in a 1-Day-Old With Obstructed Total Anomalous Pulmonary Venous Return.

treatment. Significant preoperative pulmonary venous obstruction increases surgical mortality.

Mitral Stenosis

Congenital mitral stenosis is a rare form of CHD with several subtypes. The stenosis can occur in the supravalue region, at the valve annulus, or within the mitral valve support apparatus. Typical congenital mitral stenosis is characterized by thickened leaflets, short or absent chordae tendinae, obliteration of interchordal spaces, and two separate papillary muscles. Supravalar mitral ring occurs when there is connective tissue outgrowth on the atrial surface of the mitral valve leaflets, leading to a decreased mitral valve orifice. The mitral valve orifice can also be stenotic secondary to a parachute mitral valve, when most or all chordae tendinae insert onto only one papillary muscle. Another form of obstruction occurs with a double orifice mitral valve, where a tongue of tissue connects the anterior and posterior mitral valve leaflets. A mitral arcade or hammock occurs when the leaflets are connected directly or by short chordae to the papillary muscles. Congenital mitral stenosis frequently occurs in conjunction with other left-sided obstructive lesions.

Symptoms from mitral stenosis usually occur in the first 2 years of life and may consist of shortness of breath, respiratory distress or wheezing, cyanosis, and pallor. Cardiac examination reveals a rumbling apical diastolic murmur, loud first heart sound, and loud split second heart sound. Opening snap of the mitral valve may be heard. Chest X-ray reveals left atrial enlargement and pulmonary venous congestion. ECG reveals right ventricular hypertrophy (RVH) with normal, bifid, or spiked P waves suggesting left atrial enlargement (LAE). Echocardiogram is used to define mitral valve anatomy and localize the area of obstruction. Doppler can be used to determine valve gradient and estimate right ventricular pressure.

Treatment options for congenital mitral valve stenosis include balloon mitral valvuloplasty, surgical mitral valvuloplasty, and mitral valve replacement. Despite recent improvements in outcomes, intervention and mitral valve replacement have relatively poor

short-term outcomes (Baird et al., 2015; Myers et al., 2015). Patients with additional left-sided obstructive lesions or associated defects frequently require single ventricle palliation.

Critical Aortic Stenosis

Patients with critical aortic stenosis have severe left ventricular outflow tract obstruction that limits systemic cardiac output (Fig. 55.22). The result is ductal-dependent systemic perfusion. While the obstruction can occur below the valve, at the valve, above the valve, or as a combination of these, this section focuses on outflow tract obstruction resulting from morphologic problems of the aortic valve. Severe aortic valve stenosis is defined as a Doppler-derived pressure gradient greater than 60 mmHg. Moderate stenosis is defined as gradient of 30–60 mmHg, while mild stenosis is a peak gradient less than 30 mmHg. In the setting of depressed left ventricular function, the Doppler echocardiogram-derived gradient may be significantly lower and underestimate the severity of the stenosis.

Aortic valve stenosis is detectable in utero. The long-standing pressure overload on the left ventricle causes left hypertrophy and left ventricle scarring (endocardial fibroelastosis). In some cases, as discussed above, the disease progresses to HLHS. Growing experience with fetal intervention suggests that, with proper patient selection, it is possible to alter the course of critical aortic stenosis in the fetus through early intervention (Freud et al., 2014; Friedman et al., 2015).

Clinically, critical aortic stenosis presents in the newborn period with signs of decreased systemic perfusion: pallor, decreased pulses, and prolonged capillary refill. A harsh ejection quality murmur is heard on examination in the aortic area. The volume and quality of the murmur correlate with the severity of stenosis in the setting of normal left ventricular function. If left ventricular function is depressed, the murmur may be soft despite severe stenosis. ECG reveals left ventricular hypertrophy with possible T-wave abnormalities. Heart size is typically normal on chest film, although the aortic knob may be prominent, and pulmonary congestion may be present. Echocardiogram is used to define the location and severity of the left ventricular outflow tract obstruction. Aortic stenosis is commonly found with other left-sided obstructive lesions, with possible underdevelopment of left heart structures. These

findings may alter the treatment plan and lead to single ventricle palliation. Published models have attempted to identify echocardiographic findings that predict the suitability of a two-ventricle repair in neonates with critical aortic stenosis (Rhodes et al., 1991; Lofland et al., 2001; Colan et al., 2006).

Initial management of infants with critical aortic stenosis is directed at the treatment of cardiogenic shock. Endotracheal intubation, mechanical ventilation, secure vascular access, inotropic support, sedation, and paralysis are all frequently necessary. PGE₁ maintains ductal patency and provides systemic output. A small PFO must be present for pulmonary venous return to cross the atrial septum and enter the systemic vascular via the right ventricle and ductus arteriosus. A balloon septostomy may be necessary to decompress the left atrium.

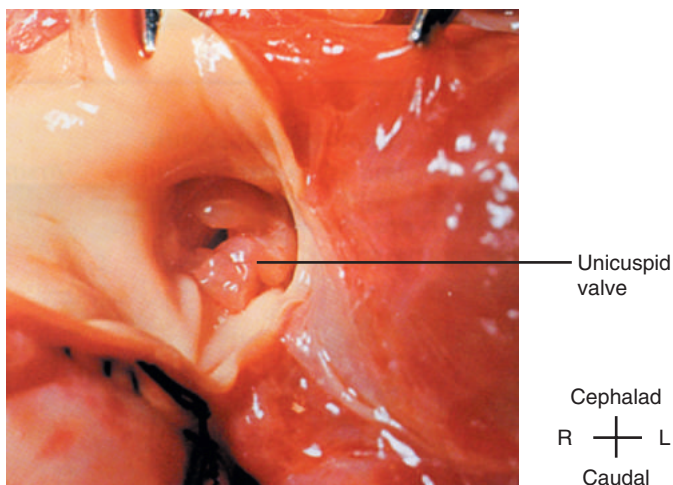
Further management depends on left ventricular size and the presence of other left heart obstructive lesions. Patients with multiple levels of left heart obstruction, a small mitral valve, hypoplastic aortic arch, or small left ventricle may be best suited for single ventricle palliation. Options for two-ventricle palliation include balloon valvuloplasty in the cardiac catheterization lab, surgical valvotomy, or neonatal Ross procedure (Fig. 55.23) (Alsoufi et al., 2007b). Outcomes of all procedures depend, in part, on relief of obstruction, presence of aortic valve regurgitation, associated cardiac lesions, and severity of end-organ dysfunction at the time of initial presentation. The mortality of each of the interventions is relatively high. Regardless of the treatment chosen, critical aortic stenosis is a lifelong illness. Patients require close follow-up and multiple procedures throughout their lifetime.

Coarctation of the Aorta

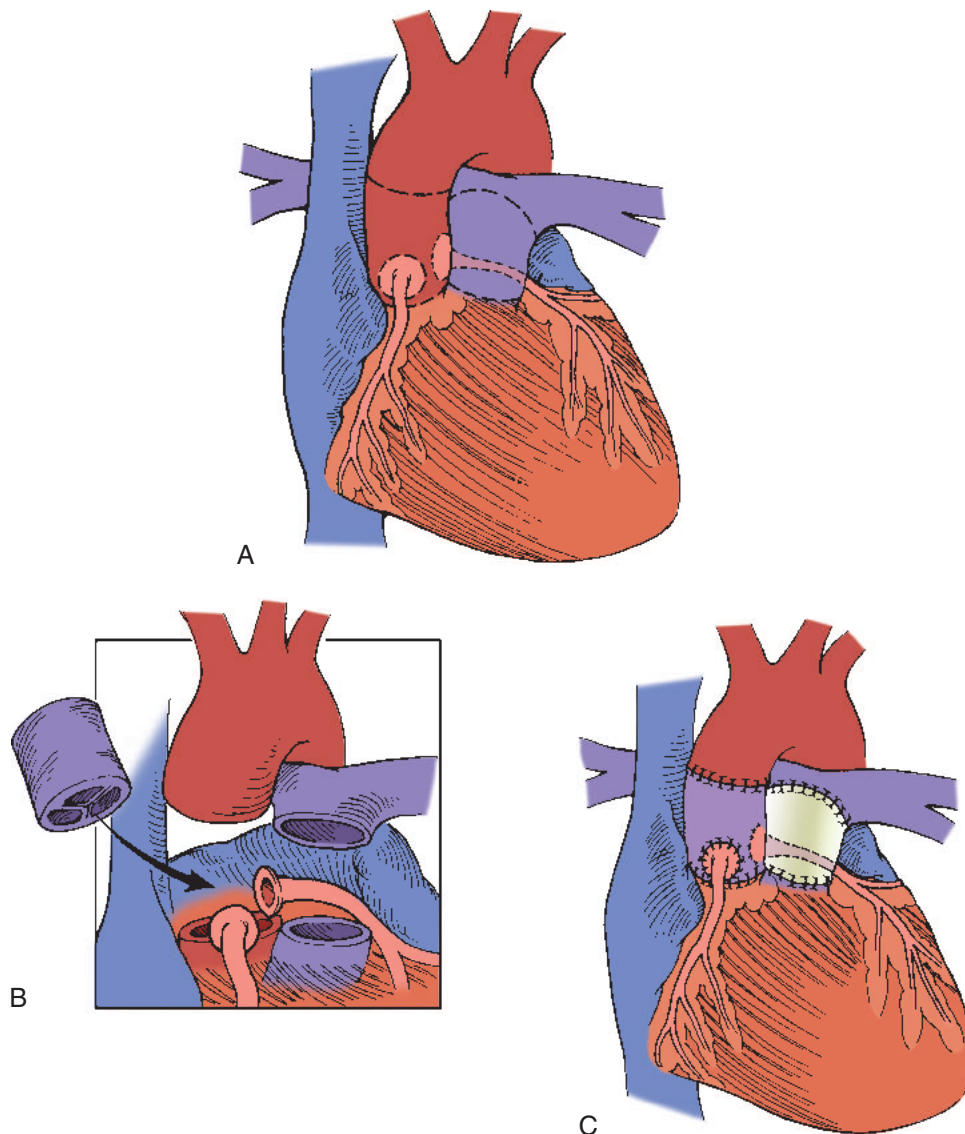
Coarctation may occur anywhere from the transverse aortic arch to the bifurcation of the iliac arteries. Most commonly, there is a discrete narrowing distal to the left subclavian artery, across from the aortic insertion of the ductus arteriosus in a *juxtaductal* position. Conversely, there can be long segment narrowing of the transverse aortic arch, otherwise referred to as a hypoplastic aortic arch. A bicuspid aortic valve occurs in 70% of cases. Other left-sided obstructive lesions tend to occur with coarctation of the aorta. There is a 2:1 male-to-female preponderance and an association with Turner syndrome.

In neonates with a discrete juxtaductal narrowing, the PDA widens the narrowed area and provides relief from the obstruction. The net shunt through the PDA is left to right. These patients have equal oxygen saturations in the upper and lower extremities. In patients with a severe coarctation or a diffusely hypoplastic arch, descending aortic flow originates from a right-to-left shunt through the ductus arteriosus. Differential upper and lower extremity oxygen saturations occur in these patients, with the upper extremities having greater saturation than the lower extremities.

The clinical presentation of coarctation of the aorta depends upon the severity of the narrowing. Mild coarctation often does not present in infancy. Detection typically occurs when upper extremity hypertension and diminished or absent femoral pulses are noted on examination. Infants with more severe coarctation or aortic arch hypoplasia present with diminished lower extremity perfusion following ductal closure. Physical examination reveals an infant with poor perfusion and absent femoral pulses. Cardiac examination may reveal a systolic ejection click if a bicuspid aortic valve is present. A systolic ejection quality murmur is heard that radiates to the back and left infraclavicular area. Chest film demonstrates a large heart with increased pulmonary vascular markings. ECG reveals



• **Fig. 55.22** Congenital Aortic Stenosis. Frontal view through opened aorta demonstrates stenotic and dysmorphic aortic valve with commissural fusion. (From Litwin SB. *Color Atlas of Congenital Heart Surgery*. St. Louis, MO: Mosby; 1996.)



• **Fig. 55.23** Ross Procedure: “Autograft” Aortic Valve Replacement. (A) Dashed lines depict surgical incisions around coronary arteries, aorta, and pulmonary artery. (B) Following removal of the coronary arteries and adjacent “buttons” and the diseased aortic valve, the patient’s native pulmonary valve (“autograft”) is positioned in the aortic root. (C) Completed repair with reimplanted coronary arteries and a cadaveric homograft valve inserted in the pulmonary position. (From Chang AC, Burke RP. Left ventricular outflow tract obstruction. In: Chang AC, Hanley FH, Wernovsky G, Wessel DL, eds. *Pediatric Cardiac Intensive Care*. Baltimore, MD: Williams & Wilkins; 1998:233–256.)

right ventricular hypertrophy. Echocardiogram is used to define the location and extent of aortic narrowing. Additional left-sided obstructive lesions are ruled out. Right ventricular hypertrophy and right ventricle hypertension are also frequently noted.

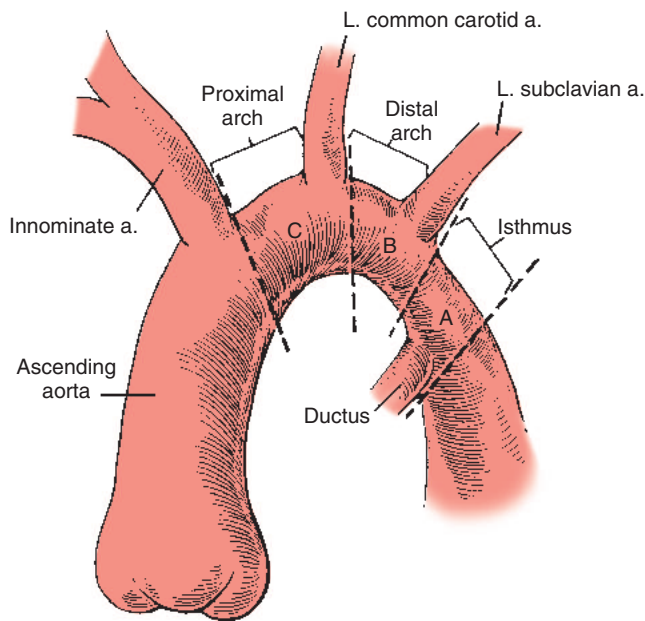
Treatment of the infant presenting with coarctation of the aorta is similar to that of infants with aortic stenosis. Intubation and mechanical ventilation are necessary. Vascular access is obtained for inotropes. PGE₁ is started to open the ductus arteriosus. Metabolic derangements are corrected. Ideally, the infant is stabilized before surgery. Surgery is typically indicated at the time of diagnosis, even in relatively asymptomatic patients. In most cases a lateral thoracotomy is performed, the area of coarctation is excised, and an end-to-end anastomosis is performed. If long segment narrowing is present, patch material may be used to augment the arch, and a more extended end-to-end anastomosis is performed. Balloon

angioplasty of native coarctation is not typically performed in infancy because of the risk of aneurysm, recoarctation, and vascular injury at the site of access.

Surgical mortality is slightly greater than 5% (Mery et al., 2015). Recoarctation occurs in 10%–15% of children and is successfully managed with balloon angioplasty. If a VSD is present, this is typically closed at the time of surgery.

Interrupted Aortic Arch

Interrupted aortic arch is a relatively rare anomaly that is defined simply as complete separation of the ascending and descending aorta. Interrupted aortic arch can be classified by the location of the interruption relative to the head and neck vessels (Fig. 55.24): type A, distal to the left subclavian artery; type B, between the



• **Fig. 55.24** Anatomic Classification of Interrupted Aortic Arch—Types A, B, and C. Dashed lines indicate the potential areas of discontinuity (interruption) in the aortic arch. See text for details. a, Artery. (From Castañeda AR, Jonas RA, Mayer JE Jr, Hanley FL. *Cardiac Surgery of the Neonate and Infant*. Philadelphia, PA: WB Saunders; 1994.)

left subclavian and left carotid; and type C between the left carotid and innominate artery. All types of interruption occur in conjunction posterior malalignment of the infundibular septum; this results in a VSD and varying degrees of left ventricular outflow tract obstruction. Aberrant arrangements of the head and neck vessels are common, with 50% of patients with type B interruption having an aberrant right subclavian artery that arises from the descending aorta. Interrupted aortic arch is associated with 22q11 deletion.

The clinical presentation of interrupted aortic arch is similar to that of other left-sided obstructive lesions. Descending aortic flow is entirely dependent upon right-to-left shunting through the PDA. Ductal closure causes cardiovascular collapse. Initial management is as described for coarctation of the aorta. PGE₁ should be started as soon as possible, as all other resuscitative efforts will have no benefit until postductal circulation is established.

Surgical repair is performed after metabolic acidosis resolves and end-organ function is improved. Continuity is established between the ascending and descending aorta via end-to-end anastomosis, homograft insertion/patch augmentation to connect the two segments, or jump grafts (Fig. 55.25). The VSD is typically closed. In patients with severe left ventricular hypoplasia, a two-ventricle repair may not be possible, and a staged repair or single ventricle palliation is performed (Tchervenkova et al., 2005). Surgical mortality is less than 10% but higher in patients with additional anomalies (Shinkawa et al., 2012). Repeat operation because of left ventricular outflow tract obstruction and balloon angioplasty for recurrent arch obstruction are both common.

Anomalous Origin of the Left Coronary Artery From the Pulmonary Artery

When the left coronary artery arises from the pulmonary artery, inadequate oxygen delivery to the left ventricle results. Coronary artery perfusion occurs primarily during diastole. As pulmonary artery pressures drop postnatally, perfusion pressure of the left

coronary artery falls resulting in ischemia and infarction of the left ventricle. If collateral vessels connect the right and left coronary circulations, flow in the left coronary artery reverses. A left-to-right shunt occurs resulting in coronary artery steal. Mitral valve regurgitation secondary to papillary muscle ischemia and left ventricular dilation develops.

Clinically, symptoms typically develop in the first month after birth. If adequate collateral vessels and myocardial oxygen delivery exist, the patient may present later in life with angina-like symptoms. In the infant, attacks of irritability, pallor, and diaphoresis with feeds are a common presentation. Cardiac examination reveals a displaced point of maximal impulse (PMI), gallop rhythm, and nonspecific murmur. If mitral regurgitation is present, a holosystolic, regurgitant quality murmur is heard. Chest X-ray reveals massive cardiomegaly. The ECG demonstrates a QR pattern and inverted T waves in leads I and aVL. Leads V₅ and V₆ may also have deep Q waves, inverted T waves, and ST segment depression. Echocardiography may suggest the diagnosis but is not always reliable as the left coronary occasionally appears to arise from the aorta. Color Doppler may demonstrate retrograde flow in the left coronary with flow into the pulmonary artery. Cardiac catheterization is diagnostic.

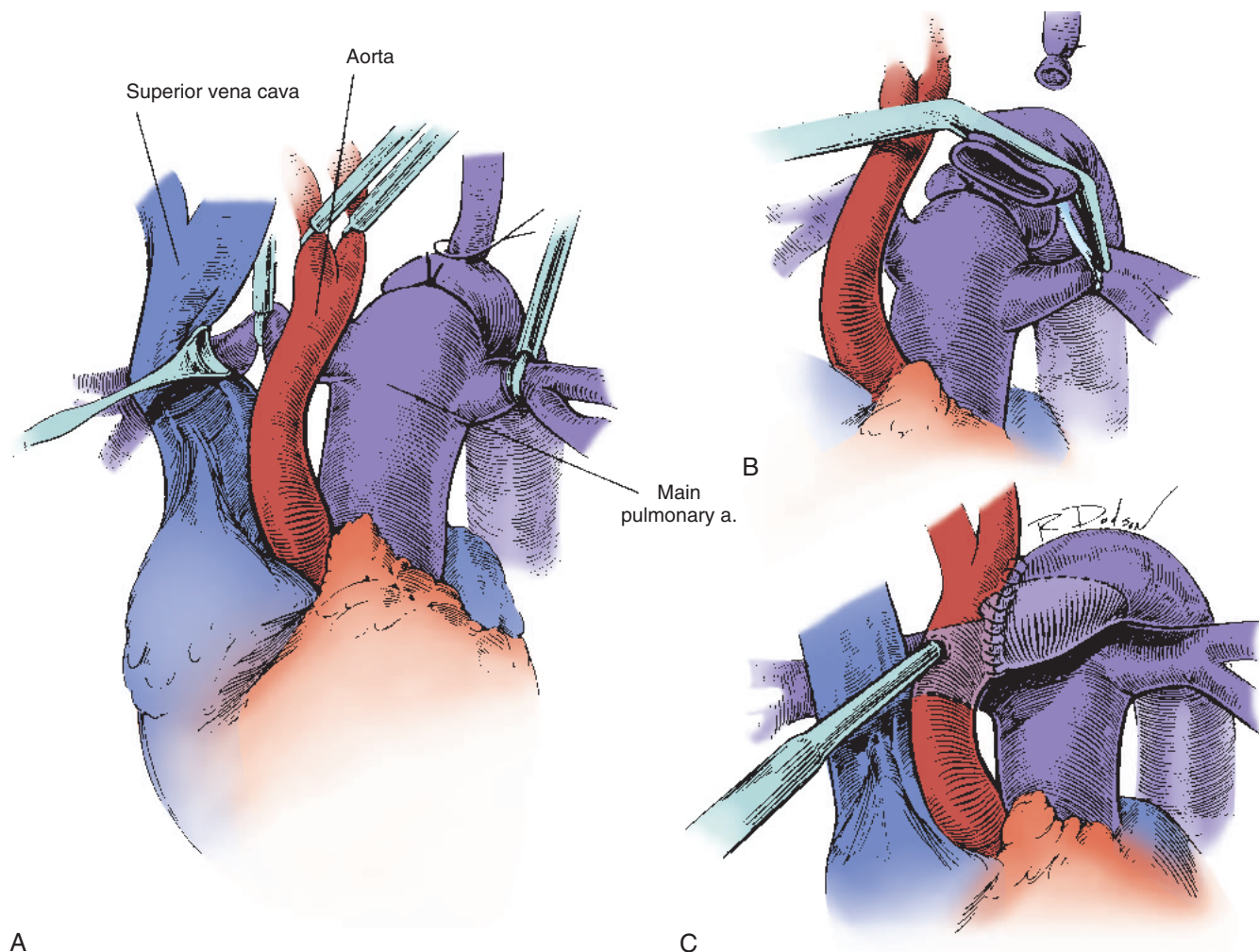
Symptoms of CHF are managed medically. Surgical reimplantation of the left coronary artery to the aorta restores normal coronary perfusion pressure. If the usual two-vessel coronary blood supply is reestablished, there is gradual normalization of left ventricular size and function, as viewed by echocardiography (Lange et al., 2007; Kuroczynski et al., 2008). If myocardial damage is severe or the left coronary artery cannot be reimplanted surgically, cardiac transplantation is performed.

Systemic Arterial Malformations

Vascular anomalies are placed in two major groups: hemangiomas and malformations. Hemangiomas are tumors that demonstrate endothelial hyperplasia and which undergo a period of proliferation and involution. Malformations result from abnormal vascular morphogenesis. They have normal endothelial cell turnover and grow accordingly with surrounding structures. Vascular malformations are further subcategorized by the type of vascular tissue involved (arterial, venous, and lymphatic). Because of their association with high output failure in the newborn period, two types of systemic vascular malformations will be discussed further: AVMs and arteriovenous fistulas (AVFs). An AVM results from multiple microfistulas between small arteries and veins. An AVF results from a connection between a large artery and vein.

Hemodynamically significant AVFs and AVMs present with high output heart failure and cyanosis in the newborn period. An effective large left-to-right shunt occurs through the direct arterial-venous connections. Heart rate, stroke volume, plasma volume, and cardiac output are increased. The fistulous connection lowers systemic vascular resistance, promoting a right-to-left shunt through the ductus arteriosus, particularly if the normal postnatal drop in PVR has not occurred. The increased systemic venous return increases right atrial pressures and promotes right-to-left shunting through the foramen ovale.

Cardiac examination reveals a hyperdynamic precordium. A prominent second heart sound, S₃, and S₄ may be heard. Systolic murmurs may be present secondary to tricuspid valve regurgitation or increased flow across the pulmonary valve. Increased tricuspid valve flow may create a diastolic sound. Bruits may be heard over



• **Fig. 55.25** Surgical Repair of Interrupted Aortic Arch, Type B. (A) The branch pulmonary arteries and arch vessels are snared, and a ligature is placed around the ductus arteriosus. (B) The proximal descending aorta is controlled with a clamp. To adequately mobilize the descending aorta, the left subclavian artery may need to be divided (as shown). Following resection of the ductus arteriosus, the proximal (pulmonary artery) end is oversewn. (C) The descending aorta is anastomosed directly to the ascending aorta. An alternative strategy is anastomosis of the left subclavian and left common carotid arteries combined with homograft patch augmentation of the inferior surface of the arch. Ventricular septal defect closure, when present, is also performed (not shown). a, Artery. (From Castañeda AR, Jonas RA, Mayer JE Jr, Hanley FL. *Cardiac Surgery of the Neonate and Infant*. Philadelphia, PA: WB Saunders; 1994.)

the vascular malformation. When a malformation is suspected, care must be taken to auscultate areas where malformations are likely such as the head, liver, and chest. Arteries proximal to the malformation are typically dilated with bounding pulses, while those distal are small with diminished pulses. ECG is nonspecific and may demonstrate right atrial and right ventricular enlargement. Chest X-ray reveals cardiomegaly with increased pulmonary vascular markings. Echocardiogram demonstrates generalized cardiomegaly. Treatment, if necessary, requires interventional closure of the anomalous vascular connections or the surgical removal of associated anatomic abnormalities.

Cardiomyopathy

A large body of literature exists describing the diagnosis and management of structural CHD in the fetus and newborn. There is a paucity of information, however, regarding the diagnosis and

management of fetal and newborn cardiomyopathy. When presented with a newborn with signs of CHF, structural heart disease should be ruled out. In the absence of structural problems, the diagnosis of cardiomyopathy should be considered. The cause of neonatal cardiomyopathy includes prenatal infections (cytomegalovirus, human immunodeficiency virus, parvovirus), familial or genetic causes, maternal autoimmune disease with anti-Ro or anti-La antibodies, prenatal drug exposure, arrhythmia-induced cardiomyopathy, and twin-twin transfusion syndrome. Postnatal evaluation should include a search for the cause.

Initial management is similar to that used for other types of heart disease. Initial stabilization may require mechanical ventilation, the use of inotropes, afterload reduction, and diuresis. Long-term treatment is dependent somewhat on the cause of the cardiomyopathy, as some forms may be reversible. Cardiac transplantation should be considered if cardiac function is poor or improvement is not noted.

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56

Perinatal Arrhythmias

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KEY POINTS

- The term *supraventricular tachycardia* (SVT) encompasses several different arrhythmias that may have different diagnostic and therapeutic requirements.
- Tachycardia mechanisms may be caused by conduction reentry, enhanced automaticity, or triggered automaticity.
- Isolated atrial and ventricular ectopy are common in the fetus and neonate and usually do not require any therapy.
- Congenital forms of complete heart block may result in slow heart rates but may not require pacemaker implantation.
- Fetal arrhythmias are rare but when present can result in significant hemodynamic compromise.
- Management of fetal arrhythmias is complex and involves evaluation of multiple factors, including: (1) severity of rhythm abnormality, (2) fetal comorbidities and signs of heart failure, (3) maternal comorbidities, and (4) gestational age at diagnosis.

The cardiac conduction system in the normal human heart enables a synchronized and orderly activation of the myocardium and chambers to provide optimal cardiac output. This sequence begins even in the earliest stages of cardiac development. However, disorders of cardiac rhythm can occur at any stage of life, from the fetus to the infant to the adult. These can range from transient and mild disturbances in rhythm to recurrent or persistent arrhythmias that can have significant hemodynamic consequences. Some abnormalities, such as isolated atrial or ventricular ectopy, can be more bothersome to the care provider than to the patient. Other disturbances, such as incessant tachyarrhythmias, can progress to heart failure and hemodynamic collapse. While the incidence of cardiac arrhythmias is increased in the setting of congenital heart disease (CHD), many of these abnormalities are commonly seen in the setting of an otherwise structurally normal heart. Knowledge of the spectrum of cardiac rhythm disturbances is important for prompt recognition, referral, and management. In this chapter, the etiology and common mechanisms of various arrhythmias, as well as differential diagnosis and treatment strategies, will be discussed in separate sections. The unique issues surrounding the diagnosis and management of arrhythmias in the fetus will also be addressed.

Conduction System of the Human Heart

To properly understand the mechanisms of arrhythmogenesis, it is important to recognize the core elements of the normal cardiac conduction system. The embryogenesis of the heart and the conduction system are beyond the scope of this chapter. However, the components of the cardiac conduction system are relatively set by the second trimester of gestation and provide the substrate for many of the observed arrhythmias ([Moorman et al., 2005](#)).

Sinus Node

The sinus node resides in the area between the vena cava and the right atrium and is predominantly located near the superior vena cava to right-atrial junction ([Christoffels et al., 2010](#)). However, there is no anatomic feature that is visible on gross inspection. Functional electrophysiology testing studies and animal studies have demonstrated that the nodal tissue can span the lateral wall of the right atrium, extending from the superior vena cava to the inferior vena cava.

The sinoatrial nodal tissue is histologically distinct from the atrial myocardium and has no contractile components. Electrical impulses are generated by automatic action potential behavior. Activation of the adjacent atrial myocardium by the sinus nodal tissue results in a wave of depolarization that propagates by cell-to-cell activation in a superior-to-inferior and a right-toward-left (in the structurally normal heart) fashion.

Atrioventricular Node

The atrioventricular (AV) node is a more organized area of conduction in the right atrium near the crux of the heart. More well-defined than the sinus node, the compact AV node is located in the posterior portion of the interatrial septum just anterior to the tricuspid valve and (in most cases) provides a singular conduction channel into the bundle of His and the distal conduction system ([Moorman et al., 2005](#)).

The majority of the atrial propagation wave depolarizes the mass of the atria. However, impulses that reach the area of the AV node enter the transition zone of conduction “input” into the AV node. Overall conduction velocity is slowed through the compact AV node but then exits the node into the specialized conduction fibers of the His–Purkinje system.

His–Purkinje System

In the normal heart, the atrial and ventricular myocardium are electrically isolated from each other by the AV rings (tricuspid and mitral annuli). The penetrating bundle of His is usually the only conductive tissue that traverses the AV rings into the ventricles. The bundle of His is insulated from ventricular myocardium until it gives off branches. The first branch from the His bundle enables activation of the septum. Next, the bundle bifurcates into the right- and left-bundle branches. The left bundle divides into anterior and posterior fascicles. At the terminus of each of these bundle branches, the specialized conduction tracts fan into an intricate network of short Purkinje fibers that insert into numerous sites on the ventricular myocardium. Electrical impulses that are conducted through the His–Purkinje system result in activation of the ventricular myocardium in a highly organized and synchronized fashion, translating into a mechanically efficient contraction of both left and right ventricles almost simultaneously.

Abnormalities in Cardiac Conduction

When the normal sequence of cardiac activation is disturbed, the result can be an irregularity in the cardiac rhythm. However, in many cases these disturbances will only be detected on electrocardiography or rhythm monitoring and have no manifestations on physical examination or otherwise. AV block can occur at any level of the conduction pathway if anything impedes propagation of the impulse through that conduction segment. This can be functional (because of negative vagal stimulation) or anatomic (as can be seen following cardiac surgery).

First-Degree Atrioventricular Block

Any conduction delay that prolongs the PR interval beyond the normal range for age is considered a first-degree AV block. By definition, impulses must still be conducted such that a 1:1 AV relationship persists. In the neonate the normal PR interval is generally between 80 and 120 ms, up to 140 ms in the first few months of life, and up to 160 ms in the first 6 months of life (Davignon et al., 1980). In general, first-degree AV block is benign and does not require any special treatment. It can be a normal variant or may be the result of influences that prolong the overall conduction time through the AV node and His–Purkinje system. Increased vagal tone is a common cause of PR prolongation. In the newborn, this can be a manifestation of vagal stimulation caused by a nasogastric or orogastric tube stimulating the oropharynx. More pathologic causes of PR prolongation can include neonatal lupus syndrome or myocarditis. Although there is evidence that significant PR prolongation has a negative impact on cardiac output in the adult heart, there is no indication that this is true in the neonate with a structurally and functionally normal heart. Even when the PR interval is extremely prolonged, there is usually no hemodynamic impact, and the cardiac examination remains essentially unchanged.

Second-Degree Atrioventricular Block

Second-degree AV block is ascribed when there is incomplete conduction from the atrium to the ventricle; that is to say, not every P wave is conducted to a QRS complex. This usually manifests as a skipped beat on physical examination or on cardiac monitoring. Mobitz type I conduction block, also known as *Wenckebach pattern*,

describes a pattern wherein the AV conduction becomes progressively prolonged with each successive beat. The PR interval becomes gradually longer and after a number of beats (usually two or three, although this can certainly be longer) there is failure to conduct for a single beat. The subsequent P wave is then conducted with a normalized PR interval, and the cycle begins anew. Mobitz type II conduction block is present when there is cyclical lack of conduction; however, the progressive PR prolongation seen in the Wenckebach pattern is lacking. Mobitz type I conduction can be seen in conditions of increased vagal tone (as described previously), whereas Mobitz type II is practically unheard of in the neonate in the absence of any other heart disease.

Third-Degree Atrioventricular Block

Third-degree, or complete AV block, occurs when there is complete lack of conduction between the atria and the ventricles. In most situations of complete AV block, there is an escape depolarization mechanism, either junctional or ventricular in origin, which ensures that cardiac output is maintained. Complete AV block can be congenital or acquired. Congenital complete AV block is a particularly challenging entity and is described in a subsequent section. The most common causes of acquired complete heart block are infections or neonatal myocarditis. In addition, acquired complete heart block can occur as a complication of neonatal cardiac surgery in 1%–3% of cases that involve surgical intervention near the interventricular septum (Gross et al., 2006).

Ventricular Preexcitation

Ventricular preexcitation occurs when the ventricular myocardium is activated abnormally, usually by an accessory bypass tract. The ventricular depolarization pattern is thus a combination (fusion) of early activation of a portion of the ventricles through an accessory pathway, and the remainder of activation occurring via the normal His–Purkinje system. In general, these bypass tracts bridge the AV groove that typically separates the atria from the ventricles and result in an anomalous electrical connection to the ventricles.

There are three electrocardiographic features that define this “Wolff–Parkinson–White” pattern: (1) short PR interval, (2) a slurred “delta wave,” and (3) widened QRS duration. How evident this pattern is on electrocardiogram (ECG) can vary depending on the location of the accessory pathway (and thus how early the preexcited portion is activated) and how quickly the ventricles are also activated by the His–Purkinje system. In the newborn heart, the rapid transit time through the AV node and His–Purkinje system can result in a very minimal amount of preexcitation being evident, and appreciation of the presence of the Wolff–Parkinson–White pattern can be delayed, sometimes for years (Cain et al., 2013). Usually, this diagnosis is made only when the newborn patient experiences a tachyarrhythmia (as discussed later).

Abnormalities in Cardiac Rhythm

Alterations in the normal cardiac rhythm can occur by one of several mechanisms and sometimes in combination. These include: (1) enhanced automaticity, (2) reentry mechanisms, and (3) triggered automaticity.

Certain areas of the heart have a tendency to exhibit spontaneous automaticity, such as the sinus node and the AV junction. Sometimes an abnormal cluster of cells can have a particular tendency toward this behavior. Gradual depolarization of the tissue during phase 0

of the action potential (electrical diastole) eventually crosses the action potential threshold, resulting in the depolarization–repolarization sequence, after which it returns to electrical diastole until the gradual depolarization occurs again (Moorman et al., 2005). After the action potential begins in the abnormal ectopic focus, the adjacent myocardium is also depolarized, which then propagates a wavefront outwards from that locus. Automatic rhythms tend to exhibit “warm-up” and “cool-down” behavior, gradually (although sometimes briskly) accelerating and then decelerating back to normal. Even when persistent, there tends to be beat-to-beat variability, and the rate can also be influenced by external influences such as autonomic tone, metabolic states, or hormonal influences. In addition, automatic rhythms can be “overdrive” suppressed when driven by a faster rhythm from another source.

Triggered automaticity occurs when excitable tissue spontaneously depolarizes to the activation threshold, beginning an action potential in that cell or cells that is then perpetuated to the adjacent myocardium. This can occur in fairly normal tissue but can be particularly enhanced by conditions of acidosis, mechanical stimulation, myocardial injury, or inflammation. Increased automaticity is often observed in the presence of certain drugs, such as inotropic medications like dopamine or epinephrine, or stimulant medications like caffeine.

Reentry rhythms are some of the more instantly recognizable tachyarrhythmias in the neonate and young infant. In contrast to the gradual nature of automatic rhythms, reentry has a very abrupt onset and termination. During the rhythm, the rate tends to be fairly stereotyped and consistent. Reentrant rhythms require several prerequisites to perpetuate. First, there must be an arrhythmia “circuit” present with both antegrade conducting and retrograde conducting limbs. Next, there must be differential conduction between the limbs of the arrhythmia circuit, whereupon one limb must exhibit slowing or unidirectional block. Finally, all elements of the arrhythmia circuit must be able to support a repetitive rhythm at a fixed rate.

Ectopic Beats

Premature Atrial Complexes

Isolated atrial ectopy is commonly seen in children and, in particular, during the newborn period. There are no ethnic predilections toward ectopy. Premature atrial complexes can be observed during fetal monitoring and have been reported to occur in up to 25%–50% of normal newborns (Nagashima et al., 1987); however, in the vast majority of cases, this resolves within the first few months of life. Premature atrial complexes are caused by an early triggered depolarization of the atria from an ectopic focus that is separate from the sinus node. On surface ECG this manifests as a P wave that is earlier than would be expected from the preceding rhythm, and the ectopic P wave has a very distinct axis and morphology from the normal sinus P wave. In this manner, one can differentiate the premature atrial complex as originating from a location separate from the sinus node.

Most atrial ectopy in the neonate is conducted normally, meaning that the premature atrial complex is followed by a normal-appearing QRS complex. The PR interval may be measured as different to normal, which is more a reflection of the atrial depolarization beginning in an abnormal location (and thus either closer or further than the sinus node) rather than indicating any defect in AV conduction. When a premature atrial complex is closely coupled to the preceding beat, the conducted QRS complex may have an abnormal appearance. This “aberrant conduction” may just be

slightly wider than normal or it may have the appearance of a complete bundle branch block. This is caused by the early impulse failing to conduct, as the refractory period for that segment has been exceeded. Aberrantly conducted premature atrial complexes are often mistaken for premature complexes because of their wider appearance but can be distinguished by the presence of a preceding P wave. Blocked premature atrial complexes can occur if the ectopic beat occurs early enough after the preceding beat. While this effect is more pronounced where this is impaired AV conduction, it is most commonly observed in the setting of normal nodal conduction and results from the early atrial impulse failing to conduct because of the refractory period of either the AV node or the His–Purkinje system. Blocked premature atrial complexes are identified by the presence of the ectopic P wave (usually with the T wave of the preceding beat) that has no QRS complex following. Often, there will be a sinus pause before the next normally conducted sinus return beat. In most instances, isolated premature atrial complexes in the newborn do not require any treatment.

Premature Ventricular Complexes

Isolated ventricular ectopy is also commonly observed in the normal neonate, although to a lesser extent than atrial ectopy (Nagashima et al., 1987). Ventricular ectopic beats are usually distinguished by a widened QRS complex with a T-wave axis that is different when compared with the normal QRS–T complex. Importantly, there is no P wave that precedes the premature QRS complex. In isolation, ventricular ectopy has no pathologic implications for infants who have no other signs or symptoms to suggest any cardiac pathology.

Tachyarrhythmias

While a number of tachyarrhythmias can be observed during childhood, only a subset of distinct arrhythmias is observed with any frequency in the fetus and neonate (Table 56.1). The most common of these are orthodromic reciprocating tachycardia (ORT), atrial ectopic tachycardia, and atrial flutter.

Orthodromic Reciprocating Tachycardia

ORT is the most common form of supraventricular tachycardia (SVT) in the fetus and the neonate (Spearman and Williams,

TABLE 56.1

Differentiating Neonatal Tachyarrhythmias

| VA Relationship | Differential Diagnosis |
|--------------------|---|
| A > V | Atrial ectopic tachycardia Atrial flutter |
| Short VA (VA < AV) | ORT (SVT) JET (VA-associated) |
| Long VA (VA ≥ AV) | Sinus tachycardia Atrial ectopic tachycardia PJRT |
| V > A | Ventricular tachycardia JET (VA-dissociated) |

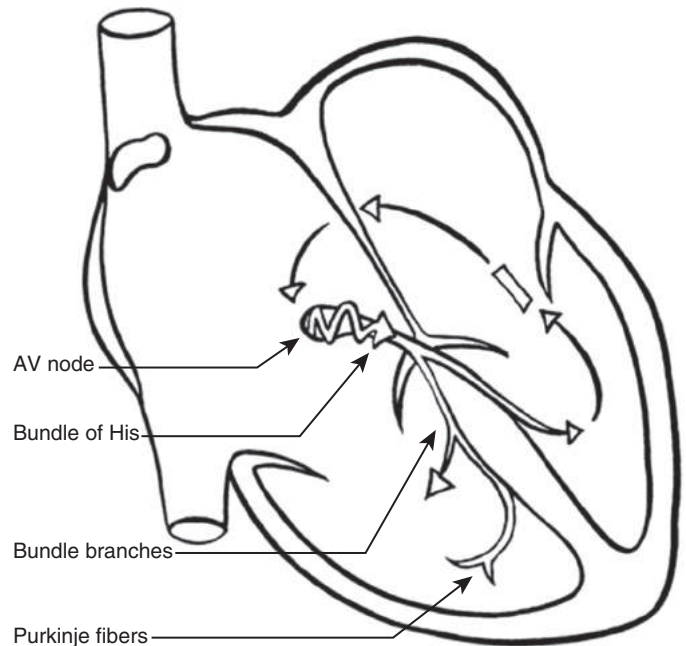
A, Atrial activity; AV, atrioventricular; JET, junctional ectopic tachycardia; ORT, orthodromic reciprocating tachycardia; PJRT, permanent form of junctional reciprocating tachycardia; SVT, supraventricular tachycardia; V, ventricular activity; VA, ventriculoatrial.

2014). It is the arrhythmia mechanism most commonly associated with SVT, so the terms are often used interchangeably. The tachycardia circuit for this reentrant arrhythmia utilizes the normal conduction system as the antegrade limb, and an accessory pathway provides the retrograde limb for the tachycardia (Fig. 56.1). When conditions are appropriate, impulses conducted through the His–Purkinje system to the ventricles are then carried in a retrograde fashion over an accessory pathway to the atria, which then reaches the AV node to start the cycle again. If not in their refractory periods, the atria, AV node and His–Purkinje system, ventricles, and accessory pathway all perpetuate a rapid repetitive SVT rhythm. In the fetal and neonatal heart, this form of SVT will manifest with very fixed rates that typically range between 240 and 300 beats per minute (bpm). So-called *retrograde* P waves may be visible on the ST segment or the T wave, as evidence of the conduction time between the activation of the ventricles, passage through the accessory pathway, and subsequent reactivation of the atria. The measured RP interval can be used to aid in the differential diagnosis of the various forms of SVT, which can consequently be used to help guide specific management and for prognosis.

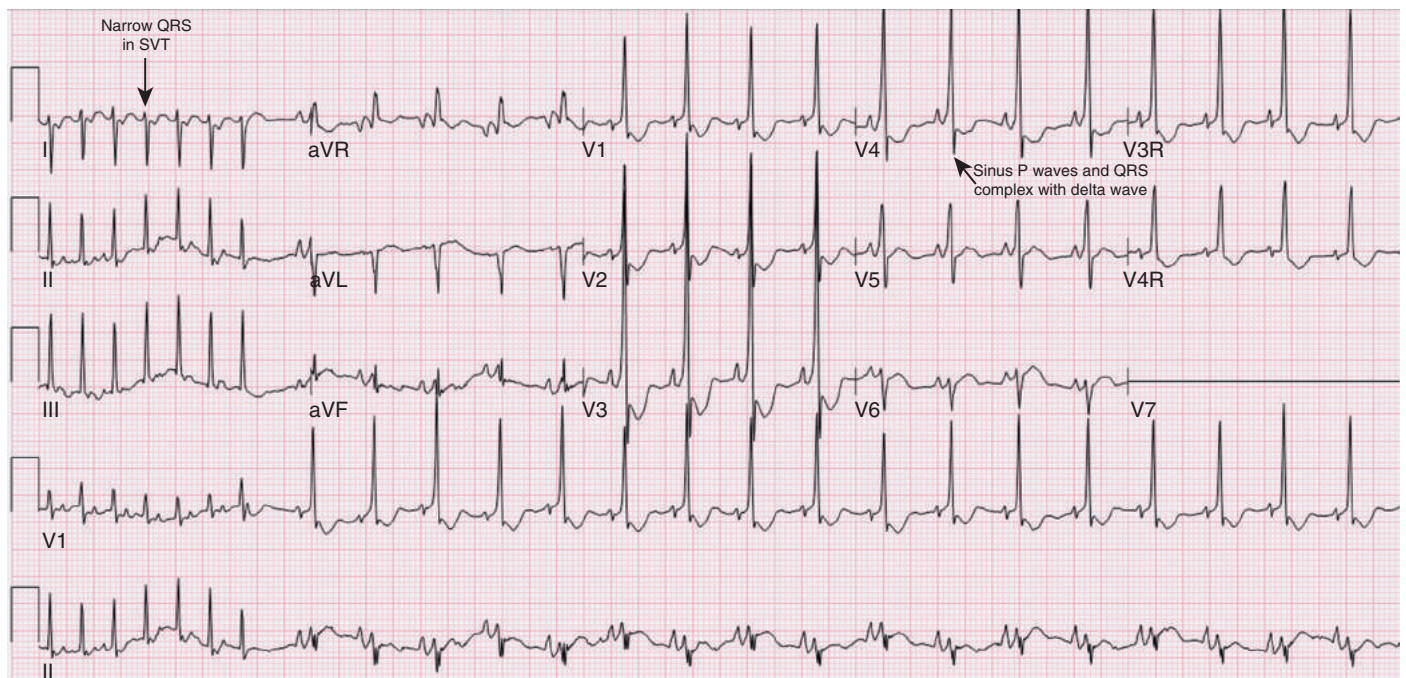
When an accessory pathway only conducts in a retrograde direction it is called a *concealed* accessory pathway; that is to say, there is no evidence of pathway conduction during normal sinus rhythm conditions, and the pathway only conducts from ventricle to atrium during reciprocating tachycardia. A manifest accessory pathway is one whose presence is known during normal sinus rhythm. These are the pathways that cause the ventricular preexcitation of Wolff–Parkinson–White syndrome (discussed previously). Manifest pathways usually conduct bidirectionally; antegrade conduction results in preexcitation on resting ECG, and retrograde conduction is utilized during reciprocating tachycardia, resulting in a narrow QRS complex without preexcitation (Fig. 56.2).

For the neonate on continuous heart rate monitoring in the intensive care unit, such episodes of SVT are usually quite apparent

by the abrupt onset and spontaneous termination of a rapid heart rate. In the absence of any monitoring the development of SVT may not be as obvious. In contrast to older children who are able to report complaints of palpitations, neonates and young infants rarely give any indication of the tachyarrhythmia occurring within. Sometimes, pallor, diaphoresis, or a change in respiratory pattern



• **Fig. 56.1** Mechanism for Orthodromic Reciprocating Tachycardia. Antegrade conduction through His–Purkinje system depolarizes ventricles. Retrograde conduction from ventricles, over an accessory pathway, to atria. (Reproduced with permission Circulation. 2008;117:2820-2840 © 2008, American Heart Association, Inc.)



• **Fig. 56.2** Wolff–Parkinson–White Syndrome. Spontaneous termination of orthodromic reciprocating tachycardia. Note change from narrow QRS in tachycardia, then wide preexcited QRS during subsequent sinus rhythm.

may be evident to the outside observer. However, despite the rapid rates, SVT is generally well tolerated. Most episodes of ORT are self-limited, persisting only for seconds or minutes at a time; sometimes, episodes can last 30–60 minutes or more before terminating spontaneously. In rare instances the SVT can be incessant, lasting for hours or sometimes a day or more. In these cases, delay in recognition and diagnosis can lead to a tachycardia-induced dilated cardiomyopathy and can possibly result in eventual cardiovascular collapse.

Acute management of ORT is directed at terminating the tachycardia and restoration of sinus rhythm. Slowing or transient blockade of AV nodal conduction is the mechanism by which most therapies work and includes vagal maneuvers (such as knee-chest position or diving reflex) and administration of adenosine. Initial adenosine doses of 0.05–0.1 mg/kg by rapid intravenous (IV) bolus are recommended for the neonate, with escalating doses as necessary. Direct current cardioversion for immediate arrhythmia termination can also be considered for the infant with impending collapse, although the risks versus benefits of this approach must be considered. For chronic medical therapy in the infant prone to recurrent episodes of SVT, β -blockade in the form of propranolol is often used as a first-line agent. In some institutions, digoxin is used alone or in combination with propranolol. While calcium channel blockers are readily used in older children and adults for this indication, this class is generally avoided in the young infant due to the potential for significant myocardial depression. Second-line agents include sotalol, flecainide, and amiodarone. These medications are discussed further in the Management Considerations for Neonatal Tachyarrhythmias section later.

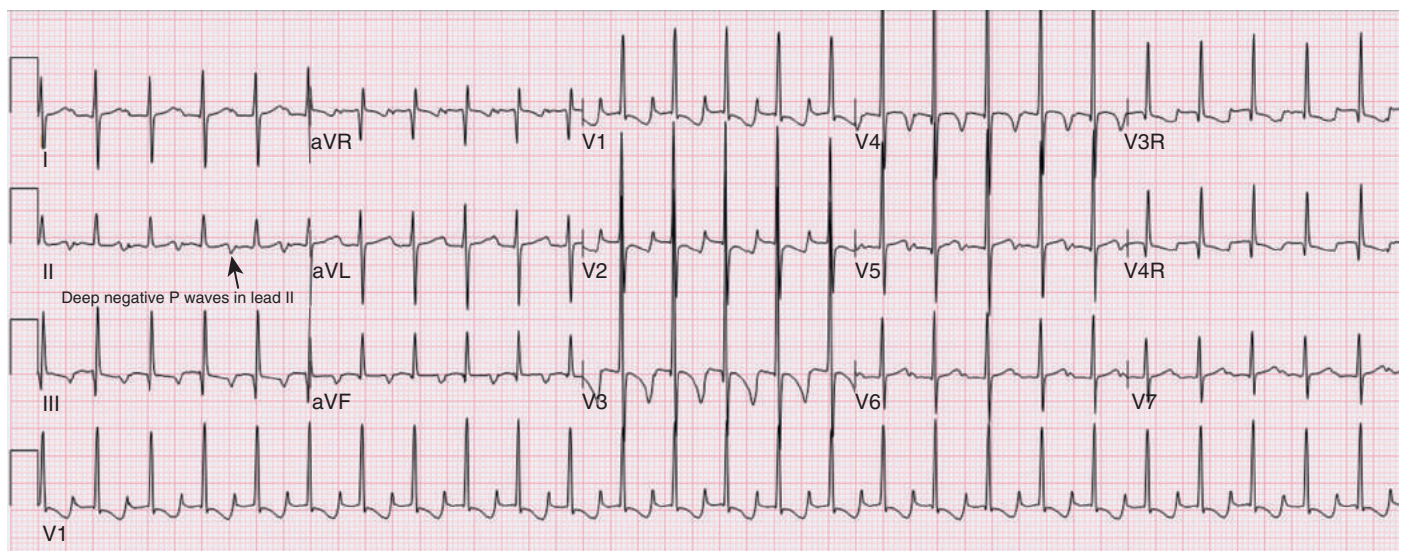
Permanent Form of Junctional Reciprocating Tachycardia

An unusual but particular vexing variant of ORT is the permanent form of junctional reciprocating tachycardia (PJRT). This is a reentry form of SVT that has rates that are usually far slower than the usual form of ORT, sometimes seemingly at the upper limits of the normal range for the newborn, around 180–200 bpm.

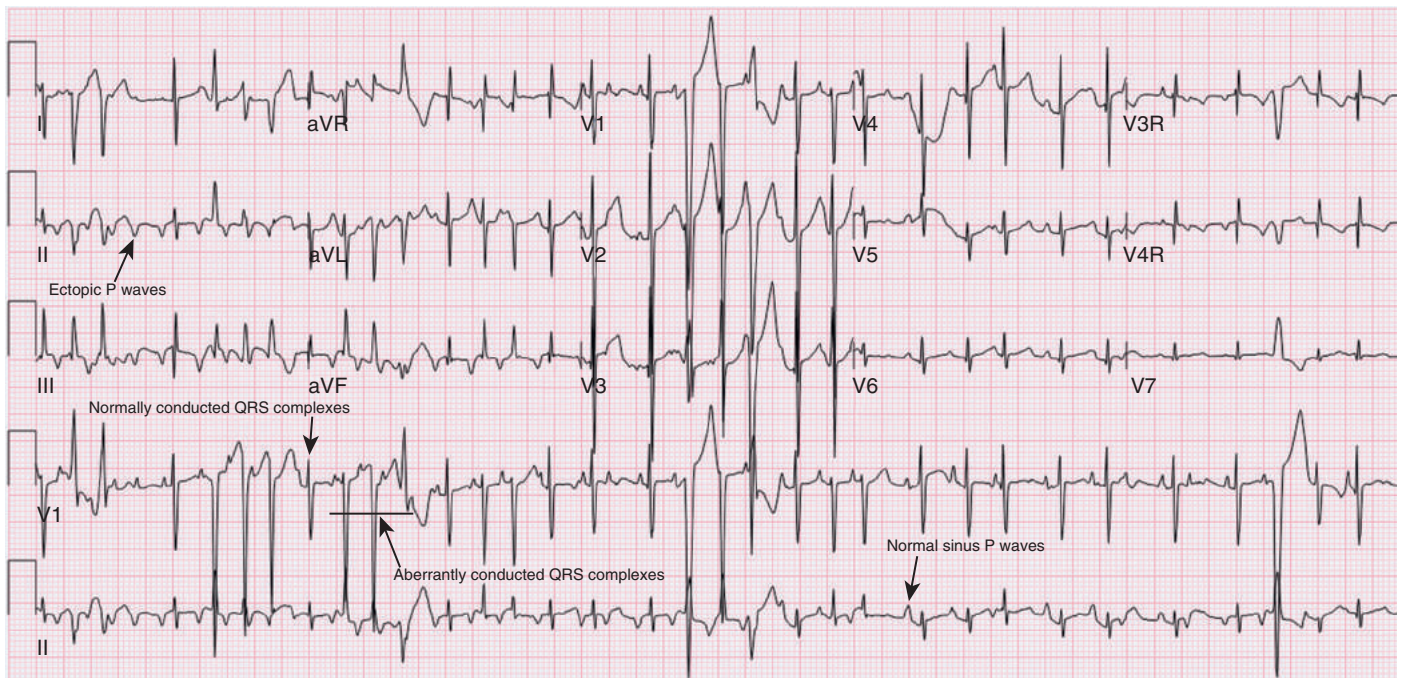
However, PJRT has a tendency toward being an incessant arrhythmia, often spontaneously reinitiating immediately after tachycardia termination. The mechanism for PJRT is usually a concealed accessory pathway, typically located in the posterior septum or posterior tricuspid annulus. The conduction velocity of this retrograde limb is slower than in most other types of accessory pathways, resulting in a very long RP interval. Another hallmark feature is the presence of strongly negative retrograde P waves in the inferior leads, particularly lead II (Fig. 56.3). These infants often escape early detection as the tachycardia rates are not dramatically elevated, which can lead to delayed diagnosis and presentation with tachycardia-induced cardiomyopathy or congestive heart failure (Kang et al., 2014). Treatment can be challenging as the arrhythmia has a tendency toward spontaneous reinitiation, even after successful termination of tachycardia. Medical therapy includes the drugs described above, although most infants with PJRT will require multiple agents for successful control of this arrhythmia.

Atrial Ectopic Tachycardia

Atrial ectopic tachycardia (AET) is a less common cause of SVT than ORT but is still well-represented among perinatal tachyarrhythmias. This is also known by other monikers such as *ectopic atrial tachycardia* or *focal atrial tachycardia*. In contrast to the reciprocating tachycardias, AET is not caused by a bypass tract but rather by a focus of cells in the atria that are more excitable than the sinus node and exhibit enhanced or triggered automaticity. As atrial activation originates from a location other than the sinus node, the P waves on surface ECG are usually quite distinctly different to normal (Fig. 56.4). The result is paroxysmal bursts of tachycardia, although prolonged and even incessant tachycardia can also be seen, which can lead to the development of tachycardia-induced cardiomyopathy (Gopinathannair et al., 2015). This arrhythmia is often catecholamine-sensitive, with increased clinical signs during periods of activity or stimulation. Heart rates often vary during sustained tachycardia, and the rates can range from slightly faster than sinus rhythm to well over 300 bpm at times. Since the tachycardia does not depend on intact AV nodal



• **Fig. 56.3** Permanent Form of Junctional Reciprocating Tachycardia. Typical appearance of permanent junctional reciprocating tachycardia, with long ventriculoatrial time (ventriculoatrial > atrioventricular) and strongly negative P waves in inferior leads II, III, and aVF.



• **Fig. 56.4 Atrial Ectopic Tachycardia.** P waves with unusual axis “march through” the tracing, with more P waves than QRS complexes on tracing. Wide aberrantly conducted QRS complexes are caused by “Ashman phenomenon” of conduction.

conduction, intermittent AV block (such as 2:1 conduction or Wenckebach pattern) can sometimes be seen, and this feature is often used to aid in the correct diagnosis. By the same token, nodal blockade does little to affect this arrhythmia. Adenosine administration usually only transiently blocks the AV nodal conduction while the atrial arrhythmia continues unabated. While direct current cardioversion may interrupt the arrhythmia, it is likely to reinstate spontaneously, because of its paroxysmal nature.

Junctional Ectopic Tachycardia

Another automatic supraventricular arrhythmia of childhood is junctional ectopic tachycardia (JET). This is caused by increased automaticity of the cells around the AV junction resulting in direct activation of the His–Purkinje system. Heart rates range from 170 to 210 bpm and demonstrate the usual warm-up and cool-down behavior. The atrial activity may be conducted retrograde from the junctional activity or may be completely dissociated from it. JET can be either acquired or congenital. The acquired form can occur in up to 20% of patients following cardiopulmonary bypass for certain congenital cardiac surgeries (Moak et al., 2013) and can cause hemodynamic embarrassment because of the inherently unstable nature of those patients, even though the arrhythmia itself is fairly self-limited. The congenital form of JET is quite rare but like atrial tachycardia can be an incessant arrhythmia. Such patients can present with tachycardia-induced cardiomyopathy (Gopinathannair et al., 2015).

Neonatal Atrial Flutter

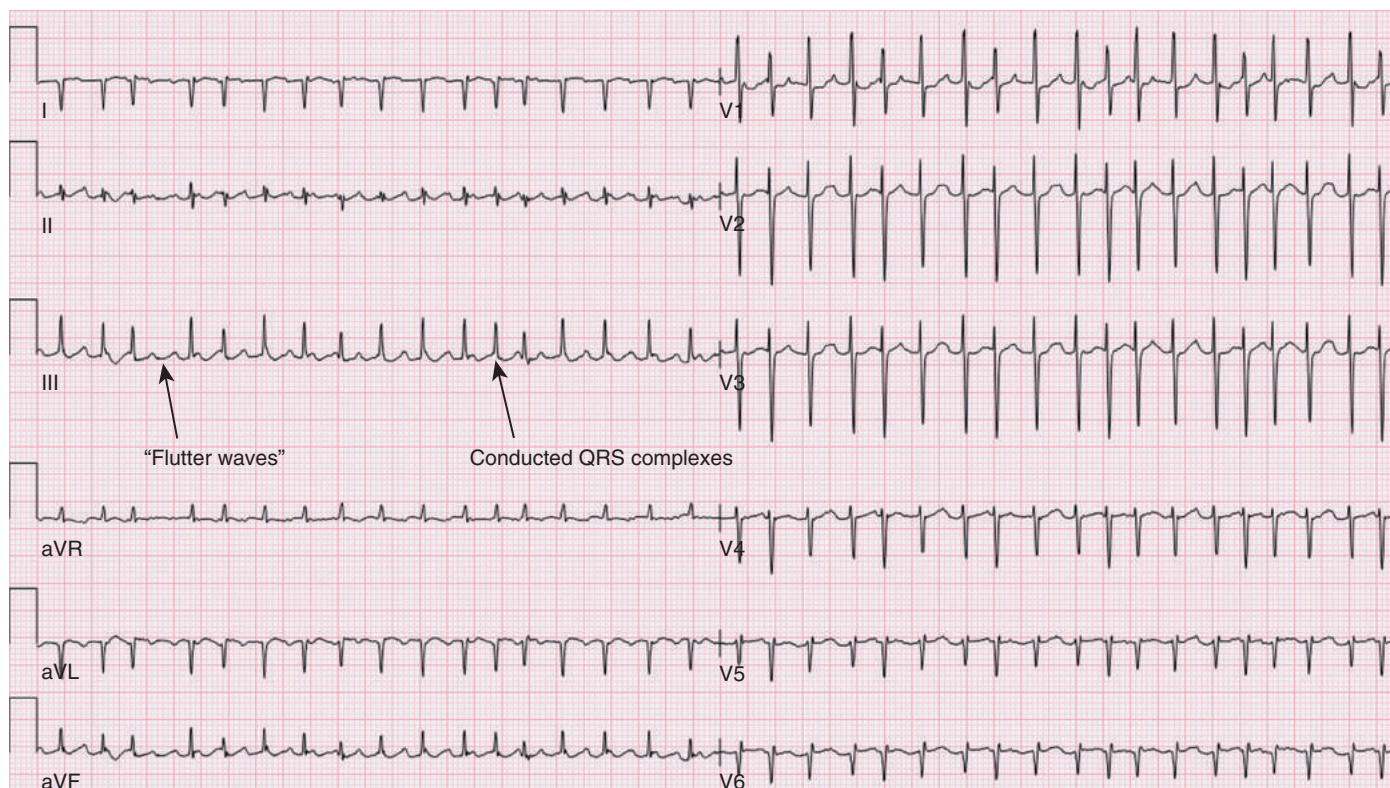
Atrial flutter is a macro-reentrant arrhythmia that occurs entirely within the atria, with a depolarization wavefront that typically runs in a counterclockwise direction around the tricuspid valve

annulus. The atrial rate is usually 300 bpm or greater, and the continuous atrial activation gives it a characteristic “sawtooth” pattern in the inferior leads, particularly lead II and III (Fig. 56.5). The atrial rate is exceedingly consistent and entirely independent of the ventricles, although varying degrees of AV conduction can be observed. While older infants and children might conduct every other (2:1) or every third (3:1) atrial impulse, the neonatal AV node has brisker conduction capability so may conduct each impulse (1:1), resulting in a very rapid ventricular rate. Administration of adenosine can be diagnostic by blocking AV nodal conduction while the flutter waves continue uninterrupted.

Medical therapy may be used to slow the ventricular rate. However, in most cases direct current cardioversion is required; this is highly effective and the recurrence risk is low (Casey et al., 1997). In the fetus there is often 1:1 conduction with a very rapid ventricular rate. When undetected and untreated, this can result in congestive heart failure, which manifests as hydrops fetalis. Detection and diagnosis may be difficult as it is unusual to be able to obtain a rhythm recording or ECG. A fetal echocardiogram may be able to demonstrate rapidly contracting atria (as discussed later). When necessary, fetal flutter may be treated by administering antiarrhythmic medications to the mother, although high maternal doses are often required to achieve adequate transplacental transfer to the fetus.

Ventricular Tachycardia

Ventricular tachycardia (VT) is a fairly uncommon arrhythmia in the perinatal period. It can take varied forms, ranging from a benign accelerated ventricular rhythm to a potentially life-threatening polymorphic VT. The latter is rarely encountered and occurs only in the setting of severe metabolic derangements or electrolyte disturbances or in the setting of an underlying congenital primary



• **Fig. 56.5** Atrial Flutter. The atrial rate is approximately 360 beats per minute, with “flutter waves” visible in leads II, III, and aVF. Note that not all flutter P waves conduct equally to the number of QRS complexes.

arrhythmia syndrome. VT may be suspected on the basis of a wide QRS complex tachycardia, although the differential diagnosis of such a rhythm includes a supraventricular rhythm conducted aberrantly, such as with preexcitation pattern (as discussed earlier) or when conducted with a bundle branch block. However, in most instances, monomorphic VT is generally a benign entity in infancy and rarely requires intervention other than medical therapy (Levin et al., 2010).

Accelerated idioventricular rhythm (AIVR) is a benign arrhythmia. Sometimes considered as “slow VT,” AIVR is caused by enhanced automaticity of the ventricles and is usually due to intrinsic catecholamine states, electrolyte disturbances, or other conditions that predispose to increased automaticity. The hallmark is a monomorphic rhythm that originates in the ventricles but at rates that are only slightly faster than the normal sinus rates. This is generally well tolerated hemodynamically, unless the infant is otherwise extremely compromised by other medical factors.

In contrast to the stability of monomorphic ventricular arrhythmias, polymorphic VT is a much more disorganized and potentially life-threatening arrhythmia and must be recognized and managed emergently. The ECG in polymorphic VT exhibits a rapid wide QRS complex, and the QRS morphology varies from beat-to-beat, indicating generally disordered ventricular depolarization. Cardiac output can be severely reduced. A particular variant of polymorphic VT is known as *torsades de pointes* (TdP). Literally, “twisting about a point,” the amplitude and axis of the QRS morphology rotate and undulate and can eventually degenerate into a more chaotic ventricular fibrillation. It is rarely seen in the neonate, except in the setting of primary arrhythmia genetic syndromes that are

associated with sudden cardiac death, such as the long QT syndrome (LQTS) (as discussed later).

Management Considerations for Neonatal Tachyarrhythmias

Appropriate treatment of neonatal tachyarrhythmias depends largely upon prompt recognition and accurate diagnosis. The most common substrates for tachycardia in this subset are ORT and AET, in addition to neonatal flutter. Orthodromic tachycardia can acutely respond to vagal maneuvers and (more reliably) to IV adenosine administration, although these methods will have little to no effect on either AET or neonatal flutter. Because of the increased likelihood of recurrent reentry tachycardia during early infancy, medical therapy is commonly utilized to decrease the possibility. Many infants can show no outward signs that they are experiencing tachycardia until incessant tachyarrhythmias result in a dilated cardiomyopathy and cardiovascular collapse. Older infants and children will usually give more outward signs of being in a tachyarrhythmia, so are less likely to present with cardiomyopathy and cardiovascular collapse.

Medical therapy for neonatal tachycardia varies considerably across institutions (Seslar et al., 2013). Digoxin selectively inhibits the sodium-potassium adenosine triphosphatase channel, which causes an increased intracellular sodium concentration. Other cardiac effects include a positive inotropic effect and an increased vagotonic effect. It is this latter effect that is suspected to contribute to the antiarrhythmic properties of the drug. In many institutions,

digoxin was historically used most commonly for acute and chronic management of SVT, although its utilization varies greatly (Guerrier et al., 2016).

Propranolol (Vaughan-Williams class II) is a nonspecific β -blocker medication that binds β -adrenergic receptors. This decreases overall sensitivity to adrenergic stimulation and has some direct effects on myocyte membrane potential. While the overall success of propranolol versus digoxin is comparable (Moffett et al., 2015), digoxin was less likely to succeed initially as monotherapy. In addition, digoxin is contraindicated in the Wolff–Parkinson–White syndrome because of enhancement of accessory pathway conduction properties, so ventricular preexcitation must be excluded before initiation of digoxin. The drug is also generally ineffective for AET. Enteral propranolol is increasingly used as first-line therapy for neonatal SVT, preferentially in high-volume treatment centers (Guerrier et al., 2016). Particularly in the preterm newborn, the risk of β -blocker-associated hypoglycemia exists so monitoring of blood glucose during initiation of propranolol therapy is recommended in this population.

Second-line agents include flecainide and sotalol, as well as amiodarone, and are highly effective in controlling the arrhythmia in incessant forms of neonatal SVT (Price et al., 2002). These drugs require closer monitoring because of their proarrhythmic effects. All three can potentially prolong the QT interval and potentially provoke ventricular arrhythmias. Flecainide (Vaughan-Williams class Ic) inhibits the fast inward sodium current of the myocardial action potential and prolongs conduction through all cardiac tissues, particularly those of the His–Purkinje system and ventricular myocardium. This can result in decreased ability of the conduction system to perpetuate SVT and is a very effective drug for treating SVT. Flecainide is bound by milk protein and significant fluctuation in drug serum levels has been reported in neonates transitioning to oral feeds (Russell and Martin, 1989); it can also prolong the QRS complex.

Vaughan-Williams class III medications used to treat perinatal SVT include sotalol and amiodarone. This class of drugs prolongs the action potential duration by extending the repolarization phase of the myocardium, thus increasing the overall refractory period of the tissue. This decreases the ability of the myocardium to support the repeated depolarizations necessary to support SVT. Sotalol exhibits β -blocker effects (class II) in lower doses and in higher doses exhibits more class III effects. Amiodarone is highly effective for acute and chronic management of tachyarrhythmias in the neonate; however, it must be used judiciously as it can have the effect of hypotension, bradycardia, heart block, cardiovascular collapse, and hypothyroidism. It has been reported to cause “neonatal gasping syndrome” and to leach plasticizers from polyvinyl chloride containers and syringes (Centers for Disease Control and Prevention, 1982). Because of the pharmacokinetics of amiodarone, it is usually administered by loading doses (in both IV and enteral dosing) for 7–10 days, after which the dose is decreased to a maintenance dose.

While commonly performed in older children, percutaneous catheter ablation for supraventricular arrhythmias is rarely performed in neonates and small infants. Technical issues and catheter size limit conventional mapping and ablation techniques. Late complications and deaths caused by injury to adjacent coronary artery structures have also been reported (Al-Ammouri and Perry, 2006).

While most ventricular arrhythmias in the neonate are controllable and self-limited, certain conditions may predispose to more life-threatening forms of VT. Particularly in cases in which defibrillation must be performed, for hemodynamically unstable VT or ventricular fibrillation, additional therapies must be considered.

Reversible causes of TdP such as electrolyte disturbances or drug-induced TdP should be addressed by aggressive correction of the imbalance or removal of the offending agent(s). However, when no such cause can be identified, the etiology is often found to be a congenital arrhythmia syndrome, commonly LQTS (as discussed later). In more-affected infants, treatment with β -blocker medications may be used, and in severe cases with recurrent episodes of TdP or ventricular fibrillation in which the anticipated risk of sudden cardiac arrest is excessive, implantation of a pacemaker or a defibrillator might be considered; however, this must be weighed against the acute implantation issues and longer-term complications.

Bradyarrhythmias

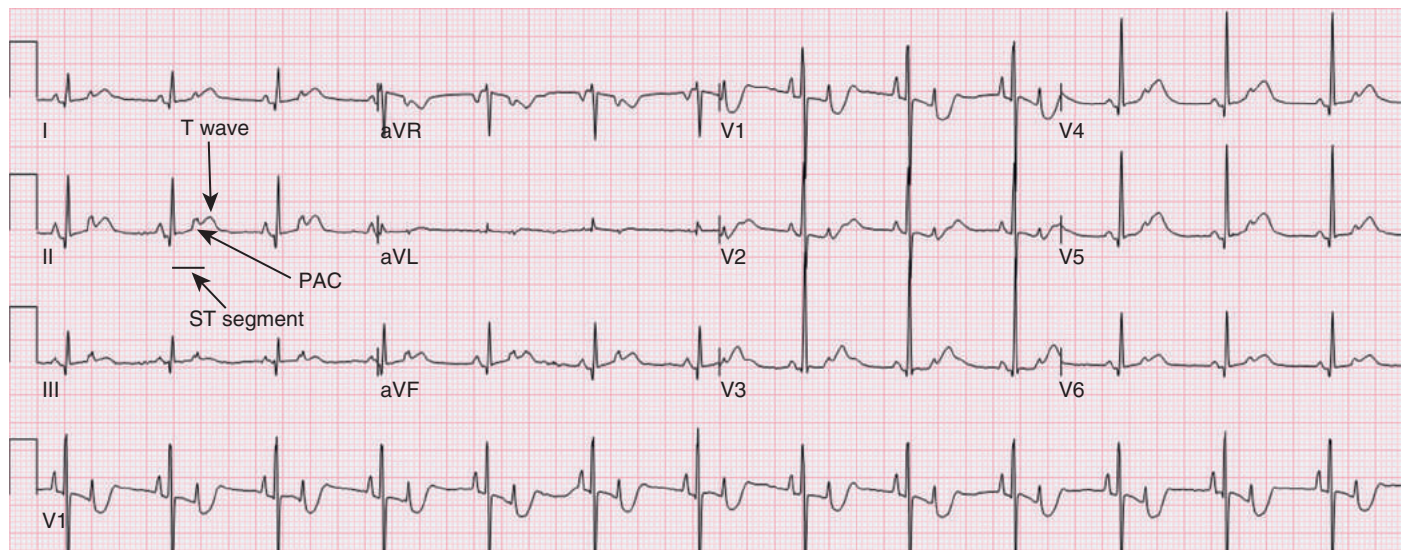
Neonatal forms of bradycardia are few. In general, bradycardia is defined as sustained heart rates less than 100 bpm. This can be the result of normal physiologic causes or secondary to arrhythmias or abnormalities in conduction. Sinus bradycardia represents the most common mechanism in the neonate. This can be a normal physiologic variant (it is not uncommon to encounter a normal term neonate with a sinus rate of 90 bpm in the first 24–48 hours after birth, especially during sleep) or secondary to other causes (such as therapeutic hypothermia). Another common mechanism is vagal stimulation, which can be provoked by the presence of oral or nasal feeding tubes, endotracheal tubes, or episodes of gastroesophageal reflux.

Blocked Premature Atrial Complex

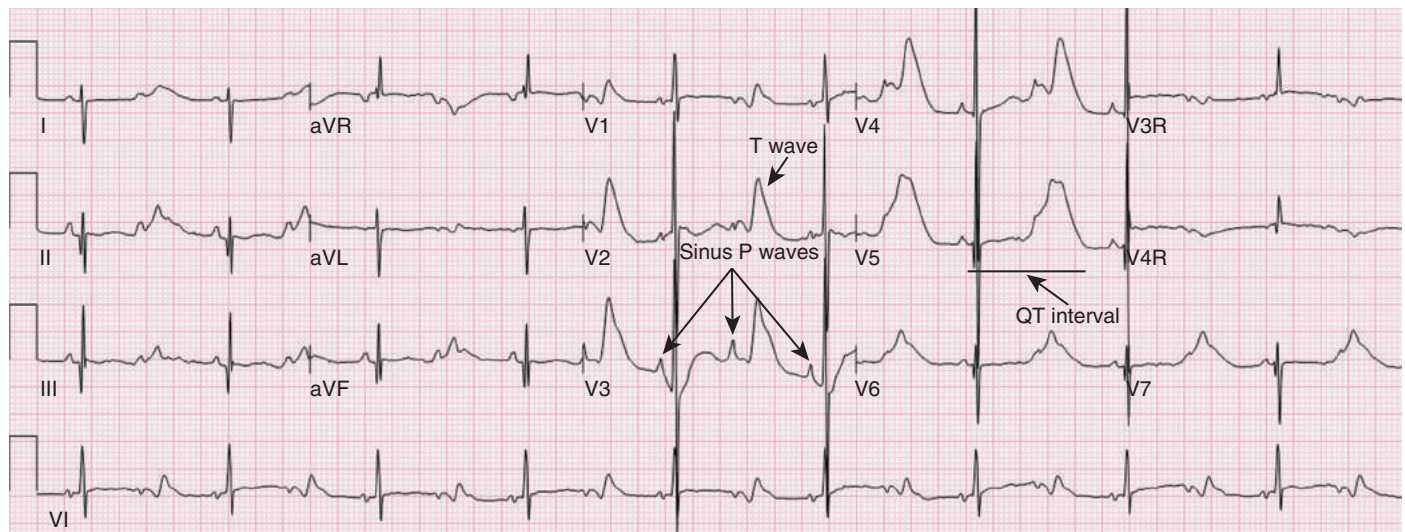
Atrial ectopy is commonly encountered in the fetus and newborn (discussed previously). In most instances, the premature atrial complex (PAC) conducts normally to a normal-appearing QRS complex. However, PACs that occur earlier in relation to the preceding QRS complex may find the conduction system or ventricular myocardium refractory to stimulation. Thus the PAC is not followed by any ventricular depolarization. This is described as a “blocked PAC.” Most often, this is observed when a PAC occurs in the early portion of the preceding T wave, such that the ventricles are still in their refractory period and cannot depolarize. Conditions that affect the refractoriness of either the AV nodal conduction or myocardial refractoriness, such as increased vagal tone or metabolic acidosis, can also increase the likelihood that a PAC is blocked. Following the premature atrial depolarization, there is typically a delay before the subsequent sinus return beat. When atrial ectopy is frequent, as in blocked atrial bigeminy, the net effect is a functional “halving” of the sinus rate (Fig. 56.6).

Long QT Syndrome

In severe forms of congenital LQTS the QT prolongation can result in bradycardia in the fetus or the newborn. When the repolarization phase becomes significantly prolonged, the subsequent sinus P wave may fail to conduct normally or at all as the ventricular myocardium is still refractory to stimulation. As a result, the functional heart rate is halved since the ventricular rate is conducted 2:1 from the sinus rate. This can have significant implications as the bradycardia may not be tolerated by the patient. Chronotropic medications for this condition may do little to improve the functional heart rate as increasing the frequency of sinus P waves does not shorten the refractory period of the ventricular myocardium. Permanent pacemaker implantation may be necessary. In addition, the functional



• **Fig. 56.6** Premature Atrial Complexes. Premature atrial complexes occurring on ST segments are failing to conduct to ventricles resulting in “blocked atrial bigeminy.”



• **Fig. 56.7** Long QT Syndrome With 2:1 Functional Heart Block. The sinus rate is 110 bpm. The QT interval is profoundly prolonged ($QT_c = 751$ ms), so every other sinus P wave cannot conduct because of the ventricular refractory period.

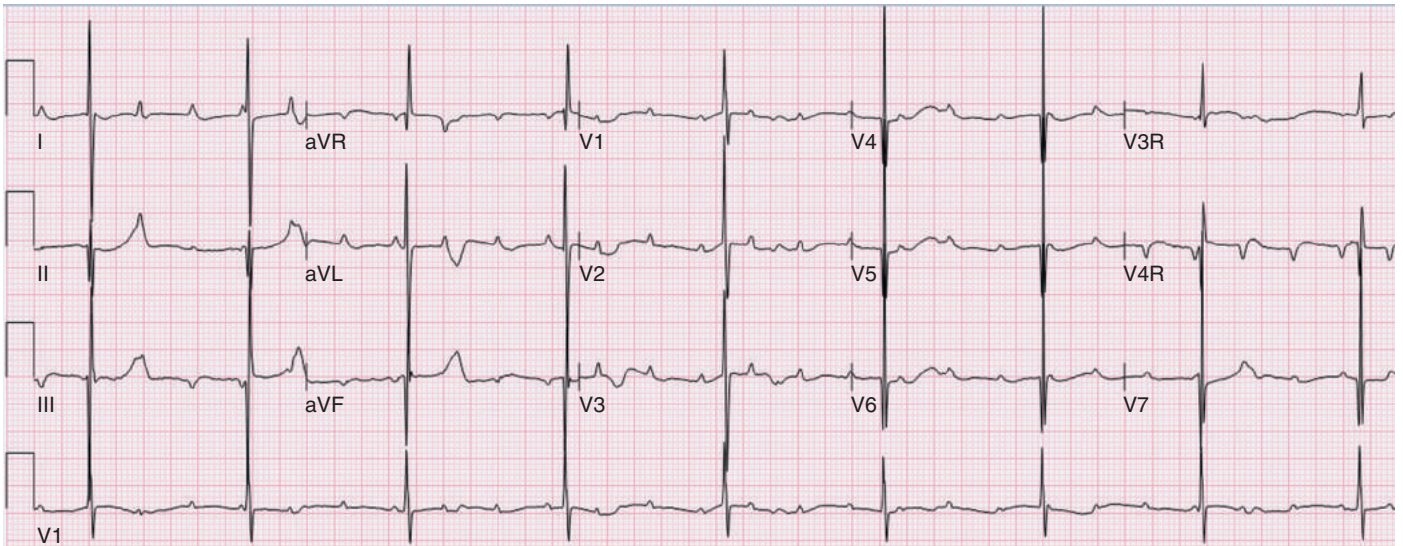
nature of this “2:1” heart block can result in irregularities in the conduction pattern (Fig. 56.7). This can result in profound QT prolongation as well as triggering VT and TdP.

Congenital Complete Atrioventricular Block

Congenital complete AV block (CCAVB) is the absence of AV conduction. This is rather uncommon, occurring in only 1 in 15,000 to 1 in 20,000 births (Bordachar et al., 2013). It is commonly associated with maternal lupus, although it also occurs in the absence of any known maternal disease. Fetal exposure to maternal autoantibodies (most commonly anti-SSA/Ro and anti-SSB/La antibodies) results in fibrosis of the AV nodal structures, leading to a progressive destructive process. There is some evidence that early treatment with high-dose maternal steroids could delay

or even arrest this process (Jaeggi et al., 2004); however, subsequent studies have not been able to demonstrate an improvement in conduction in treated fetuses (Fesslova et al., 2009; Hayashi et al., 2009), so the risk–benefit ratio of this therapy must be weighed on an individual basis. When detected, the fetus is monitored frequently for the development of hydrops fetalis, a sign of congestive heart failure in the fetus.

Complete heart block can also occur in the fetus or newborn in the setting of CHD, most commonly those defects that involve “L-transposition of the great arteries” or “ventricular inversion.” In these conditions, fetal looping of the heart results in the His bundle being a far more superficial structure than normal and therefore prone to fibrosis. Development of complete heart block in this setting can occur at any stage of development and often does not occur until later childhood or adulthood.



• **Fig. 56.8** Congenital Complete Atrioventricular Block. The sinus nodal rate is approximately 150 bpm, but there is complete atrioventricular dissociation with a junctional escape rate of 50 bpm.

In most cases of CCAVB, the region around the bundle of His remains intact and can exhibit normal properties of automaticity. This “junctional escape rhythm” typically results in a normal narrow QRS complex and ventricular contractility, despite the lack of AV conduction. It has the appearance of a normally conducted sinus beat but is completely dissociated from the sinus nodal P waves occurring above (Fig. 56.8). The escape rate in the fetus and neonate is often 60–80 bpm and can provide adequate cardiac output. There are many instances in which CCAVB is completely undetected in the newborn period and remains undetected for many years. The QRS complex may be wide (greater than 70 ms in the newborn) if there is extensive damage to the junctional region, implying that the escape rhythm arises from a location lower on the septum or from the ventricular myocardium itself.

However, a slower junctional escape rhythm, usually when the rate is below 50 bpm, is inadequate to support normal cardiac output. Signs of congestive heart failure may become manifest. In the fetal circulation, where the pulmonary vascular bed is largely bypassed by the ductus arteriosus, this will manifest as hydrops fetalis or intrauterine fetal demise. Management of the fetus with inadequate junctional escape rate and signs of poor cardiac output are addressed in the Fetal Rhythms section in this chapter. For the postnatal patient, signs of low cardiac output would include respiratory distress (with pulmonary congestion), poor perfusion, metabolic acidosis, low urine output, and frank edema. This can present a clinical challenge. Heart rate and blood pressure may be augmented by inotropic and chronotropic drip medications such as isoproterenol, epinephrine, and dobutamine. In some centers, temporary transvenous pacing may be utilized for emergent rescue, but this can be challenging because of the size disparity between commercially available temporary pacing leads and the small vessel size in this population (Doshi and Lokare, 2011). Permanent pacemaker implantation is indicated if there is evidence of ventricular dysfunction or low cardiac output in the neonate with a heart rate less than 55 bpm (or less than 70 bpm in the setting of concurrent CHD) or if there is evidence of a wide QRS complex escape rhythm (Epstein et al., 2008). Box 56.1 summarizes the absolute and relative indications for pacemaker implantation in the neonate with CCAVB.

• BOX 56.1 Indications for Permanent Pacing in Congenital Complete Atrioventricular Block

- Permanent pacemaker implantation is indicated for congenital third-degree AV block in the infant with a ventricular rate less than 55 bpm or with congenital heart disease and a ventricular rate less than 70 bpm.
- Permanent pacemaker implantation is indicated for congenital third-degree AV block with a wide QRS escape rhythm, complex ventricular ectopy, or ventricular dysfunction.
- Permanent pacemaker implantation is reasonable for congenital third-degree AV block beyond the first year of life with an average rate less than 50 bpm, abrupt pauses in ventricular rate that are two or three times the basic cycle length, or are associated with symptoms due to chronotropic incompetence.
- Permanent pacemaker implantation may be considered for congenital third-degree AV block in asymptomatic children or adolescents with an acceptable rate, a narrow QRS complex, and normal ventricular function.

AV, Atrioventricular; bpm, beats per minute.

From Epstein AE, DiMarco JP, Ellenbogen KA, et al. ACC/AHA/HRS 2008 guidelines for device-based therapy of cardiac rhythm abnormalities: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to revise the ACC/AHA/NASPE 2002 guideline update for implantation of cardiac pacemakers and antiarrhythmia devices) developed in collaboration with the American Association for Thoracic Surgery and Society of Thoracic Surgeons. *J Am Coll Cardiol*. 2008;51:e1–e62.

Fetal Rhythms

Fetal arrhythmias occur in 1%–2% of pregnancies, with resultant hemodynamic compromise, hydrops fetalis, and fetal demise occurring in 10% of cases (Naheed et al., 1996; Simpson, et al., 1997). The arrhythmias may develop late in the second or third trimester; this is particularly true for premature contractions, atrial tachycardias, and VTs, which often do not manifest before 25–26 weeks' gestation and in some cases only in the third trimester (van Engelen et al., 1994; Simpson et al., 1998). Fetal arrhythmias require close monitoring, as even benign rhythms may have a small risk of complications. The standard modality for prenatal evaluation of fetal arrhythmias is via fetal echocardiography, focusing on the

mechanical atrial and ventricular systoles and their relationship to one another (Fig. 56.9A,B) as a surrogate for AV synchrony/dyssynchrony (Jaeggi et al., 1998; Fouron et al., 2003; Carvalho et al., 2007). Investigation of the fetus referred for an abnormal rhythm should focus on causes of impaired AV conduction and causes of abnormal atrial or ventricular rhythms, including varying degrees of AV block, congenital LQTS, myocarditis, intracardiac tumors, and structural CHD. Differentiation between types of arrhythmia mechanism is helpful in determining the most optimal therapy and the likelihood of success of arrhythmia treatment.

Benign Arrhythmias

One of the most common reasons for referral to the fetal cardiologist is irregular fetal heart rhythms, noted in 1%–3% of pregnancies (Strasburger, 2005). The causes of these irregular rhythms are often isolated premature atrial contractions (Srinivasan and Strasburger, 2008). Rarely, they may be ventricular in origin. The vast majority of these atrial ectopic beats are benign; premature atrial contractions have a small risk (0.5%–1%) of developing into a fetal tachycardia (Wacker-Gusmann et al., 2014). However, 2% of cases may be associated with fetal LQTS, atrial flutter, and second-degree AV block (Cuneo et al., 2006).

Management of Benign Arrhythmias

The 2014 American Heart Association scientific statement for the diagnosis and treatment of fetuses with irregular rhythms (Donofrio et al., 2014) recommends that fetuses with frequent ectopic beats (bigeminy, trigeminy, or more than every 3–5 beats on average) should have a baseline fetal echocardiogram to assess cardiac structure and function and to determine the mechanism of the arrhythmia (Fig. 56.10). Medical treatment is not recommended for premature atrial contractions. If frequent ectopy continues, weekly heart rate monitoring, to assess for progression to tachycardia, should be performed in the obstetric office until it resolves. In fetuses with less frequent extra systoles, fetal echocardiogram is indicated if the irregular rhythm persists beyond 1–2 weeks or if there is difficulty differentiating a benign rhythm from a pathologic one (Donofrio et al., 2014). A postnatal ECG should be performed if an irregular rhythm is auscultated after birth.

Fetal Tachycardias

Fetal tachycardia constitutes a rare but important cause of perinatal morbidity and mortality. Sustained SVT includes ORT (70%), atrial flutter (30%), and rare tachyarrhythmias and usually occurs at rates greater than 220 bpm (Strasburger, 2005). The type of SVT can be determined using the mechanical PR interval derived from either the spectral Doppler of the mitral inflow and aortic outflow or the superior vena cava and aorta. Management for a given fetus can then be assessed based on the type of suspected SVT. VT is a much less common cause of fetal tachycardia as are chaotic or multifocal atrial tachycardia.

In general, the goal of in utero treatment of fetal tachycardias is not conversion to 100% sinus rhythm but instead to establish sufficient sinus rhythm to allow resolution of hydrops and ventricular dysfunction. Management depends on the gestational age at presentation, degree of fetal compromise, maternal conditions, and potential risks to the mother from therapy (Donofrio et al., 2014). Emergent delivery should be reserved for the hydropic fetus who exhibits persistent tachycardia refractory to medical

management (Srinivasan and Strasburger, 2008). Delivery can be considered as a primary management strategy if the fetus is near term and if delivery incurs no significantly increased risk to the neonate (Donofrio et al., 2014).

Orthodromic Reentrant Tachycardia

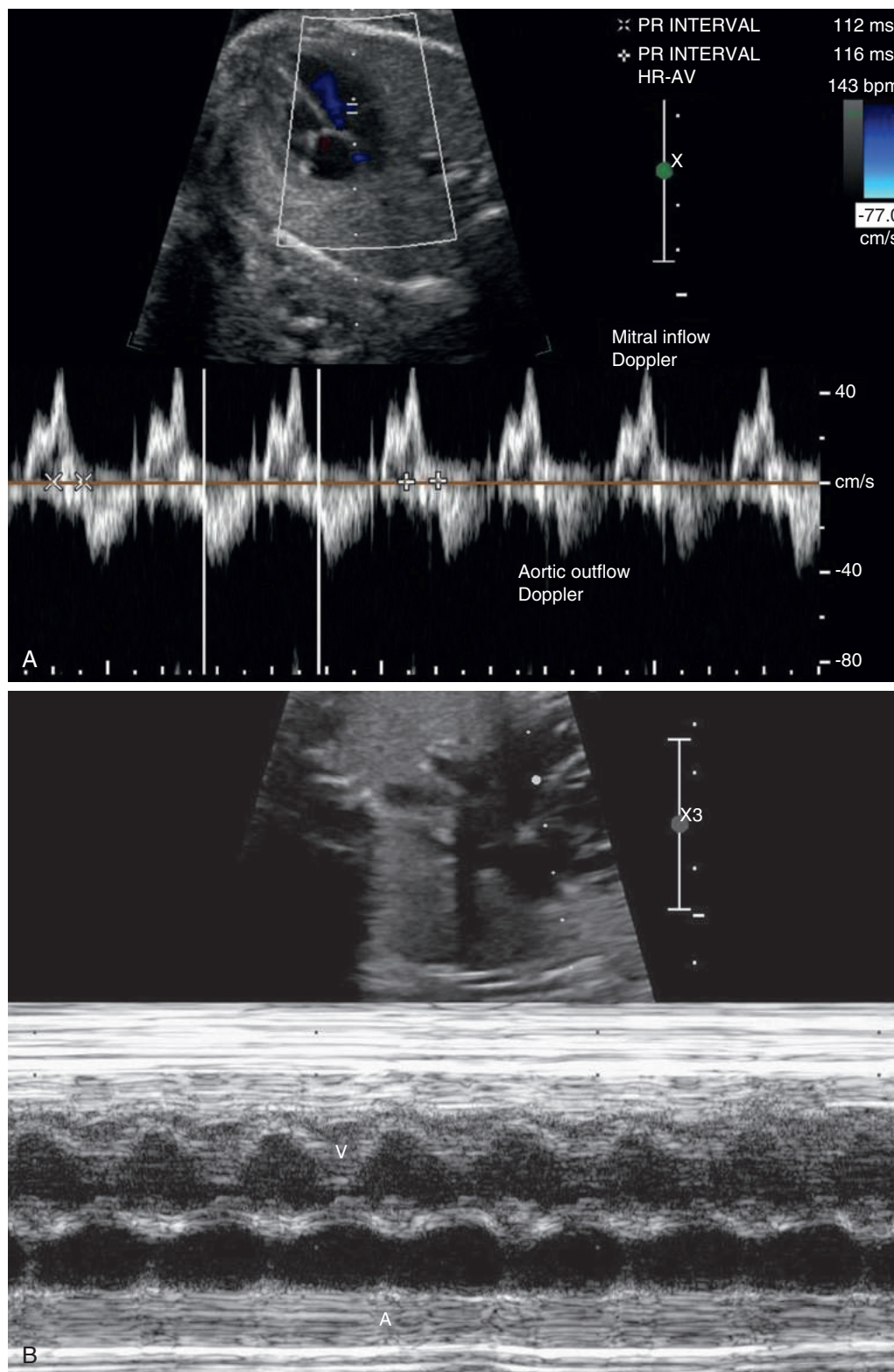
ORT accounts for approximately 70% of fetal tachycardias (Strasburger, 2005). It usually presents with intermittent or sustained heart rates in the range of 240–260 bpm. It is identified on fetal echocardiogram by the short RP interval (ventriculoatrial [VA] activation time), 1:1 AV relationship, and fast ventricular rate (Fig. 56.11). This rhythm results from conduction down the AV node to the ventricles and retrograde through an accessory pathway to the atrium. Generally, reentrant ORT presents between 28–33 weeks' gestation (Hornberger and Sahn, 2007). Progression to hydrops is not uncommon, with risk factors being tachycardia duration, fetal immaturity, and concurrent structural heart disease (Naheed et al., 1996; Hornberger and Sahn, 2007).

Intrauterine fetal treatment is recommended if delivery does not offer lower risk. The first-line and second-line antiarrhythmic therapy choices are controversial, as are the management strategies after initial treatment failure. The use of combination therapies presents greater risk for maternal and/or fetal complications, and thus monotherapy is recommended. Digoxin, flecainide, and sotalol as monotherapy have all been used successfully as first-line therapies for reentrant SVT (Oudijk et al., 2000; Jaeggi et al., 2011; Shah et al., 2012), though the use of digoxin is controversial because of the very small risk of atrial fibrillation with rapid antegrade conduction across an accessory pathway (Kleinman and Nehgme, 2004). There is no study to date that supports one as the superior treatment option. All three of these medications, as well as amiodarone, have also been used as second-line therapy (Donofrio et al., 2014). Amiodarone has a more significant toxicity profile for the mother and fetus and should be reserved as a third-line treatment for life-threatening arrhythmias. It should be discontinued once hydrops resolves. Verapamil and procainamide are no longer used to treat fetal tachyarrhythmias (Donofrio et al., 2014). Transplacental transfer of drugs is reduced in the setting of hydrops, thus direct fetal treatment (to the fetal buttock/thigh or intracordal) concomitantly with transplacental therapy has been described for restoration of sinus rhythm. These strategies should be limited to severely hydropic fetuses who cannot be delivered because of early gestational age, as there is an associated risk of fetal death (Hansmann et al., 1991; Cuneo and Strasburger, 2000).

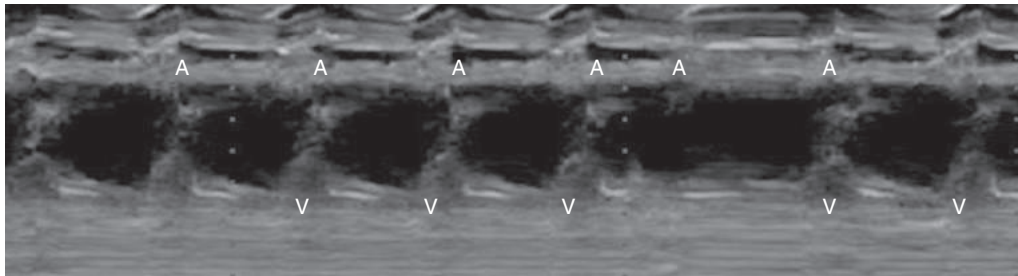
After delivery, up to 50% of reentrant SVT cases will not have postnatal recurrence (Naheed et al., 1996). Thus medical treatment must be reassessed relative to the length of time since the last occurrence and the mechanism of clinical tachycardia.

Atrial Flutter

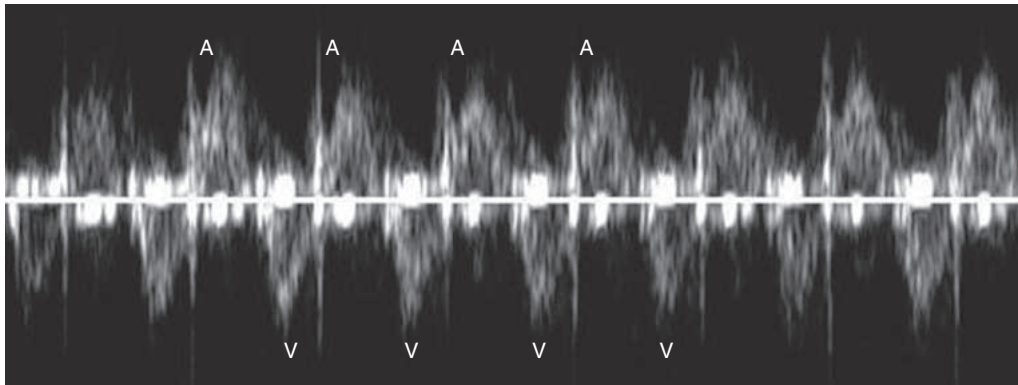
Atrial flutter accounts for approximately 30% of fetal tachycardias (Strasburger, 2005). It is associated with fetal myocarditis, structural CHD, and in utero exposure to SSA/SSB antibodies. It is typically characterized by atrial rates of 300–500 bpm and slower ventricular rates (150–170 bpm) because of a physiologic block in the AV node. It is best identified on fetal echocardiogram using an M-mode across an atrial free wall, interventricular septum, and ventricular free wall (Fig. 56.12). Atrial flutter is more likely to present later in pregnancy than reentrant SVT, at around 31–34 weeks' gestation. It is generally a well-tolerated rhythm because of the lower



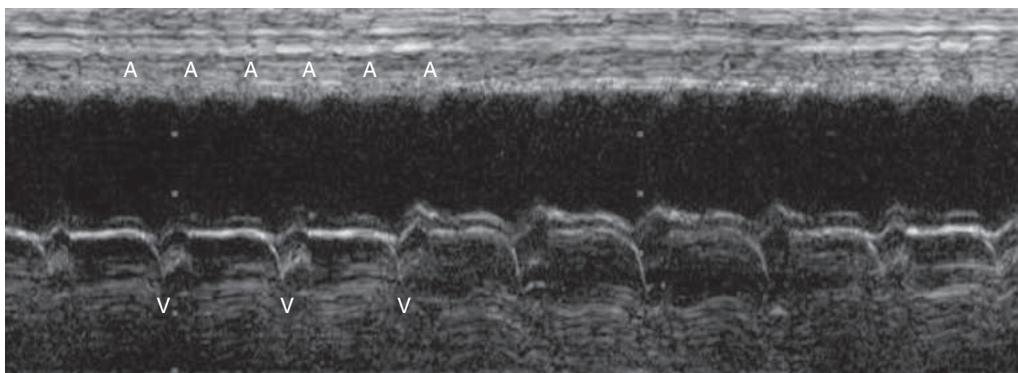
• **Fig. 56.9** Fetal echocardiogram performed from a coronal view of the fetus demonstrating a four-chamber view of the heart with normal fetal heart rate (146 beats per minute) with 1:1 atrioventricular conduction via (A) normal mitral valve inflow and aortic valve outflow spectral Doppler. The measurement between the inflow and outflow is the PR interval (XX and ++ = 116 ms). (B) M-mode diagonally across the four-chamber heart measures the movement of the atrial (A) and ventricular (V) free wall. *bpm*, Beats per minute; *HR-AV*, heart rate measured from aortic valve Doppler.



• **Fig. 56.10** M-mode of the Fetal Heart Demonstrating an Isolated Premature Atrial Contraction. There is normal atrioventricular conduction at a normal rate until the fifth labeled atrial beat (A) that is early (ectopic) and results in a blocked ventricular beat (V).



• **Fig. 56.11** Fetal Mitral Inflow and Aortic Outflow Spectral Doppler Demonstrating Reentrant Supraventricular Tachycardia. There is 1:1 atrioventricular conduction (A and V labels) at a steady rate of 238 beats per minute. The RP interval is 100 ms.



• **Fig. 56.12** M-mode of the fetal heart demonstrating 2:1 atrial flutter with an atrial rate of 120 beats per minute and a ventricular rate of 260 beats per minute. A, Atrial flutter waves; V, ventricular activation.

ventricular rates (Jaeggi et al., 1998; Strasburger, 2005). However, the rhythm can remain undiagnosed prenatally because of the relatively normal fetal ventricular rates (Fouron et al., 2003). The lack of variability in the heart rate provides a clue to the diagnosis. Atrial flutter can cooccur with reentrant SVT in 12%–33% of affected fetuses (Casey et al., 1997).

Sotalol has been shown to be effective in converting 50%–80% of fetuses with atrial flutter without mortality (Shah et al., 2012) and is considered first-line therapy. Digoxin is also recommended as a first-line therapy, and amiodarone may be considered; however, procainamide is contraindicated (Strasburger et al., 2004). Transesophageal pacing of synchronized cardioversion is recommended after delivery to restore sinus rhythm. Sinus node suppression may

rarely occur due to in utero therapy, and back-up external pacing should be available after cardioversion. Postnatal medical treatment should be reassessed given that atrial flutter may not recur (Donofrio et al., 2014).

Sustained Ventricular Tachycardia

Fetal VT is associated with AV block, cardiac tumors, myocarditis, and ion channelopathies. LQTS should be suspected when tachyarrhythmia and bradyarrhythmia coexist (Cuneo et al., 2003). Fetuses with LQTS can develop rapid TdP and monomorphic VT with subsequent development of ventricular dysfunction, AV valve insufficiency, and hydrops. Fetal magnetocardiography or ECG can confirm

the diagnosis by identifying a prolonged QTc interval (Strasburger and Wakai, 2010; Cuneo et al., 2013; Arya et al., 2015). Intrauterine IV magnesium is recommended as first-line therapy for VT greater than 200 bpm, and treatment should be limited to less than 48 hours. If maternal magnesium levels are less than 6 mEq/L and there are no signs of toxicity, redosing can be considered. IV lidocaine, oral propranolol, or mexiletine may also be considered in conjunction with magnesium, especially in the setting of hydrops. If LQTS can be excluded, then sotalol, flecainide, and amiodarone are alternative therapies and have been shown to be successful in terminating fetal VT. Dexamethasone and IV infusion of immunoglobulin (IVIG) have been used in the setting of antibody-mediated or myocarditis-associated VT (Donofrio et al., 2014).

Rare Tachycardias

Less common tachycardias such as AET and PJRT can be differentiated by the long RP interval (VA time) on fetal echocardiogram. The two can be differentiated by the gradual warm-up phase and variable AV nodal block at faster rates characteristic of AET and the sudden onset and consistent heart rate of PJRT. AET generally occurs at rates of 180–250 bpm and is most refractory to treatment both before and after birth (Strasburger, 2005). These fetal tachycardias occur in the late second or third trimester. Multifocal atrial tachycardia is rare (associated with Costello syndrome) and usually occurs in the last weeks of pregnancy (Lin et al., 2011). JET is commonly associated with SSA antibody exposure in the fetus and can be seen in the presence or absence of AV block. For AET and multifocal atrial tachycardia, treatment is recommended for average heart rates greater than 200 bpm with normal cardiac function or greater than 160 bpm with cardiac dysfunction (Donofrio et al., 2014). Digoxin is recommended as the first-line therapy, though flecainide and sotalol may be considered. Flecainide or sotalol are recommended for PJRT or rapid AET. Similar therapies can be used for the medical management of fetal JET, though amiodarone has also been used. Fetuses that develop JET in the setting of anti-SSA antibody exposure can be treated with dexamethasone as well. After delivery of a fetus with these rare tachycardias, continued medical treatment is usually required.

Sinus Tachycardia

Sinus tachycardia at rates of 180–190 bpm may mimic other pathologic tachycardias; however, ventricular dysfunction and hydrops are uncommon. Sinus tachycardia is associated with maternal infection, anemia, drug/medication use, hyperthyroidism, or trauma. Treatment of the underlying cause is recommended (Donofrio et al., 2014).

Arrhythmia Medications

Relatively high doses of antiarrhythmic agents must be administered during pregnancy because maternal circulating blood volume and renal clearance are both increased. In most cases, treatment should be initiated in the hospital with close observation of maternal and fetal well-being. A baseline maternal ECG and electrolytes, as well as a cardiology consultation, should be obtained before the initiation of medications to assess for maternal risk factors such as preexcitation or LQTS. Serial maternal ECG, electrolyte concentrations, and drug levels should be monitored throughout the duration of therapy. Oral medication administration is recommended, except for IV

magnesium and lidocaine, and for digoxin loading doses (Donofrio et al., 2014). Most arrhythmia medications demonstrate diminished transplacental transfer in the setting of hydrops, with the efficacy of digoxin decreasing to 25% (Srinivasan and Strasburger, 2008). However, flecainide and sotalol have been shown to be efficacious in the setting of hydrops (Donofrio et al., 2014). If the maternal PR, QRS, or QT intervals are noted to prolong during therapy with digoxin, flecainide, or sotalol, respectively, doses should be decreased and close observation is recommended. Serious maternal adverse reactions are rare and have resolved with discontinuation of therapy (Donofrio et al., 2014).

Fetal Bradycardia

Fetal bradycardia is defined as a persistent heart rate of less than 120 bpm. Causes include sinus bradycardia secondary to sinus node dysfunction, channelopathies, maternal exposures/conditions, or fetal central nervous system involvement and blocked atrial bigeminy, which is associated with a 10% risk for conversion to SVT. The most common cause of fetal bradycardia is congenital AV block, occurring in approximately 1:20,000 live births (Kertesz et al., 1997). Approximately 50% of fetal AV block is secondary to structural heart disease, 40% is due to immune-mediated mechanisms (Schmidt et al., 1991; Machado et al., 1988), and an additional 10% is idiopathic. Fetal congenital heart block at low heart rates is associated with significant morbidity and mortality, especially when associated with maternal autoimmune disorders. Management of fetal bradycardia is focused on frequent fetal echocardiographic evaluations to assess for the development of ventricular dysfunction, hydrops, or fetal heart rate less than 55 bpm. Treatment of nonimmune-mediated heart block is directed at augmenting fetal ventricular rates. For immune-mediated AV block, several therapies may be considered and are controversial (Jaeggi et al., 2004).

Benign Fetal Bradycardia

Maternal treatment with β -blockers, sedatives, and other medications has been associated with sinus node suppression. Fetuses with exposure to maternal anti-SSA/SSB antibodies or those with myocarditis may develop inflammation and fibrosis of the sinus node. Finally, fetuses with heterotaxy syndrome may develop bradycardia secondary to a low-atrial rhythm in left-atrial isomerism or dual sinoatrial nodes in right-atrial isomerism. In these conditions, fetal heart rates range between 90 bpm and 130 bpm. No treatment is recommended (Donofrio et al., 2014).

Blocked atrial bigeminy may result in fetal heart rates between 75 and 90 bpm with 2:1 AV conduction and can be mistaken for second-degree AV block. There is an increased risk of the development of fetal SVT, occurring in approximately 10% of fetuses with blocked atrial bigeminy (Donofrio et al., 2014). Fetal echocardiography may demonstrate normal AV conduction followed by an early atrial beat coupled to the preceding ventricular beat in which case the AV node is refractory. This results in failure of conduction to the ventricle (see Fig. 56.10). Management of blocked atrial bigeminy is the same as for isolated premature atrial contractions.

Ion Channelopathies

Congenital LQTS should be considered in the setting of persistent bradycardia in an otherwise asymptomatic fetus (Horigome et al., 2010). Measurement of the QTc can be performed by magnetocardiogram or

fetal ECG (Strasburger and Wakai, 2010; Cuneo et al., 2013; Arya et al., 2015). Fetal treatment is not recommended; however, the development of VT requires treatment as delineated in the Fetal Tachycardia section. Management involves close observation prenatally, correction of maternal electrolyte abnormalities, and avoidance of maternal exposure to QT-prolonging medications. Postnatal evaluation includes an ECG and continued observation and management of arrhythmias associated with LQTS (Donofrio et al., 2014).

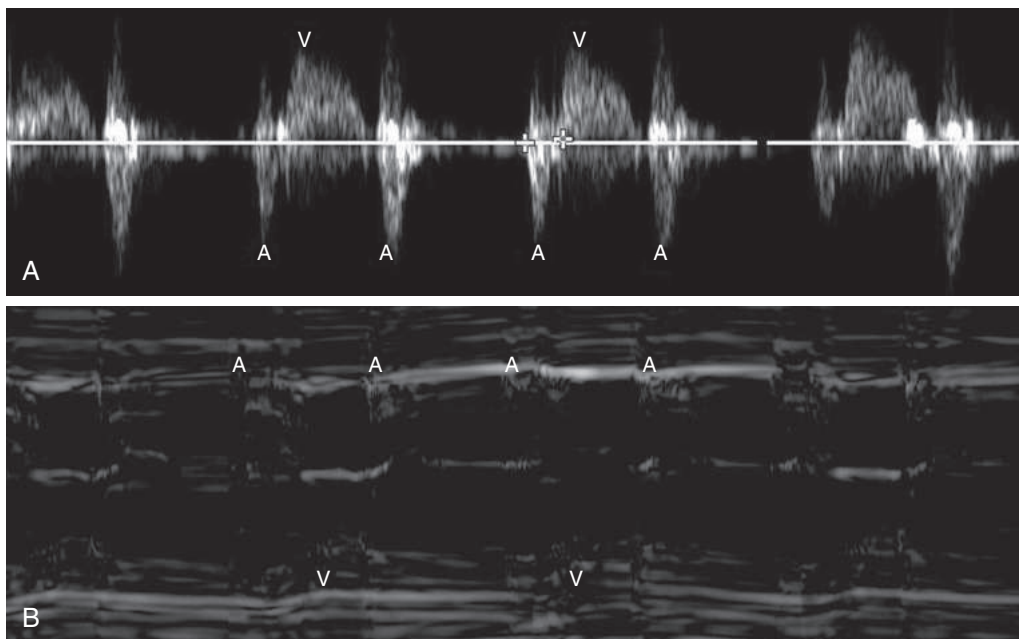
Atrioventricular Block

Structural CHD accounts for 50% of fetal AV block. Fetuses with heterotaxy syndrome with left-atrial isomerism, congenitally corrected transposition, and AV canal defects are most at risk for the development of AV block. In a fetus the combination of congenital AV block, complex structural CHD, and hydrops fetalis is associated with a high rate of in utero demise (Anandakumar et al., 1996). AV block can be diagnosed by fetal echocardiogram by evaluating the atrial and ventricular rates and the AV relationships (Fig. 56.13A,B).

Maternal anti-SSA (Ro) and anti-SSB (La) antibodies are present in the majority of fetuses that develop isolated AV block. The presumed mechanism is that the fetal cardiac structures are a target for immune complex deposition, namely the AV node and the myocardium. Ro-52-specific antibody has shown a very close association with immune-mediated congenital AV block (Sonesson et al., 2004). The risk of heart block in mothers with anti-SSA/SSB antibodies is 2%–3%, with a recurrence rate after one affected child as high as 12%–17% (Buyon et al., 1998; Costedoat-Chalumeau et al., 2004). Autopsy specimens typically reveal progressive fibrosis of the AV nodal structures in affected fetuses. The process typically spares the His–Purkinje system so that the slower “escape rhythm” tends to be relatively reliable. Damage to the ventricular myocardium and the presence of endomyocardial fibroelastosis are also found in affected fetuses (Krishnan et al.,

2014). The risk of transfer of maternal antibodies to the fetus, and therefore the risk for development of AV block, is highest between 16 and 26 weeks’ gestation (Buyon et al., 1998). Close follow-up with fetal echocardiogram to evaluate fetal heart rate, PR interval for prolongation, and ventricular function is recommended every other week during this critical time period for all fetuses with exposure to anti-SSA/SSB antibodies. Fetal heart rate monitoring should be performed at standard intervals by the obstetrician.

Treatment of AV block depends on the cause, ventricular rate, and degree of heart failure. β -Sympathomimetics such as terbutaline, salbutamol, and isoprenaline are reasonable to use in fetuses with heart rates less than 55 bpm or with higher heart rates if there is significant CHD or signs of cardiac dysfunction or hydrops. Although terbutaline may increase the fetal heart rate, improvement in survival has not been demonstrated. Terbutaline is well tolerated; however, maternal heart rates of up of 100–120 bpm and benign ectopy have been reported (Cuneo et al., 2007). Immune-mediated AV block may benefit from dexamethasone (4–8 mg/day) or IVIG therapy. Dexamethasone has been shown to reduce inflammation, reverse or stabilize second-degree block, and improve hydrops or myocardial fibrosis and function (Krishnan et al., 2014). Risks associated with the use of dexamethasone include fetal growth restriction, oligohydramnios, effects on the central nervous system, and maternal diabetes. A trial of dexamethasone may be considered in fetuses with second-degree AV block or first-degree AV block with signs of cardiac inflammation (valve insufficiency, ventricular dysfunction, effusion) to prevent progression to complete AV block, though its usefulness has not been well established (Donofrio et al., 2014). The treatment of mothers with hydroxychloroquine before 10 weeks’ gestation has been associated with a reduced risk of anti-SSA/SSB antibody-mediated fetal cardiac disease and may be used in high-risk pregnancies (Izmirly et al., 2012).



• **Fig. 56.13** (A) Fetal mitral inflow and aortic outflow spectral Doppler and (B) M-mode of the fetal heart demonstrating 2:1 atrioventricular block with an atrial rate of 120 beats per minute and ventricular rate of 60 beats per minute. A, Sinus atrial activity; V, ventricular depolarization.

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Long-Term Neurologic Outcomes in Children With Congenital Heart Disease

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KEY POINTS

- Congenital heart disease (CHD) is a common birth defect with improved survival of neonates with complex lesions requiring heart surgery.
- Neurodevelopmental (ND) abnormalities are common in school-age children and adolescents after neonatal heart surgery.
- Magnetic resonance imaging (MRI) studies have demonstrated evidence of brain injury and delayed brain development, even before having a corrective operation, in patients with hypoplastic left heart syndrome and transposition of the great arteries.
- Fetal MRI studies suggest this delayed development begins in the third trimester.
- Aberrant fetal physiology resulting in decreased oxygen and substrate supply to the brain may result in these imaging and ND abnormalities in addition to other perioperative risk factors.
- Children with complex CHD have a prevalence of pervasive but subtle cognitive problems termed *the neurodevelopmental signature of complex congenital heart disease*.

Severe congenital heart disease (CHD) requiring a corrective operation occurs in 6 to 8 per 1000 live births, with up to half of the cases requiring an operation in the neonatal period to survive (Hoffman and Kaplan, 2002). With improved surgical technique, mortality for CHD has steadily declined (Karamlou et al., 2010). In fact, the number of adults living with CHD has surpassed the number of children with CHD in the United States (Gilboa et al., 2016).

Given the changing epidemiology of CHD, considerable effort has been devoted to evaluating both neurodevelopmental (ND) outcomes and quality of life for patients with CHD. Evidence suggests that although children with CHD may no longer have overt signs of neurologic dysfunction, they may exhibit deficits in multiple domains including visual-spatial skills, memory, executive function, speech and language, and gross and fine motor function (Newburger et al., 2012; Sananes et al., 2012; von Rhein et al., 2012). Long-term follow-up data suggest that these deficits continue into adolescence and young adulthood with potential significant impact on societal contribution (Schaefer et al., 2013; Bellinger et al., 2009, 2011, 2015a,b).

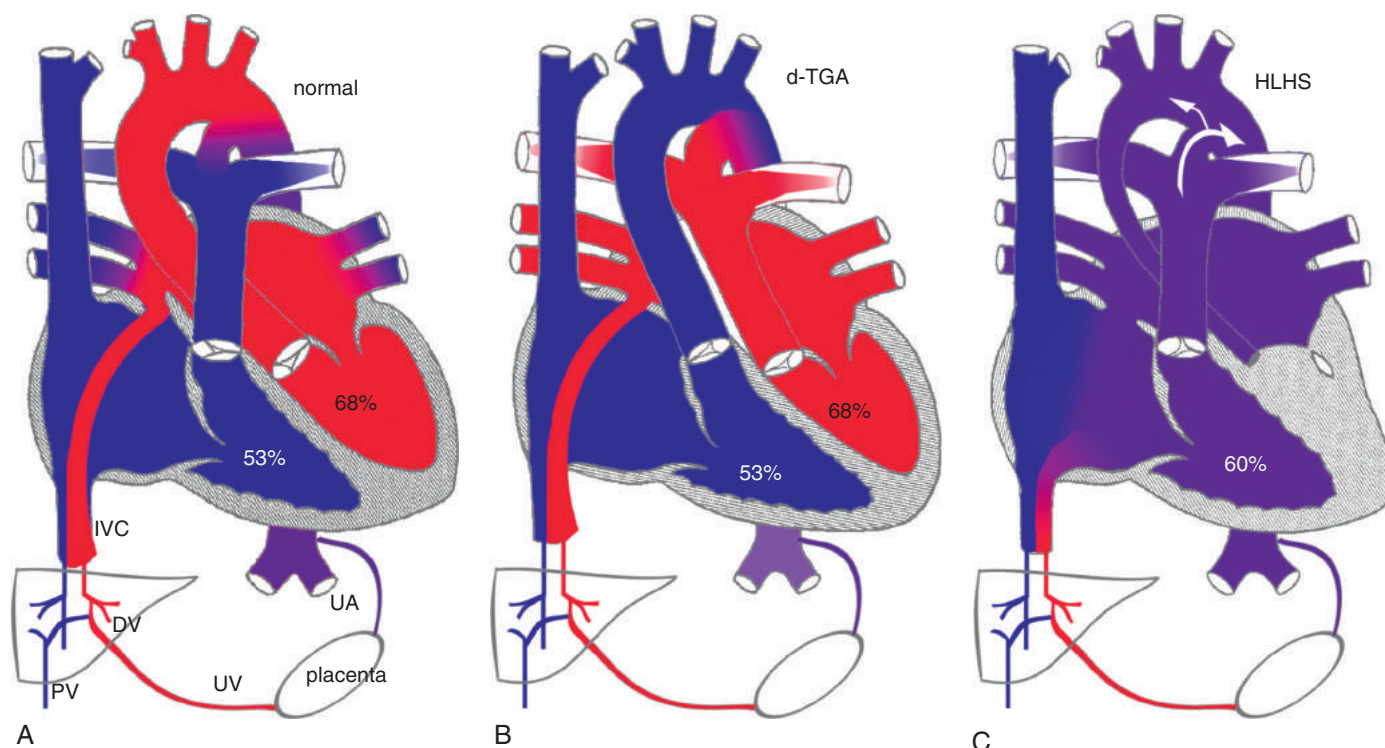
A natural assumption has been that these adverse outcomes were directly related to brain injury sustained during neonatal cardiac surgical interventions. However, in the recent era it has become apparent that patient-specific risk factors play essential roles in determining the ultimate ND outcome and, furthermore, that the interplay between the brain and the circulation is complex, occurring at many levels throughout fetal and postnatal development. This chapter will review mechanisms influencing neurologic outcome in CHD including (1) the physiologic effects of congenital heart lesions on brain blood flow, (2) brain development in the context of CHD, (3) the timing, appearance, and mechanism of acquired brain injuries, and (4) current knowledge on ND outcomes in critical CHD in the short and long term.

Structural and Developmental Abnormalities of the Brain in Congenital Heart Disease

Human cardiac development is largely complete by gestational week 7 (Srivastava, 2006). In contrast, brain development extends over a much longer time period, with morphologic events (cell proliferation, migration, axon pathfinding, and target selection) occurring predominantly in the first two trimesters, followed by a prolonged period of refinement of circuits that begins in the third trimester and extends into infancy. This stage of brain development includes dramatic growth, myelination, and increasing neuronal electrical activity and depends upon receiving an adequate supply of nutrients and oxygen. Consequently, blood flow to the developing brain increases and is estimated to be a quarter of the combined ventricular output in the third trimester demonstrating the unique heart-brain interplay that is critical for normal brain development (Rudolph, 2011).

Fetal Circulation in Congenital Heart Disease: Effects on Cerebral Blood Flow

The fetal circulation is unique in a number of respects that can impact cerebral blood flow and development. In the normal fetus, cerebral blood flow is supplied by highly oxygenated blood from the ductus venosus preferentially streaming across the foramen ovale to



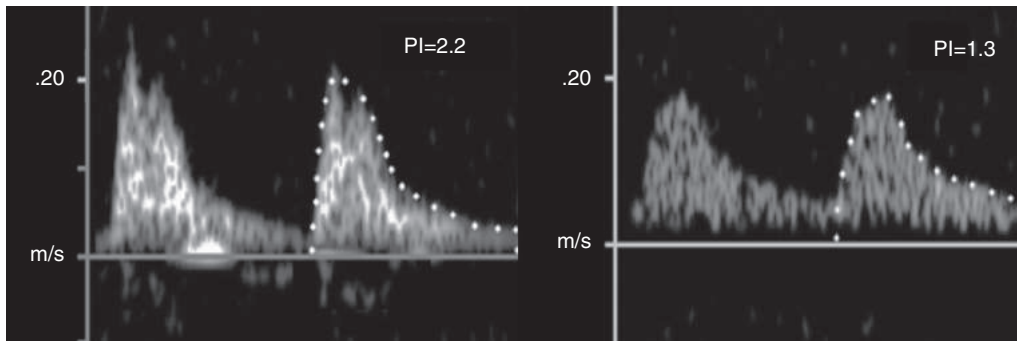
• **Fig. 57.1** Normal and Altered Fetal Circulation. (A) Normal fetal blood flow; (B) d-transposition of the great arteries (d-TGA); (C) hypoplastic left heart syndrome (HLHS) with aortic atresia. Deoxygenated blood (blue–purple) flows to the placenta through the umbilical artery. Blood with higher oxygen content (red) returns through the umbilical vein and ductus venosus to the inferior vena cava (IVC). The higher saturated blood from the IVC is preferentially directed toward the foramen ovale into the left ventricle, ascending aorta, and head and neck vessels in the normal fetus. Desaturated blood also returns to the right side of the heart and is directed to the descending aorta through the ductus arteriosus. In the fetus with d-TGA, the aorta arises from the right ventricle such that the brain receives less oxygenated blood, while the higher saturated blood is directed to the descending aorta through the ductus arteriosus. In HLHS, reduced or absent left-ventricular ejection results in elevated left-atrial pressure, limiting or reversing flow at the foramen ovale and resulting in complete mixing of desaturated and well-saturated blood in the right atrium and ventricle. Blood flow to the head and neck may occur in a retrograde fashion from the ductus arteriosus across the aortic isthmus. *d-TGA*, d-transposition of the great arteries; *DV*, ductus venosus; *HLHS*, hypoplastic left heart syndrome; *IVC*, inferior vena cava; *PV*, portal vein; *UA*, umbilical artery; *UV*, umbilical vein. (Reprinted with permission from McQuillen PS, Goff DA, Licht DJ. Effects of congenital heart disease on brain development. *Prog Pediatr Cardiol*. 2010;29(2):79-85.)

the left atrium and ventricle (Fig. 57.1). In contrast, in fetuses with transposition of the great arteries (TGA), the aorta and pulmonary artery are transposed and thus the higher oxygenated blood reaches the pulmonary vasculature as opposed to the cerebral vasculature. Similarly, in hypoplastic left heart syndrome (HLHS), inadequate left heart structures lead to reversal of blood flow in the foramen ovale with mixing of oxygenated and deoxygenated blood in the right ventricle and, in cases of aortic atresia, retrograde flow in the ascending aorta. The effects of these abnormal flow patterns on brain development are uncertain but may involve different mechanisms, despite the fact that both decrease the oxygen content of the blood delivered to the brain. Other mechanisms may be responsible such as inadequate substrate delivery (glucose) because of decreased perfusion pressure and flow to the brain (Rudolph, 2016). In d-transposition of the great arteries (dextroposition [d]-TGA), the pulsatility and perfusion pressure of the cerebral circulation are normal. However, in HLHS, the hypoplastic isthmus and aortic arch may function as resistors, potentially decreasing the pulsatility and perfusion pressure to the cerebral circulation. In contrast, in d-TGA, decreased pulsatility and perfusion to the brain can result

from preferential blood flow to the pulmonary vasculature because of a lower pulmonary vascular resistance than usual.

Cerebral Doppler ultrasound can assess fetal cerebral vascular resistance in the middle cerebral artery (MCA) and provide insight into fetal cerebral blood flow patterns. By calculating the MCA pulsatility index (PI, a measure of vascular resistance in the circulatory bed downstream from the point of Doppler sampling), studies have identified a pattern of “brain-sparing” in fetuses with intrauterine growth restriction and placental insufficiency as a mechanism of autoregulation of fetal cerebral blood flow (Wladimiroff et al., 1987; Mari and Deter, 1992). In normal pregnancies, the cerebral/umbilical PI ratio is more than 1.0, whereas in many growth-restricted fetuses the ratio is less than 1.0 and predicts adverse perinatal and neurologic outcomes (Rizzo et al., 1989; Gramellini et al., 1992). This autoregulatory mechanism is thus paradoxically a harbinger for poor outcome in the setting of fetal growth restriction.

There have been several studies examining in utero blood flow patterns in human fetuses with CHD. These have demonstrated lower MCA PI in fetuses who have lesions with the most intracardiac



• **Fig. 57.2** Middle cerebral artery Doppler patterns in a normal fetus and a fetus with hypoplastic left heart syndrome (HLHS). The pulsatility index (peak systolic velocity–end diastolic velocity/mean velocity) in the HLHS fetus is lower (*right panel*), suggesting decreased impedance in the cerebral vasculature. *PI*, Pulsatility index.

mixing, such as HLHS (Fig. 57.2). In fact, fetuses with HLHS have been shown to have the lowest cerebral/umbilical PI ratio among different types of CHD (Donofrio et al., 2003; Kaltman et al., 2005). This is likely secondary to the lower oxygen content of blood delivered to the brain as well as abnormalities in cerebral perfusion with a hypoplastic aortic isthmus.

Cerebral blood flow characteristics have been shown to predict ND outcomes in fetuses with CHD. In a retrospective multicenter study of infants with HLHS, a lower MCA PI in utero predicted a better ND outcome at 14 months of age as assessed by the Bayley Scales of Infant Development (BSID) II (Williams et al., 2013). These findings suggest that the autoregulatory response of cerebral vasodilation in the setting of HLHS may be sufficient and adaptive to a state of chronic hypoxemia, which is in contrast to what is seen in the context of fetal growth restriction. Further large prospective studies are needed to understand the predictive utility of cerebral blood flow patterns in fetuses with CHD.

Preoperative Evidence of Delayed Brain Development by Magnetic Resonance Imaging

Structural brain malformation in neonates with CHD has been identified, even in the absence of a defined genetic syndrome. Autopsy studies have revealed multiple congenital brain anomalies in neonates with HLHS including microcephaly, abnormal cortical mantle formation, and overt central nervous system malformations such as agenesis of the corpus callosum or holoprosencephaly (Glauser et al., 1990).

In addition to structural abnormalities, there is magnetic resonance imaging (MRI) evidence that brain development is delayed in CHD prior to corrective surgery. Quantitative MRI techniques such as diffusion tensor imaging (DTI) measure the direction and magnitude of water movement and thus microstructural brain development (Hüppi and Dubois, 2006). During normal brain development, the magnitude of water diffusion motion decreases (apparent diffusion coefficient [ADC]) and regional directionality increases in white matter (fractional anisotropy [FA]). Similarly, metabolic brain development can be measured with magnetic resonance spectroscopy (MRS) by measuring major metabolic compounds such as *N*-acetylaspartate (NAA), choline (Cho), creatine (Cr), and lactate. Using these techniques, researchers have discovered that newborns with CHD (d-TGA and single ventricle lesions) have findings suggesting an immature brain with abnormal DTI (4% higher ADC and 12% lower FA) and MRS (10% lower NAA/

Cho ratio). Comparing these findings with those obtained in premature newborns without CHD, full-term newborns with CHD appear approximately 1-month delayed (Miller et al., 2007). These observations have been replicated in studies assessing brain development by semiquantitative morphologic scoring, specifically the brain total maturation score (TMS). The TMS was found to be significantly lower in full-term newborns with d-TGA and HLHS compared with a normal cohort of newborns (Licht et al., 2009). Finally, morphometry studies have demonstrated lower total and regional brain volumes in newborns with CHD compared with controls (von Rhein et al., 2015). These findings help explain the abnormal somatic growth seen in the newborn with CHD. Specifically, those with d-TGA and HLHS have smaller head circumferences that are out of proportion to their weight (Rosenthal, 1996). These brain MRI findings in the neonate with CHD, seen before any corrective operations are performed, have led to the theory that abnormal brain development and susceptibility to acquired injury begin in utero.

Fetal Brain Magnetic Resonance Imaging Identifies Developmental Abnormalities in Congenital Heart Disease

Technical advances in fetal MRI have made it an important tool in the clinical evaluation of fetuses with suspected cerebral abnormalities. A study comparing brain volumes and MRS between normal fetuses and fetuses with CHD between 25 and 37 weeks' gestation showed definitive evidence for delayed fetal brain development (Limperopoulos et al., 2010). During the third trimester, a progressive impairment of brain volumes was observed, particularly in those fetuses with left-sided obstruction. Additionally, larger delays in the expected increase in NAA/Cho ratio and greater impairment of growth in brain volume were noted in fetuses with aortic atresia, who have no antegrade blood flow in the aortic arch. These observations support the concept that brain development is altered during fetal life because of impaired fetal cerebral blood flow, oxygen, and substrate delivery and that compensatory mechanisms (i.e., brain-sparing effect) may not be adequate.

Recently, novel fetal cardiac MRI techniques have been utilized to understand the heart–brain interplay in CHD. These techniques enable measurements of flow and oxygen saturation in fetal blood vessels. By combining fetal brain MRI and cardiovascular magnetic resonance, researchers found a correlation between fetal cerebral

oxygen consumption and brain size (estimated brain weight) among 30 fetuses with CHD in late gestation (Sun et al., 2015). There was a direct correlation between estimated brain weight and cerebral oxygen consumption. In addition, there was a modest association between cerebral oxygen delivery and brain size. The predictive value of these findings for postnatal outcomes such as brain injury and ND outcomes remains unknown. Further exploration to potentially identify targets and methods of intervention to modify ND outcome in CHD is warranted.

Trajectory of Brain Development in Congenital Heart Disease

Although much of the literature has been focused on the identification of abnormal brain development in the fetus and newborn with CHD, studies on older patients have emerged suggesting that these delays continue into adolescent years. In a study of single ventricle patients who had undergone a Fontan operation, the frequency of any structural abnormality on MRI was 11 times higher than in a normative cohort (Bellinger et al., 2015b). Similarly, in a brain MRI study of adolescents with d-TGA, FA was significantly reduced in several regions of the white matter compared with a normative cohort (Rivkin et al., 2013). Many have postulated that the trajectory of brain development over time may be dependent on the specific cardiac lesion and whether the surgical management is corrective (i.e., d-TGA) or largely palliative (i.e., HLHS). In particular, patients with aortic atresia have been found to have the least robust microstructural brain development (Sethi et al., 2013). Another study demonstrated, at 2.5 years of age, more cortical atrophy and lower brain volumes in those with HLHS compared with those with d-TGA, suggesting a lesion-specific influence on the long-term trajectory of brain development (Ibuki et al., 2012).

Acquired Brain Injury With Congenital Heart Disease: Characteristics and Risk Factors

Neonates with CHD are also at risk for newly acquired brain injury. Delayed brain development itself may be a risk factor for new brain injury, particularly preoperative injury.

Focal brain injury in the term newborn can be clearly and reliably detected with conventional MRI and with greater resolution than with either ultrasound or computed tomography. The most common brain injuries observed in newborns with CHD are focal white matter injury (WMI) and small focal strokes (less than one-third to two-thirds of the arterial distribution; Fig. 57.3). These injuries are largely clinically silent and can be overlooked with routine clinical screening cranial ultrasounds.

WMI has specific imaging characteristics defined by punctate periventricular lesions seen as hyperintensity on T1-weighted MRI. Identification of this pattern of injury was unexpected because it was thought to be restricted to premature newborns with brain injury (periventricular leukomalacia). Risk factors for brain injury are summarized below and in Table 57.1.

Risk Factors for Preoperative Brain Injury

Several large prospective studies have been performed using preoperative and postoperative brain MRI to determine the frequency of acquired brain injury and associated risk factors in newborns with CHD (Table 57.2). Preoperative brain injury in the form of WMI or stroke is present in 28%–39% of these newborns (Table 57.2) (Mahle et al., 2002; Licht et al., 2004; Miller et al., 2004; McQuillen et al., 2007; Mulkey et al., 2014). This was a surprising finding given the focus on operative factors causing ND abnormalities in these patients. Risk factors for preoperative brain injury include hypoxemia and time to surgery (Petit et al., 2009), preoperative base deficit, preoperative cardiac arrest, and the need for balloon atrial septostomy (McQuillen et al., 2006). Other patient-specific risk factors identified include male sex and the presence of aortic atresia (lack of antegrade flow in the aorta) in those with HLHS (Goff et al., 2014).

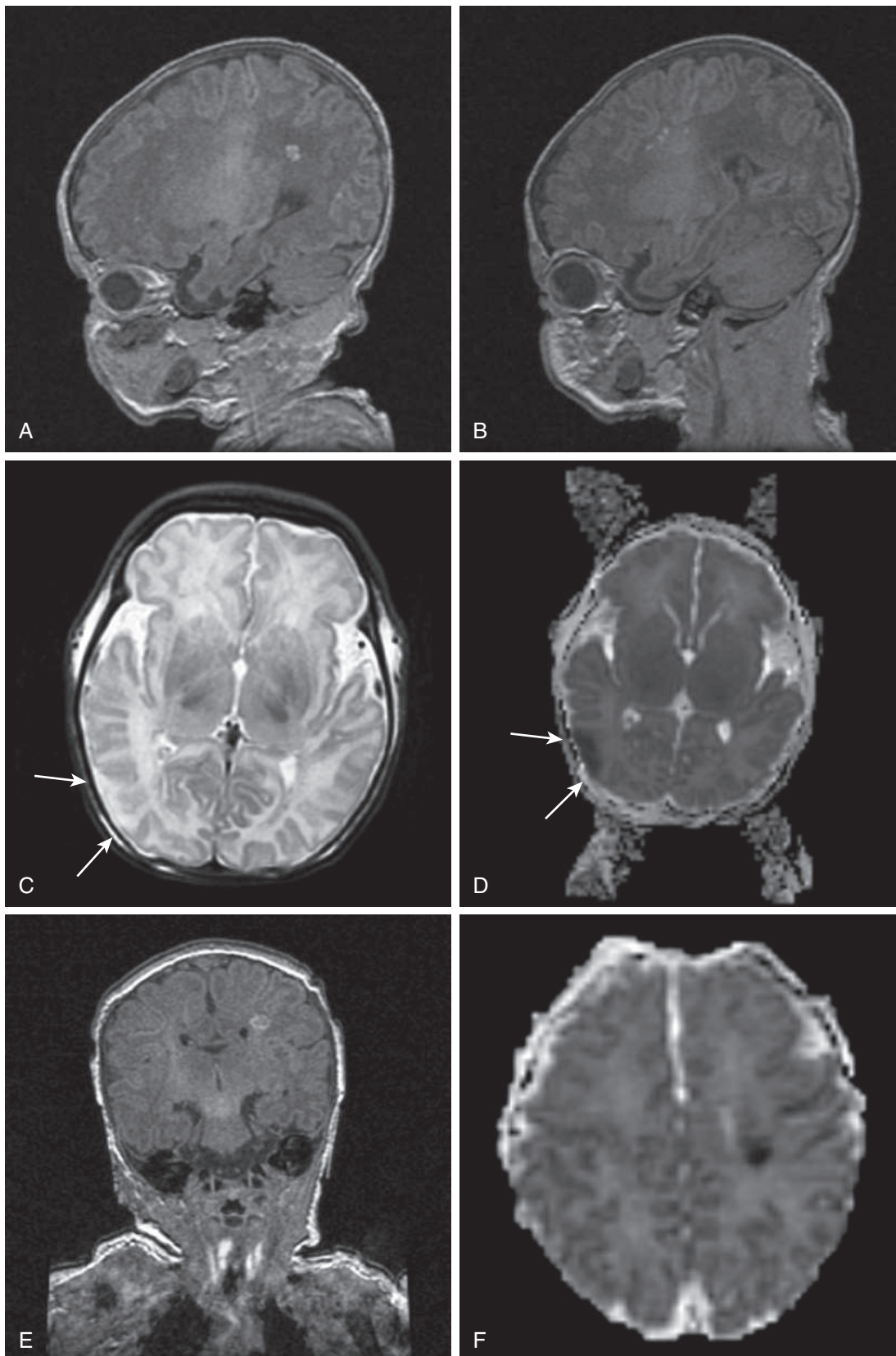
Risk Factors for Intraoperative Brain Injury

Proposed risk factors for intraoperative brain injury relate predominantly to the methods of cardiopulmonary bypass and/or hypothermic total circulatory arrest. The Boston Circulatory Arrest Trial compared two methods of vital organ support in infants

TABLE 57.1 Risk Factors for Brain Injury

| Preoperative | Intraoperative | Postoperative |
|--|---|--|
| Low arterial hemoglobin saturation | Prolonged total circulatory arrest (>40 minutes) | Low blood pressure |
| Length of time to surgery | Decreased cerebral oxygen saturation (NIRS) | Low arterial PaO ₂ |
| Catheter-based procedure (e.g., balloon atrial septostomy) | Cardiopulmonary bypass strategy (regional cerebral perfusion) | Prolonged cerebral regional oxygen saturation (NIRS <45% for >3 hours) |
| Preoperative base deficit | Air or particulate emboli | Morphologically immature brain (total maturation score) |
| Preoperative cardiac arrest | Inflammation | Single ventricle physiology |
| Morphologically immature brain (total maturation score) | | |
| Male sex | | |
| Aortic atresia | | |

NIRS, Near-infrared spectroscopy; PaO₂, partial pressure of oxygen.



• **Fig. 57.3** Magnetic Resonance Imaging Patterns of Brain Injury in Congenital Heart Disease. (A, B) Moderate white matter injury (WMI) in a newborn with hypoplastic left heart syndrome (HLHS) is seen on sagittal T1 images in the postoperative scan. WMIs appear as small focal areas of T1 hyperintensity (brightness). (C, D) Term newborn with HLHS imaged postoperatively at day 17 of life, after a modified Norwood procedure. A small middle cerebral artery distribution infarct is seen as cortical T2 hyperintensity (white arrows in [C]) and corresponding reduced diffusion (white arrows in [D]) in the right parietal-occipital lobe. (E, F) Term newborn with transposition of the great arteries imaged preoperatively after a balloon atrial septostomy. A single focus of T1 hyperintensity is seen in the peratrial white matter on the coronal sequence (E). This same focus has reduced water diffusivity on the average diffusivity map (F, dark spot). This spot is larger than the typical solitary white matter lesion and may represent a small embolic stroke.

TABLE 57.2 Magnetic Resonance Imaging Evidence of Preoperative Brain Injury in Newborns With Congenital Heart Disease

| Study | Sample | CHD | Findings | Risk Factors |
|-------------------------|-------------------------|-------|--|---|
| Mahle et al. 2002 | CHD = 24 | Mixed | PVL = 16% Infarct = 8% Elevated brain lactate = 53% | N/A |
| Miller et al. 2004 | CHD = 10 Control = 5 | TGA | Brain injury (stroke) = 40% Elevated brain lactate = 2.7 × higher than controls | N/A |
| Licht et al. 2004 | CHD = 25 | Mixed | PVL = 28% | <ul style="list-style-type: none"> Decreased cerebral blood flow Hypercarbia |
| McQuillen et al. 2007 | CHD = 62 | Mixed | WMI = 18% Stroke = 21% IVH = 8% | <ul style="list-style-type: none"> Low Apgar score at 5 min BAS |
| Petit et al. 2009 | CHD = 26 | TGA | PVL = 38% | <ul style="list-style-type: none"> Lower preoperative oxygen saturation Longer time to surgery |
| Andropoulos et al. 2010 | CHD = 68 | Mixed | WMI = 16% Infarct = 18% | <ul style="list-style-type: none"> Low total maturation score (brain immaturity) |
| Glass et al. 2011 | CHD = 127 | Mixed | WMI = 24% | <ul style="list-style-type: none"> Bloodstream infection in TGA subjects |
| Goff et al. 2014 | CHD = 57 | HLHS | PVL = 19% | <ul style="list-style-type: none"> Male sex Aortic atresia Low total maturation score (brain immaturity) |
| Peyvandi et al. 2016 | CHD = 153 | Mixed | WMI = 24% Stroke = 20% Hypoxic-ischemic = 1% | Postnatal diagnosis of CHD |

BAS, Balloon atrial septostomy; CHD, congenital heart disease; HLHS, hypoplastic left side heart syndrome; IVH, intraventricular hemorrhage; N/A, not applicable; PVL, periventricular leukomalacia; TGA, transposition of the great arteries; WMI, white matter injury.

undergoing open heart surgery to repair d-TGA with an arterial switch operation (Newburger et al., 1993). This operation is “corrective” since normal cardiovascular physiology is reestablished with low mortality and excellent long-term cardiac functional outcomes. Although deep hypothermic circulatory arrest, which provides the surgeon with an empty and relaxed heart, clearly allowed intricate surgeries to be performed, there was concern at the time regarding late adverse neurologic outcomes and the “safe” duration of circulatory arrest was unknown. An alternative method (low-flow bypass) was felt to maintain some amount of brain oxygen delivery, while still allowing the surgeon a relatively bloodless field. This landmark study enrolled 171 infants into a single-center, randomized clinical trial comparing deep hypothermic total circulatory arrest with low-flow cardiopulmonary bypass. All of the early outcome variables pointed to a benefit from low-flow bypass compared with circulatory arrest. Specifically, the circulatory arrest group had more frequent postoperative seizures, higher serum levels of brain-specific enzymes (creatin kinase), worse 1-year motor outcome (BSID—Psychomotor Development Index [PDI]), and abnormalities on neurologic exam. In contrast, no differences were found in cognitive development (BSID—Mental Development Index [MDI]) or MRI at 1 year of age. Importantly, these differences between the groups disappeared when the patients were assessed at older ages, but both groups remained below population norms for performance on standardized tests (detailed later in this chapter) (Wypij et al., 2003). Other studies have identified circulatory arrest as a risk factor for new postoperative WMI identified on MRI (Beca et al., 2013).

Additional variables examined included hypothermic blood pH management (alpha stat versus pH stat), hemodilution/hematocrit (25% vs 35%), and maintenance of regional cerebral perfusion during aortic arch reconstruction (Jonas et al., 2003; Wypij et al., 2008). Very few of these studies have identified definitively improved neurologic outcomes. In fact, patients who underwent regional cerebral perfusion tended to have worse outcomes, and this technique was associated with new postoperative injury on brain MRI.

These results suggest that although risks remain during the intraoperative period, a major burden of risk for acquired injury occurs outside of the operative period. However, the possibility that unidentified intraoperative risk factors contribute to brain injury cannot be excluded. Analysis of combined cohorts of subjects requiring neonatal cardiac surgery over a 13-year period did not identify substantial improvement in early ND outcomes despite improvements in survival (Gaynor et al., 2015).

Risk Factors for Postoperative Brain Injury

Risk factors for postoperative brain injury include hypotension and hypoxemia related to low cardiac output syndrome, defined as a combination of clinical signs (tachycardia, oliguria, cold extremities, or cardiac arrest) and a greater than 30% difference in arterial–mixed venous oxygen saturation or lactic acidosis. Multiple studies have identified hypotension as a risk factor for new postoperative WMI, including low systolic blood pressure on admission, low mean blood pressure during postoperative day 1,

and low diastolic blood pressure during postoperative days 1 to 2 (Galli et al., 2004; McQuillen et al., 2007).

Studies utilizing cerebral near-infrared spectroscopy have also suggested that low regional cerebral oxygen saturation (<45%) for more than 3 hours was a risk factor for new ischemic injury (Hoffman et al., 2013). In general, patients with single ventricle lesions carry a higher risk of postoperative brain injury, which correlates with the higher postoperative hemodynamic instability, morbidity, and mortality.

Brain Immaturity as a Risk Factor for Brain Injury

The relationship between brain immaturity and brain injury has been explored in the literature although with variable results. Qualitative MRI (TMS) techniques have suggested an association between brain immaturity and the risk of preoperative and postoperative brain injury (Andropoulos et al., 2010). However, quantitative MRI techniques (DTI and MRS) have demonstrated an association between brain immaturity and the risk of preoperative brain injury but not postoperative brain injury (Dimitropoulos et al., 2013). Both studies suggest that brain immaturity may be a risk factor for brain injury with slightly different results. These differences likely relate to the method of measuring brain development. MRS and DTI exhibit changes with brain development and can be influenced by acquired brain injury based on severity, timing, and mechanism. Although overt brain injury has not been identified in the fetal period, indolent brain injury may be present influencing developmental changes and further injury in the preoperative and postoperative period.

Neurodevelopmental Outcomes

There is an increasing body of literature reporting short-term and long-term ND outcomes in patients with various types of CHD. Despite heterogeneity among these reports because of various methodologies for age at follow-up, assessment tools used, and type of cardiac lesion, these outcome data have provided us with useful knowledge on ND outcomes and provide the foundation for understanding imaging and clinical data in the fetal and neonatal period. Studies assessing the predictive value of fetal and neonatal imaging studies are lacking, and longitudinal studies are needed to assess the typical trajectory of brain growth and pattern of injury as these children grow older.

Immediate Neurologic Outcomes After Surgical Repair

Although the prevalence of overt neurologic dysfunction postoperatively has declined, a small percentage of infants continue to exhibit neurologic abnormalities including clinical seizures, hypotonia, and asymmetry of tone. Combining several reports, the prevalence of postoperative subclinical seizures appears to be 4%–11% and has been detected by continuous electroencephalogram monitoring in up to 20% of patients in the immediate postoperative period (Ehyai, et al., 1984; Newburger et al., 1993; Miller et al., 1995; Clancy et al., 2005; Gaynor et al., 2005; Gaynor et al., 2006). In a more recent study, a large percentage of young infants had evidence of ND abnormalities prior to surgery, suggesting that fetal and neonatal factors play a significant role in brain injury and development (Majnemer et al., 2009). In addition, there is a higher prevalence of feeding abnormalities (swallow or

suck dysfunction) in neonates undergoing cardiac surgery, which may be an early indicator of abnormal neurodevelopment later in life (Medoff-Cooper et al., 2015).

Short-Term and Long-Term Neurologic Outcomes After Surgical Repair

Although there have been several reports of ND outcomes in a mixture of CHD types, it is important to recognize that outcomes can vary significantly by cardiac lesion. Outcome studies have been carried out in two specific high-risk patient populations: those with d-TGA and those with defects requiring single ventricle palliation (i.e., HLHS).

In patients with d-TGA after an arterial switch operation, the Boston Circulatory Arrest Trial (described previously) has demonstrated that intelligence quotient (IQ) scores, although below the national average, fall within the normal range at 8 and 16 years of age (Bellinger et al., 2009; Bellinger et al., 2011). However, d-TGA patients at 16 years of age continue to exhibit deficits in academic achievement, memory, executive function, visual-spatial skills, attention, and social cognition. A high percentage of children were judged to have behavioral problems by parents and teachers; 37% required remedial education services and 10% had repeated a grade. Interestingly, in a multivariable regression model, socioeconomic status was a strong predictor of ND outcome in these patients at 16 years of age. These studies demonstrate that early ND testing may underestimate the ultimate burden of functional impairment in subjects with subtle but diffuse brain injuries.

Patients with single ventricle lesions, in particular HLHS, are at highest risk of ND abnormalities based on the underlying physiology, hemodynamics, and the complexity of surgical repair. These children must undergo a series of palliative surgical procedures typically culminating in a Fontan operation. Various studies have demonstrated that children with HLHS tend to have lower IQs (typically below the general population mean) and problems with visual–motor skills, expressive language, attention, and externalizing behavior. In the largest series reported to date, children with HLHS at 12 months of age had a median MDI of 90 (range 50–129) and a lower PDI of 73 (range 50–117) (Newburger et al., 2012). Risk factors for poor outcome were mainly patient specific and included genetic syndromes, earlier gestational age at delivery, and perioperative instability. Other studies have assessed the impact of growth and feeding issues on ND outcomes. Infants requiring device-assisted feeding and with lower weight, length, and head circumference at 3 months of age were at increased risk for ND delay at 6 and 12 months of age (Medoff-Cooper et al., 2015). Long-term studies have identified persistent ND abnormalities in adolescents with Fontan physiology including lower IQ and abnormal neuropsychological testing compared with a normative population (Bellinger et al., 2015b).

Genetic Susceptibility to Neurodevelopmental Abnormalities

An important point to consider is the impact of genetic comorbidities on ND outcome in the context of CHD. Approximately one-third of children with CHD have an underlying genetic disorder such as aneuploidy or deletion syndromes (i.e., 22q11.2 deletion syndrome). Studies have shown greater impairments in cognition, IQ, motor skills, hearing, and visual skills in those children, compared with children with CHD who do not have a genetic comorbidity (Pierpont et al., 2007; Marino et al., 2012). Several

studies have been performed in children with CHD who also have 22q11.2 deletion syndrome. This microdeletion encompasses three megabases of DNA, representing 30 to 40 genes, and the syndrome is highly heterogeneous with a variable phenotype. These patients have also been noted to have psychiatric disorders such as anxiety, attention deficit hyperactivity disorder, and psychosis. Interestingly, the presence of CHD does not change the prevalence of these psychiatric abnormalities (Yi et al., 2014).

Studies have also identified patient-specific genetic risk factors that can modify ND outcome. This has been described for alleles of apolipoprotein E (*APOE*) in which the ApoE ε4 allele is associated with adverse outcomes in many adult conditions (e.g., Alzheimer's disease, traumatic brain injury, stroke, and subarachnoid hemorrhage). This has been explored in infants with CHD and the ApoE ε2 allele is associated with worse outcome (Gaynor et al., 2014). Overall, it is likely that multiple genes and environmental factors influence ND outcome in these patients.

Neurodevelopmental Signature of Congenital Heart Disease

Although children with complex CHD have intelligence testing results in the range of the normal population, there is a prevalence of pervasive but subtle cognitive problems that some have termed a *neurodevelopmental signature of complex congenital heart disease*. These children show behavioral and attention problems that are often not detected on standardized testing but result in poor school performance. Among a large cohort of children who were followed prospectively after undergoing cardiac surgery as infants, abnormalities on neurologic exam at school entry were present in 28%, although less than 5% were severe. Most of the abnormalities involved fine motor coordination and tone. Cognitive difficulty and behavioral problems were identified in 30%. Certainly, survivors of CHD exhibit a “developmental profile” that changes in each stage of life and affects quality of life and the ability to perform everyday tasks (Majnemer et al., 2009). It is important to note that the American Heart Association released a statement paper recommending serial ND assessments for at-risk children with CHD (Marino et al., 2012). This includes neonates requiring open heart surgery (before 30 days of life) and other cyanotic heart lesions such as tetralogy of Fallot that may not require a neonatal operation. In addition, serial evaluation is recommended whenever CHD is seen in combination with prematurity (<37 weeks' gestation), developmental delay recognized in infancy, suspected genetic anomaly, history of extracorporeal life support, heart transplantation, the need for cardiopulmonary resuscitation, prolonged perioperative hospitalization, perioperative seizures, and abnormal neuroimaging findings.

Conclusions

Advances in prenatal diagnosis and care and cardiovascular surgical techniques have contributed to the overall increased survival of

neonates born with CHD. Given this improvement in survival, greater emphasis is now being directed toward improving ND outcomes. More specifically, understanding the predictive value of imaging studies both in the fetus and neonate provides an opportunity for potential intervention trials to improve outcomes among this patient population. In addition, as the prenatal detection of CHD increases, informing families about potential noncardiac adverse outcomes is critical to ensure recognition and timely intervention for ND abnormalities.

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Central Nervous System Development

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KEY POINTS

- The brain of the preterm and term newborn is actively developing, with several key steps occurring during the third trimester, such as the end of neuronal migration, programmed cell death, the generation of axons and dendrites, and the first wave of synaptogenesis.
- These processes are controlled by genetic programs but are also very sensitive to environmental factors.
- Epigenetic mechanisms appear to play a central role in these processes, with long-term and potentially transgenerational consequences.

Brain development results from the accomplishment of successive genetic programs during the different ontogenic stages. It starts with the individualization of the neural plate at the beginning of the third week postconception and is mostly complete by adolescence. However, some neuronal production persists lifelong: this late neurogenesis has been well described at the level of the olfactory bulbs and the dentate gyrus of the hippocampus; its importance to the associative neocortex remains to be shown.

The principal stages of brain development can be summarized as follows; induction of the neuroectoderm, formation of the neural tube followed by the telencephalon, neurogenesis (production of neuronal progenitors and then of mature neurons), neuronal migration, programmed neuronal death, generation of neurites (axons and dendrites), elimination of superfluous neurites, synaptogenesis, elimination and selective stabilization of synapses, angiogenesis, gliogenesis (production of astrocytes and oligodendrocytes), and myelination (Fig. 58.1). These different stages of the development and maturation of the brain are controlled by intrinsic factors (determined genetically) and modulated by extrinsic environmental factors. This modulation by environmental factors could bring epigenetic mechanisms into play.

The perturbation of the unfolding of any of these different stages of brain development leads to a deficit in brain growth and/or brain malformations. The functional consequences to the child depend on the developmental stage in question. This raises the notion of critical periods.

Neuronal Production and Migration

The expansion of the cortex occurs through two processes that take place in parallel: a lateral expansion process that allows the

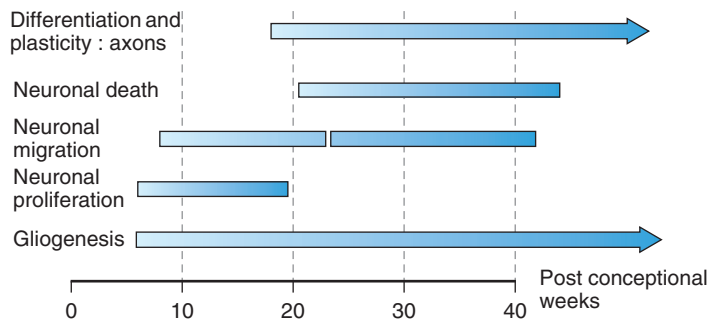
surface of the cortex to grow and a radial expansion process that leads to an increase in its thickness. The total number of neurons in a mature human brain is estimated at between 3 and 100 billion. The cortex is initially generated from a monolayer of proliferative neuroepithelial cells lining the walls of the lateral ventricles—the ventricular zone (VZ). These cells are oriented radially. At approximately the seventh gestational week (GW) in humans, a second proliferative zone, the inner subventricular zone (SVZ), appears, derived from precursors in the VZ (Fietz and Huttner, 2011). The cells of the inner SVZ do not adopt a radial conformation. More recently, a third proliferative zone has been identified in the developing neocortex: the outer SVZ, which appears in humans at approximately GW 11. In humans, this zone displays prolonged proliferation whereas in rodents these cells only undergo a single division. This difference in the behavior of neuronal precursors could explain the impressive evolutionary expansion and folding observed in the surface of the neocortex.

These proliferative zones, situated on the dorsal side of the lateral ventricles, give rise to the excitatory (glutamatergic) neurons of the different cortical layers (the cortex in mammals consists of six layers), as well as a portion of inhibitory neurons (γ -aminobutyric acid [GABA]ergic interneurons). The other inhibitory neurons derive from another proliferative structure located on the lateral wall of the lateral ventricles, the ganglionic eminence, which is also the source of thalamic neurons. Migrating neurons can adopt a radial trajectory by migrating in contact with specialized glial cells, the radial glia, which serve to guide them (Gressens and Evvard, 1993; Haydar et al., 2003). In contrast, neurons originating in the medial eminence adopt an initially tangential trajectory, independent of radial glia, though there is some evidence of a final stage of radial migration.

After exiting the mitotic cycle, neurons migrate from the proliferative zones toward the future cortex (Rakic, 1971; Marin-Padilla, 1978; Rakic, 1978; Nieuwenhuys et al., 2007; Bystron et al. 2008; Fietz and Huttner, 2011). The first wave of migratory neurons forms the primitive cortical plate or preplate (Fig. 58.2). The second wave of migratory neurons then splits this primitive plate into two, at approximately GW 7, giving rise to a three-layered structure; layer I, which contains the Cajal–Retzius neurons, is located just below the meninges, layer VI, which contains neurons that have already completed their migration, and finally the subplate (SP)—a transient structure located below the future neocortex. Consecutive waves of migratory neurons subsequently cross the SP and the cortical layers already in place but stop below layer I, thus successively forming layers V, VI, III, and II along what is

known as an “inside-out” gradient (Angevine and Sidman, 1961) (see Fig. 58.2). Until recently, it was thought that the migration of neurons to the neocortex was complete by approximately GW 24 (Sidman and Rakic, 1973). However, recent studies suggest that GABAergic interneurons continue to be added to the neocortex practically until term (Xu et al., 2011). Premature birth could thus have an impact on this late migration (Leviton and Gressens, 2007). Interestingly, in a model of premature birth in baboons, when compared with fetuses of the same developmental age, a rarefaction of interneurons in the primary occipital cortex has been demonstrated (Verney et al., 2010).

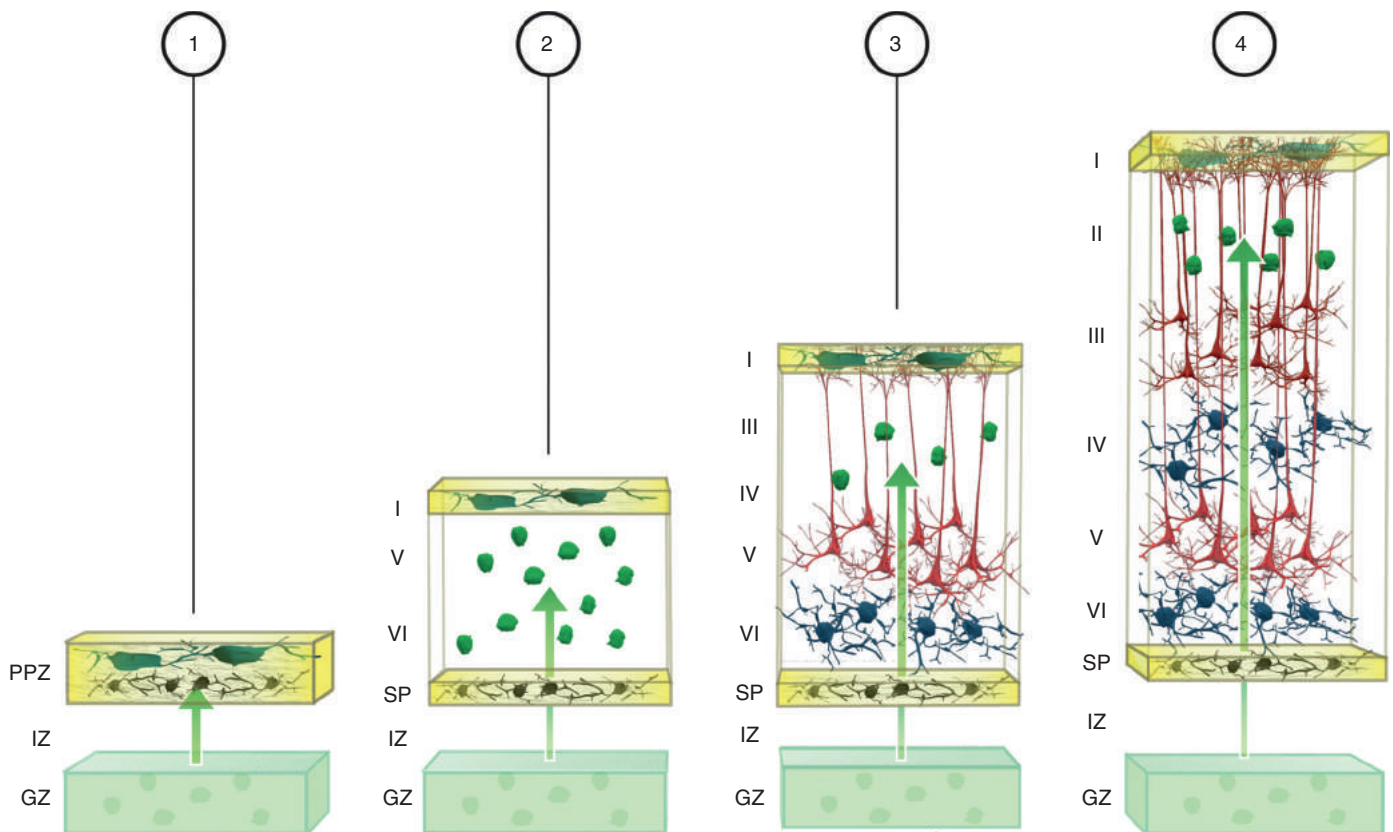
Several molecules involved in controlling neuronal migration and their navigation to the appropriate destinations have been



• **Fig. 58.1** Schematic representation of the major ontogenic events taking place in the human neocortex.

identified. These molecules can be schematically divided into four categories (Gressens, 2006; Diaz and Gleeson, 2009).

1. Cytoskeletal molecules, which play an important role in the initiation and progression of neuronal movement (extension of the apical process and nucleokinesis). Molecules controlling initiation include Filamin-A (an actin-binding protein that is implicated in periventricular nodular heterotopias) and Arfgef2 (a molecule that plays a role in vesicular trafficking and is involved in periventricular nodular heterotopias associated with microcephaly). Among the molecules controlling progression are Doublecortin (a microtubule-associated protein [MAP] implicated in double cortex syndrome), Lis1 (a MAP implicated in Type 1 lissencephaly and Miller–Diecker syndrome), and α -1 Tubulin (involved in the formation of tubulin heterodimers).
2. Signaling molecules that play a role in lamination, such as Reelin (a glycoprotein implicated in a human disorder combining lissencephaly with cerebellar hypoplasia)
3. Molecules modulating glycosylation that provide a stop signal to migrating neurons, such as POMPT1 (protein *O*-mannosyltransferase, associated with Walker–Warburg syndrome), POMGnT1 (protein *O*-mannose β -1,2-N-acetylglucosaminyltransferase, implicated in muscle–eye–brain disease) and Fukutin (a glycosyltransferase implicated in Fukuyama congenital muscular dystrophy). These three human disorders display Type 2 lissencephaly.
4. In addition to these three principal groups of molecules, neuronal migration can be modulated by other factors such as certain neurotransmitters (glutamate and GABA), molecules derived



• **Fig. 58.2** Schematic illustration of mammalian neocortical formation and neuronal migration. GZ, Germinative zone; I, cortical layer I or molecular layer; II to VI, cortical layers II to VI; IZ, intermediate zone (prospective white matter); PPZ, primitive plexiform zone; SP, subplate. Arrows and green circles indicate migrating neurons.

from peroxisomal metabolism, and certain environmental factors (ethanol and cocaine).

Programmed Neuronal Death

Depending on the brain region under consideration, 15%–50% of the neurons initially produced die through a physiologic process termed programmed cell death or apoptosis. Approximately 70% of the neurons that disappear seem to die between GWs 28 and 41 in humans (Bhutta et al., 2001).

Programmed cell death is a complex mechanism bringing into play a balance between cell death and survival-inducing signals, genetic programs involved in cell death or survival, and effectors of cell death and inhibitors of these effectors (Polleux et al., 2001). Under the influence of a combination of endogenous and exogenous factors, genetic programs are activated (as reflected in the term “programmed cell death”) and are capable of overcoming the natural defense mechanisms of the neuron. Once the cell is dead, it is rapidly phagocytosed by neighboring glial cells without inducing inflammatory phenomena or scar formation. This process is facilitated by changes in the composition of the apoptotic cell membrane, which occurs very early in the apoptotic process. Within the cell death process, the activation of caspases (proteolytic enzymes) in the form of a cascade is a key stage that culminates in DNA fragmentation and the death of the neuron.

Electrical activity appears to be a critical factor for neuronal survival. During the peak period of brain growth in rodents, the administration of substances that block electrical activity induces a serious aggravation of developmental neuronal death in various brain regions. These substances include *N*-methyl-D-aspartate receptor inhibitors (MK-801 or ketamine), GABA-A receptor agonists such as antiepileptics (phenytoin, phenobarbital, diazepam, clonazepam, vigabatrin, and valproic acid), and anesthetics (a combination of midazolam, nitric oxide, and isoflurane) (Turski and Ikonomidou, 2012).

Organization of the Central Nervous System

Subplate Neurons

SP neurons constitute a transient structure during brain development (Kostovic and Judas, 2002). They are generated at approximately GW 7, and the SP itself appears at approximately GW 10. This structure, located under the neocortical plate, reaches its maximum thickness between GWs 22 and 36.

SP neurons express various neurotransmitters, neuropeptides, and growth factors. They receive synapses and form connections with cortical and subcortical structures. These neurons play an important role during brain development; (1) they produce axons for the internal capsule, which act as guides for neurons from layers V and VI, (2) between GWs 25 and 32, they produce axons for the corpus callosum, and (3) they constitute a “waiting zone” for thalamocortical axons (with which they establish synapses) before the latter invade the neocortical plate to reach layer IV. This waiting area is necessary for the adequate targeting of thalamocortical afferents.

SP neurons may be destroyed in preterm newborns presenting with lesions of the periventricular white matter (Sarnat and Flores-Sarnat, 2001), findings which have been confirmed in animal models of periventricular white matter injury (McQuillen et al., 2003; Sfaello et al., 2005). This damage to the SP could participate in the thalamocortical connection anomalies recently demonstrated

by magnetic resonance imaging in preterm infants (Ball et al., 2013), as well as in the associated cognitive and/or motor disorders.

Axonal and Dendritic Growth

The final morphology of the neurons and the formation of connections between neurons depend on complex cellular interactions. Mature neurons develop dendrites and axons. In parallel, growing axons migrate toward their target neurons, which secrete chemoattractant or chemorepulsive factors and adhesion molecules, in order to form appropriate synapses. These chemoattractant or chemorepulsive axonal guidance molecules interact with receptors present on the growth cone and induce it to advance or to retract. The interaction between these different ligands and their receptors leads to modifications in calcium levels at the growth cone, which play a key role in growth cone motility and orientation. This ontogenic stage occurs largely, but not exclusively, during the second half of pregnancy and extends into the postnatal period.

The formation of corticospinal projections occurs at early stages from the SP and then from neurons that populate the deep cortical layers (V and VI). Corticothalamic projections also appear, from both the SP and the deep cortical layers. Corticocortical projections appear later.

It has been shown that a number of transcription factors (e.g., Ctip2, Satb2, Fezf2) regulate the commissural, corticothalamic, and corticospinal projections of the cortex. Even though genetic programs control the molecular mechanisms of axonal and dendritic growth and determine the initial pattern of these connections, experience and the environment then sculpt this global pattern to generate the final set of connections (through brain plasticity).

Synaptogenesis

The concept of synaptic stabilization (by the elimination of nonstabilized synapses) was first proposed by Changeux and Edelman (Changeux and Danchin, 1976). During brain development, there is a systematic overproduction of labile synapses in successive phases that leads to redundant connections in a fairly random manner. This stage is mainly controlled by genetic factors. Each wave of overproduction is followed by a period of stabilization of synapses that are of functional value and the elimination of redundant or useless ones. This period of stabilization and elimination is strongly influenced by environmental stimuli and experience. In this model, the moderate increase in the number of genes involved in synaptogenesis during the course of evolution has resulted in a richer substrate upon which the environment and experience act to generate a more complex network.

In the occipital neocortex of the monkey, five successive waves of synaptogenesis have been described (Bourgeois, 1997). Based on data obtained from the human occipital cortex (Lagercrantz and Ringstedt, 2001), the following chronology has been proposed for the human cortex: (1) a first phase starting at GW 6–8 and limited to the deep layers, such as the SP, (2) a second phase starting at GW 12–17, with relatively few synapses produced in the cortex, (3) a third phase starting at around the middle of pregnancy and finishing at around the 8th month after birth (this phase is characterized by a rate of production of new synapses estimated at around 40,000 per second in the monkey), (4) a fourth phase that extends up to puberty and is also characterized by a high rate of synapse formation, and (5) a final phase that extends up to adulthood but is somewhat masked by the significant

loss of synapses with age. Experimentally, the two first phases are not influenced by the deprivation of sensory stimuli. The third phase is partly dependent on sensory input whereas the fourth phase is strongly controlled by sensory stimuli and experience.

Glial Proliferation, Differentiation, and Myelination

Astrocytes

Neocortical astrocytes are of two origins (Gressens et al., 1992). At the end of neuronal migration, radial glial cells (which are both neural stem cells and play a role in guiding migrating neurons) are transformed into astrocytes, which then reside in the deep cortical layers and the white matter. In contrast, the astrocytes of the superficial cortical layers are primarily derived from glial precursors that multiply in the SVZ and then migrate into the cortex.

In the human neocortex, astrocytic proliferation probably begins at approximately GW 24, with a peak at approximately GW 26–28. The exact date at which the production of astrocytes ends is not known, but it could be supposed that the major part of astrocytic production is over by the end of a normal pregnancy. This peak in the production of astrocytes at a GW 26–28 could be of particular importance for preterm newborns. Indeed, astrocytes play several important roles, including axonal guidance, stimulation of neuronal growth, synapse formation, transfer of metabolites between blood vessels and neurons, establishment of the pattern of certain brain structures, production of extracellular matrix components, production of trophic factors, neuronal survival, myelination, and maintenance of the blood–brain barrier. For example, experimentally blocking astrocyte production temporarily in the neocortex of rodents induces an increase in programmed cell death in neurons and long-term changes in neocortical synaptic equipment (Zupan et al., 2000).

Oligodendrocytes and Myelination

Oligodendrocytes can be divided into four types depending on their degree of maturation.

1. Oligodendrocyte precursors that arise from the SVZ are bipolar and mitotically active; their differentiation into preoligodendrocytes occurs during their migration into the future/putative white matter.
2. Preoligodendrocytes are multipolar cells that retain their proliferative capacity. This second cell type is predominant in the periventricular white matter during the second half of pregnancy.
3. Immature oligodendrocytes are multipolar cells that appear during the third trimester and wrap axons in preparation for their myelination.
4. The last stage is their differentiation into myelinating and extremely multipolar mature oligodendrocytes.

The oligodendrocyte precursors and preoligodendrocytes, the predominant varieties in the brain of preterm newborns, are extremely vulnerable to oxidative stress, the excitotoxic cascade, and hypoxic–ischemic insults (Volpe, 2001). This death of preoligodendrocytes has been implicated in cystic periventricular leukomalacia. This type of severe lesion has progressively decreased in incidence, to be replaced by more diffuse lesions (Ferriero and Miller, 2010). Recent neuropathologic and experimental data suggest that, in this new type of white matter lesion in preterm infants,

the primary phenomenon is a blockade of oligodendrocyte maturation (Billiards, et al. 2008; Verney et al., 2010; Buser et al., 2012), in which the Wnt pathway plays a role (Fancy et al., 2011).

Myelination occurs during a prolonged period, persisting into childhood. The chronology and the degree of myelination vary according to the brain structure studied. There is no detectable myelination in the prosencephalon before the seventh month of pregnancy. Myelination in the telencephalon is most intense during the third trimester and postnatally and is mostly complete by the age of 2–3 years. The olfactory and auditory pathways and the sensorimotor cortex are the first to be myelinated, while projection and association pathways (in particular the prefrontal cortex) are the last (Girard et al., 2007).

Microglia

Microglia constitute 5%–15% of the total number of brain cells. Recent work in rodents suggests that cerebral microglia are derived from a pool of monocytic precursors that invade the amniotic sac early on and, from this pool, infiltrate the developing brain (Ginhoux et al., 2010). During the first trimester of pregnancy in humans, microglia that penetrate the brain have an amoeboid morphology. This morphology evolves progressively toward an intermediate and then a mature phenotype, with a small cell body and long processes. Around mid-pregnancy, microglial populations are principally detected in white matter fascicles such as the internal and external capsules, the corpus callosum, and axonal fascicle crossings (Monier et al., 2007). It is precisely in these regions of high microglial density that preterm infants develop the most white matter lesions (Verney et al., 2010). Experimental data support the hypothesis that microglia play an important role in the origin of these lesions (Tahraoui et al., 2001). Experimental studies show that during brain development, these cells participate to a significant extent in the physiologic phenomena of synaptic and neurite remodeling (Graeber, 2010). It is unknown how the fetal inflammatory response syndrome, which induces microglial activation, influences these developmental phenomena.

The Environment and Epigenetics

The different stages of brain development are finely controlled by diverse genetic programs. Nevertheless, experience, the environment, or stimulation can modulate, adapt, or refine the initial pattern to allow the brain to adjust to its environment. These adaptive processes are the expression of the great plasticity of the developing brain and allow the acquisition of new skills throughout childhood and adolescence. This is true even with regard to developmental stages that were initially thought to be almost completely controlled by genetic programming, such as the proliferation of neuronal precursors; experimental studies have shown that a maternal factor (vasoactive intestinal peptide) can change the proliferative capacity of these precursors by up to 20% (Passemard et al., 2011). However, the environment can also have deleterious effects on brain maturation. In particular, preterm newborns are exposed to numerous stimuli that exist only sparingly or not at all for a fetus of the same age, such as excessive or repeated sensory stimulation, painful stimuli, stress, several neuroactive drugs, as well as the brutal withdrawal of maternal and placental factors. Any environmental factor or drug that affects the brain is capable of altering a few or several stages of brain development.

The major mechanisms by which these environmental factors (positive or deleterious) act on brain maturation depend on the

epigenetic events that control the organization and compaction of the genome and thus the expression of genes. Chromosomes in eukaryotes result from a complex between DNA and a protein sheath called *chromatin*, whose basic unit is the nucleosome, around which the DNA is wrapped. Each nucleosome is composed of an octamer of histones (H2A, H2B, H3, and H4). The compaction of DNA—and thus the accessibility of genetic information to the transcriptional machinery—is regulated on the one hand by covalent modifications of the DNA and on the other by posttranslational modifications that decorate the N-terminal regions (tails) of histones (acetylation, phosphorylation, and methylation). The combination of these two types of events governs the opening or closing of chromatin (and thus the transcription or repression of the gene in question).

The methylation of DNA at CpG islands located in transcriptional regulatory regions of genes represses their transcription by blocking the binding of transcription factors to the DNA (Jones, 2012). DNA methyltransferases catalyze the methylation of DNA by using S-adenosyl-methionine (SAM) as a methyl group donor. In addition, the information deposited by the methylation of the DNA is “read” and “translated” by proteins that recognize methylated DNA: the methyl-CpG-binding proteins (MBPs). These MBPs recruit histone-deacetylases (HDACs), (and thus constitute the link between DNA methylation and the posttranslational modifications of histones), as well as other chromatin remodelers, and contribute to the establishment of transcriptionally inactive chromatin. The crucial role of histone acetylation in brain development is illustrated, for example, by the role of the histone-acetyltransferase CREB-binding protein (CBP), whose mutation in the heterozygous state is characteristic of Rubinstein–Taybi syndrome in humans and leads to anomalies of cortical gyration, a reduction of white matter, and regional hypoplasia in association with intellectual disability (Graff et al., 2011).

Remarkably, the level of DNA methylation is much higher in the brain than in other tissues and is essential for brain development in mouse models (Jones, 2012). DNA methylation especially affects neuronal differentiation and survival and the neurogenesis/gliogenesis “switch.” In humans, several syndromes with intellectual disability have a developmental origin linked to the DNA methylation pathway. This is the case with Rett syndrome, which is associated with mutations of the MBP MeCP2, and immunodeficiency–centromeric instability–facial dysmorphism syndrome, caused by mutations in *Dnmt3b* (Xu et al., 1999; Graff et al., 2011).

Finally, numerous imprinted genes (i.e., those expressed uniquely from the paternal or maternal allele), which are affected by epigenetic regulation, are expressed in the brain, and the deregulation of their expression is often associated with intellectual disability. This is the case, for example, with the neurodevelopmental syndromes, Prader–Willi and Angelman syndromes (Chamberlain and Lalonde, 2010).

Interestingly, fetal stress modifies the pattern of DNA methylation of key genes involved in neurodevelopment and the integrity of

the adult brain (such as *BDNF*, *Reelin*, and *GAD67*) (Graff et al., 2011). Thus women suffering from depression or anxiety-related disorders during the third trimester of pregnancy give birth to newborns in whom the promoter region of the glucocorticoid receptor gene is hypermethylated. Additionally, neuronal plasticity, learning, and memory rely on the establishment of epigenetic events and neuronal activity in particular modifies patterns of DNA methylation (Guo et al., 2011).

In summary, one could thus speak of “neuro-epigenetics” and all that this entails in terms of future therapeutic possibilities, since an in-depth knowledge of epigenetic events and their reversibility opens the door to the search for compounds that could remodel the epigenome of the brain and reprogram neural cells. In this context, it is of interest to note that diets poor in methionine (the precursor of SAM) and treatment with HDAC inhibitors (such as valproic acid) have been widely prescribed for psychiatric disorders such as schizophrenia or bipolar disorder, as well as in diverse forms of addiction (Graff et al., 2011).

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Congenital Malformations of the Central Nervous System

SARAH BAUER HUANG AND DAN DOHERTY

KEY POINTS

- Brain malformations are a substantial source of morbidity and death in neonates.
- Advances in imaging and genetics now allow more specific diagnoses.
- Early and specific diagnosis allow more precise prognostic and recurrence information to be obtained and improved monitoring for complications and can guide treatment
- Early diagnosis and management of patients with brain malformations require multidisciplinary team management.

This chapter will focus on congenital malformations of the central nervous system (CNS), including developmental disorders of the spinal cord and forebrain, midbrain, and hindbrain. When possible, examples of imaging in the fetal or neonatal period will be used to highlight these anomalies. Within each section we will review relevant embryology, discuss the known genetic or environmental risk factors, and briefly discuss management, if applicable. Categorization of brain malformations has evolved over time. Initial categories were based solely on clinical and pathologic features. Brain imaging has added greatly to our ability to distinguish different malformations, followed by remarkable advances in genetics and the elucidation of molecular mechanisms. Classifications will continue to evolve with further developments in all of these areas.

Prosencephalic Cleavage and Related Events

Normal Prosencephalic Development

The prosencephalon refers to the future forebrain, which includes the telencephalon and the diencephalon; these structures give rise to the cerebral hemispheres and the thalamus/hypothalamus. The prosencephalon develops after closure of the anterior neuropore, through processes that induce the bifurcation of the rostral extent of the fluid-filled neural tube (Fig. 59.1) to form the right and left forebrain structures (Rubenstein and Beachy, 1998). During the fifth and sixth weeks of development, the structure of the forebrain is defined by cleavage along three major planes. As the

anterior neuropore is closing, the first major event is formation of the optic vesicles and nasal placodes, separated along the *horizontal* plane. When the embryo has reached a length of about 5 mm, both neuropores have closed, isolating the developing ventricular system from the amniotic fluid. The retinal and lens placodes also develop at this time. In the hindbrain the cerebellum begins to form, along with somatic and visceral efferent nuclei, the common afferent tract, and the ganglia for most of the cranial nerves. At about day 32 of gestation, when the embryo is 5 to 7 mm long, the forebrain divides in the *sagittal* plane to give rise to the paired structures. Specific areas, including the hypothalamic, amygdala, hippocampal, and olfactory regions, can be defined at this time. The third major event in forebrain development occurs shortly thereafter when the forebrain divides in the *coronal* plane. This event separates the telencephalon from the diencephalon, defining the epithalamus, subthalamus, and hypothalamus. During the remainder of the second and third months of gestation, multiple midline structures form, including the corpus callosum, anterior and hippocampal commissures, optic nerves, optic chiasm, and hypothalamus.

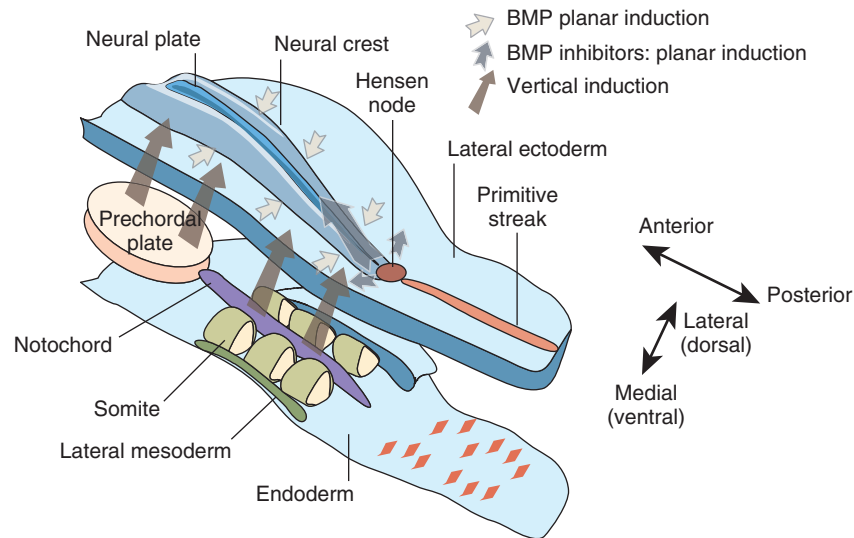
Disorders of Structures Derived From the Prosencephalon

Disorders of development of the prosencephalon include the severe malformations: atelencephaly, aprosencephaly, and holoprosencephaly (HPE). Milder defects include agenesis of the corpus callosum (ACC), septo-optic dysplasia (SOD), and isolated absent cavum septi pellucidi (CSP).

Aprosencephaly and Atelencephaly

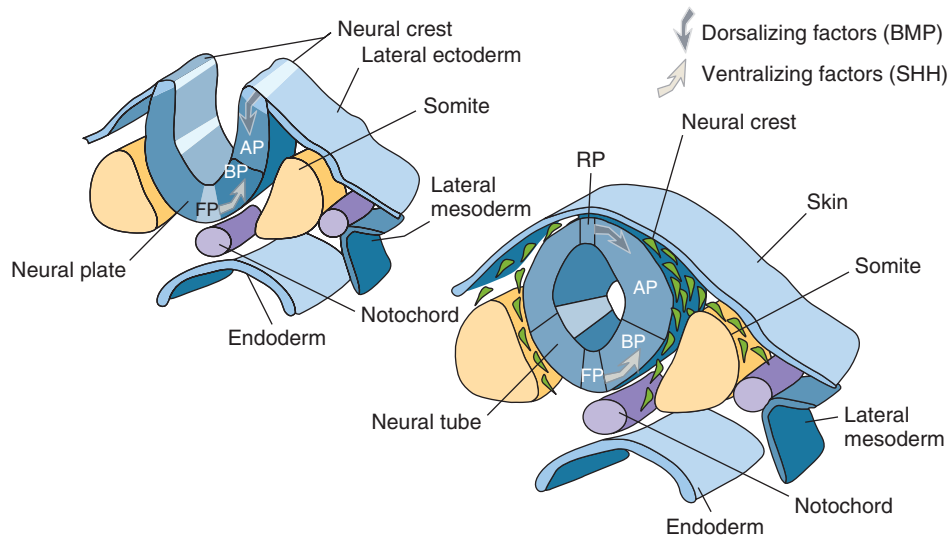
Aprosencephaly and atelencephaly are two rare and very severe cerebral malformations. In aprosencephaly, neither prosencephalic nor diencephalic structures develop. In atelencephaly, there remains a rudimentary prosencephalon. Craniofacial abnormalities are secondary to deformation rather than malformation (Siebert et al., 1987). These disorders may result from possible autosomal recessive inheritance versus an abnormality of chromosome 13 (Ippel et al., 1998). Aprosencephaly/atelencephaly has also been reported in a family with mutations in the *SIX* gene, which has also been

GASTRULATION



A

NEURULATION



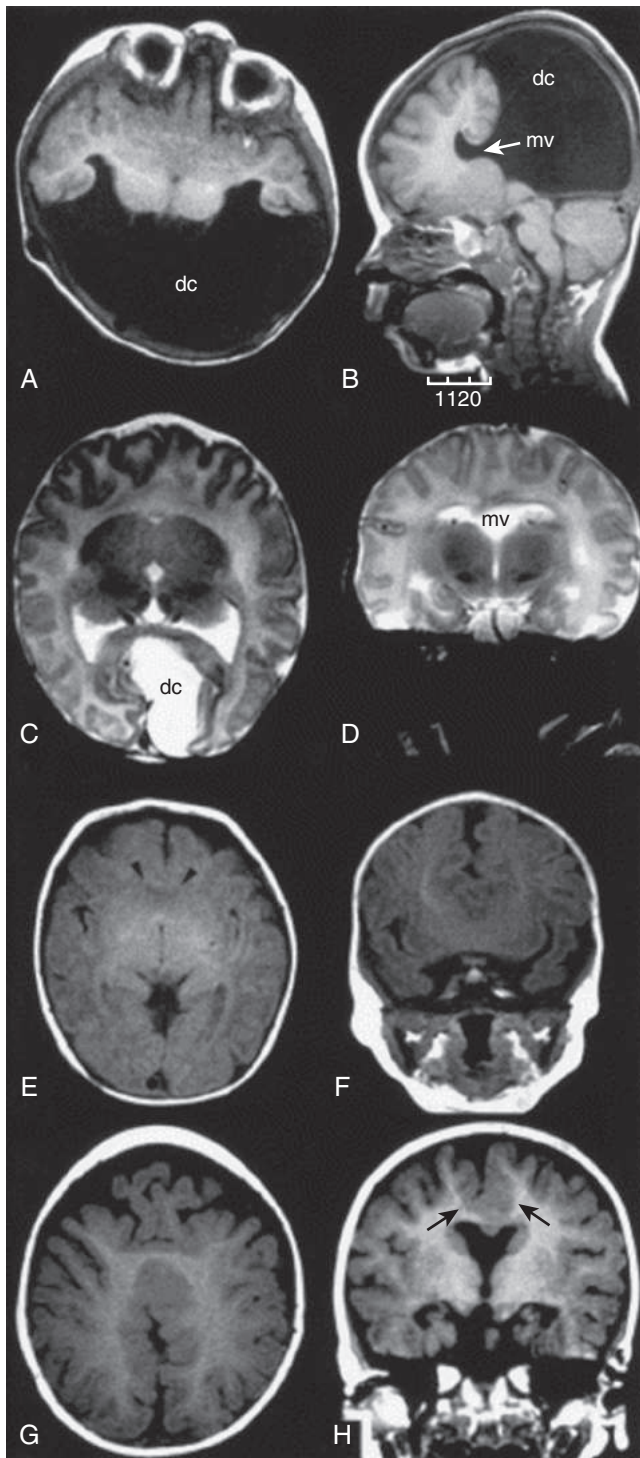
B

• **Fig. 59.1** Formation of the Neural Tube. (A) During gastrulation, at the neural plate stage, dorsoventral polarity and early anteroposterior regionalization are defined by a process of vertical induction by fibroblast growth factor 8 and other factors (*long gray arrows*) derived from mesendoderm (notochord and prechordal plate). Planar induction occurs via bone morphogenetic proteins (BMPs) and BMP inhibitors that are derived from lateral ectoderm (*short light gray arrows*) and the Hensen node (*short dark gray arrows*) respectively. (B) The process of neurulation proceeds with the approximation of the neural folds toward the dorsal midline. Before closure of the neural tube, neural crest cells delaminate and migrate from the neural folds. Dorsalizing factors (BMPs; *dark gray arrow*) derived from the dorsal midline roof plate (RP) and ventralizing factors (sonic hedgehog [SHH]; *light gray arrow*) from the floor plate (FP) establish dorsal-ventral gradients of these key signaling molecules that induce formation of the alar plate (AP) and the basal plate (BP) from the lateral wall of the neural tube. (Adapted from Vieira C, Pombero A, García-López R, Gimeno L, Echevarría D, Martínez S. Molecular mechanisms controlling brain development: an overview of neuroepithelial secondary organizers. *Int J Dev Biol.* 2010;54:7–20, courtesy of Dr. Salvador Martínez, Institute of Neuroscience, Universidad Miguel Hernández, San Juan de Alicante, Spain.)

associated with HPE (Pasquier et al., 2005). Typically, these are diagnosed prenatally by ultrasound and fetal magnetic resonance imaging (MRI) (Nagaraj et al., 2016). Although considered “lethal” malformations, it is important for the neonatologist to be prepared for infants with severe malformations to live longer than expected and even go home from the hospital.

Holoprosencephaly

HPE represents a spectrum of defects in forebrain development and is the most common brain malformation (Norman et al., 1995; Barr and Cohen, 1999), although most affected fetuses are miscarried early in gestation. HPE represents a variable degree of



• **Fig. 59.2** The Spectrum of Holoprosencephaly as Demonstrated by Magnetic Resonance Imaging. (A, B) Magnetic resonance imaging (MRI) of the brain in a patient with alobar holoprosencephaly. The T1-weighted axial image (A) reveals lack of separation of the two hemispheres and deep gray nuclei. A large dorsal cyst (dc) is observed posteriorly. The T1-weighted sagittal image (B) reveals a midline monoventricle (mv) that communicates posteriorly with the dorsal cyst (dc). (C, D) MRI of a patient with semilobar holoprosencephaly. The T2-weighted axial image (C) indicates separation of the hemispheres posteriorly but not anteriorly. Anterior horns of the lateral ventricles are absent, whereas the posterior horns are well formed and separated. Note the formation of a posterior dorsal cyst (dc). There is also an incomplete separation of the basal ganglia. The T2-weighted coronal image (D) reveals a lack of interhemispheric fissure and a monoventricle (mv). (E, F) MRI of the brain in a patient with lobar holoprosencephaly. The T1-weighted axial image (E) reveals that the two hemispheres are fairly well separated as manifested by the presence of an interhemispheric fissure both anteriorly and posteriorly. Note that the frontal horns of the lateral ventricles are only rudimentary (black arrowheads). The T1-weighted coronal image (F) documents incomplete separation of the inferior frontal lobes near the midline. (G, H) MRI of the brain in a patient with the middle interhemispheric variant of holoprosencephaly. The T1-weighted axial (G) and coronal (H) images demonstrate the continuity of gray matter in the posterior frontal lobes across the midline (arrows). (From Hahn JS, Plawner LL. Evaluation and management of children with holoprosencephaly, *Pediatr Neurol.* 2004;31:79–88.)

incomplete separation of the prosencephalon along one or more of its three major planes (discussed earlier). The DeMyer classification scheme includes “alobar,” “semilobar,” and “lobar” divisions (DeMyer et al., 1964) and a milder middle interhemispheric variant subtype, also referred to as *syntelencephaly*; however, HPE can be difficult to classify in a given patient. In *alobar* HPE (Fig. 59.2), a single anterior ventricle is contained within a holosphere with complete lack of separation of the prosencephalon. Alobar defects may be classified by fetal imaging findings. Depending on the

severity of the defect, other midline structures such as olfactory bulbs/tracts, the corpus callosum, anterior commissure, and the optic nerves may be affected. In addition, midline deep structures such as the basal ganglia, hypothalamus, and thalamic structures are fused, and vascular malformations may also be present (Winter et al., 2015). The milder *semilobar* and *lobar* forms still have distinct hemispheres and the presence of a portion of the posterior corpus callosum. Semilobar forms are suggested if the frontal lobes are more than 50% fused, and the thalami and hypothalamus may also be fused. Lobar HPE is associated with the fissure along almost the entire midline and separation or near separation of the thalami and absence of the CSP. A middle hemispheric variant has been described in which the posterior frontal and parietal lobes fail to separate, with absence of the body of the corpus callosum (Barkovich and Quint, 1993; Lewis et al., 2002). Typically, patients with the middle interhemispheric variant are not identified in the neonatal period unless the variant was diagnosed prenatally. Finally, a septo-preoptic type has been suggested, where the nonseparation is restricted to the septal and preoptic areas (reviewed in Solomon et al., 2013).

Diagnosis

With the advent of prenatal ultrasonography, patients with HPE are usually identified before birth. Alobar HPE may be identified as early as 10 weeks’ gestation and can be reliably identified by 14 weeks. False-positive findings by ultrasonography are common (Kaliaperumal et al., 2016), so fetal MRI can be very useful for clarifying the diagnosis and severity of the malformation, particularly if pregnancy termination is being considered. Subtle findings such as absence of CSP may suggest the milder forms of HPE, although absent CSP can be isolated or associated with ACC (Winter et al., 2015). Given the high rate of chromosome abnormalities, amniocentesis for chromosome array and possibly DNA sequencing is indicated (Solomon et al., 2013).

Clinical Features

Up to 80% of children with HPE have a craniofacial anomaly (reviewed in Solomon et al., 2013). The findings may range from cyclopia (a single central eye) with a nose-like structure (proboscis) above the eye, to cebocephaly (a flattened single nostril situated centrally between the eyes), to median cleft lip. Mild cases may have a single central incisor or hypotelorism. The concept of “the face predicts the brain,” refers to the fact that more severe facial malformations are often associated with more severe brain malformations; for example, patients with alobar HPE can have normal facies, while those with lobar or semilobar HPE can have severe facial malformations (DeMyer et al., 1964; Cohen, 1989; Plawner et al., 2002).

Newborns with HPE present with low tone and microcephaly, unless hydrocephalus occurs (Hahn and Plawner, 2004) due to the blockage of cerebrospinal fluid (CSF) flow through the fused thalami and is often associated with a large dorsal cyst (Simon et al., 2001). Ventriculoperitoneal shunts can relieve symptoms of increased intracranial pressure (Levey et al., 2010). Over time, patients may develop spasticity and dystonia. Oromotor dysfunction is frequent, and many children with HPE require tube feedings.

As with other midline brain defects, endocrinologic abnormalities are very common. Diabetes insipidus occurs in up to 70% of patients, with hypothyroidism, hypoadrenocorticism, and growth hormone deficiency being less common (Hahn et al., 2005). These endocrine abnormalities can develop over time, requiring periodic monitoring. Hypothalamic dysfunction may also cause irregularities of sleep, temperature regulation, appetite, and thirst.

Only about 40% of children have epilepsy, but one-third of these will have intractable epilepsy (Plawner et al., 2002; Levey et al., 2010). Seizures may also be provoked by endocrinologic abnormalities such as hypernatremia or hypoglycemia. Given the range of possible medical issues, a multidisciplinary approach to the care of these children is necessary, as is continued surveillance for potential complications such as hydrocephalus, seizures, and pituitary insufficiency.

Prognosis is related to the severity of the defect, involvement of other organ systems, and the genetic cause (Winter et al., 2015). Fetal or neonatal death is typical for most individuals with chromosome abnormalities (Croen et al., 1996). In contrast, more than 50% of cytogenetically normal patients with all types of HPE are alive at 12 months (Olsen et al., 1997; Barr and Cohen, 1999). Survival into late adolescence and adulthood has been reported (Plawner et al., 2002; Levey et al., 2010).

Epidemiology and Etiology

The live birth prevalence of HPE is 1 in 10,000 to 1 in 20,000 (Croen et al., 1996; Rasmussen et al., 1996; Croen et al., 2000; Orioli and Castilla, 2010). The prevalence is higher in miscarried embryos and fetuses, representing as many as 50 per 10,000 pregnancies.

The cause of HPE is multifactorial, with both genetic and environmental factors appearing to contribute to the variable spectrum of presentations (Norman et al., 1995; Golden, 1998; Muenke and Beachy, 2000). Up to 45% of HPE is caused by chromosomal abnormalities detectable by standard karyotyping (Winter et al., 2015). The most common chromosome abnormalities are trisomies 13 and 18 (Solomon et al., 2010c). Chromosome microarrays can identify smaller copy number variants in 10%–20% of individuals with HPE with normal karyotypes (Bendavid et al.,

2009). Mutations in at least 14 genes have been associated with HPE, accounting for up to 25% of syndromic forms (e.g., Smith–Lemli–Opitz, Meckel, Rubenstein–Taybi, Kallman, and Pallister–Hall syndromes) (Muenke and Beachy, 2000). Six genes have been well established to cause nonsyndromic HPE: *SHH*, *ZIC2*, *SIX3*, *TGIF*, *GLI2*, and *PTCH1*. *SHH* accounts for up to 40% of familial cases of HPE (Roessler et al., 1996; Nanni et al., 1999).

Genotype–Phenotype Variability

HPE is characterized by extreme intrafamilial variability. Asymptomatic or mildly affected family members may carry a deletion for a gene associated with HPE, whereas in a subsequent generation, offspring with the same deletion may be severely affected. Incomplete penetrance can result in “microforms,” including hypotelorism, midface hypoplasia, or a single central incisor (reviewed in Petryk et al., 2015). One explanation for these observations comes from studies suggesting that the mode of inheritance for HPE may be multigenic (Ming and Muenke, 2002). The severity of expression of the HPE phenotype throughout a given family may be influenced by the additive contributions from multiple genetic factors and environmental or teratogenic effects.

Some genotype–phenotype correlations have emerged. Patients with *ZIC2* mutations can have a characteristic facial appearance (bitemporal narrowing, upslanting palpebral fissures, large ears, and a short nose with anteverted nares). *ZIC2* mutations also appear to be the most common de novo mutation and have a high penetrance (Solomon, Gropman, and Muenke, 2013).

Environmental Factors

Environmental factors (e.g., ethanol, vitamin A toxicity) can produce cyclopia during the early phase of gastrulation. Maternal diabetes mellitus increases the risk of HPE to approximately 1%–2% of all pregnancies. Cholesterol-lowering agents have been associated with HPE (Edison and Muenke, 2003), presumably because of effects on *SHH* signaling. Smith–Lemli–Opitz syndrome is caused by a defect in the terminal step of cholesterol biosynthesis, and HPE manifests itself in approximately 5% of affected individuals. Additionally, ingested plant alkaloids have caused epidemics of cyclopia in sheep by processes that inhibit cholesterol biosynthesis. Other environmental associations (cytomegalovirus [CMV] infection, medications, assisted reproductive technologies) have been suggested by case reports or animal studies (reviewed in Petryk et al., 2015).

Agensis of the Corpus Callosum

The corpus callosum consists of approximately 190 million axons and is formed by a complex, multistep process that involves cellular proliferation and migration, axonal growth, and midline patterning (Raybaud, 2010). Formation of the corpus callosum is mediated by many genes, and completion takes at least 11 weeks (O’Driscoll et al., 2010). Development continues through adolescence as these connections are refined (Palmer and Mowat, 2014). Genetic and environmental factors affect callosal formation and can result in complete ACC or hypoplasia. Genetic syndromes associated with ACC also have additional malformations such as cerebellar hypoplasia, microcephaly, and polymicrogyria (PMG) (O’Driscoll et al., 2010).

ACC results in abnormal gyration of the medial portion of each hemisphere, eversion of the cingulate gyri, and sulcation perpendicular to the long axis of the hemisphere (Barkovich and Norman, 1988). The external angles of the lateral ventricle are

oriented parallel and upward, and the fornices are widely separated. If present, a useful distinguishing feature is Probst bundles (Fig. 59.3), which are fiber bundles that run parallel to the ventricle in an anterior-to-posterior direction. ACC can be partial, more often involving loss of posterior segments (Schaefer et al., 1991; Roessmann, 1995). In addition to ACC, additional findings can be present, such as cysts or lipomas (see Fig. 59.3). CNS malformations seen in association with ACC can include heterotopia, sulcation, commissural and white matter abnormalities, and malformations of the posterior fossa (Hetts et al., 2006).

Epidemiology and Etiology

The prevalence of ACC ranges from 0.5 in 10,000 in the general population to 600 in 10,000 in children with neurodevelopmental disability (reviewed in Palmer and Mowat, 2014). ACC can also be associated with prenatal infections, vascular, and teratogen effects (Palmer and Mowat, 2014). However, infection usually causes thinning rather than agenesis and is a rare cause of isolated ACC. Ethanol exposure has the strongest association with ACC, which was reported in 7% of children with fetal alcohol syndrome in one series (Roebuck et al., 1998).

ACC is a feature of hundreds of different disorders, and all modes of inheritance have been observed. Glass et al. (2008) found that callosal anomalies were associated with a chromosomal abnormality 17% of the time, commonly aneuploidy (chromosomes 13, 18, and 21). ACC may be associated with either inherited or de novo copy number variants, some overlapping with those found in patients with autism (Sajan et al., 2013). ACC is also a primary feature of several important disorders, such as Mowat–Wilson, Aicardi, acrocallosal syndromes, *LICAM*-related spastic paraplegia, and X-linked lissencephaly with ACC and ambiguous genitalia. Given the number of genetic syndromes associated with ACC, Palmer et al. (2014) have suggested a subdivision of groups based on the associated conditions (craniofacial, metabolic, ocular, ciliopathies), which aids in the diagnostic evaluation of these patients.

Prenatally, fetal MRI at 20 to 22 weeks' gestation maximizes the identification of additional cerebral findings when pregnancy decision making is still an option. Earlier MRI scans yield more false-negative results and miss structural changes. Additional diagnostic options include genetic (chromosomal microarray, cell-free fetal DNA) and infectious (serologic tests, polymerase chain reaction testing on amniotic fluid) disease testing. Given the challenges with accurate prenatal diagnosis, newborns should be evaluated by brain MRI shortly after birth (when sedation is not required) and by subspecialty consultations (neurology, ophthalmology, genetics, developmental pediatrics, audiology). Laboratory testing (chromosome microarray, DNA sequencing, metabolic testing) should be considered, depending on the examination and MRI findings. Surveillance for neurodevelopmental issues, visual impairment, and pituitary insufficiency is recommended (Palmer and Mowat, 2014).

Prognosis

The outcomes of ACC are variable. A systematic literature review revealed that up to 75% of patients with MRI-confirmed isolated ACC were developing typically at early school age, while 11% had severe disability (Sotiriadis and Makrydimas, 2012). Microcephaly, epilepsy, cerebral palsy, and cerebral dysgenesis are associated with higher risk of abnormal neurodevelopmental outcome. Neuropsychological studies have shown specific impairment in abstract reasoning, problem solving, and category fluency (Fischer et al.,

1992; David et al., 1993). Difficulty with higher-level language such as comprehension of syntax and linguistic pragmatics has also been demonstrated (Banich and Brown, 2000). These findings underscore the need for continued evaluation for learning and language difficulties in these patients.

Septo-Optic Dysplasia

Classic SOD is the triad of the absence of the septum pellucidum, optic nerve hypoplasia, and pituitary dysfunction. The diagnosis of SOD is made when two or more features of the triad are present (Webb and Dattani, 2010); 30% of patients have all three features (Morishima and Aranoff, 1986). SOD is a clinically and etiologically very diverse group of disorders and overlaps with isolated optic nerve hypoplasia (Garcia-Filion and Borchert, 2013), and this diversity makes it difficult to provide counseling about prognosis and associated medical issues.

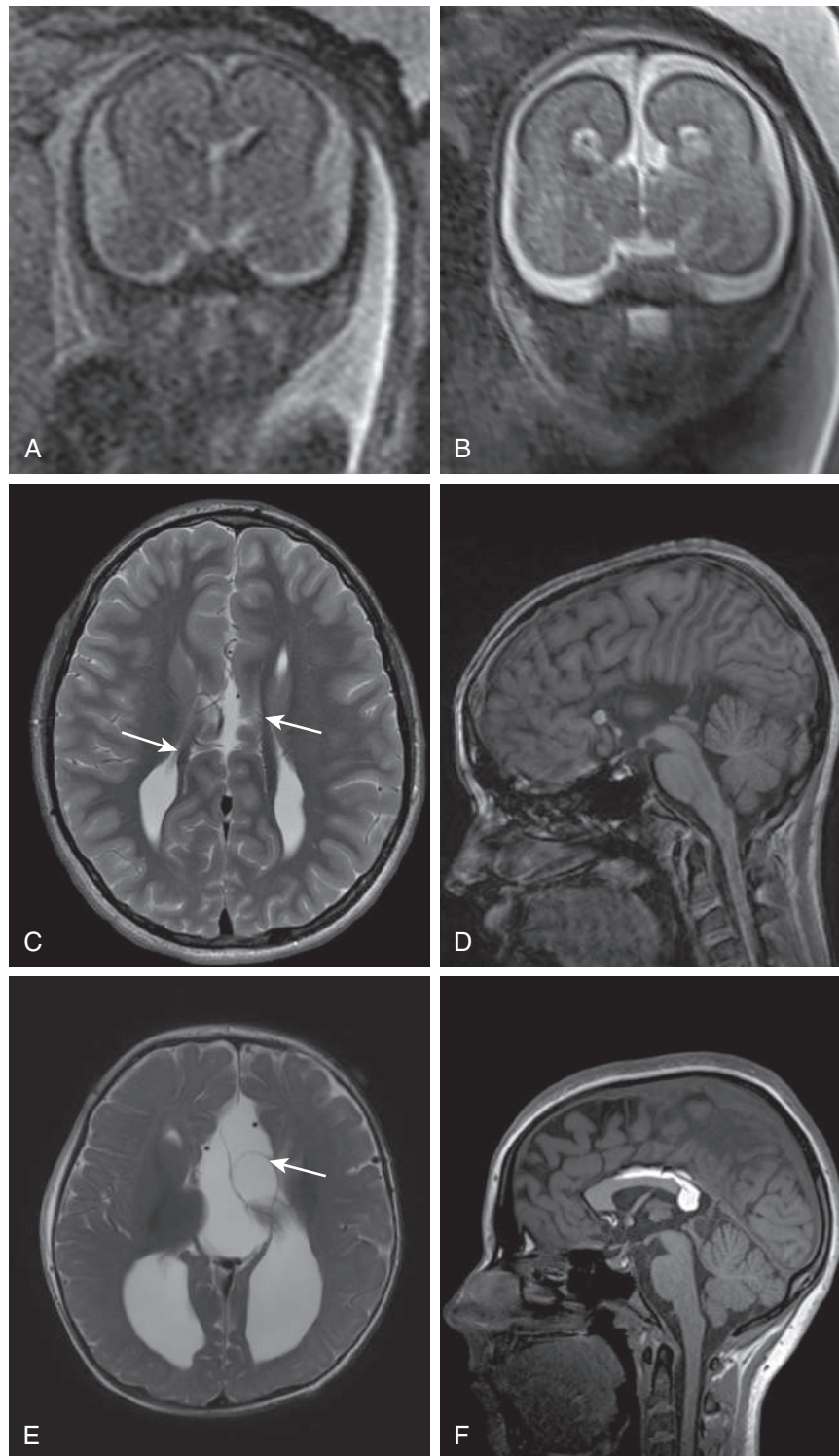
Diagnosis and Prognosis

Prenatally, SOD may be suspected in fetuses with absent CSP or ACC, although most fetuses with isolated absent CSP do not have SOD, posing a challenge for counseling. Postnatally, SOD may be suspected in a patient with growth failure, visual abnormalities, or genital abnormalities. Early diagnosis is important to reduce the risk of adrenal crisis and hypoglycemia and requires brain MRI, ophthalmologic evaluation, and laboratory testing for pituitary insufficiency.

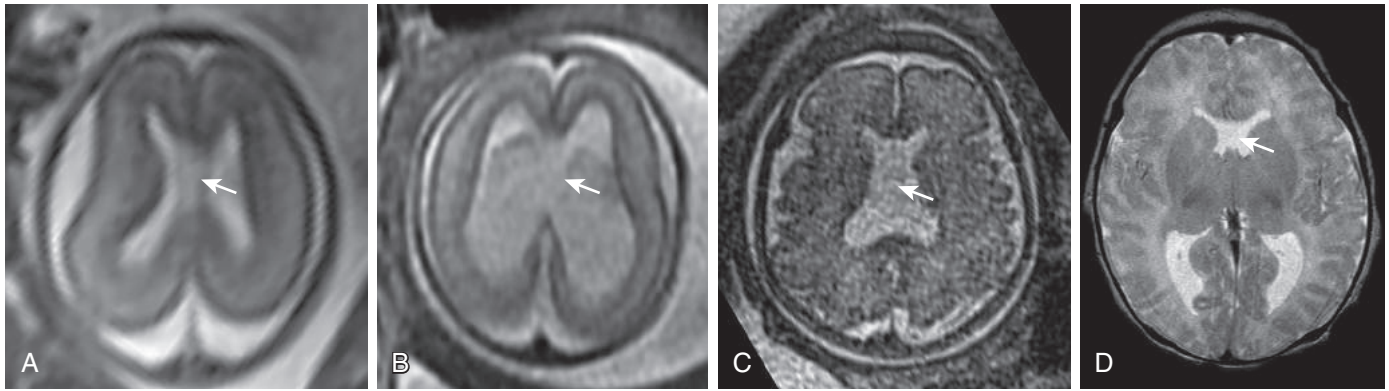
Children with SOD may be blind or have visual impairment, presenting with roving gaze, nystagmus, or strabismus. Neurodevelopmental prognosis is controversial and may be correlated with the presence of other brain abnormalities. In early studies, cerebral palsy was found in 57% of children with SOD, intellectual disability was found in 71%, epilepsy was found in 37%, and behavioral problems were found in 20% (Acers, 1981; Margalith et al., 1984). A later neurodevelopmental study of seven children with unilateral or bilateral optic nerve hypoplasia with the absence of CSP found normal cognitive development, intact neurologic status, normal language development, and age-appropriate behavior in six of the seven children (Williams et al., 1993). Conversely, a descriptive series of three children with SOD plus cortical dysplasia showed that all three had abnormal development and neurologic examination findings (Miller et al., 2000). Developmental, neurology, endocrinology, and ophthalmology specialists should follow up these children. Hypopituitarism is seen in up to 80% of patients, with growth hormone deficiency being most common. Lifelong monitoring is needed, because of evolution of pituitary insufficiency over time (Webb and Dattani, 2010).

Epidemiology and Etiology

The prevalence of SOD is 1 in 10,000 births, with increased risk in younger mothers (Murray et al., 2005). Mutations in several genes have been associated with SOD (e.g., *HESX1*, *SOX2*, *SOX1*, *OTX2*) (Webb and Dattani, 2010; McCabe et al., 2011); however, a genetic origin is not identified in most patients, suggesting environmental or complex genetic causes. The disease is sporadic in most patients, and SOD can exhibit dominant and recessive modes of inheritance. Some instances of SOD have been associated with prenatal drug and alcohol exposure or a vascular pathogenesis (Lippe et al., 1979; Lubinsky, 1997; Stevens and Dobyns, 2004); however, these associations remain unproven. The recurrence risk is less than 1% in the absence of consanguinity.



• **Fig. 59.3** Findings Associated With Agenesis of the Corpus Callosum as Demonstrated by Magnetic Resonance Imaging. (A) Coronal fetal magnetic resonance imaging (MRI) demonstrating the presence of the corpus callosum. Note that the medial cortex curls toward the midline. (B) Coronal fetal MRI demonstrating agenesis of the corpus callosum (ACC). Note that the medial cortex curls away from the midline. (C) Postnatal axial T2-weighted image of ACC demonstrating colpocephaly and classic Probst bundles (arrows). (D) T1-weighted sagittal section demonstrating near complete ACC (rostrum remains). (E) Postnatal axial T2-weighted image demonstrating a multiloculated cystic structures (arrow) that can be seen with ACC. (F) T1-weighted sagittal image demonstrating a lipoma (bright white area) that can be seen with ACC.



• **Fig. 59.4** Fetal and Postnatal Magnetic Resonance Imaging With Absent Cavum Septi Pellucidum. (A) Fetal magnetic resonance imaging (MRI) demonstrating expected cavum septi pellucidum (CSP) at 21 weeks' gestation (arrow) (B-D) MRI demonstrating absent CSP (arrow) at 20 weeks' gestation (B), 34 weeks' gestation (C), and birth (D). The fetus in (B) also has mild ventriculomegaly.

Absent Cavum Septi Pellucidum

CSP is a common finding on ultrasonography and MRI of fetuses and premature infants. This normal finding represents a fluid-filled space between the lamellae of the septi pellucidum, which typically fuse as the fetal brain matures. The absence of the CSP, when normally expected, may be associated with neuroanatomic anomalies (Damaj et al., 2010). It may be an isolated finding (Fig. 59.4), but fetal MRI is recommended for further evaluation (Hosseinzadeh et al., 2013). In addition, a larger than expected CSP width should prompt a detailed ultrasound evaluation as this anomaly can be seen in fetuses with aneuploidy (Abele et al., 2013).

Cortical Defects in Size and Organization

Defects in proliferation and neuronal survival include microcephaly and macrocephaly as well as cortical dysplasia. Migration defects result in disruption of the layered cortical structure, which can be seen grossly (as with lissencephaly) or microscopically (focal dysplasia). We will also discuss cortical defects such as heterotopia, PMG, cobblestone cortex, schizencephaly, and other destructive lesions. We will not discuss disorders of neuronal crest migration (e.g., neuroblastoma, Hirschsprung disease).

Projection neurons originate at the periventricular zone and subventricular zones and undergo symmetric and asymmetric cell divisions to populate the neural progenitor cells (reviewed in Lui et al., 2011). The cerebral cortex develops in an inside-out pattern, with early-born cells forming the deepest layers and later-born cells populating the superficial layers. This process occurs during weeks 7 to 11 of gestation. Stop signals direct appropriate arrest of migration and positioning of the final cell layers. Disruption of all of these processes can result in the malformations we discuss (reviewed in Pang et al., 2008). Most interneurons are born in the median ganglionic eminences and migrate tangentially to populate the brain, and the origin of astrocytes and other cell types is under investigation. Brain malformation disorders primarily affecting these cell types have not been described.

These disorders may be genetic or caused by in utero insults (hypoxia, ischemia, metabolic derangements, toxins, and infection), and diagnosis is based on clinical presentation, imaging, pathology, and genetic testing. The malformations may result in epilepsy and intellectual disability and may be part of syndromic disorders with other organ involvement. Management includes both medical and

surgical interventions for seizure control, therapy support for motor and cognitive disabilities, and appropriate surveillance and involvement of specialists if needed. The framework of cerebral cortex development has been used to classify cortical malformations and is being updated on the basis of expanding genetic knowledge (Barkovich et al., 2012).

Cortical Defects in Proliferation and Neuronal Survival

Microcephaly

Disorders caused by abnormal proliferation include microcephaly (small brain) and megalencephaly (large brain). Microcephaly (occipitofrontal circumference greater than two standard deviations [SDs] below the mean) can be defined as primary (evident at birth) or secondary (normal to small occipitofrontal circumference at birth with progression to greater than two SDs below the mean after birth). The causes can be genetic or acquired and may be associated with other extracranial malformations (syndromic microcephaly) or may be an isolated finding. Von der Hagen et al. (2014) described a retrospective cohort of 680 children with microcephaly and found that 28.5% had genetic causes of various types (aneuploidies, de novo copy number variants, autosomal dominant, autosomal recessive, and X-linked). The remaining cases were secondary to injury around or after birth (which can include birth complications, infection, maternal disease, teratogen exposure, infarction, craniosynostosis), but 40% of the cases did not have an identified cause.

Imaging may suggest a normal brain, but oligogyria, gray matter disruption, and hypomyelination may be present microscopically. Patients with microcephaly can also have other cortical malformations, suggesting an overlap in mechanisms. Genetic defects that cause primary microcephaly affect cell cycle progression, cell proliferation, mitotic spindle formation, and DNA repair. The most common genetic causes (such as *ASPM* mutations) affect centrosome function and cell division (Verloes et al., 2013). Secondary microcephaly can be due to postmigrational microcephaly and is associated with a number of syndromes, including Rett and Angleman, and genes involved in protein synthesis and transfer ribonucleic acid synthetases have been implicated (reviewed in Jamuar and Walsh, 2015). Many causes are recessive, so the recurrence risk is often 25%.

Primary microcephaly can be detected in utero by ultrasonography, and when severe, it is associated with a high risk of abnormal neurodevelopmental outcomes. Additional prenatal evaluation may include fetal MRI, infectious evaluation, and genetic testing. Recently, the Zika virus has been implicated as the causative agent of microcephaly in infants whose mothers were exposed to the virus during pregnancy (Plosa et al., 2012; Mlakar et al., 2016). Diagnosis of secondary microcephaly occurs via routine head measurements after birth, and then further evaluation can include brain imaging (ultrasonography or MRI), infectious disease evaluation, and subspecialty consultation (e.g., neurology, genetics, ophthalmology) as indicated.

Prognosis is related to the severity of microcephaly (Ashwal et al., 2009), as well as the presence of other brain abnormalities, somatic malformations, and genetic diagnosis. Developmental disabilities and imaging abnormalities are seen in approximately 80% of children with severe microcephaly, which is defined as a head circumference greater than three SDs below the mean, while the risk is much lower in children with a head circumference greater than two SDs below the mean. Comorbid conditions include intellectual disability, epilepsy, cerebral palsy, and ophthalmologic disorders.

Macrocephaly and Megalencephaly

Macrocephaly refers to a large head size (greater than two SDs above the mean) that can be due to a variety of causes, such as hydrocephalus, thick skull, or megalencephaly (large brain size). Megalencephaly is rare in neonates since it is usually due to increasing brain size over time, but it is often associated with developmental delay and epilepsy (Winden et al., 2015). Increasing head circumference in infancy needs to be followed closely, and imaging should be considered to evaluate the infant for causes of increased intracranial pressure. In the absence of other malformations or neurodevelopmental issues, accelerated head growth with increased extra-axial fluid during the first year or two after birth is generally thought to be benign and may be due to an imbalance in CSF production versus CSF absorption by immature subarachnoid granulations (Neveling and Truex, 1983; Barlow, 1984).

Macrocephaly can be seen in a diverse group of conditions characterized by a large brain, most commonly manifesting itself as an isolated finding in familial and sporadic cases. At birth, the head circumference is greater than 90%. Macrocephaly can also be seen in fragile X syndrome and Klinefelter syndrome and in neuroendocrine disorders (Beckwith–Wiedemann syndrome, cerebral gigantism, and achondroplasia) (DeMeyer, 1972; Dodge et al., 1983). The prognosis and recurrence risk depend on the cause.

The causes of megalencephaly can be divided into metabolic and anatomic categories. Metabolic causes include organic acidurias (e.g., glutaric aciduria), lysosomal storage diseases, and leukoencephalopathies, while anatomic megalencephaly can be due to mutations in genes affecting the pathways involved in cell growth such as *MTOR*, *Ras*/*MAPK*, and *SHH* (reviewed in Winden et al., 2015). In rare cases, megalencephaly is part of a syndrome called *megalencephaly, polymicrogyria, polydactyly, and hydrocephalus (MPPH) syndrome* (Colombani et al., 2006; Pisano et al., 2008). Megalencephaly–capillary malformation (MCAP) syndrome is also part of megalencephaly-related syndromes. Three genes have been implicated: *AKT3*, *PIK3R2*, and *PIK3CA* (Lee and Gleeson, 2010; Riviere et al., 2012; D’Gama, et al., 2015). This spectrum of disorders (including focal cortical dysplasia, hemimegalencephaly, and megalencephaly) are due in part to pathway mutations in the

PI3K/AKT pathway (Jansen et al., 2015). *PIK3CA* is also associated with other overgrowth disorders, including MCAP, but also including congenital lipomatous overgrowth, vascular malformations, epidermal nevi, and skeletal/spinal abnormalities (CLOVES) syndrome and fibroadipose hyperplasia (Mirzaa et al., 2013).

Hemimegalencephaly results in overgrowth of partial, one hemisphere, or bilateral overgrowth, resulting in cortical dysplasia, abnormalities of the white matter and cell types, and abnormal gyral patterns. This finding has been associated with a number of genetic syndromes and is associated with intellectual disability and intractable epilepsy, with onset usually in infancy (reviewed in Pang et al., 2008). An improved outcome may be achieved in select patients after hemispherectomy as early as the neonatal period (Battaglia et al., 1999). Hemimegalencephaly may also be associated with neurocutaneous syndromes such as sebaceous nevus syndrome, hypomelanosis of Ito, facial lipoma, and tuberous sclerosis (Montagna et al., 1991; Dodge and Dobyns, 1995; Guerra et al., 2007; Leventer, et al., 2008; Gowda et al., 2015). The prognosis and recurrence risk for macrocephaly and megalencephaly depend on the underlying cause.

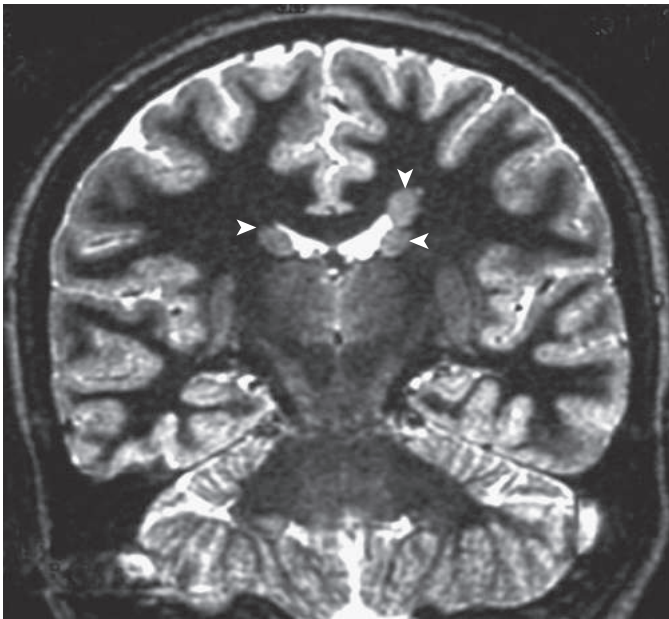
Cortical Defects in Migration

In addition to appropriate proliferation, cortical development requires appropriate neuronal migration. Disruption in the initiation, continuation, or arrest of neuronal migration results in a number of disorders, including heterotopia, lissencephaly, and cobblestone cortex. These abnormalities are often accompanied by other malformations, such as hypoplasia or ACC, pointing to a complex interplay between the mechanisms that determine neuronal migration and axon path finding. Structural imaging has identified many defects in radial migration of projection neurons, while defects in tangential migration of interneurons are not typically identified.

Heterotopia

Heterotopia can either be periventricular (suggesting disruption of initiation of migration), subcortical (as in subcortical band heterotopia), caused by misregulation of migration, or transmantle (as seen with injury). Periventricular heterotopia (PVH) is characterized by masses of gray matter adjacent to the walls of the lateral ventricles (Fig. 59.5). Patients often present with partial seizures. Bilateral PVH may be associated with other malformations (such as hypoplasia of the corpus callosum). *FLNA* mutations cause bilateral contiguous PVH in females or rarely in males if they are hemizygous, as this is usually lethal in males. The involvement of the *FLNA* gene suggests a disruption of cell motility as a mechanism for this malformation (reviewed in Leventer et al., 2008). Among cases of familial PVH, 80% have *FLNA* mutations. Twenty percent of sporadic cases have *FLNA* mutations, indicating the importance of environmental influences, or unidentified genetic causes (Lu and Sheen, 2005). Given associated cardiac problems with *FLNA*-related PVH, echocardiogram and cardiac surveillance are recommended. Neurodevelopmental outcomes are varied and may be associated with learning disabilities (Chen and Walsh, 2015). Mutations in *ARFGEF2* have also been identified in patients with PVH (Bardon-Cancho et al., 2014).

Subcortical band heterotopia is characterized by a band of heterotopic gray matter within the white matter (Fig. 59.6). Somatic mutations in *DCX* and *LIS1* have been associated with subcortical band heterotopia (reviewed in Jamuar and Walsh, 2015). Patients typically present with developmental delay, intellectual disability,



• **Fig. 59.5** Magnetic Resonance Image Scan Demonstrating Periventricular Nodular Heterotopia. Periventricular nodular masses (arrowheads) are visualized adjacent to the lateral ventricles in this T2-weighted coronal image. (Courtesy of Dr. Martin Salinsky, Department of Neurology, Oregon Health and Science University, Portland, OR.)

and/or epilepsy but have less severe disability than those with lissencephaly, and 25% have normal or near normal intelligence. Neurologic deficits roughly correlate with the thickness and extent of the subcortical band (Dobyns et al., 1996).

Lissencephaly

The lissencephalies are characterized by reduced or absent gyri, giving the cortical surface a smooth or nearly smooth appearance (Kato and Dobyns, 2003). Patients have intellectual disability, hypotonia, epilepsy, and feeding difficulties (reviewed in Leventer et al., 2008). The classification of the lissencephalies reflects the rapidly evolving molecular basis of these disorders (Barkovich et al., 2001; Jissendi-Tchofo et al., 2009; see Fig. 59.6). Most patients are included in the classic lissencephaly/subcortical band heterotopia spectrum (previously type 1), which is associated with deletions or missense mutations in *LIS1* (Torres et al., 2004). Other associated genes include *DCX* (Hehr et al., 2011), *YWHAE*, and several tubulin genes. These gene products have roles in cell motility, neurogenesis, and microtubule polymerization (des Portes et al., 1998; Gleeson et al., 1998). Mutations in the *ARX* gene, which encodes a transcription factor, result in another X-linked lissencephaly with abnormal genitalia and are characterized by abnormal basal ganglia, immature white matter, and ACC (Kitamura et al., 2002).

Lissencephaly can also be seen with cerebellar hypoplasia (Barkovich et al., 2001; Ross and Walsh, 2001; Kato and Dobyns, 2003; Lu and Sheen, 2005; Jissendi-Tchofo et al., 2009). The most common cause of this phenotype is dominant mutations in several tubulin genes, which can often be distinguished by characteristic basal ganglia, brainstem, and cerebellar dysplasia (Bahi-Buisson and Cavallin, 2016). Less frequently, lissencephaly with a moderately thick cortex, severe global cerebellar hypoplasia, and a malformed hippocampus is caused by recessive mutations in the

RELN gene, which encodes a large extracellular matrix protein involved in signaling via the apolipoprotein E and very low-density lipoprotein receptors (Hong et al., 2000; Ross and Walsh, 2001). Mutations in *VLDLR* cause a milder phenotype with mildly thickened and simplified cortical gyri and cerebellar hypoplasia (Boycott et al., 2005).

Clinical Features

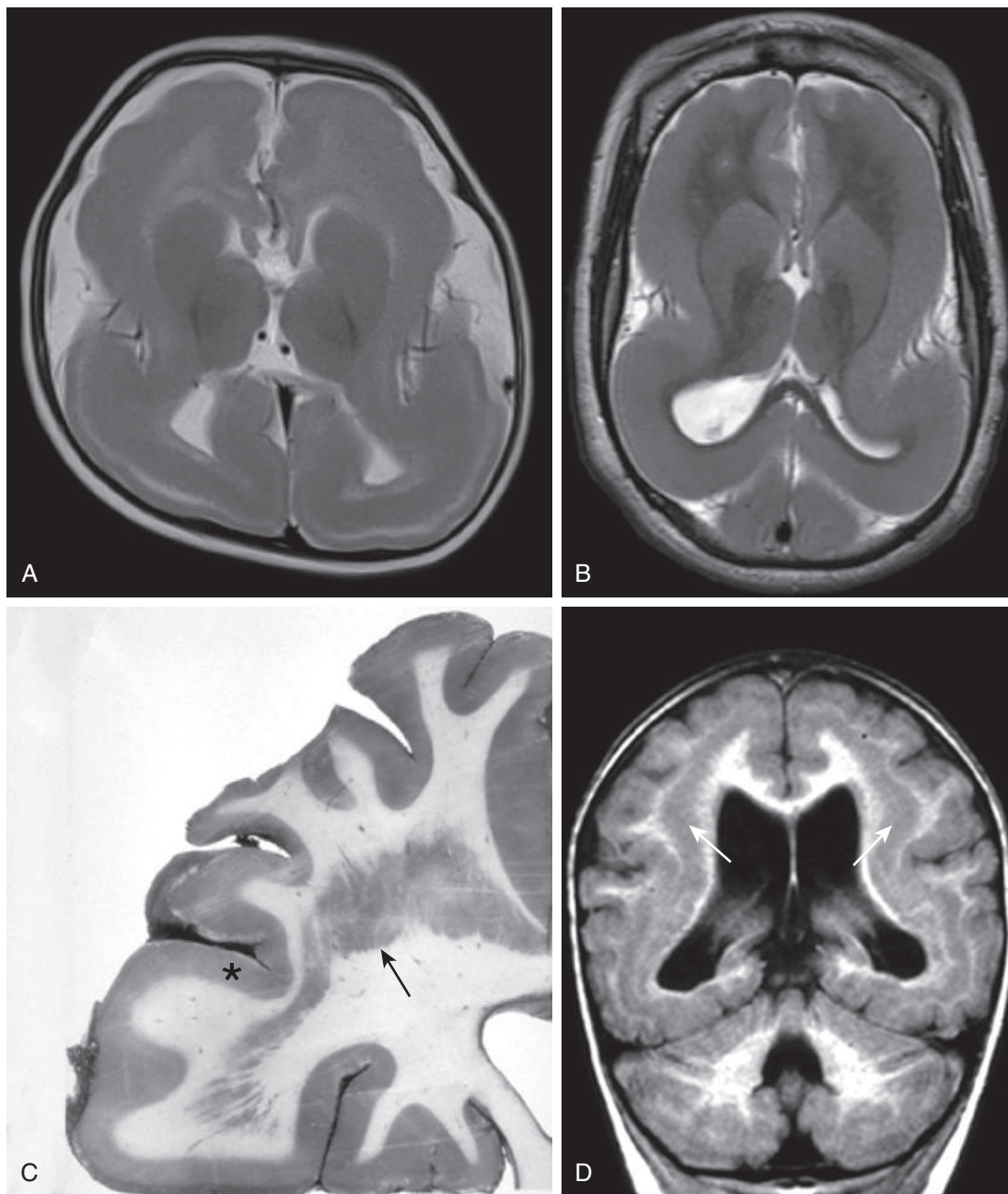
Lissencephaly is diagnosed in utero or patients with lissencephaly present with hypotonia, developmental delay, and/or seizures during infancy. Spasticity may develop later in the first year. Isolated lissencephaly carries a poor long-term prognosis dominated by cognitive disability, spastic quadriplegia, and epilepsy. Craniofacial anomalies can be subtle in patients with *LIS1* mutations or more pronounced as in Miller–Dieker syndrome caused by deletion of *LIS1* and *YWHAE* (Dobyns et al., 1984). Miller–Dieker syndrome is characterized by severe lissencephaly, a high forehead with bitemporal hallowing, short nose, upturned nares, downturned vermian border, and small jaw (Dobyns and Das, 2014). Head circumference is typically normal at birth, but progressive microcephaly occurs during the first year. Neonatal seizures may occur, and severe myoclonic epilepsy typically develops in the latter half of the first year (e.g., infantile spasms or Lennox–Gastaut syndrome) (Guerrini and Carrozzo, 2001).

Cobblestone Complex Syndromes

Cobblestone cortex (previously known as *lissencephaly type II*) is characterized by a nodular appearance of the cortex, which is due to disruption of the normal arrest of cells at the pial surface (reviewed in Pang et al., 2008). On MRI, T2/fluid-attenuated inversion recovery signal is often markedly increased in cortical white matter. Cobblestone cortex is seen in the congenital muscular dystrophies: Walker–Warburg syndrome, muscle–eye–brain disease, and Fukuyama congenital muscular dystrophy (reviewed in Sparks et al., 2012). These autosomal recessive disorders (Cormand et al., 2001) belong to a class of glycosylation-deficient muscular dystrophies, the dystroglycanopathies, that are related to mutations in enzymes that catalyze the posttranslational O-glycosylation of a small number of mammalian glycoproteins (Grewal and Hewitt, 2003; Toda et al., 2005).

Walker–Warburg syndrome is the most severe of the three (Warburg, 1987). Macrocephaly is present at birth or develops in the first year and is due to hydrocephalus. Kinked brainstem, cerebellar hypoplasia, and elevated creatine kinase level are strongly suggestive of the diagnosis. Ocular anomalies include retinal detachment, optic nerve hypoplasia, microphthalmia, and coloboma; muscular weakness is typically severe (Volpe, 2000). Survival beyond the first year is uncommon. The brain malformations and neurodevelopmental outcome in patients with muscle–eye–brain disease are less severe, but patients still have substantial disability and most often experience seizures. Individuals with Fukuyama congenital muscular dystrophy can have variable cortical and eye findings. Hypoplasia, cysts, and PMG can be seen in the cerebellum. Moderate cognitive disability can be seen, and some patients may walk independently (reviewed in Sparks et al., 2012).

Mutations in the laminin subunit β_1 (encoded by *LAMB1*), which localizes to the pial basement membrane, also result in cobblestone cortex and other brain conditions, but these malformations do not exhibit the ocular or muscular abnormalities seen in the syndromes mentioned above (Radmanesh et al., 2013). Similarly, *GPR56* mutations can cause a recessive syndrome with

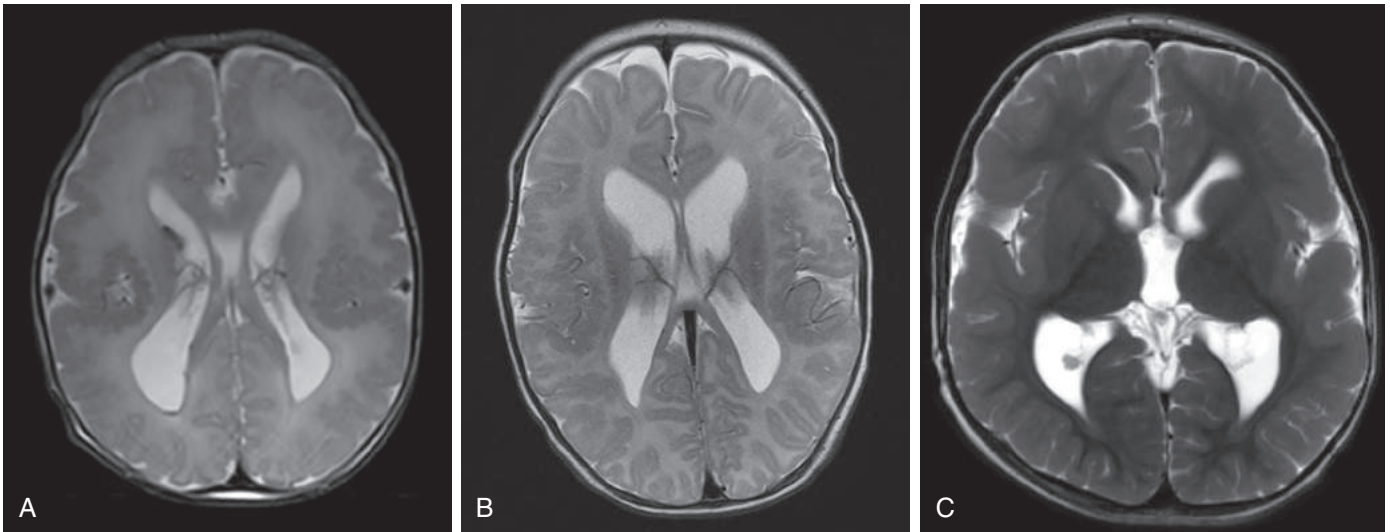


• **Fig. 59.6** Classic Lissencephaly/Subcortical Band Heterotopia Spectrum. (A) Axial T2-weighted magnetic resonance image (MRI) of the brain in a child with *LIS1* mutation. (B) Axial T2-weighted MRI of the brain in a child with a *TUBA1A* mutation. (C) Histologic specimen demonstrating the presence of a broad band of heterotopic gray matter (*arrow*) that is situated within the cerebral white matter and distinct from the cerebral cortex (*asterisk*) in a female patient with a *DCX* mutation. (D) T1-weighted axial MRI demonstrating the circumferential nature of the subcortical band heterotopia (*arrows*) in another female patient with a *DCX* mutation. (Courtesy of Dr. Joseph G. Gleeson, Department of Neurology, School of Medicine, University of California, San Diego, CA; M. Elizabeth Ross, Department of Neurology, University of Minnesota, Minneapolis, MN; Christopher A. Walsh, Department of Neurology, University of Minnesota; and Christopher A. Walsh, Department of Neurology, Harvard Medical School, Boston, MA.)

cobblestone-like cortex, abnormal white matter signal, and cerebellar cysts (Quattrocchi et al., 2013). Bilateral frontoparietal PMG may be associated with mutations in the G protein-coupled receptor 56 (Piao et al., 2002). Neuroimaging shows bilateral white matter changes, small brainstem, and small dysmorphic cerebellum, which is unique for the PMG syndromes (Chang, et al., 2003; Barkovich, 2010).

Polymicrogyria

PMG was originally defined by histopathologic findings. With the advent of brain MRI, the term is now commonly used to refer to a variety of appearances with an irregular gray-white border and many very small gyri or a thickened cortical ribbon (Fig. 59.7). PMG or PMG-like appearances can be caused by mutations in a



• **Fig. 59.7** Spectrum of Findings With Polymicrogyria. T2-weighted axial images (A-C) showing the varied magnetic resonance findings with polymicrogyria: (A) frontal and perisylvian polymicrogyria; (B) predominantly perisylvian; (C) globally thickened cortex.

variety of genes, as well as by infection (CMV, toxoplasmosis), ischemic/vascular anomalies, and metabolic disorders. A variety of other malformations, such as schizencephaly, can be seen in association with PMG. Recently, some ischemic etiologies have been found to be genetic in origin (*OCN* or *COL4A1* mutations, 22q11 deletions, and Sturge–Weber syndrome) (Squier and Jansen, 2014).

Historically, two major types of PMG have been proposed: layered and unlayered. *Layered PMG* has four rather than six distinct cortical layers. This “classic” form is localized adjacent to regions of encephalomalacia and is thought to be frequently caused by injury. *Unlayered PMG* is cortex that lacks distinct cortical layers and is associated with other migrational disturbances, such as subcortical nodular heterotopia, lissencephaly, and schizencephaly. More recently, multiple types of PMG have been demonstrated in the same patient (Judkins et al., 2011), and the term has been used to refer to a variety of appearances on structural MRI without correlation to pathologic findings (see Fig. 59.7). PMG is usually sporadic, but it can be seen in association with several syndromes, especially if it is bilateral, and also has been seen in multiple modes of inheritance in rare familial cases (Barkovich et al., 1999; Ross and Walsh, 2001).

Clinical Features

The clinical presentation relates to the extent of cortical dysplasia and the underlying cause but is difficult to predict on the basis of imaging findings alone. Bilateral PMG or involvement of more than half of a single hemisphere carries a high risk of moderate-to-severe developmental disability and significant motor dysfunction (Barkovich et al., 2005). Hemiparesis or quadriplegia is often seen. Refractory epilepsy with partial complex seizures or multiple generalized seizure types may be delayed in onset beyond the neonatal period.

Bilateral perisylvian PMG is the most commonly observed pattern. Patients with bilateral perisylvian PMG are likely to have epilepsy and intellectual disability (Guerrini et al., 1992; Kuzniecky et al., 1993; Guerreiro et al., 2000). In bilateral frontoparietal PMG, patients have global developmental delay, dysconjugate gaze/

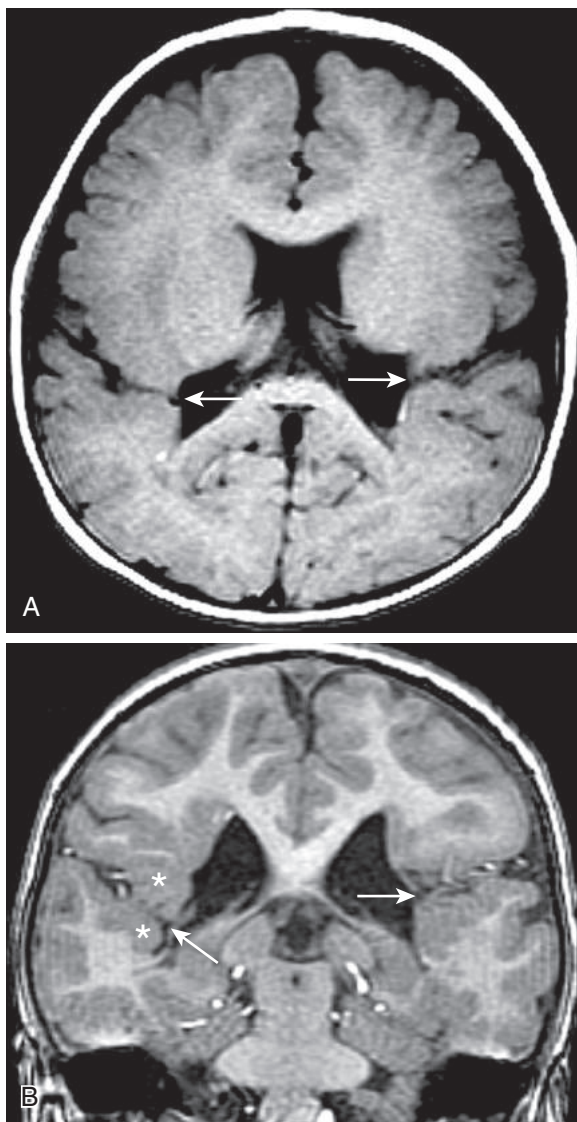
esotropia, and bilateral pyramidal and cerebellar motor signs (Chang et al., 2003).

Tubulinopathy-Related Dysgyria

Mutations in α - and β -tubulin genes have been implicated in a range of cortical malformations (Cushion et al., 2013). Mutations in six tubulin genes have been shown to cause tubulinopathies usually because of de novo dominant mutations, with rare examples of recurrence caused by germline mosaicism (Bahi-Buisson and Cavallin, 2016). A more subtle malformation, called *tubulinopathy-related dysgyria*, shows a subtle irregular cortical gyral pattern with basal ganglia and brainstem dysplasia, associated with *TUBA1A*, *TUBB2B*, and *TUBB3* mutations that occur de novo or are inherited from a mosaic parent (Oegema et al., 2015). Patients can present with motor, cognitive, and language delay that can be mild, abnormal eye movements, behavioral issues, and seizures.

Destructive Lesions

Schizencephaly, porencephaly, and hydranencephaly are malformations that result from destructive lesions during development. Schizencephaly may be unilateral or bilateral and “open-lipped” or “closed-lipped” (Fig. 59.8). When it is severe, both lateral ventricles communicate widely with the extra-axial space (bilateral open-lipped). Unsurprisingly, patients have significant cognitive, motor, and communication difficulties, and epilepsy is common (reviewed in Granata et al., 2005). The severity of the epilepsy is not correlated with the severity of the brain malformation, and two studies found that patients with unilateral schizencephaly had earlier age of seizure onset and more refractory epilepsy than those with bilateral schizencephaly (Granata et al., 1996; Denis et al., 2000). Diagnosis is by MRI, and the findings may include other brain malformations, such as cortical dysplasia, corpus callosum dysgenesis, and absent septum pellucidum. Presumed causes include destructive events such as ischemia and infection. Recurrence within rare families suggests a genetic origin (reviewed in Leventer et al., 2008). Schizencephaly may also result from an extreme failure of



• **Fig. 59.8** Magnetic Resonance Image Scan Demonstrating Bilateral Open-Lip Schizencephaly. (A) Axial T1-weighted image. Note that the bilateral clefts (arrows) extend to the lateral ventricles. (B) Coronal T1-weighted image. Note that the clefts (arrows) are lined with gray matter (asterisks). (Courtesy of Dr. A. James Barkovich, Department of Radiology, School of Medicine, University of California, San Francisco, CA.)

neurons to migrate. This is supported by histology showing that the schizencephalic cleft has features of a migrational disturbance, such as large neuronal heterotopia bordered by adjacent PMG (Bird and Gilles, 1987). Pituitary insufficiency is seen in a substantial subset of patients, particularly if optic nerve hypoplasia is also present.

Porencephaly is a fluid-filled cavity within the cerebral hemispheres, caused by loss of tissue secondary to trauma, infection, or hemorrhage. This can develop prenatally and postnatally. Mutations in *COL4A1* are associated with an autosomal dominant form of hereditary porencephaly. Mutations in *COL4A1* can also cause lesions of the kidneys, eyes, cardiac muscle, or skeletal muscle (reviewed in Meuwissen et al., 2015). *COL4A2* has been associated with familial and sporadic porencephaly. These two genes encode collagen subunits that help form the basement membrane. It is felt that collagen-related porencephaly is secondary to vascular

events. *COL4A1* and *COL4A2* mutations can be associated with stroke and other highly variable manifestations in family members with the same mutation, so the affected infant and at-risk family members should undergo neurologic, ophthalmologic, renal, and cardiac screening.

Hydranencephaly is due to replacement of the hemispheres with a fluid-filled sac and may be considered an extreme form of porencephaly. This rare condition has a number of proposed mechanisms, including infarction, leukomalacia, necrosis, and infection versus thrombotic material from a cotwin. In addition to the etiologies previously mentioned, there may be an association of destructive lesions, such as porencephaly, hydranencephaly, and schizencephaly, with in utero exposure to vasoactive drugs such as cocaine, heroin, and methamphetamine (Eller and Kuller, 1995).

A rare genetic disorder called *Fowler syndrome*, caused by mutations in *FLVCR2*, causes severe hydrocephaly but also hypokinesia, CNS vasculopathy, and arthrogryposis. This disorder is also called *proliferative vasculopathy and hydranencephaly–hydrocephaly (PVHH) syndrome*. Although Kvarnung et al. (2016) described two siblings that survived beyond infancy, most infants do not live beyond the first year (Gentry and Connell, 2013). Hydrocephalus and feeding difficulties are frequent complications, and decisions about whether to treat the infant with CSF diversion or tube feeding can be difficult given the limited developmental potential and life span.

Malformations of Structures in the Posterior Fossa

Normal Midbrain and Hindbrain Development

The brainstem comprises the midbrain, pons, and medulla, while the cerebellum is composed of a vermis and hemispheres. The development of these structures occurs after closure of the neural tube and coincides with prosencephalic development (Ten Donkelaar and Lammens, 2009). The development of the cerebellum, like that of the cortex, is influenced by both intrinsic (genetic) and extrinsic (inductive/environmental) factors (Leto et al., 2016). The midbrain structures derive from the mesencephalon and myelencephalon, and the hindbrain derives from the rhombencephalon by gestational week 4. The cerebellar vermis derives from both the caudal third of the mesencephalon and the hindbrain (Shekdar, 2011). Cerebellar development continues into the second postnatal year, making the cerebellum particularly vulnerable in premature infants. The posterior fossa is evaluated by ultrasonography or MRI during the second and third trimesters of pregnancy, but MRI is the modality of choice if abnormalities are suspected (Bosemani et al., 2015).

For this section, we will characterize posterior fossa malformations on the basis of imaging findings (Doherty et al., 2013). Embryonic and genetic classifications have been used (Barkovich et al., 2007, 2009). Alternatively, posterior fossa malformations can be classified on the basis of inherited (metabolic, structural, neurodegenerative, or the spinocerebellar ataxias) versus acquired causes (vascular, hypoxic-ischemic encephalopathy infection, disrupted development, toxic, neoplastic, or teratogenic) (Klein et al., 2016). Clinical features of posterior fossa abnormalities are nonspecific but include hypotonia, developmental delay, nystagmus, and decreased visual attention. Depending on the malformation, seizures and apnea may also be present, as may evidence of cranial nerve dysfunction. The combination of clinical, imaging, and genetic testing information is required for accurate diagnosis.

Malformations With Major Cerebellar Involvement

Dandy–Walker Malformation

Dandy–Walker malformation (DWM) is typified by aplasia or hypoplasia of the vermis of the cerebellum, cystic dilation of the fourth ventricle, and enlargement of the posterior fossa with upward displacement of the lateral sinuses, tentorium, and torcular (Benda, 1954; D’Agostino et al., 1963; Hart et al., 1972; Friede, 1989; Fig. 59.9). The brainstem may be affected by hypoplasia of the pons (Shekdar, 2011). Diagnosis can occur in utero by ultrasonography and fetal MRI. Prenatal ultrasonography and MRI are increasingly used, but both have continued challenges for the precise anatomic definition of these malformations (Malinge et al., 2009). The enlargement of the posterior fossa and displacement of its contents may be related to communication of the fourth ventricle with a retrocerebellar cyst, often of considerable size. Hydrocephalus

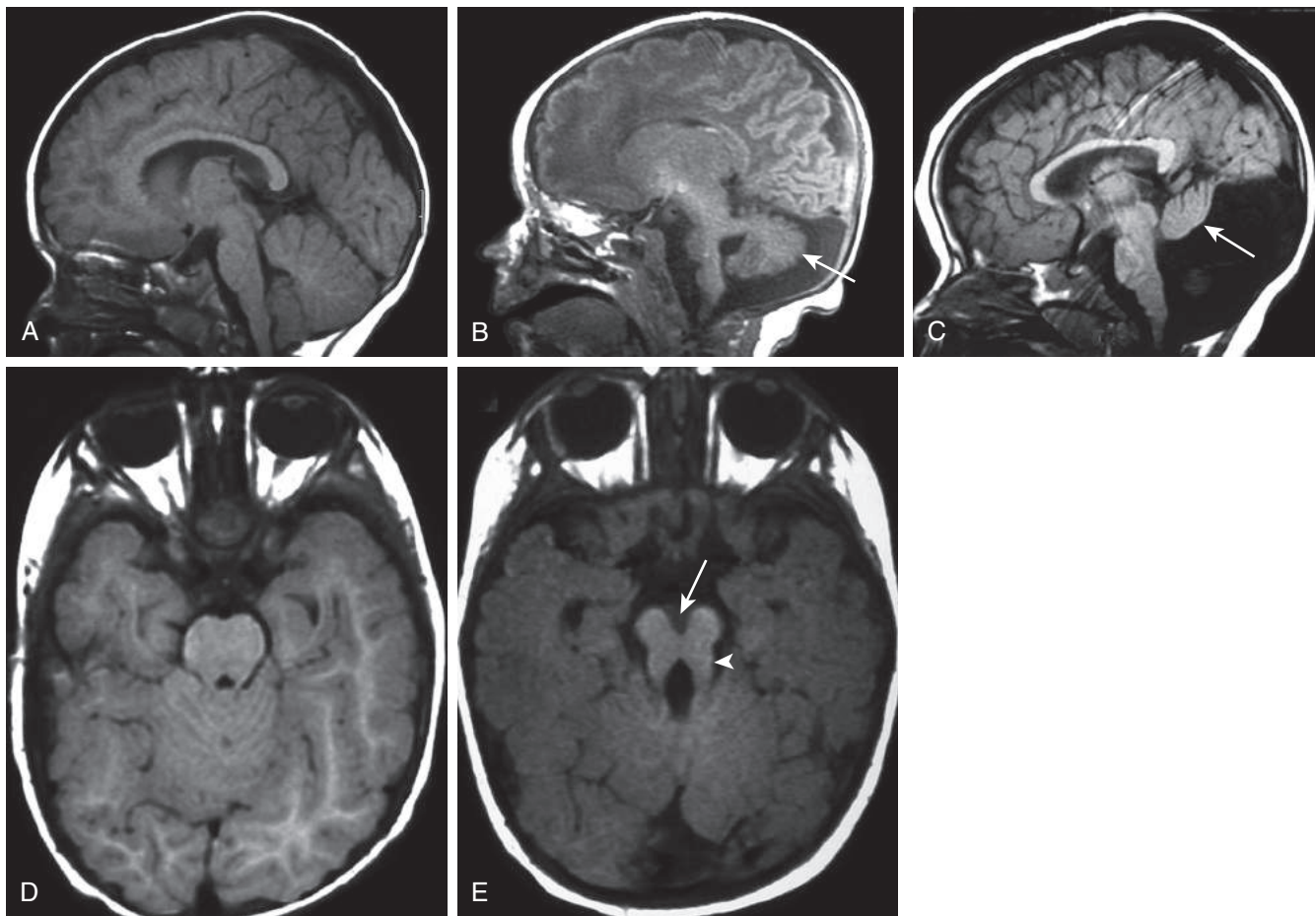
is also a common feature that may present in the neonatal period with macrocephaly (Hart et al., 1972; Costa and Hauw, 1995).

Epidemiology and Etiology

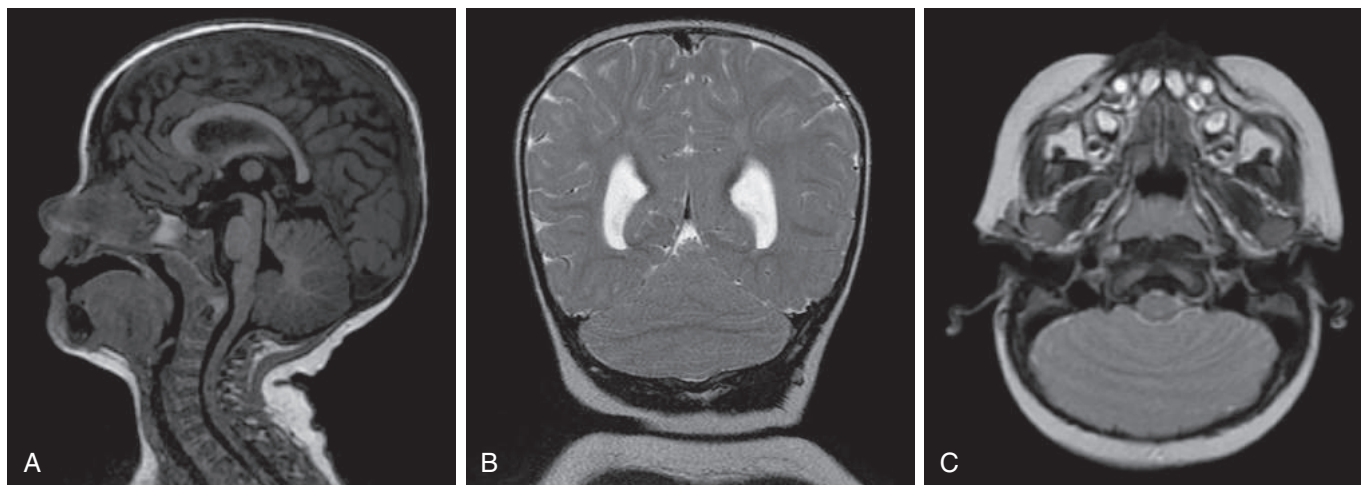
Estimates of the prevalence of DWM are affected by the lack of agreement regarding the definition of DWM. DWM appears to occur in at least 1 in 5000 liveborn infants (Parisi and Dobyns, 2003). DWM may be isolated or associated with a number of syndromes (e.g., Walker–Warburg syndrome, Meckel syndrome) as well as trisomies 9, 13, and 18. Given the low recurrence risk of 1%–3%, DWM is likely due to de novo mutations, environmental insults, and vascular and multifactorial causes. Mutations in *FOXC1* and *ZIC1/ZIC4* have been identified in small numbers of patients, but in general a specific genetic cause cannot be identified.

Clinical Features and Management

DWM can be associated with other brain imaging abnormalities, particularly ACC and hydrocephalus. In addition, cardiac and



• **Fig. 59.9** Posterior Fossa Abnormalities. Sagittal images of a normal brain (A), the brain of a child with Joubert syndrome (B), and the brain of a child with Dandy–Walker malformation (C) illustrate the features of these hindbrain malformations. Note the presence of cerebellar vermis hypoplasia (arrow) in both (B) and (C). By contrast, the patient with Joubert syndrome (B) has only a slightly enlarged fourth ventricle, whereas in the Dandy–Walker malformation (C) the fourth ventricle is massively dilated. (D, E) Molar tooth sign. Comparison of axial images from a normal brain (D) with that of a child with Joubert syndrome (E). Note two key features of the molar tooth sign: a deepened interpeduncular fossa (arrow) and the elongated superior cerebellar peduncles (arrowhead). ([A–C] Courtesy of Dr. Joseph G. Gleeson, Department of Neurology, School of Medicine, University of California, San Diego, CA, and Dr. William B. Dobyns, University of Chicago School of Medicine, Chicago, IL; [D, E] courtesy of Dr. Joseph G. Gleeson, Department of Neurology, School of Medicine, University of California, San Diego, CA.)



• **Fig. 59.10** Rhomboencephalosynapsis as Demonstrated by Magnetic Resonance Imaging. (A) T1-weighted sagittal image showing excessive white matter centrally in the cerebellum and absent primary and horizontal fissures. The T2-weighted coronal (B) and axial (C) show continuous folia across the midline and the smooth dorsal cerebellar surface caused by vermian agenesis and hemisphere fusion.

other organ malformations can be present. Management is similar to that for other neurodevelopmental conditions and can include surgical correction of hydrocephalus with shunt placement within the lateral ventricles and/or posterior fossa. The outcome is broad, with higher risk of abnormal neurodevelopmental outcome in patients with additional malformations and specific genetic conditions.

Rhomboencephalosynapsis

Rhomboencephalosynapsis (RES) is unique among the cerebellar vermis hypoplasias. Instead of the cerebellar hemispheres being widely separated, the hemispheres, white matter, and deep cerebellar nuclei are variably fused (Paprocka et al., 2012; Fig. 59.10). Clinically, patients with RES may present with ataxia, muscle hypotonia, abnormal eye movements, dysarthria, head shaking, and developmental delay with variable severity. RES may be isolated or seen in combination with other syndromes such as vertebral, anal, cardiac, tracheoesophageal, renal, and limb anomalies (VACTERL) and may be associated with other CNS abnormalities, such as hydrocephalus (Ishak et al., 2012; Tully et al., 2012; Poretti and Boltshauser, 2015). No definitive causes have been identified, but proposed causes include genetic defects and environmental factors (such as maternal diabetes and other exposures) (Paprocka et al., 2012).

RES can be diagnosed prenatally by fetal MRI and should be suspected in fetuses with ventriculomegaly and cerebellar hypoplasia identified by ultrasonography. In the absence of ventriculomegaly or other imaging abnormalities, RES is rarely diagnosed prenatally. In addition, when aqueductal stenosis is suspected prenatally or postnatally, the cerebellum should be closely scrutinized for RES (Ishak et al., 2012).

Malformations With Both Cerebellar and Brainstem Involvement

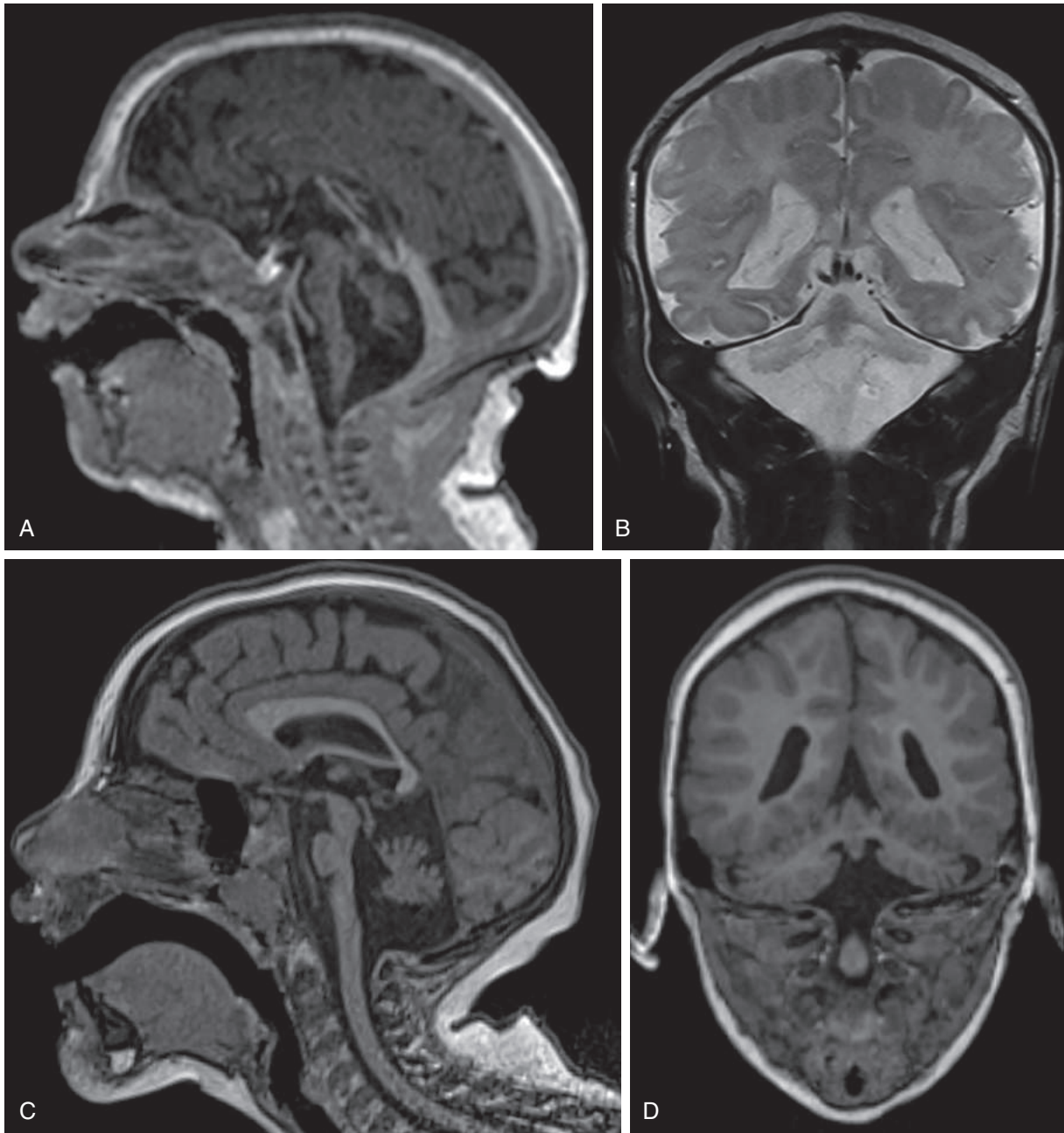
Joubert Syndrome and Related Disorders

Joubert syndrome (JS) is an autosomal recessive disorder usually presenting with hypotonia, abnormal eye movements, and

developmental delays. The prevalence has been estimated to be 1 in 100,000. Apnea and tachypnea may be notable in infancy (Romani et al., 2013), and early presentation may include head titubation (Poretti et al., 2014), which is a subtle nodding of the head seen in JS and other posterior fossa malformations. JS is related to a number of other disorders that can be defined by the “molar tooth sign” on imaging (see Fig. 59.9): cerebellar vermis hypoplasia/aplasia, deep interpeduncular fossa, and thick, elongated superior cerebellar peduncles. Disorders with the molar tooth sign on imaging have associated gene products related to ciliopathies, suggesting a common pathway. Historically, several other names have been used to refer to JS, including cerebellar ocular renal syndrome, cerebellar hypoplasia/aplasia, oligophrenia, cerebellar vermis hypoplasia/aplasia, oligophrenia, congenital ataxia, ocular coloboma, and hepatic fibrosis (COACH) syndrome, and Varadi–Papp syndrome (Sattar and Gleeson, 2011). Mutations in more than 30 genes have been identified in more than 50% of patients with JS (Parisi and Glass, 2013), with five genes accounting for approximately 40% of patients (*AHI1*, *CC2D2A*, *CEP290*, *TMEM67*, and *C5ORF42*) (Bachmann-Gagescu et al., 2015). Distinguishing JS from other hindbrain malformations is essential, since the recurrence risk is 25%, and substantial subsets of patients develop progressive retinal dystrophy, fibrocystic kidney disease, and liver fibrosis, requiring lifelong monitoring and treatment. Some genetic causes are associated with higher risk of complications (e.g., loss of *CEP290* is associated with retinal dystrophy), therefore genetic testing can be used to guide monitoring (Bachmann-Gagescu et al., 2015).

Pontocerebellar Hypoplasia

Pontocerebellar hypoplasia (PCH) is a diverse group of largely autosomal recessive disorders characterized by a variable degree of pons and cerebellar hypoplasia (Fig. 59.11). Ten overlapping subtypes have been proposed, many presenting in the neonatal period (reviewed in Klein et al., 2016). PCH can occur in association with congenital disorders of glycosylation, mitochondrial diseases, and congenital muscular dystrophies. Multiple genetic causes have been identified, most commonly mutations in *TSEN54*, but up to 40% of patients do not have an identified cause (Dyment et al.,



• **Fig. 59.11 Pontocerebellar Hypoplasia.** (A) T1-weighted sagittal and (B) T2-weighted coronal images of a patient with *TSEN45*-related pontocerebellar hypoplasia (PCH). *TSEN54* mutations have been identified in PCH types 1, 2, 4, and 5 and can be associated with additional malformations, including atrophic cortex and cerebellar hemisphere hypoplasia more than vermin hypoplasia, cerebellar hemisphere more than vermin hypoplasia. (C) T1-weighted sagittal and (D) T1-weighted coronal images of a patient with *CASK*-related PCH. *CASK*-related PCH may be associated with mild small pons and cerebellum, with proportionate hypoplasia of the cerebellar vermis and hemispheres.

2013). Given the phenotypic and genetic overlap in these subtypes, use of a combination of genetic, clinical, and imaging findings is more specific in describing these disorders (Table 59.1; Aldinger and Doherty, 2016). In patients with *TSEN*-related PCH, the vermis is often relatively preserved compared with the hemispheres, in contrast to most other PCH disorders. Outcome data are sparse and depend somewhat on the type of PCH, but typically patients have significant developmental delays, tone abnormalities, feeding issues, and respiratory compromise (Klein et al., 2016).

Malformations With Brainstem Involvement

Moebius Sequence Disorders

A number of disorders fall under the congenital cranial dysinnervation disorders, which are sporadic or familial neuromuscular disorders involving abnormal eye, eyelid, or facial movement caused by primary or secondary dysinnervation of cranial nerves. Moebius sequence represents a group of disorders with unilateral or bilateral

TABLE 59.1 Pontocerebellar Hypoplasia*

| Pontocerebellar Hypoplasia | Clinical Findings | Radiologic Findings | Associated Genes (Major Listed First) |
|----------------------------|---|--|--|
| Type 1 | Spinal muscular atrophy | Mild hypoplastic pons, with proportional hypoplasia of the vermis and hemispheres | <i>EXOSC3</i> , <i>RARS2</i> , <i>TSEN54</i> , <i>VRK1</i> |
| Type 2 | Neonatal encephalopathy, progressive microcephaly, increased tone, dyskinesia, seizures, cortical visual impairment | Postmigrational microcephaly, small pons and cerebellum, atrophic cortex with thin corpus callosum, vermis less affected than hemispheres | <i>TSEN54</i> , <i>TSEN2</i> , <i>TSEN34</i> |
| Type 3 | Hypotonia, hyperreflexia, infantile seizures, developmental delay | Small pons and cerebellum, reduced amount of cerebral white matter | <i>PCLO</i> |
| Types 4 and 5 | Severe type 2 | As type 2 | <i>TSEN54</i> , <i>TSEN2</i> , <i>TSEN34</i> |
| Type 6 | Elevated CSF lactate level | Small pons and cerebellum, vermis affected more severely than hemispheres | <i>RARS2</i> |
| Type 8 | Acquired microcephaly, increased tone and contractures, moderate to severe developmental delay | Small pons and cerebellum with proportionate hypoplasia of the vermis and hemispheres | <i>CHMP1A</i> |
| Type 9 | Progressive microcephaly, seizure onset in infancy, hypertonia, and visual impairment. Severe developmental delay | Small cerebellum with atrophy, pons with ventral flattening, mega cisterna magna and brainstem with a figure-of-eight appearance, atrophy of cerebral cortex, and corpus callosum hypoplasia | <i>AMPD2</i> |
| Type 10 | Progressive microcephaly and neurodegeneration by 6 months, absent or delayed speech, progressive spasticity, spontaneous seizures, severe developmental impairment | Small cerebellum, pons, and corpus callosum with atrophy | <i>CLP1</i> |

*All with autosomal recessive inheritance.
 CSF, Cerebrospinal fluid.
 Modified from Aldinger KA, Doherty D. The genetics of cerebellar malformations. *Semin Fetal Neonatal Med.* 2016;21(5):321–332.

facial weakness, loss of abduction of the eye, and other cranial nerve dysfunction and may also include orofacial malformations, limb anomalies, and chest wall anomalies (Briegel, 2006; Oystreck et al., 2011; Gutowski and Chilton, 2015).

Moebius sequence disorders have a prevalence of 2 in 10,000 to 20 in 10,000 (reviewed in Briegel, 2006). Moebius sequence may be secondary to an ischemic event affecting the tegmental watershed zone (Sarnat, 2004). Other vascular causes may include disruption of primitive subclavian arteries before establishment of the blood supply to the brainstem. This may be represented by calcifications in the brainstem (Briegel, 2006).

A number of teratogens have been implicated in Moebius sequence, including hyperthermia, electric shock, alcohol, drugs (benzodiazepines, alcohol, and cocaine), and medications such as misoprostol (da Silva Dal Pizzol et al., 2006). A number of genes have been hypothesized to play a role, including *MBS1*, *MBS2*, and *MBS3*. Infants may have feeding difficulties, requiring special bottles or tube feeding. Given the association with Kallman syndrome, evaluation of the heart and renal system is needed. Management includes therapies for feeding, speech, and other issues, as well as surgical remediation of nerve and muscle abnormalities. Challenges for older children include speech, language, and intellectual disabilities and autism (Briegel, 2006).

HOXA1A Mutation Syndromes

Mutations in *HOXA1*, which encodes a transcription factor, have been found to cause two related syndromes: Bosley–Salih–Alorainy syndrome in a Saudi Arabian family (and a Turkish individual) and Athabascan brainstem dysgenesis syndrome in an Athabascan Native American population (Erickson, 1999). Bosley–Salih–Alorainy syndrome is typified by bilateral Duane retraction syndrome type 3, deafness, cerebral vascular malformations, and autism, while Athabascan brainstem dysgenesis syndrome presents with horizontal gaze restriction, deafness, intellectual disability, weakness, central hypoventilation, cerebral vascular malformations, and cardiac malformations. Not all patients have horizontal gaze restriction or hearing difficulties. Bilateral Duane retraction syndrome or heart anomalies may be the sole representation of *HOXA1* mutations, and other clinical manifestations may cooccur (such as deafness, autism, and cerebrovascular abnormalities) (Bosley et al., 2008).

Chiari Malformations

Historically, three numbered types of Chiari malformations have been defined, but they are not etiologically related.

Chiari type I malformation is characterized by inferior ectopia of the cerebellar tonsils into the spinal canal, often with compression

of the tonsils and restricted CSF flow. It is uncommonly diagnosed in neonates. Diagnosis is based on clinical and imaging findings; patients may have headache and lower cranial nerve, cerebellar, and brainstem dysfunction. Herniation of the tonsils greater than 5 to 10 mm in the presence of symptoms is diagnostic, but similar herniation can be seen in the absence of symptoms. Other findings include syringomyelia and scoliosis (Cotes et al., 2015). Mild inferior tonsillar ectopia without compression of the tonsils or restricted CSF flow is common and is not usually associated with symptoms. Chiari type I malformation may be either congenital or acquired: the congenital form presents in children and young adults, while the acquired form may have a later onset. In acquired Chiari malformations the posterior fossa size is normal.

Chiari type II malformation is seen almost exclusively in association with open neural tube defects (NTDs) and is defined by displacement of the cerebellar vermis, medulla, and fourth ventricle into the spinal canal and is associated with a small posterior fossa and frequently hydrocephalus. MRI can be used to confirm these findings and often demonstrates cerebellar hypoplasia and ACC, although these features have not been associated with a higher risk of neurodevelopmental issues. Rarely, migration defects, HPE, and interhemispheric cysts can also be seen (Cotes et al., 2015).

The term *Chiari type III malformation* has been used to refer to a variety of hindbrain malformations that include occipital encephalocele and often kinking of the brainstem or cervical spinal cord. In the absence of good diagnostic criteria and associated outcome data, we recommend referring to specific features in each patient. The medical literature on outcomes is extremely limited, but several reported patients have had limited life span and substantial disability.

Neural Tube Defects and Spinal Cord Dysraphisms

Open NTDs result from a failure of primary neural tube closure during the fourth week of gestation (Roessmann, 1995). Anencephaly results from failure of anterior neuropore closure, while myeloschisis and myelomeningocele (MMC) result from failure of posterior neuropore closure. Craniorachischisis totalis occurs with complete failure of neural tube closure and typically results in spontaneous abortion during embryogenesis or early fetal development. The remainder of the NTDs to be discussed result in malformations of the CNS and the overlying axial skeleton, meninges, and skin that are associated with various degrees of viability in the newborn period.

Epidemiology and Etiology

NTDs remain one of the most common congenital malformations encountered in newborns, with 0.5 to 2 in 1000 pregnancies affected worldwide (Mitchell, 2005; Au et al., 2010). The prevalence differs widely and is particularly influenced by ethnicity, geographic area, and socioeconomic status (Frey and Hauser, 2003). In the United States, for example, the risk of NTDs is higher in people of Hispanic descent but lower in people of African-American descent. After the first affected pregnancy, the risk of recurrence increases at a disproportionately higher rate with each subsequent pregnancy. The risk may nearly triple after each subsequent pregnancy (Elwood et al., 1992).

A marked decline in birth prevalence of both anencephaly and spina bifida has occurred in recent decades. In 1960 the prevalence

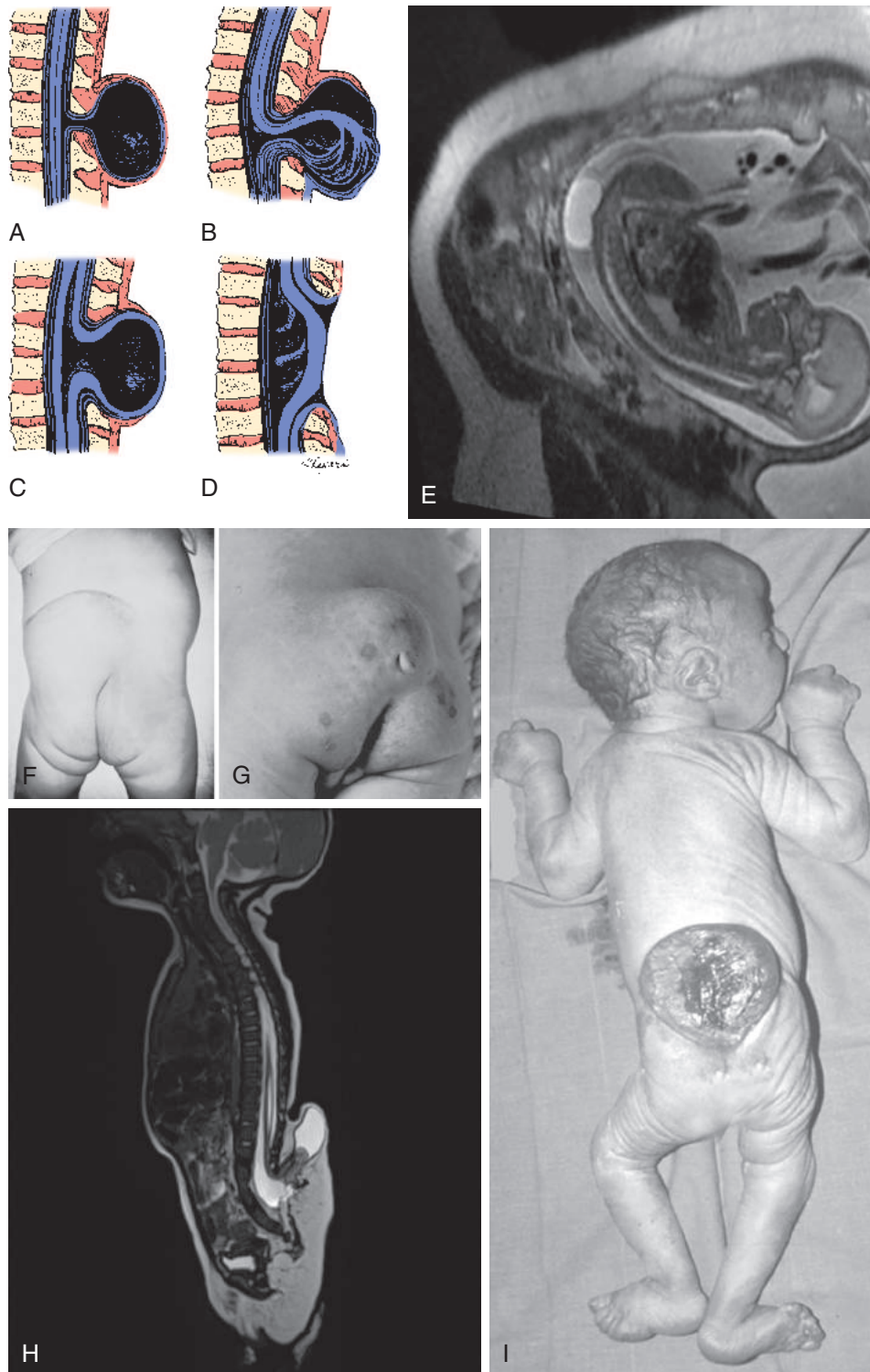
for England and Wales was about 6 in 1000 births; in 1990 this rate dropped to about 1 in 1000. The lower rates reflect two major interventions: in utero diagnosis with termination of affected pregnancies and maternal periconceptional folate therapy. Maternal folate therapy is estimated to prevent approximately 60%–70% of NTDs (Smithells et al., 1981; Czeizel and Dudas, 1992; MRC Vitamin Study Research Group, 1991; Oakley et al., 1994; Castilla et al., 2003; Frey and Hauser, 2003; Mills and Signore, 2004; Zohn and Sarkar, 2008). Approximately 200 genes are known to be required for neurulation, many of which are involved in folic acid metabolism or transport (Harris and Juriloff, 2007). Yet, up to one-third of NTDs are not prevented by folic acid therapy, so a combination of interventions may be required to optimally prevent NTDs (Greene and Copp, 2014), and other factors need to be considered. For example, inositol deficiency can also trigger NTDs in rodent models, and inositol supplementation prevents a significant proportion of these defects (reviewed in Greene et al., 2017). Inositol therapy may reduce the risk of developing gestational diabetes, may improve the metabolic state of the mother, and has not been associated with complications (Noventa et al., 2016). Yet, only folate supplementation is currently recommended for women of childbearing age in the United States (U.S. Preventive Services Task Force, 2009). Most countries now recommend a healthy diet and folate supplementation before conception through the 12th week of pregnancy, with additional supplementation based on risk (Gomes et al., 2015).

Gene–Environment Association of Neural Tube Defects

The etiology of NTDs is complex and multifactorial, and both genetic and environmental factors interact to determine risk; most defects occur sporadically (Blom 2009; Au et al., 2010). Environmental risk factors linked to NTDs include maternal hyperthermia, hyperglycemia, lower socioeconomic status, dietary factors, and prenatal exposure to a number of drugs, including antiepileptic medications such as valproate and carbamazepine (Jones et al., 1989; Frey and Hauser, 2003; Yerby, 2003; Blom, 2009; Au et al., 2010; Wilde et al., 2014). In addition, the fungal toxin fumonisin has been associated with NTDs. A fourfold to fivefold higher prevalence of NTDs occurred in a Mexican-American population because of fumonisins in corn flour used to make tortillas (Suarez et al., 2012; Wilde et al., 2014). NTDs have been associated with genes implicated in diabetes mellitus, obesity, and glucose and oxidative stress. Also, epigenetic mechanisms (such as histone modification, methylation, and nucleosome remodeling) may play a role in neurulation (Wilde et al., 2014).

Fetal Diagnosis of Neural Tube Defects

Diagnosis of open NTDs may be suspected in the case of maternal serum elevation of alpha fetoprotein (AFP) level and can be confirmed by fetal ultrasonography or MRI (Fig. 59.12; Cameron and Moran, 2009). AFP screening is designed to detect open NTDs and Down syndrome (Anderson and Brown, 2009). The optimal time for determination in maternal serum is at 16 to 18 weeks' gestation and in amniotic fluid is at 14 to 16 weeks' gestation. In open NTDs (anencephaly, open spina bifida, and open encephalocele), fetal AFP leaks directly into the amniotic fluid, indirectly increasing maternal serum protein levels. By contrast, skin-covered NTDs are not associated with elevated maternal serum AFP level (Milunsky et al., 1980). A false-positive AFP result can



• **Fig. 59.12 Neural Tube Defects.** (A) Meningocele. Through the bony defect (spina bifida), the meninges herniate and form a cystic sac filled with spinal fluid. The spinal cord does not participate in the herniation and may or may not be abnormal. (B) Myelomeningocele. Spina bifida with myelomeningocele; the spinal cord is herniated into the sac and ends there or may continue abnormally further downward. (C) Myelocystocele or syringomyelocele. The spinal cord shows hydromyelia; the posterior wall of the spinal cord is attached to the ectoderm and undifferentiated. (D) Myelocele. The spinal cord is araphic; a cystic cavity is in front of the anterior wall of the spinal cord. (E) Fetal magnetic resonance image of lumbosacral dysraphism. Note the ventriculomegaly and crowded posterior fossa. (F–H) Three examples of skin-covered neural tube defects. (F, G) Lesion presented in the left buttock as a firm, well-circumscribed, lobulated tumor that became tense when the infant cried. (G) Macular erosions and a congenital skin tag and dimple over the surface. This last feature may be a pilonidal dimple displaced by the tumor. (H) T2-weighted image of a lipomyelomeningocele. (I) Newborn with a large thoracolumbar myelomeningocele. The distal musculature in the lower extremities was weak. ([A–D] From Benda CE. *Developmental Disorders of Mentation and Cerebral Palsies*. New York, NY: Grune and Stratton; 1952; [F–H] courtesy of Dr. Marjorie Grafe, Department of Pathology, Oregon Health and Science University, Portland, OR.)

occur with misdating of the fetus (if older than predicted) and a multiple-gestation pregnancy (Brock, 1976). Other causes of a high maternal serum AFP level include contamination of the amniotic fluid by fetal blood (which may occur in cases of esophageal and duodenal atresia), omphalocele, gastroschisis, congenital nephrosis, polycystic kidneys, renal agenesis, annular pancreas, or fetal demise.

Ultrasonography can be used as early as the end of the first trimester for evaluation of the radiographic signs of NTDs, but more typically the diagnosis is made during the second trimester. Abnormal head and cerebellar shape (the “lemon” and “banana” signs) are more sensitive ultrasound indicators for NTD diagnosis than spinal imaging findings. Historically, fetal MRI has not been superior to ultrasonography for identifying the spinal cord defect; however, for those patients electing to have in utero surgery, MRI is used for surgical planning.

Open Neural Tube Defects

Typically, NTDs are characterized as either open lesions (craniorachischisis, anencephaly, MMC, or myelochisis) or closed lesions (see later).

Anencephaly

Anencephaly results after failed anterior closure where exencephaly converts to anencephaly by degradation of the neural tissue (Copp et al., 2013). This is the severest disorder of anterior neural tube closure. Anencephaly accounts for roughly half of all open NTDs (Au et al., 2010). As an early neurulation defect, it occurs no later than 24 days' gestation. Anencephaly can be diagnosed by fetal ultrasound examination (Fig. 59.13) during the first or second trimester (Goldstein and Filly, 1988; Crane, 1992); it is frequently associated with polyhydramnios (Nichols and Schrepfer, 1966).

Anencephaly most commonly involves the forebrain and upper brainstem. It is characterized by absence of the calvaria, and the intracranial contents are replaced by vascularized, disorganized glial tissue (area cerebrovasculosa) (Menkes, 1991; Roessmann, 1995). The hypothalamus and cerebellum are usually malformed,

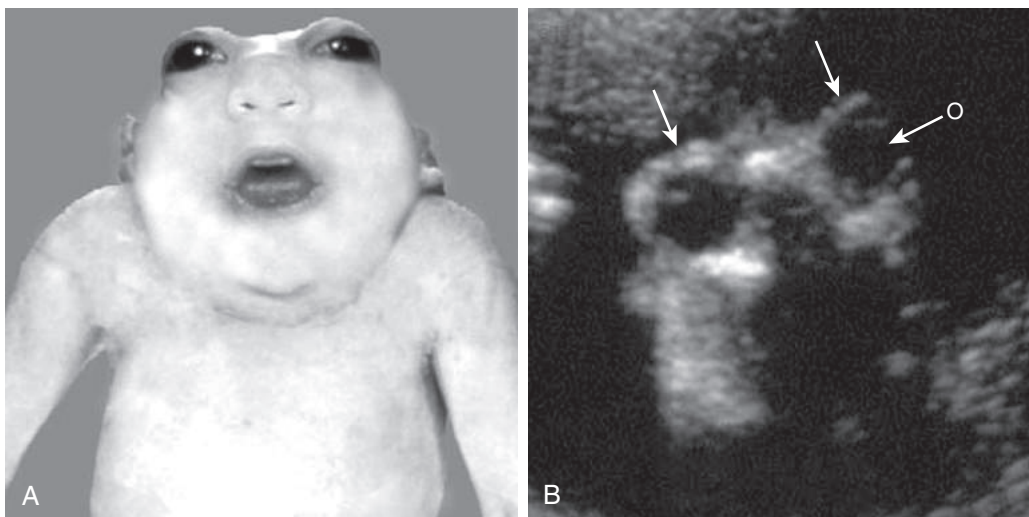
the anterior lobe of the pituitary is present, and the internal carotid arteries are hypoplastic, which may be secondary to abnormal brain formation. Because the anencephalic infant has a period of exencephaly, where brain tissue extrudes through the unformed calvaria and is degraded by exposure to the amniotic fluid, some investigators have hypothesized that the primary defect is the abnormal skull formation. There are some cases in which remnants of calvarial bones are present, with brain tissue under the protective bones (Roessmann, 1995).

Most neonates are stillborn, and the physical examination is notable for a lack of the brain and cranial vault (Copp et al., 2013). Liveborn neonates live less than 1 or 2 months at the most (Baird and Sadovnick, 1984; Peabody et al., 1989). Without intensive care, survival has not been reported beyond the neonatal period. In a series of 211 women who reported choosing to deliver fetuses with an in utero diagnosis of anencephaly, 72% were liveborn, approximately 67% of the liveborn infants died within 24 hours, and six infants lived for 6 days or more (maximum of 28 days). This study suggested that continuation of pregnancy is a preferred option for some women after they have been counseled about the expected outcomes (Jaquier et al., 2006).

Myelomeningocele

MMC and other associated malformations of the spinal cord arise from failure of posterior neuropore closure, probably no later than day 26 of gestation. Open spinal NTDs are exposed lesions on the back without vertebral or dermal covering (see Fig. 59.12; Menkes, 1991). The spectrum of these spinal cord malformations includes MMC and meningocele. MMC is characterized by herniation of the meninges and spinal cord at the site of the defect. MMCs are about four times more common than meningoceles (skin-covered lesion) (Friede, 1989; Menkes, 1991) and most often occur in the lumbar or lumbosacral regions.

The diagnosis is usually made prenatally by ultrasonography and/or increased AFP level (reviewed in Copp et al., 2013). MMC is usually accompanied by other clinically significant CNS abnormalities, including Chiari type II malformation and hydrocephalus (Del Bigio, 2010). On the basis of historical data, 60% of patients with occipital, cervical, thoracic, or sacral lesions develop



• **Fig. 59.13** Anencephaly. (A) Infant with anencephaly. (B) Ultrasonogram of a fetus with anencephaly. Note the absence of the normal cranial structures (arrows) superior to the orbits (O). (Courtesy of Dr. Marjorie Grafe, Department of Pathology, Oregon Health and Science University, Portland, OR.)

hydrocephalus, in contrast to 90% of those with thoracolumbar, lumbar, or lumbosacral lesions (Lorber, 1961). Because of decompression caused by leakage of CSF from the MMC at birth, hydrocephalus and increased pressure may become evident only after surgical closure of the back (Stein and Schut, 1979).

Clinical management of the newborn with an NTD must be individualized. At present, surgical closure is advocated for most infants, resulting in decreased infection and improved cognitive abilities, ambulation, a lower prevalence of incontinence, and lower mortality (Hunt and Holmes, 1975; Stein et al., 1975; McLone, 1992). Usually, surgical management of the spinal defect occurs within 48 to 72 hours of birth. Involvement of a plastic surgeon should be considered, particularly with wide or complex lesions. CSF diversion may be needed concurrently or after the back has healed. Additional surgical procedures may be required for shunt malfunction or infection, sequelae of Chiari type II malformation (apnea, stridor, dysphagia), tethered cord, or syringohydromyelia. Initial evaluation of all neonates with a suspected NTD should include assessment of lower extremity sensory and motor function. Particular attention should be paid to spontaneous movements, response to touch and pain, and voiding and stooling pattern (particularly urinary retention and dribbling). Babies should be closely evaluated for other malformations, including congenital heart disease, unless they have been excluded by prenatal ultrasonography. Monitoring neonates for Chiari symptoms (apnea, stridor, poor feeding) and signs of increased intracranial pressure is also required. The overall approach should be multidisciplinary with involvement of the neurosurgery, urology, and physical therapy departments at a minimum. Long-term care of the child with MMC also requires symptom management for neurogenic bladder and bowel, as well as endocrine, orthopedic, rehabilitative, and neuropsychiatric care (reviewed in Apkon et al., 2014). Developmental pediatric, neurologic, or rehabilitation medicine specialists are often the primary point of contact for long-term management, depending on the resources available.

Fetal surgery has become an option for surgical repair of MMC. A randomized trial demonstrated that fetal surgery was associated with a lower rate of shunt placement and possibly better motor function at approximately 3 years of age but was also associated with premature delivery and maternal complications (Adzick et al., 2011; Grivell et al., 2014). In addition, mothers can require prolonged bedrest during the pregnancy and cannot deliver subsequent children vaginally. No improvements in neurogenic bowel/bladder have been observed as compared with those with standard postnatal care (Holmes et al., 2001), and the effects on long-term mobility, cognition, and other outcomes are under ongoing study. In light of these positive results, some centers promote fetal surgery as the standard of care; however, the American College of Obstetrics and Gynecology recommends an approach that balances the risks and benefits to both the fetus and the mother (ACOG, 2013).

Myeloschisis

Myeloschisis differs from MMC in that there is no CSF space beneath the neural placode that is against the anterior wall of the spinal cord; this defect also lacks overlying vertebrae and skin. This results in a continuous CSF leak. Depending on the anatomic level of the defect, neurologic deficits can include the lower extremities, bladder, and bowel. Closure may be more difficult than with MMC, and postclosure hydrocephalus may result (Jeelani and McComb, 2011). Like, MMC, this defect is initially diagnosed by elevated AFP level and by prenatal ultrasonography.

Skin-Covered Neural Tube Defects

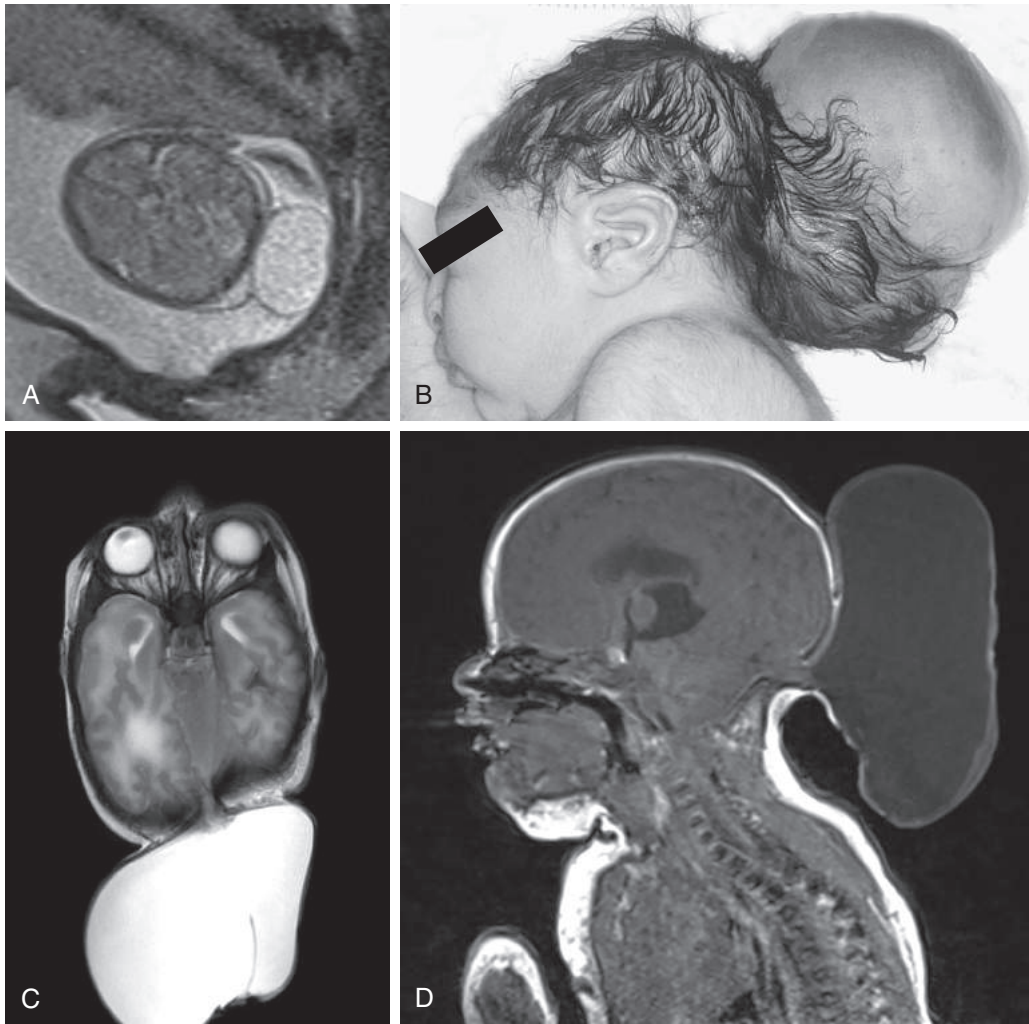
Encephalocele

Encephalocele is considered a postneurulation disorder and likely occurs by mechanisms distinct from those for open NTDs (Copp et al., 2013). Encephaloceles typically present as cranial skull defects through which brain tissue protrudes. Less severe defects include cranium bifidum, where there is a failure of midline fusion of the skull, and cranial meningoceles that contain meningeal but not neural tissue. In up to 80% of patients, encephaloceles are occipital (Fig. 59.14), with the remainder in the parietal, frontonasal, intranasal, or nasopharyngeal regions. Geographic or ethnic-genetic factors influence the location of the lesion. Frontal encephaloceles are more prevalent in Southeast Asia, and occipital lesions are more common in Western populations (Mahapatra and Agrawal, 2006). A role for genetic factors is supported by a number of autosomal recessive syndromes associated with encephalocele (e.g., Meckel and Joubert syndromes; see earlier) (Brown and Sheridan-Pereira, 1992). Overall, about half of infants with encephaloceles have other major congenital anomalies, including microcephaly, arrhinencephaly, anophthalmia, cleft lip or palate, craniosynostosis, complex congenital heart disease, and other systemic abnormalities (Brown and Sheridan-Pereira, 1992).

Encephaloceles are also associated with various other CNS defects that influence surgical management and the severity of the outcome. In a series of 129 patients with anterior encephaloceles (including frontoethmoidal, orbital, transethmoidal, transellar, and interfrontal), 22 had associated hydrocephalus, and ACC was seen in 16 (Mahapatra, 2011). In addition to hydrocephalus, other anomalies include anomalous draining veins and nodular heterotopia (Diebler and Dulac, 1987; Menkes, 1991). The occurrence of lower occipital lobe encephalocele with skull base defects and malformations of the cerebellum and lower brainstem characterizes Chiari type III malformations.

Evaluation of the infant with an encephalocele can be aided by transillumination, skull radiographs, cranial ultrasonography, computed tomography scan, and MRI. Typically, MRI with magnetic resonance angiography and magnetic resonance venography is the study of choice, particularly for surgical planning. Frontonasal encephaloceles pulse or bulge with brief bilateral jugular vein compression, indicating communication with the subarachnoid space. Nasal gliomas, dermoids, and teratomas can all occur in the same region. Intranasal encephalocele should be suspected when an intranasal mass is found in a child with a broad nasal bridge and widely spaced eyes. Some of these children may also present with recurrent meningitis (Menkes, 1991). Basal encephaloceles are not usually diagnosed until childhood and can be located in the nasopharynx, sphenoid sinus, or posterior orbit.

In most patients, neurosurgical management is indicated early in life; however, large lesions or other severe CNS anomalies may preclude intervention, since typically, brain tissue exterior to the skull cannot be salvaged. Early treatment is imperative for those infants at high risk of meningitis caused by lesions that externally communicate and leak CSF. Surgical repair goals include dural closure and improving cosmesis. Complications can include postoperative CSF leakage or pseudomeningocele formation (Hervey-Jumper et al., 2011). For patients who are not surgical candidates, a palliative course should be pursued, although it can be quite challenging to decide which interventions are appropriate for a given patient. Ongoing discussions involving the family, subspecialists, and, ideally, dedicated palliative care providers are essential for provision of good care.



• **Fig. 59.14** Fetal and Postnatal Images of Occipital Encephalocele. (A) Fetal axial magnetic resonance image. (B) Newborn with a large occipital encephalocele. (C) Postnatal T2-weighted axial magnetic resonance image of a patient with an occipital encephalocele. (D) Postnatal T1-weighted sagittal magnetic resonance image of a patient with an occipital encephalocele. ([A, B] Courtesy of Dr. Marjorie Grafe, Department of Pathology, Oregon Health and Science University, Portland, OR.)

Encephaloceles can be associated with medically intractable seizures, which may be responsive to surgical resection (Faulkner et al., 2010). Survival and outcome remain difficult to predict because of the variability of presentation and surgical selection bias. One study of a series of children with encephaloceles reported overall mortality of 29% (45% in infants with posterior defects; 0% in infants with anterior defects) (Brown and Sheridan-Pereira, 1992). Neurologic deficits were severe in 33% of survivors. Mild neurologic deficits were found in 17% of survivors with anterior defects and in 50% of survivors with posterior defects. Intellectual disability prognosis depends on the amount of neural tissue within the defect (reviewed in Hervey-Jumper et al., 2011), and outcome can be quite favorable in patients with normal brain anatomy.

Meningocele

Meningocele is a skin-covered lesion containing meningeal tissue that has herniated through the posterior vertebral column (Copp and Greene, 2013). This occurs more commonly in the lumbosacral spine than in the cervical spine, and while the spinal cord does not extend into the defect, it may still be tethered and eventually

cause symptoms. Postnatally, meningoceles may be evaluated by spinal ultrasonography or MRI to evaluate the spine for tethering, syringohydromyelia, and diastematomyelia (Ladino Torres and DiPietro, 2014). At centers without extensive ultrasound expertise, MRI is the study of choice, and computed tomography is used mainly to delineate bony landmarks. Chiari type II malformation is not typically seen with posterior meningocele (Gupta et al., 2013).

Occult Spinal Dysraphisms

Occult spinal dysraphisms are closed defects of the distal part of the spinal cord with an intact dermal covering and are a result of defects of caudal neural tube formation (secondary neurulation). In some instances these defects are truly occult without any overlying abnormalities of the skin and may go undetected until they become symptomatic. Prenatally, they may be detected by fetal MRI (Schwartz and Rossi, 2015). In the vast majority of newborns, an occult spinal dysraphism is accompanied by cutaneous stigmata. Such lesions include abnormal hair tufts, hemangiomas, pigmented spots, skin tags, aplasia cutis congenita, cutaneous dimples or tracts

(particularly with CSF leak), or a subcutaneous mass, often apparent because of an asymmetric gluteal cleft (Hall et al., 1981; Albright et al., 1989; Scatliff et al., 1989). Sacral dimples or deep gluteal clefts between the buttocks without other features are rarely associated with spinal dysraphism, while dimples clearly above the gluteal cleft are associated with higher risk of spinal dysraphism.

Spinal dysraphisms are classified by a clinical, imaging, and developmental approach (Schwartz and Rossi, 2015). MRI is indicated for evaluation and diagnosis, when available. For closed dysraphisms, the initial distinction is based on the presence of a subcutaneous mass (Badve et al., 2011). Skin-covered lesions without a subcutaneous mass should be examined clinically for skin tags, evidence of spinal cord dysfunction, and anorectal malformations. Defects with subcutaneous masses are classified by the tissue types involved: lipomyelocele (fat and meninges), lipo-MMC (fat, nerve, and meninges), meningocele (meninges only), and terminal myelocystocele, which is a complex lesion with persistence of the terminal syringohydromyelic cavity (Schwartz and Rossi, 2015).

Other spinal cord lesions associated with occult dysraphism include diastematomyelia–diplomyelia, lipoma, teratoma, and other tumors, dermal sinus with or without dermoid or epidermoid cyst formation, and tethered cord (Menkes, 1991; Anderson and Brown, 2009). More severe lesions include neurenteric cysts, anterior meningocele, and caudal regression syndrome (dysraphia of sacrum and coccyx, atrophy of muscles and bones of the legs, fusion of spinal nerves and sensory ganglia, or agenesis of the distal part of the spinal cord) (Towfighi and Housman, 1991). As for other NTDs, infants of diabetic mothers are at increased risk of these lesions (Becerra et al., 1990). Abnormal conus and a thickened filum are usually present, and a symptomatic tethered cord is a common presentation after surgical repair of both skin-covered lesions and MMC (Bowman et al., 2009). Thickened filum with or without a small amount of fat can be seen frequently on spinal MRI and is usually not significant in the absence of a low-lying conus and neurologic symptoms.

Clinical Features and Diagnosis

Progressive functional impairment can occur as the cord is stretched against the fixed filum (Lew and Kothbauer, 2007), with neurologic impairment rarely presenting in the newborn period (Hertzler et al., 2010). Atypical voiding and stooling (particularly continuous dribbling) should prompt bladder volume measurement and urology and neurosurgical referrals. Delay in walking, disturbed sphincter control, contractures of the feet or legs, and pain in the back or legs may present in infancy or childhood, while gait and sphincter abnormalities, foot deformities, and scoliosis are more common in older patients. Rarely, recurrent meningitis and acute loss of function are seen.

Because prophylactic surgical intervention may prevent deterioration, early diagnosis is a necessity (Gower et al., 1988; Scatliff

et al., 1989; Hertzler et al., 2010). In neonates this evaluation may be facilitated by spinal ultrasonography, which permits a dynamic evaluation of lower spinal cord mobility, and MRI to define structural anomalies of the cord. Evaluation should occur as soon as a closed spinal dysraphism is suspected. The timing of surgical management depends on the presence or progression of neurologic symptoms and signs. The care of these children requires a multidisciplinary approach for management of neurosurgical issues and comorbidities (Cornette et al., 1998).

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Complete references used in this text can be found online at www.expertconsult.com

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Brain Injury in the Preterm Infant

STEPHEN A. BACK AND STEVEN P. MILLER

KEY POINTS

- Intraventricular hemorrhage (IVH) remains a common cause of chronic neurologic morbidity. Despite a gradual decline in the incidence of most grades of IVH, the increased survival of very low birth weight infants has resulted in an increase in the absolute number of infants with IVH.
- Preterm infants are currently at much lower risk of severe white matter injury (WMI) that typically results in focal cystic necrosis and secondary gray matter degeneration. Cystic WMI is commonly associated with cerebral palsy, cortical visual impairment, and a spectrum of cognitive and learning disabilities.
- Most preterm infants commonly display less severe diffuse WMI that results primarily in myelination disturbances related to death of oligodendrocyte progenitors (preOLs). Both cranial ultrasonography and magnetic resonance imaging (MRI) appear to underdiagnose the extent of diffuse WMI.
- Chronic diffuse WMI results in reduced cerebral white matter growth related to a series of dysmaturation events that result in regeneration of preOLs that fail to differentiate to myelinating oligodendrocytes. Chronic diffuse WMI is also accompanied by reduced cerebral gray matter growth that appears to be related to widespread disturbances in neuronal maturation rather than loss of developing neurons.
- Diffuse WMI, often reflected in punctate WMI on diagnostic MRI, is linked to a broad spectrum of persistent neurobehavioral disabilities that include impairments in motor and cognitive skills. Punctate WMI is most readily diagnosed on early-preterm MRI scans. The burden of punctate WMI (lesion volume and location) predicts motor and cognitive dysfunction. The spectrum of neurodevelopmental impairments that follow WMI in the preterm neonate are consistent with the dysmaturation described in white matter and cerebral gray matter.
- These recently recognized forms of cerebral gray and white matter dysmaturation present new challenges for diagnosis and suggest new therapeutic strategies to promote reversal of the processes that cause dysmaturation of neurons and preOLs.

General Principles of Preterm Brain Injury

The preterm brain is susceptible to a broad spectrum of injury that ranges from diffuse nonnecrotic lesions to hemorrhage to severe necrotic tissue destruction. Brain injury is initiated by two major *upstream mechanisms*, *ischemia* and *infection/inflammation*, that may interact and potentiate each other. Because the developing

brain is rapidly evolving, the susceptibility to injury is critically related to the timing and severity of the insult. As brain development progresses, distinct populations of cells are selectively more vulnerable to injury, whereas others display greater resistance. Patterns of cell vulnerability shift as the brain matures. Moreover, a wide variety of additional factors may sensitize the brain's susceptibility to injury. The preterm brain may be exposed to a variety of subclinical factors that in isolation may not be injurious but in combination may synergize to potentiate injury. Such factors include nutritional status, systemic illnesses, exposure to glucocorticoids, sedatives, or drugs of abuse, the burden of painful procedures, and other sources of neonatal stress. Equally important to consider is the concept of tolerance in which an antecedent subinjurious insult may reduce the severity of a subsequent one. For example, a low-grade fetal infection may be protective against a subsequent more severe hypoxic-ischemic insult.

This chapter addresses three common and frequently overlapping forms of preterm cerebral injury: intraventricular hemorrhage (IVH), white matter injury (WMI), and gray matter injury. The impact of preterm cerebral injury is considerable. Among children born very preterm, even with modern neonatal intensive care, 5%–10% have major motor deficits, including cerebral palsy related to significant WMI, and more than half have significant cognitive, behavioral, or sensory deficits (Vohr, 2014). These cognitive and neurobehavioral deficits are increasingly observed in the absence of significant motor impairments or cerebral palsy (Gonzalez and Miller, 2006), which has also suggested *primary* involvement of multiple gray matter structures. Gray matter injury was previously attributed to cystic necrotic WMI that led to *secondary* cortical and subcortical gray matter degeneration. Although contemporary cohorts of preterm survivors commonly display less severe injury, these milder forms of injury are associated with both reduced cerebral gray matter growth and reduced cerebral white matter growth. The impairment in cerebral growth arises from complex and disparate responses of neurons and glia that fail to fully mature during a critical window in development of neural circuitry. These observations support the notion that much of the spectrum of motor and neurobehavioral disabilities currently seen in preterm survivors is related to cerebral maturational disturbances rather than overt injury (Back and Miller, 2014). Thus preterm children with a broadly normal intelligence quotient currently display motor dyspraxia (Miller et al., 2000; Sanger et al., 2006), processing deficits in attention and executive functions (e.g., cognitive flexibility,

inhibitory control, working memory) (Anderson and Doyle, 2004; Marlow et al., 2005; Diamond et al., 2007), and visually based challenges with information processing and language (Grunau et al., 1990; Whitfield et al., 1997; Grunau et al., 2002; Taylor et al., 2004; Saavalainen et al., 2007; Luu et al., 2009). These cognitive and behavior problems persist to young adulthood (Curtis et al., 2002; Hack et al., 2002; Grunau et al., 2004; Taylor et al., 2004; Lindstrom et al., 2007; Nosarti et al., 2007; Saavalainen et al., 2007). Hence these highly prevalent neurocognitive impairments support widely distributed disturbances in brain growth and connectivity that involve both gray matter and white matter (Doesburg et al., 2011).

Intraventricular and Periventricular Hemorrhage in the Preterm Infant

Pathogenesis

IVH is a common injury in the preterm brain, originating in the subependymal germinal matrix (Ballabh, 2010). Cortical neuronal and glial cell precursors develop from the germinal matrix and adjacent ventricular germinal zone during the late second and early third trimesters. The subependymal germinal matrix is a highly vascularized region whose arterial supply is derived from the anterior and middle cerebral arteries as well as the anterior choroidal artery. These arteries feed an elaborate capillary network of thin-walled vessels that is continuous with a deep venous system that terminates in the vein of Galen. The terminal, choroidal, and thalamostriate veins course anteriorly to form the internal cerebral vein, which courses posteriorly to join the vein of Galen, thus leading to a U-shaped turn in the direction of blood flow. Involution of the germinal matrix occurs with advancing gestation.

The predisposition of the preterm infant to IVH is due to several factors. A pressure-passive state exists because of the lack of autoregulation of blood flow in the cerebral arterioles of the preterm brain. In the presence of a highly vascularized subependymal germinal matrix, the risk of IVH is enhanced by the lack of a supporting basement membrane for the matrix blood vessels, an increased amount of matrix fibrinolytic activity, and a decrease in extravascular tissue pressure in the first few days of extrauterine life. Thus IVH may occur in the setting of elevated venous pressure or an increase in fluctuations in cerebral blood flow (CBF) velocity triggered by factors that include respiratory distress, pneumothorax, asphyxia, myocardial failure, patent ductus arteriosus, hypotension, hypothermia, and hyperosmolality (Ment and Schneider, 1993). Fluctuating pressure passivity is common in preterm infants and may be associated with IVH (Soul et al., 2007; O'Leary et al., 2009).

IVH has been produced experimentally after hypotension followed by reperfusion (Goddard-Finegold et al., 1982; Ment et al., 1982). These studies support the observation that IVH is more likely when an early period of prolonged hypotension is followed by an increase in blood pressure (Miall-Allen and Whitelaw, 1987; Miall-Allen et al., 1987). Isolated hypertension associated with seizures, intubation, and suctioning also predisposes the brain to IVH. Even gavage feeding and surfactant administration can lead to changes in cerebral hemodynamics, as measured by near-infrared spectroscopy, that lead to IVH (Roll et al., 2000; Baserga et al., 2003; Kaiser et al., 2004). **Box 60.1** lists key factors that may interact to produce IVH.

• BOX 60.1 Pathogenic Factors Leading to Intraventricular Hemorrhage

Increase in cerebral blood flow
Fluctuation in cerebral blood flow
Increase in cerebral venous pressure
Endothelial injury
Vulnerable germinal matrix capillaries
Coagulation disturbances
Increased fibrinolysis

Cellular injury in infants with grade III or grade IV (periventricular hemorrhagic infarction; PVHI) IVH may occur from antecedent ischemic injury, a decrease in cerebral blood flow, increased intracranial pressure, or vasospasm. More severe IVH is associated with cerebral WMI, cerebellar injury (see later), and less frequently pontine neuronal necrosis (Volpe, 2008). In this setting, venous infarction leads to neuronal death as well as glial death. Posthemorrhagic hydrocephalus (PHH) is a common sequela of severe IVH and of PVHI. The optimal treatment of PHH to promote optimal neurodevelopmental outcomes remains controversial, with recent data favoring earlier intervention (de Vries et al., 2002; Srinivasakumar et al., 2013).

Site, Incidence, and Timing of Hemorrhage

In preterm infants, germinal matrix hemorrhage is most commonly seen at the junction of the terminal, choroidal, and thalamostriate veins in the germinal matrix overlying the body of the caudate nucleus at the level of the foramen of Monro. Parenchymal hemorrhage occurs most commonly in the frontoparietal region, in approximately 15% of cases, where it appears not to be an extension of the IVH but rather a separate process—a hemorrhagic infarction. The hemorrhage is more often unilateral or, in less than 30% of cases, asymmetrically bilateral.

The incidence and severity of IVH increase with decreasing gestation. IVH occurs in only approximately 5% of term newborns. IVH previously occurred in 40%–60% of very low birth weight (VLBW) infants (less than 1500 g birth weight). An overall reduction in the incidence of IVH in VLBW infants from 15% to 25% has occurred in the past 2 decades (Paneth et al., 1993; Vohr et al., 2000; McCrea and Ment, 2008), yet the rate of PVHI appears stable (Hamrick et al., 2004b). The rates of grade III IVH and PVHI remain at 16% in VLBW infants (Stoll et al., 2010). As these smaller infants are surviving in higher numbers, severe IVH and PVHI still contribute importantly to poor neurodevelopmental outcomes.

Although there has been a gradual decline in the incidence of most grades of IVH, the increased survival of VLBW infants has resulted in an increase in the absolute number of infants with IVH.

The risk period for the occurrence of IVH is highest in the first 3 or 4 days of life. Hemorrhage is rarely seen at birth, although it has been reported as early as the first hour of life (Ment et al., 1984). Prenatal hemorrhages can occur, especially in the setting of neonatal alloimmune thrombocytopenia. Twenty-five percent of hemorrhages occur by the sixth hour of life, and 50% of hemorrhages occur during the first 24 hours of life. Less than 5% of newborns develop IVH after the fourth or fifth day of life (Ment and Schneider, 1993). Extension of the hemorrhage may occur

during the first few days because of disturbances in cerebral blood flow.

Clinical Presentation

The clinical presentation of IVH in the newborn depends on the extent of the hemorrhage. It may range from *asymptomatic* to a sudden and catastrophic deterioration that manifests itself with neurologic signs such as stupor or coma, seizures, decerebrate posturing, or apnea. A tense fontanel together with a sudden drop in hematocrit, hyperglycemia, hyperkalemia, hypotension, or bradycardia may herald an IVH. Inappropriate secretion of antidiuretic hormone may occur. The more common presentation, however, is that of a gradual clinical deterioration with an altered level of consciousness, hypotonia, abnormal extremity, or eye movements. In 25%–50% of cases, clinical signs are lacking (Dubowitz et al., 1998).

Grading of Intraventricular Hemorrhage

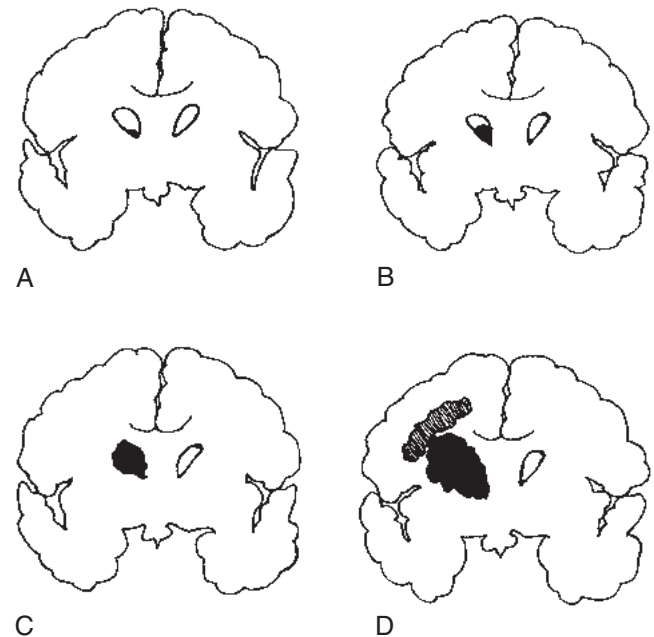
Ultrasound examination is a reliable and sensitive bedside technique for evaluation of the severity of IVH in the newborn nursery. Papile et al. (1978) adapted the standard grading system originally applied to computed tomographic images of IVH to ultrasound images. They classified IVH into four grades of severity related to the location and extent of the hemorrhage (Fig. 60.1, Table 60.1). This classification system was previously used widely for outcome studies. Currently, the more widely used grading system is that proposed by Volpe (2008), which relies on the cranial ultrasound examination to define the extent of ventricular hemorrhage. Because parenchymal involvement is a distinct process, it is not included in the continuous grading of IVH severity (Table 60.1). PVHI appears to arise from venous infarction of the periventricular white matter rather than from a direct extension of the IVH into the parenchyma (Volpe, 2008). Hence the presence of intracerebral hemorrhage or parenchymal lesions is described separately and is not designated as grade IV. Intraparenchymal hemorrhage is followed in 1 to 8 weeks by tissue destruction and formation of a porencephalic cyst. Serial ultrasound examinations are especially important in linking the severity of IVH with neurodevelopmental outcomes (Rademaker et al., 2005).

In addition to various forms of cerebral WMI, IVH may cause graded injury to the cerebellum (Fig. 60.2) that manifests itself as magnetic resonance imaging (MRI) defined changes in microstructure (Tam et al., 2009) and reductions in cerebellar growth

(Tam et al., 2011b). This reduced growth appears to be related to a large population of proliferative external granule cells that are the precursors that generate the internal granule cell layer, which accounts for most of the cells in the human brain. These cerebellar progenitors may be particularly vulnerable to the toxicity of IVH-derived blood products, which are detected by MRI as hemosiderin deposition on the surface of the brainstem and cerebellum (Volpe, 2009). The heightened vulnerability of cerebellar progenitors is consistent with serial neuroimaging studies of human preterm survivors that demonstrated disrupted cerebellar growth in response to postnatal glucocorticoid exposure (Tam et al., 2011a).

Outcome and Prognosis

Isolated grade I and II hemorrhages generally resolve without evolution. Grade III hemorrhages evolve over a period of 1 to 3



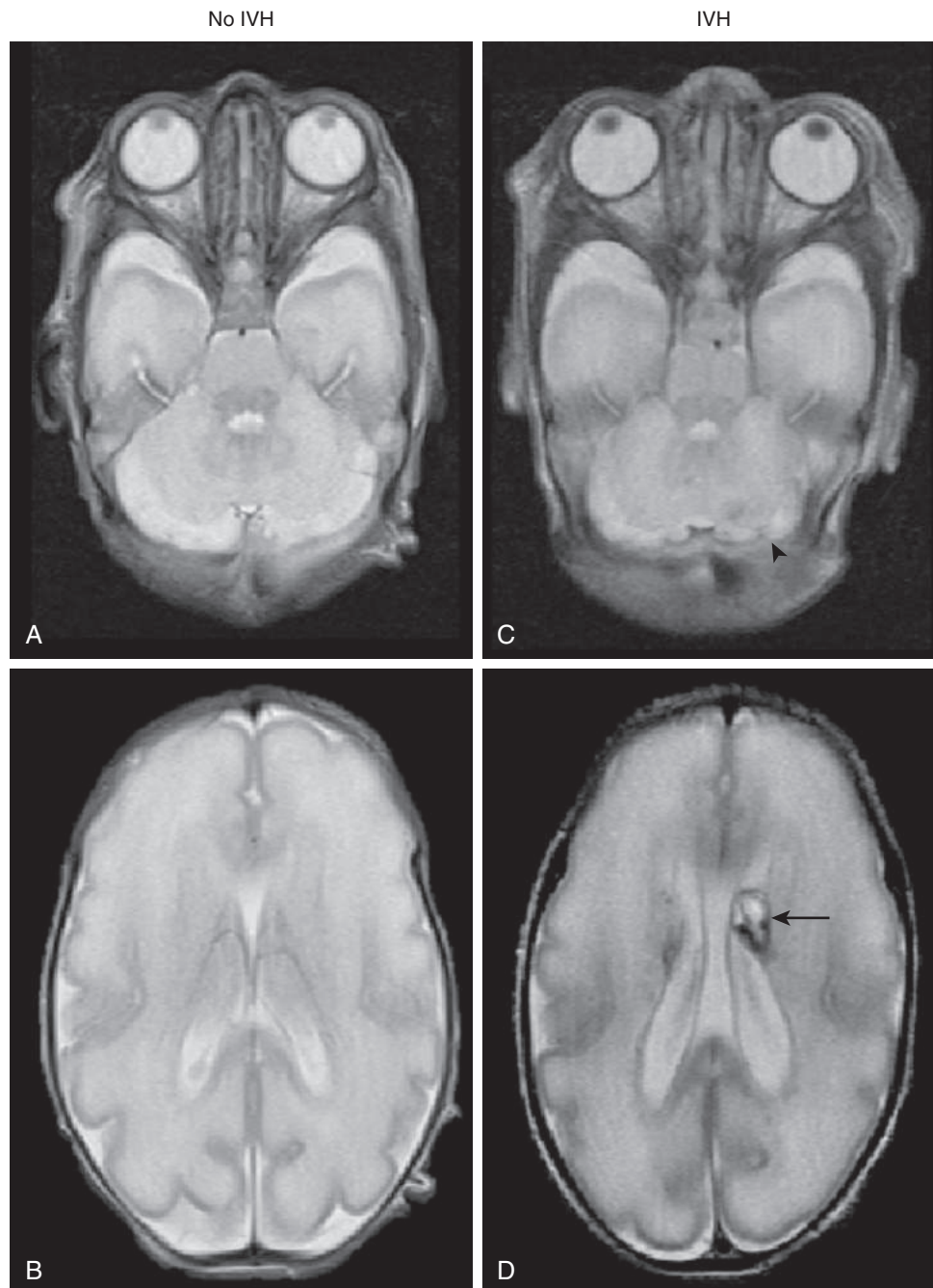
• **Fig. 60.1** The Progressive Grades of Intraventricular Hemorrhage from Mildest to Most Severe. (A) Grade I hemorrhage involves less than 10% of the ventricular volume of the lateral ventricles. (B) Grade II involves 10%–50% of the ventricular volume. (C) Grade III involves more than 50% of the ventricular volume and is frequently associated with ventricular dilatation. (D) Hemorrhage is associated with parenchymal infarction.

TABLE 60.1

Grading of Intraventricular Hemorrhage by Cranial Ultrasound Examination Findings

| Papile Grading System | | Volpe Grading System ^a | |
|-----------------------|--|-----------------------------------|---|
| I | Subependymal hemorrhage with minimal or no IVH | I | Germinal matrix hemorrhage <10% IVH |
| II | Definite IVH without distention of the ventricles | II | IVH 10%–50% |
| III | Enlargement of the ventricles secondary to distention with blood | III | IVH >50%, usually with distention of lateral ventricle |
| IV | Extension of the hemorrhage into the parenchyma along with IVH and enlargement | Separate notation | Periventricular echodensity signifying parenchymal lesion |

^aBy cranial ultrasound examination.
IVH, Intraventricular hemorrhage.



• **Fig. 60.2** Intraventricular Hemorrhage Is Associated With Impaired Growth of the Cerebellum. (A, B) The normal appearance of the brain and cerebellum in a preterm neonate born at 28 weeks' gestational age who was imaged by magnetic resonance imaging (MRI) at 30.3 weeks. (C, D) Smaller cerebellum (arrowhead) associated with intraventricular hemorrhage (IVH) (arrow) in a preterm neonate born at 28 weeks' gestational age who was imaged by MRI at 30.1 weeks.

weeks and may produce a fibrotic reaction that obliterates the subarachnoid space with subsequent ventricular dilatation and hydrocephalus. Clinical symptoms of progressive hydrocephalus, such as rapid head growth, a full anterior fontanel, or separation of cranial sutures, often appear days or weeks after the onset of ventricular dilatation. The delayed onset of clinical symptoms is related to the presence of a large subarachnoid space as well as the paucity of myelin in premature infants.

Outcomes differ between studies, but in general, mortality rates are not significantly increased in infants with a grade I or grade

II hemorrhage. In VLBW infants, 10%–15% sustain more severe hemorrhages (McCrea and Ment, 2008). These grade III or grade IV hemorrhages are associated with increased mortality. In 15% of cases of IVH, areas of PVHI occur that are associated with mortality rates that approach 50% greater than those in infants without IVH (Brouwer et al., 2008; Sarkar et al., 2009; Bolisetty et al., 2014).

Several studies have focused on the relationship between IVH and subsequent neurodevelopmental outcomes. In extremely low birth weight (ELBW) survivors, even grade I and grade II IVH

have been associated with a modest increase in morbidity relative to those infants without IVH in some studies (Patra et al., 2006; Vavasseur et al., 2007; Klebermass-Schrehof et al., 2012) but not all studies (Payne et al., 2013). Given the considerable morbidity associated with extreme prematurity, it can be challenging to independently assess the increased risk of neurologic complications associated with lower grades of IVH. One more recent study of nearly 1500 ELBW survivors found that grade I and grade II IVH were associated with a 7% greater rate of poor neurodevelopmental outcomes after exclusion of other cranial ultrasound abnormalities such as WMI or porencephaly (Bolisetty et al., 2014). The prognosis for infants with grade I and grade II IVH thus may not be benign if there is comorbid brain injury. This increased risk of abnormal outcome may be related to underrecognized WMI or gray matter injury (Inder et al., 1999; O'Shea et al., 2012). Follow-up studies have shown that the degree of IVH at birth and the presence of ventriculomegaly are predictors of neurologic status at a corrected age of 24 months. Neurologic sequelae occur in up to 35% of infants with grade III IVH and 55% with grade IV IVH (Brouwer et al., 2008). In a longitudinal study of preterm infants with a birth weight less than 1750 g, only infants with grade III or grade IV IVH differed in neurologic examination findings from term control infants at 2 years of age (Vohr et al., 2000). Unlike motor function, cognitive function as assessed by the Bayley scores deteriorated in the first 18 months of life. The persistence of ventriculomegaly in grade III or grade IV IVH is associated with greater risk of more severe neurologic sequelae that include seizures, cerebral palsy, and severe impairment of vision or hearing (Ancel et al., 2006). The presence of hydrocephalus with or without shunting at term increases the risk of a poor neurodevelopmental outcome (Radic et al., 2015). Adverse outcomes are significantly worse for ELBW infants with PHH that required a shunt, highlighting the importance of appropriate and timely intervention (Adams-Chapman et al., 2008).

Prevention

Prevention of preterm birth is the most effective method of reducing the incidence of IVH. In the event of preterm labor, it is advisable that the neonate be born at a center specializing in high-risk deliveries. The risk of IVH is higher in neonates who are outborn and transported after birth. Prenatal administration of steroids is associated with a decreased risk of IVH and cerebral palsy (Roberts and Dalziel, 2006), but prenatal administration of magnesium sulfate does not reduce the rates of IVH (Canterino et al., 1999; Rouse et al., 2008). Optimal neurologic intensive care of the preterm infant includes reduced exposure to hyperventilation, hypocarbia, or hypoxia, as well as maintenance of adequate mean arterial pressure (MAP). Abrupt elevations in CBF may be precipitated by excessive handling or tracheal suctioning. Other risks for IVH include pneumothorax, acidosis, rapid infusions of sodium bicarbonate, and volume expanders.

Because of the increased risk of neurodevelopmental sequelae in infants with IVH, several clinical trials have been performed to evaluate the role of prolonged neuromuscular paralysis in preterm infants. A metaanalysis of five trials concluded that although neuromuscular paralysis with pancuronium may help decrease the risk of IVH and pneumothorax in asynchronously breathing infants, its routine use was not recommended because of concerns about safety and long-term pulmonary and neurologic effects (Cools and Offringa, 2000).

A low MAP or increased fluctuations of blood pressure have been associated with an increased risk of IVH (Perlman and Volpe, 1982). Although close monitoring of the MAP is recommended, there is no evidence that pharmacologic manipulation of systemic blood pressure (e.g., with pressors, steroids, or volume expanders) to achieve a set goal (e.g., MAP >30 mmHg) alters the incidence of IVH or improves neonatal outcome. In early studies, phenobarbital administration appeared to be beneficial by preventing fluctuations in blood pressure (Donn et al., 1981; Arroyo-Cabrales et al., 1998). However, a subsequent multicenter trial of prenatal administration of phenobarbital (10 mg/kg) to 110 women provided no reduction in the postnatal frequency of IVH in preterm infants between 24 and 33 weeks' gestational age (Kaempf et al., 1990). A larger trial confirmed these findings, and long-term follow-up at 18 to 22 months found no difference in neurodevelopmental outcomes (Shankaran et al., 1997, 2002). A metaanalysis of 10 trials of postnatal administration of phenobarbital showed no difference in the rates of severe IVH or ventriculomegaly (Whitelaw and Odd, 2007). Therefore postnatal administration of phenobarbital does not appear to be beneficial for prevention of IVH.

Pharmacologic doses of vitamin E, an antioxidant, were associated with a reduction in the incidence of IVH in low birth weight infants when given intramuscularly (Speer et al., 1984). However, after reports of the association of such large doses of vitamin E with sepsis and necrotizing enterocolitis, its use for prevention of IVH was curtailed (Finer et al., 1984; Johnson et al., 1985).

Indomethacin, a prostaglandin synthase inhibitor, was originally found to decrease the incidence of IVH in infants weighing less than 1250 g (Ment et al., 1985). However, later studies, on the long-term effects of indomethacin prophylaxis in these ELBW infants, showed no increase in the rate of survival without neurosensory impairment, despite a reduction in the rate of severe IVH (Schmidt et al., 2001; Vohr et al., 2003). A metaanalysis of 19 trials and 2872 infants found an increased risk of oliguria, which was, however, not associated with major renal impairment. There was no difference in the incidence of necrotizing enterocolitis, a modest reduction in the number of infants with severe IVH and no difference in long-term neurosensory impairments (Fowlie and Davis, 2002). Prenatal administration of indomethacin, as a tocolytic to arrest preterm labor, is associated with increased risks of severe IVH and WMI (Hammers et al., 2015). Yet, recent evidence suggests decreased risk of WMI with prolonged postnatal indomethacin exposure in the preterm neonate (Miller et al., 2006; Gano et al., 2015).

Prenatal administration of steroids (Elimian et al., 2000; Smith et al., 2000; Canterino et al., 2001; Eriksson et al., 2009; Abbasi et al., 2010; Wong et al., 2014) and surfactant replacement therapy (Soll and Dargaville, 2000; Kribs et al., 2015) decrease the incidence of IVH as well as neonatal mortality in low birth weight infants. However, an increase in the incidence of IVH may occur with surfactant administration as a result of the mode of instillation (Cowan et al., 1991; Hellstrom-Westas et al., 1991, 1992) and the drop in PaCO₂ with improved ventilation. Since a significant drop in MAP and CBF volume can occur during surfactant administration, attention should be paid to the speed and volume of instillation.

Mechanical ventilation may be associated with an increased risk of IVH (Cools et al., 2015). Several studies evaluated the early use of high-frequency ventilation versus conventional ventilation for infants with respiratory distress syndrome. These studies found either an increased risk of IVH with high-frequency oscillatory ventilation (HIFI Study Group, 1989; Cools and Offringa, 1999;

Moriette et al., 2001) or no increase in the risk of IVH (Clark et al., 1996; Keszler et al., 1997). The results of a recent metaanalysis of 19 studies and 4096 infants found no increased risk of severe IVH or WMI in preterm infants treated with high-frequency versus conventional ventilation (Cools et al., 2015).

It is important to avoid both hypocarbia ($\text{PCO}_2 < 30$ mmHg) and hypercarbia ($\text{PCO}_2 > 55$ mmHg) because of their significant effects on CBF (Young and Yagel, 1984; Gleason et al., 1989; Pryds et al., 1989, 1990; Habgood et al., 1991; Pryds, 1991). Avoiding low PCO_2 has been shown to be neuroprotective in animal studies (Sola et al., 1983; Vannucci et al., 1995). Hypocarbia is associated with hypotension and an increased risk of IVH and WMI (Erickson et al., 2002; Fabres et al., 2007). Hypercarbia promotes increased cerebral blood flow, which in the presence of other therapies aimed at increasing blood pressure increases the risk of IVH (Fabres et al., 2007; Vela-Huerta et al., 2009; Ambalavanan et al., 2015).

Free radicals and iron have been shown to be damaging to oligodendrocyte progenitors (preOLs) in both cell culture and animal studies (Back et al., 1998; Dommergues et al., 1998), and iron-chelating agents such as deferoxamine have been shown to be neuroprotective in animal models (Sarco et al., 2000). However, clinical studies are lacking to evaluate the benefit of antioxidants in the setting of posthemorrhagic infarction. As 80% of iron transfer occurs in the third trimester, it is also important to emphasize that iron sufficiency is required for normal brain development, and so withholding iron administration may be detrimental.

Management

Because of the high risk of IVH and ischemic WMI in the early perinatal period, short-term management should be focused on elimination of factors shown to promote excessive fluctuations in CBF (Perlman and Volpe, 1982) and the prevention of nosocomial infections (Chau et al., 2012). Clinical practices to limit fluctuations in CBF include maintenance of blood gases and metabolic status within a normal range, avoidance of excessive suctioning and handling, and detection and treatment of seizures. Systemic blood pressure should be maintained with particular attention to the rate of administration of fluids. Infants with birth weights of less than 1500 g or a gestational age of less than 32 weeks should undergo a screening ultrasound examination to detect IVH.

Serial cranial ultrasound examinations remain the optimal diagnostic modality to screen neonates for IVH and WMI at the bedside in the perinatal period, when the risk of IVH is the highest (Benders et al., 2014). An ultrasound scan on the fourth postnatal day detects 90% of lesions (de Vries et al., 2004). Because extension of a hemorrhage may occur during the next several days, a repeated ultrasound examination after 5 days is necessary to establish the extent of the IVH. Serial imaging is often necessary, because about half of infants with ventricular enlargement from IVH develop rapidly progressive ventricular dilatation during the next 4 to 8 weeks. In the setting of severe IVH, regular screening ultrasound examinations to reevaluate the neonate for slowly progressive hydrocephalus should complement measurement of head circumference, examination of the fontanel, and assessment of clinical status in the first 4 weeks of life. The decision to continue intensive care support is partly informed by the severity of the IVH and associated brain injury as assessed by a combination of cranial ultrasound and MRI examinations. MRI is the optimal imaging modality to detect smaller cerebellar hemorrhages and potentially more subtle forms of noncystic WMI (de Vries et al., 1998; Inder et al., 2003a; Miller et al., 2003).

Evidence-based guidelines are still mostly lacking for the optimal clinical management of posthemorrhagic hydrocephalus. Serial lumbar punctures or ventricular taps were previously used in the nonsurgical management of PHH to temporarily prevent progression of hydrocephalus and potentially reduce the need for shunt placement. Systematic review of four controlled trials found that the early removal of debris from liquefied blood clots did not reduce mortality, developmental disabilities, or the risk of permanent shunt dependence and was associated with an increased risk of central nervous system (CNS) infection (Whitelaw, 2001; Mazzola et al., 2014). However, more recent studies suggest that increasing ventricular size predicts more adverse neurodevelopmental outcomes and that earlier intervention with lumbar punctures and/or reservoirs may reduce the eventual need for ventriculoperitoneal (VP) shunts (de Vries et al., 2002; Srinivasakumar et al., 2013).

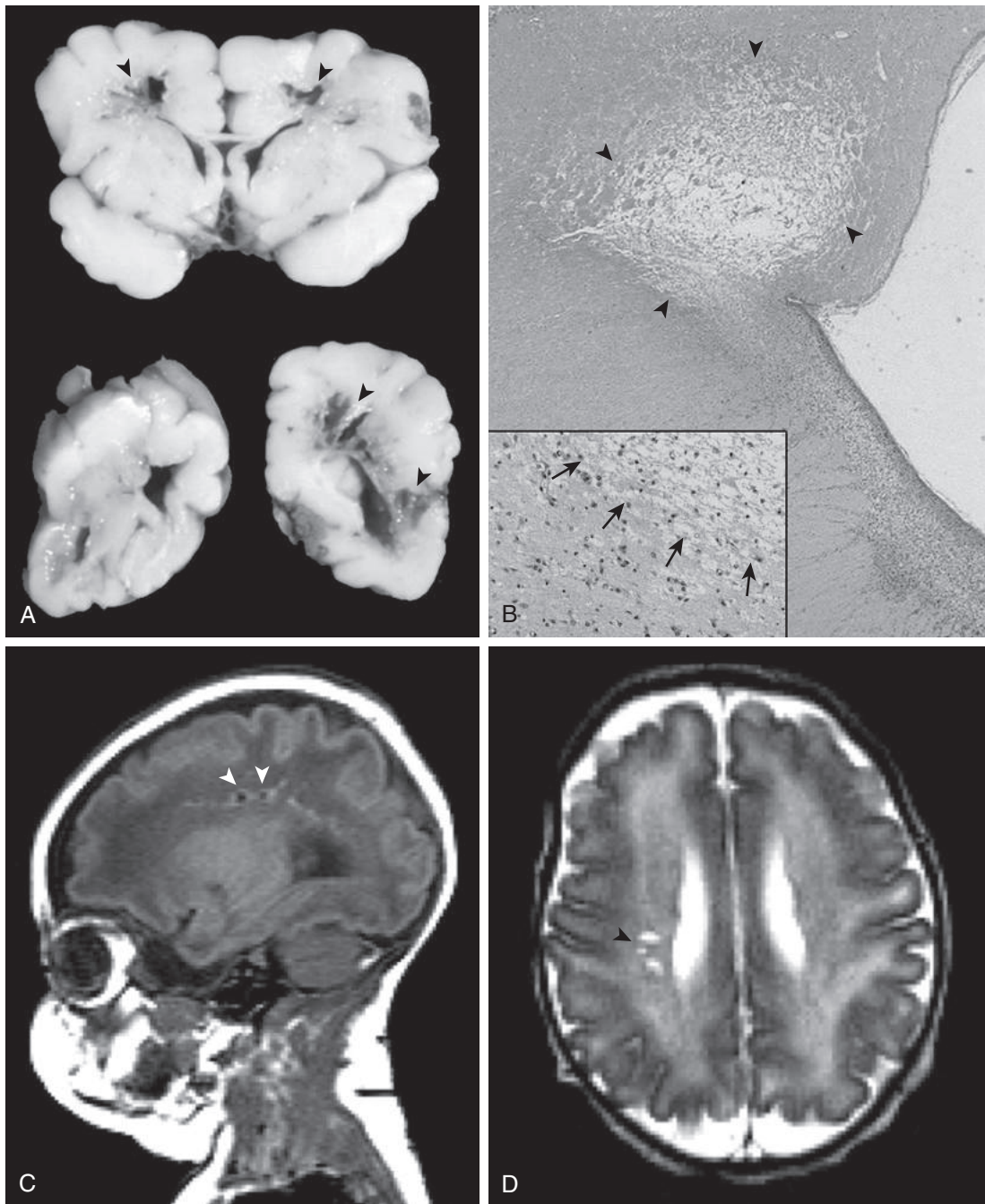
A randomized controlled trial of the combined use of furosemide and acetazolamide in 177 infants with PHH was also ineffective in reducing VP shunt placement and was associated with an increased risk of adverse neurologic outcomes (Kennedy et al., 2001). Several studies also found no benefit of early intraventricular delivery of a fibrinolytic agent (tissue plasminogen activator, urokinase, or streptokinase) to reduce complications associated with PHH (Whitelaw, 2000; Mazzola et al., 2014). In a multicenter randomized clinical trial of drainage, irrigation, and fibrinolytic therapy (DRIFT) when compared with removal of excess cerebrospinal fluid (CSF) via a reservoir, DRIFT reduced severe cognitive disability in survivors and overall death or severe disability, despite an increase in secondary intraventricular bleeding (Whitelaw et al., 2010).

Early placement of a VP shunt that diverts cerebrospinal fluid from the lateral ventricles to the peritoneal cavity in a premature infant weighing less than 2.5 kg is problematic because of the risk of skin breakdown, shunt obstruction, and infection. Placement of a ventricular access device (e.g., Ommaya/Rickham reservoir), an external ventricular drain, or a ventriculosubgaleal shunt is a temporary measure for the early management of PHH. A small retrospective historical cohort study found that ventriculosubgaleal shunts compared with ventricular access devices may reduce the need for daily CSF aspiration (Lam and Heilman, 2009). However, both approaches had similar complication rates, and similar numbers of infants ultimately required VP shunt placement (Limbrick et al., 2010). Either placement of VP shunts or endoscopic third ventriculostomy is a long-term treatment option for PHH, and both have similar clinical outcomes (Limbrick et al., 2014).

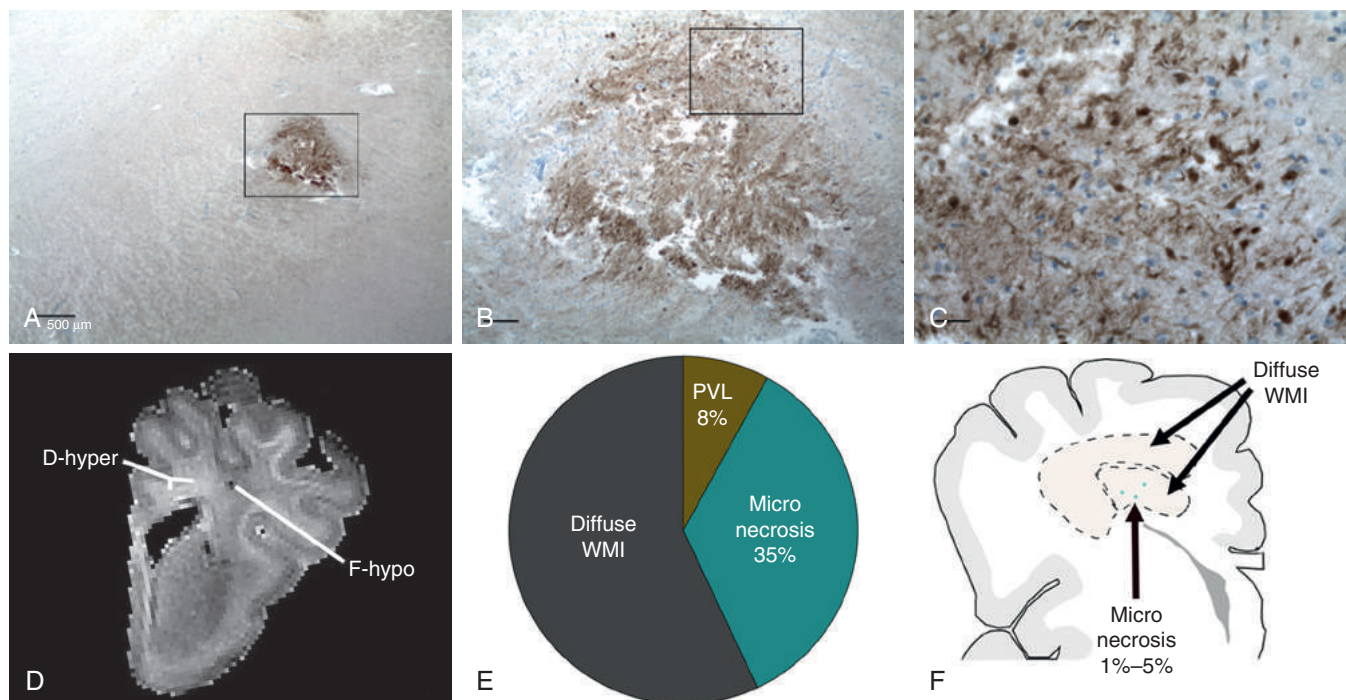
White Matter Injury

Spectrum of Human White Matter Injury

The spectrum of human WMI includes three major identifiable forms of disease: focal cystic necrosis, focal microscopic necrosis, and diffuse nonnecrotic lesions. Large cystic necrotic lesions greater than about 1 mm in diameter are the most severe, but their incidence has decreased markedly in recent years (Fig. 60.3). In several series, focal cystic lesions were detected by MRI in less than 5% of cases (Maalouf et al., 2001; Counsell et al., 2003; Inder et al., 2003a; Miller et al., 2003; Hamrick et al., 2004a; Groenendaal et al., 2010). In fact, the incidence of all forms of necrotic WMI were found to have decreased by approximately 10-fold in contemporary cohorts relative to retrospective cases from earlier decades (Buser et al., 2012). Although the incidence of large necrotic lesions has decreased markedly, discrete small foci of microscopic necrosis



• **Fig. 60.3** Severe White Matter Injury Results in Focal or Diffuse Tissue Destruction (Periventricular Leukomalacia). (A) This autopsy brain, from an infant who died of complications of prematurity, shows large foci of severe cystic necrosis (*arrowheads*) in frontal (*upper specimen*) and parietal (*lower specimens*) periventricular white matter. (B) Histologic analysis of the frontal lesion (stained with hematoxylin and eosin) shows a large focus of necrosis (*arrowheads*) adjacent to the lateral ventricle. The *inset* shows a high-power detail of the edge of the lesion (*arrows*), where marked rarefaction of the tissue can be appreciated adjacent to a region of gliosis at the *lower left*. (C) Appearance of cystic necrotic white matter injury on magnetic resonance imaging. Images from a preterm infant born at 33 weeks' gestational age and scanned at 5 weeks of age (38 weeks' adjusted gestational age). Small areas of cavitation (*arrowheads*) are appreciated as hypointensity on the sagittal T1-weighted image and as hyperintensity on the axial T2-weighted image (D). (Courtesy of Dr. Marjorie Grafe, Oregon Health & Science University, Portland, OR; courtesy of Dr. Ken Poskitt, Children's and Women's Hospital, University of British Columbia, Vancouver, BC.)



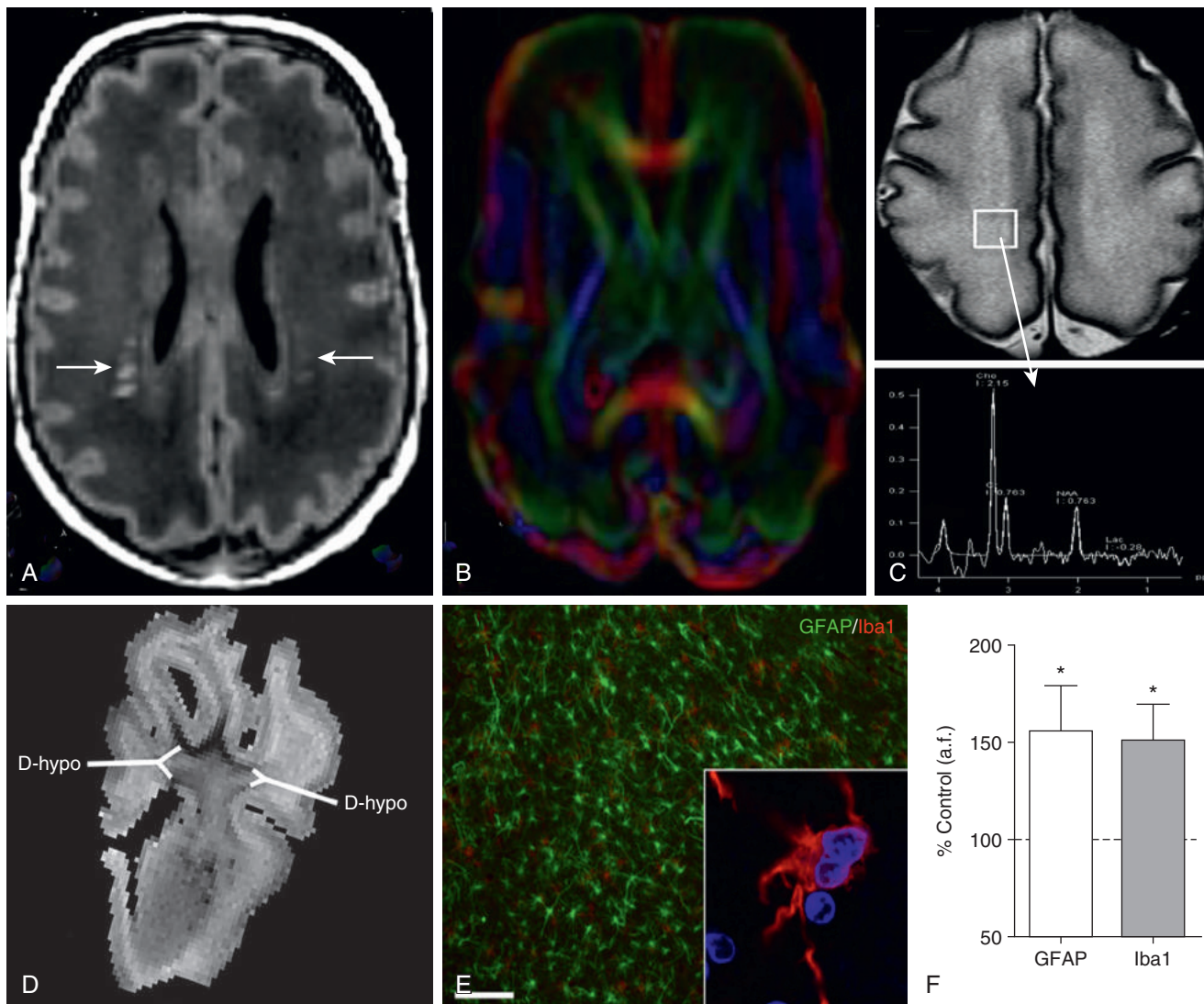
• **Fig. 60.4** Microscopic Necrosis Occurs Commonly but Constitutes a Small Fraction of the Total Burden of Preterm Cerebral White Matter Injury. (A–C) Typical sparse distribution of microcysts visualized with a marker of degenerating axons in a human autopsy brain at 32 weeks' postconceptional age. (B, C) Higher-power detail of the degenerating axons in the microcyst seen in the box in (A). (D) A microcyst visualized by high-field (12 Tesla) ex vivo magnetic resonance imaging as a focal hypointense lesion (F-hypo) on T2. This lesion was detected 2 weeks after global cerebral ischemia in a fetal sheep model of preterm white matter injury (WMI) (Riddle et al., 2011). Note the surrounding diffuse WMI, which is seen as a diffuse hyperintense signal (D-hyper). (E) Pie chart showing the relative percentages of human diffuse WMI, cystic periventricular leukomalacia (PVL), and microscopic necrosis. Note that microcysts often overlap in distribution with regions of diffuse WMI. (F) The relative burden of human microcysts (blue dots) compared to diffuse WMI (tan shading), which typically constitutes more than 80% of the total burden of WMI. Scale bars: (A) 500 μm; (B) 100 μm; (C) 25 μm. (From Back SA, Miller SP. Brain injury in premature neonates: a primary cerebral dysmaturation disorder? *Ann Neurol*. 2014;75:469–486.)

(microcysts) less than 1 mm in diameter appear to be much more common (Pierson et al., 2007). Similarly to cystic necrosis, microcysts are destructive lesions enriched in cellular debris, degenerating axons, and phagocytic macrophages (Fig. 60.4A–C). Because of their small size, microcysts are typically not detected on clinical MRI scans at field strengths of 3 Tesla (T) but can be detected experimentally at higher magnetic field strengths of 12 T (Riddle et al., 2011; see Fig. 60.4D). In a study of human archival and contemporary autopsy cases, microcysts were observed in at least 30% of cases, but they constituted approximately 1%–5% of the total burden of WMI (Buser et al., 2012). Hence although microscopic necrosis occurs with high incidence, the burden is typically low (see Fig. 60.4E–F). It should nevertheless be emphasized that the clinical significance of these small necrotic lesions remains an important but clinically inaccessible question, since microcysts are not readily detected by clinical MRI. It is thus possible that microcysts may be clinically silent or a significant contributor to motor or cognitive disabilities, depending on the extent to which they localize to functionally important regions of white matter.

Diffuse WMI is the most frequently observed form of injury in contemporary cohorts of preterm newborns (Buser et al., 2012). Diffuse WMI is much more widely distributed than previously appreciated and comprises activated astrocytes and microglia that

extend widely beyond the foci of necrosis (see Fig. 60.4E–F). In contrast to necrotic injury, diffuse WMI is defined by selective degeneration of late preOLs, whereas axons are mostly spared except in necrotic foci (Haynes et al., 2008; Riddle et al., 2012). Human preOLs are the precursors of all the myelinating cells of the CNS and are particularly susceptible to significant oxidative damage of a magnitude seen with hypoxia–ischemia (HI) in the clinical context (Back et al., 2005b). The susceptibility to preterm WMI peaks at approximately 23 to 32 weeks' postconceptional age and coincides with a developmental window when preOLs predominate in human cerebral white matter. Paradoxically, it was shown experimentally that ischemia is necessary but not sufficient to cause WMI. Even under conditions of moderately severe ischemia, some regions of white matter may be relatively spared. The regions of particular vulnerability to WMI are defined by both the timing of the appearance and the distribution of susceptible preOLs (Riddle et al., 2006).

Although cranial ultrasound examination is the preferred bedside imaging technique for diagnosing necrotic WMI, it has limited sensitivity for diagnosing diffuse WMI (Maalouf et al., 2001; Inder et al., 2003a; Miller et al., 2003). MRI is the preferred method to visualize diffuse WMI (Fig. 60.5A–C). On diagnostic MRI scans, WMI is visualized as either discrete focal areas or more diffuse areas of magnetic resonance signal abnormalities (Chau



• **Fig. 60.5** Diagnostic and Experimental Magnetic Resonance Imaging Approaches to Define Dysmaturation Processes Related to Diffuse White Matter Injury. (A) Diffuse human white matter injury (WMI) on diagnostic magnetic resonance imaging (MRI) (1.5 Tesla (T); T1) has the appearance of bilateral multifocal signal hyperintensities (*arrows*). (B) Diffusion tensor imaging defines the microstructure of white matter tracts and can be used to follow the long-term progression of diffuse WMI. (C) Magnetic resonance spectroscopic imaging can be applied to define biochemical and metabolic abnormalities associated with diffuse WMI. Both diffusion tensor imaging and magnetic resonance spectroscopic imaging detect abnormalities beyond the areas of signal abnormality on T1-weighted images. (D) Diffuse WMI defined by high-field ex vivo MRI at 12 T in a preterm fetal sheep model of global cerebral ischemia. Diffuse WMI was visualized as extensive hypointense regions (*D-hypo*) on a T2-weighted image at 1 week after ischemia. (E) The typical histopathologic features of diffuse WMI seen on MRI. Note the pronounced staining of reactive astrocytes (*green*) and microglia/macrophages (*red* and *inset*) indicative of a diffuse inflammatory response quantified in (F); the *asterisks* indicate $P < .05$. Scale bar in (E) 100 μ m. GFAP, Glial fibrillary acidic protein.

et al., 2013). The spectrum of noncystic WMI or “punctate” lesions has recently been described as linear or cluster lesions with different evolutions over time (Kersbergen et al., 2014). There is, however, unexplained variability in the nature of lesions detected at different centers, which may reflect differences in management, clinical acuity, or modes of detection. In particular, MRI may not fully define early diffuse lesions, and disparities between clinical presentation and the distribution of lesions on MRI have been widely noted. Experimental studies (see Fig. 60.5D–F) demonstrated that early WMI is particularly well visualized at high magnetic field

strength (12 T) (Riddle et al., 2011), which suggests that currently used clinical MRI field strengths of 1.5 to 3 T may be a limiting factor to detect both diffuse WMI and microcysts.

It should also be emphasized that preterm survivors frequently display diffuse abnormalities in gray and white matter maturation by virtue of their preterm birth that may be more common than focal or diffuse WMI (Chau et al., 2013). These diffuse abnormalities are apparent on MRI as enlarged subarachnoid spaces, a reduction in the amount of white matter, ventriculomegaly, and impaired gyral development (Skranes et al., 2013; Engelhardt et al., 2015).

However, many premature newborns do not have these dramatic abnormalities, and up to 20% with adverse outcomes do not have significant qualitative abnormalities on MRI (Miller et al., 2005; Boardman et al., 2007).

Physiologic Factors Related to the Pathogenesis of White Matter Injury

Role of Hypoxia–Ischemia

HI contributes to the pathogenesis of preterm cerebral injury via several maturation-dependent mechanisms. Since measurements of CBF are technically challenging in human preterm neonates, experimental studies have often been used to define the role of CBF disturbances in the generation of cerebral WMI. The developmental epoch when CBF disturbances occur is a critical factor that influences susceptibility to hypoxia–ischemia. For example, acute injury to the cerebral cortex is relatively low compared with that to the white matter in the preterm fetal sheep, even with prolonged ischemia, whereas severe panlaminar cortical necrosis occurs in term animals (Reddy et al., 1998; Riddle et al., 2006). After global cerebral hypoperfusion, midgestation sheep displayed a predilection to periventricular and subcortical WMI, whereas near term animals displayed predominantly parasagittal cortical neuronal injury (Reddy et al., 1998; Raad et al., 1999; Riddle et al., 2006). Cerebral ischemia in conjunction with hypoxia appears to be a critical factor to generate significant preterm WMI. WMI was infrequently observed when a restriction in uteroplacental blood flow resulted in decreased oxygen delivery to and mild acidemia in the fetus without systemic hypotension or cerebral hypoperfusion (Rees et al., 1997; Mallard et al., 1998; Rees et al., 1999). Similarly, in models of maternal fetal infection, preterm ovine WMI was observed only when repeated systemic fetal endotoxin exposure resulted in both transient hypoxemia and hypotension (Duncan et al., 2002; Dalitz et al., 2003).

Pressure–Passive Circulation

Disturbances in cerebral autoregulation appear to be a key factor that predisposes preterm neonates to cerebral WMI from hypoxia–ischemia. *Cerebral autoregulation* refers to the maintenance of constant CBF over a range of changes in systemic arterial blood pressure or cerebral perfusion pressure (Lassen and Christensen, 1976; Paulson et al., 1990; Greisen, 2009). This autoregulatory range has both upper and lower limits. When blood pressure changes above or below these limits, CBF fails to remain constant and increases or decreases passively, along with changes in arterial blood pressure. Preterm infants are particularly prone to display a “pressure–passive” circulation, especially in the setting of critical illness. Cerebral autoregulation disturbances in premature infants were initially studied by means of xenon clearance and Doppler and more recently by near-infrared spectroscopy and spatially resolved spectroscopy (du Plessis, 2008; Greisen, 2009). Severe perinatal asphyxia, hypoxia, head trauma, and hypercapnic acidosis, even when relatively mild, attenuate or even abolish autoregulation (Busija and Heistad, 1984; Tweed et al., 1986; Jones et al., 1988). Cerebral autoregulation appears to involve an intrinsic property of arterial smooth muscle cells that respond to changes in transmural pressure to modify muscle tone. Autoregulation may be mediated in part by a balance between endothelial cell–derived constricting and relaxing factors (Iadecola and Nedergaard, 2007). Fundamental questions regarding cerebral autoregulation remain unanswered,

including the optimal clinical practices for blood pressure regulation (Greisen, 2009).

Factors That Influence the Distribution of White Matter Injury

Multiple factors that influence CBF or white matter metabolism determine the severity of WMI. It was originally proposed that the topography of more severe WMI was determined by the distribution of arterial end and border zones within the white matter. This attractive hypothesis provided an explanation for the propensity of more cystic necrotic lesions to localize to the deeper periventricular white matter. It was proposed that when periventricular white matter flow falls below a critical threshold, periventricular white matter would sustain greater WMI relative to a putatively better-perfused cerebral cortex. It has been feasible to test this hypothesis experimentally, but not in humans. Quantitative measurements of fetal CBF were done in utero in histopathologically defined regions of cerebral cortex and white matter in preterm fetal sheep (McClure et al., 2008). In the setting of moderately severe ischemia, no pathologically significant gradients of fetal blood flow were detected between cerebral cortex and periventricular white matter during either ischemia or reperfusion. Moreover, although WMI preferentially localized to deeper white matter regions, they were not susceptible to greater ischemia (Fig. 60.6). Neither were less vulnerable superficial regions of white matter characterized by greater blood flow during ischemia. As discussed earlier, the distribution of WMI was explained by the relatively higher density of preOLs in susceptible regions of white matter.

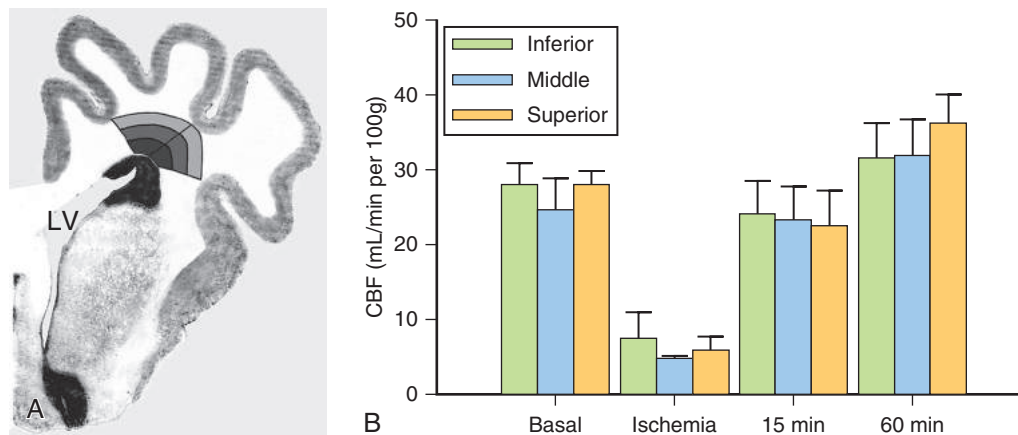
It is also currently unclear if recurrent HI predisposes to more severe WMI. In preterm-equivalent neonatal rats, a pronounced increase in cell death was observed in chronic WMI after recurrent HI (Segovia et al., 2008). However, in preterm fetal sheep, whose lesions more closely resemble those of humans, recurrent HI did not trigger enhanced WMI, which suggested that recurrent HI may confer protection against more severe WMI through an ischemic tolerance-like mechanism (Hagen et al., 2014).

Clinical Factors Related to the Severity of White Matter Injury

Several common clinical conditions have been associated with increased risk of more severe WMI (Box 60.2). Among these is neonatal sepsis, which occurs in up to 25% of preterm newborns who weigh less than 1500 g at birth (Stoll et al., 2002b; Bizzarro

• BOX 60.2 Pathogenic Factors Contributing to More Severe White Matter Injury

- Postnatal sepsis may promote postnatal cerebral hypotension or modify the response to hypoxia–ischemia.
- More severe hypoxemia coupled with hypotension may increase the magnitude of energy failure mediated by hypoxia–ischemia.
- Hypocarbica may exacerbate hypoxia–ischemia by promoting more severe hypoperfusion.
- Hypoglycemia may exacerbate energy failure associated with hypoxia–ischemia.
- Steroid exposure (e.g., dexamethasone) may increase the risk of white matter injury.
- Exposure to multiple painful or stressful procedures may increase the risk of white matter injury.



• **Fig. 60.6** Periventricular White Matter Injury Is not Explained by Gradients of Reduced Cerebral Blood Flow in Cerebral White Matter. (A) Analysis of fetal sheep cerebral blood flow (CBF) in preterm cerebral white matter during hypoxia–ischemia and reperfusion. CBF measurements were made in inferior, middle, and superior regions of the white matter, as illustrated (gray gradient). Typically, white matter injury localized to the inferior region of the white matter. (B) There were no differences between baseline CBF values and those obtained during ischemia or reperfusion at 15 or 60 minutes after ischemia in inferior, middle, and superior regions of the white matter. Hence white matter injury was not preferentially associated with regions that sustained the most severe ischemia. LV, Left ventricle. (From McClure M, Riddle A, Manese M, et al. Cerebral blood flow heterogeneity in preterm sheep: lack of physiological support for vascular boundary zones in fetal cerebral white matter. *J Cereb Blood Flow Metab.* 2008;28:995–1008.)

et al., 2005; Makhoul et al., 2005) and is associated with a significantly increased risk of WMI (Glass et al., 2008; Shah et al., 2008; Chau et al., 2009). Postnatal infections have been linked to altered development of white matter pathways (Inder et al., 2003b; Adams et al., 2010) and widespread impairments in brain development (Chau et al., 2012). Even without culture-proven sepsis, infections have been associated with increased rates of cerebral palsy and other neurodevelopmental disabilities that are consistent with neonatal brain imaging findings (Stoll et al., 2004; Miller et al., 2005; Shah et al., 2008; Chau et al., 2012). Recurrent postnatal infection is an important risk factor for progressive WMI (Glass et al., 2008). Postnatal infections and hypotension, but not histologically defined chorioamnionitis, were associated with increased risk of MRI-defined WMI (Chau et al., 2009). Despite the lack of strong associations between prenatal chorioamnionitis and adverse neurodevelopmental outcomes, chorioamnionitis may indirectly increase the risk of postnatal infections and hypotension as independent risk factors for cerebral ischemia and WMI. In a single center, the incidence of punctate WMI appears to be decreasing over time; in this cohort prolonged exposure to indomethacin predicted reduced WMI (Gano et al., 2015).

Bronchopulmonary dysplasia (BPD) and the number of days of mechanical ventilation have been associated with adverse white matter and cortical development (Anjari et al., 2009; Kaukola et al., 2009; Ball et al., 2010). Although a history of BPD is a strong predictor of cognitive outcome, even after birth weight and neurologic morbidity have been controlled for (Short et al., 2003), a causative role for isolated hypoxemia in WMI remains unclear from clinical or experimental studies (Back and Rosenberg, 2014). Postnatal exposure to corticosteroids for the prevention and treatment of chronic lung disease may impair brain growth, and this effect may be more pronounced with dexamethasone treatment (Murphy et al., 2001; Lodygensky et al., 2005). Postnatal exposure to corticosteroids, used for the treatment of BPD or low blood pressure, is also associated with impaired growth of the cerebellum (Tam et al., 2011a).

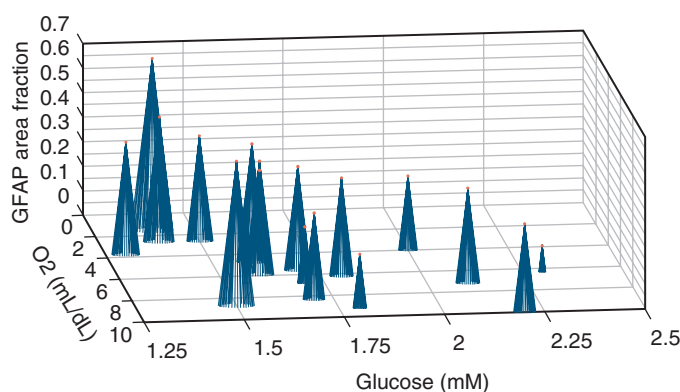
Hypoxemia, as an independent risk factor for WMI, was proposed on the basis of a suspected association between cystic WMI and BPD (Ment et al., 1998). However, a direct association between hypoxemia from chronic lung disease and severe WMI is difficult to establish, because cystic WMI is also commonly associated with IVH and PVHI. The response of the fetus to acute hypoxemia without ischemia has been extensively studied in preterm fetal sheep. Transient hypoxemia was found to cause variable degrees of combined WMI and gray matter injury in the midgestation or near term sheep (Penning et al., 1994; Rees et al., 1997, 1999). However, WMI was notably more severe when significant hypotension was observed, which resulted in *both* cerebral hypoxia and ischemia (Ting et al., 1983). Midgestation chronic hypoxemia, generated by chronic placental insufficiency, caused disturbances in hippocampal, cerebellar, and white matter development (Penning et al., 1994; Rees et al., 1999). Exposure of neonatal rodents to chronic hypoxia results in a spectrum of somatic and cerebral growth retardation that is accompanied by disturbances in white matter development that include ventriculomegaly, reduction in white and gray matter volumes, and disrupted myelination (Ment et al., 1998; Turner et al., 2002; Back et al., 2006; Scafidi et al., 2014). However, central features of human preterm WMI, such as preOL degeneration and reactive gliosis, are not observed in rodents. It thus appears that chronic hypoxemia is related to disturbances in white matter maturation that are distinct from the injury generated by acute hypoxia–ischemia.

Hyperoxia is also a potential complication of neonatal resuscitation and ventilation of the preterm infant during intensive care, but a direct association with human preterm WMI has not been demonstrated. A rabbit model of neonatal resuscitation found no increase in IVH or cerebral injury in response to hyperoxia (Chua et al., 2010). Tissue culture models of hyperoxia have demonstrated enhanced vulnerability of preOLs to cell death from oxidative stress, and transient disturbances in myelination in neonatal rodents were observed (reviewed in Back and Rosenberg, 2014).

Both hypocarbia and hypercarbia have been associated with more severe WMI and IVH, which appear related to disturbances in CBF (Greisen and Vannucci, 2001). A dramatic decline in the incidence of cystic periventricular leukomalacia appeared to be related to a decrease in the number of days of mechanical ventilation (Hamrick et al., 2004b), possibly by avoiding hypocarbic alkalosis, which can promote hypotension. Hypocarbia was identified as a potential risk factor for more severe WMI in early studies (Fujimoto et al., 1994; Dammann et al., 2001; Okumura et al., 2001; Giannakopoulou et al., 2004). Cumulative exposure to hypocarbia but not hyperoxia (Wiswell et al., 1996) was associated with an increased risk of more severe cystic WMI. The deleterious effect of cumulative hypocarbia but not hyperoxia was confirmed in a large study of nearly 800 infants (Shankaran et al., 2006). Hypercarbia promotes increased CBF and WMI in association with more severe IVH (see earlier). Permissive hypercapnia has been widely used as a strategy to deliver a high partial pressure of carbon dioxide to reduce lung injury in mechanically ventilated preterm infants. Trials of permissive hypercapnia found no evidence of cystic WMI by cranial ultrasound evaluation (Mariani et al., 1999; Carlo et al., 2002). Although a more recent small study similarly found no increase in overt WMI in response to hypercarbia, abnormal white matter microstructure was identified when ELBW infants were evaluated at term by diffusion tensor MRI (Ou et al., 2014).

WMI is a prominent and common feature of symptomatic hypoglycemia in term infants that has a predilection for the posterior limb of the internal capsule and posterior cerebral regions. WMI often occurs in association with cortical or subcortical gray matter abnormalities seen on MRI (Burns et al., 2008). Experimental studies support the observation that even mild hypoglycemia exacerbates cerebral injury from HI (Yager et al., 1992; Vannucci and Vannucci, 2001). Studies in preterm fetal sheep (Fig. 60.7) found that hypoglycemia was the most significant factor associated with more severe WMI generated by global cerebral HI (Riddle et al., 2013).

Preterm infants are often routinely exposed to multiple painful and stressful procedures that have been linked to altered brain



• **Fig. 60.7** Blood Glucose and Oxygen Levels Influence the Severity of White Matter Injury. Studies in preterm fetal sheep analyzed the severity of white matter injury (WMI) in chronic lesions after hypoxia-ischemia. The spectrum of WMI severity was estimated by quantification of the magnitude of reactive astrocytes (glial fibrillary acidic protein [GFAP] area fraction on the z-axis) in the lesions. Those sheep with the greatest degree of WMI showed a combination of reduced CaO_2 and blood glucose levels, while those sheep with less severe WMI had higher CaO_2 and blood glucose levels. (From Riddle A, Maire J, Cai V, et al. Hemodynamic and metabolic correlates of perinatal white matter injury severity. *PLoS One*. 2013; 8:e82940.)

maturation that involves white and gray matter structures, as well as impaired brain function (Smith et al., 2011; Brummelte et al., 2012). Procedural pain has also been linked with impaired postnatal growth (Vinall et al., 2012), a predictor of poor cortical development (Vinall et al., 2013a). More recently, analgesic and sedative medication exposure in the preterm neonate have been linked to regional dysmaturation, specific to the exposure: increasing midazolam exposure predicts slower growth of the hippocampus (Duerden et al., 2016), whereas increasing morphine exposure predicts slower growth of the cerebellum (Zwicker et al., 2016). Exposure to painful procedures is also associated with more adverse neurodevelopment, which is influenced by parent–child interactions (Tu et al., 2007; Grunau et al., 2009), as well as parental stress and anxiety (Perlman, 2002; Benzie et al., 2013). Importantly, experimental and human studies support the potential of parent–infant interactions to compensate for compromised early brain maturation and adversity (Vazquez et al., 2000; Tu et al., 2007; Brummelte et al., 2011; Bagot et al., 2012; McManus and Poehlmann, 2012; Vinall et al., 2013b).

Pathogenesis of Chronic White Matter Injury

Emerging Roles for Myelin in Brain Development, Learning, and Memory

Myelination disturbances are one of the central features of chronic WMI. Myelination begins in the preterm brain and normally progresses in well-defined sequences that continue for years postnatally. The central role of myelin is to wrap axons to ensure optimal nerve conduction throughout the CNS. However, emerging studies are challenging the long-held belief that myelin is a stable structure that simply provides static insulation to nerve fibers. Recent studies report that myelin sheaths are dynamic structures that contribute importantly to learning and memory by remodeling the thickness of myelin sheaths so as to enhance or diminish the relative strength of nerve conduction. Hence adaptive myelin plasticity appears to provide a mechanism whereby the nervous system can strengthen or weaken the flow of information along competing pathways so as to optimize new learning (Fields, 2015). Whereas synaptic plasticity operates on millisecond dynamic timescales to fine-tune learning and memory, myelin plasticity occurs on longer timescales to enable the nervous system to solidify the acquisition of new skills. For example, in adult animals, generation of new oligodendrocytes and myelin is required for the learning and acquisition of new motor skills (Gibson et al., 2014; McKenzie et al., 2014).

Hence the definition of these new roles for myelin underscores the potential impact that WMI may have on the disruption of the timing and sequences of myelination during a critical period in preterm brain development. Preterm brain development involves multiple cell maturational and activity-dependent events that coincide with sequential waves of late neurogenesis, gliogenesis, glial and neuronal maturation, synaptogenesis, and myelination. Aberrant myelination is likely to disrupt both gray matter and white matter development by causing enduring disturbances in the ultimate establishment of neural networks and connectivity that are integral to normal brain function.

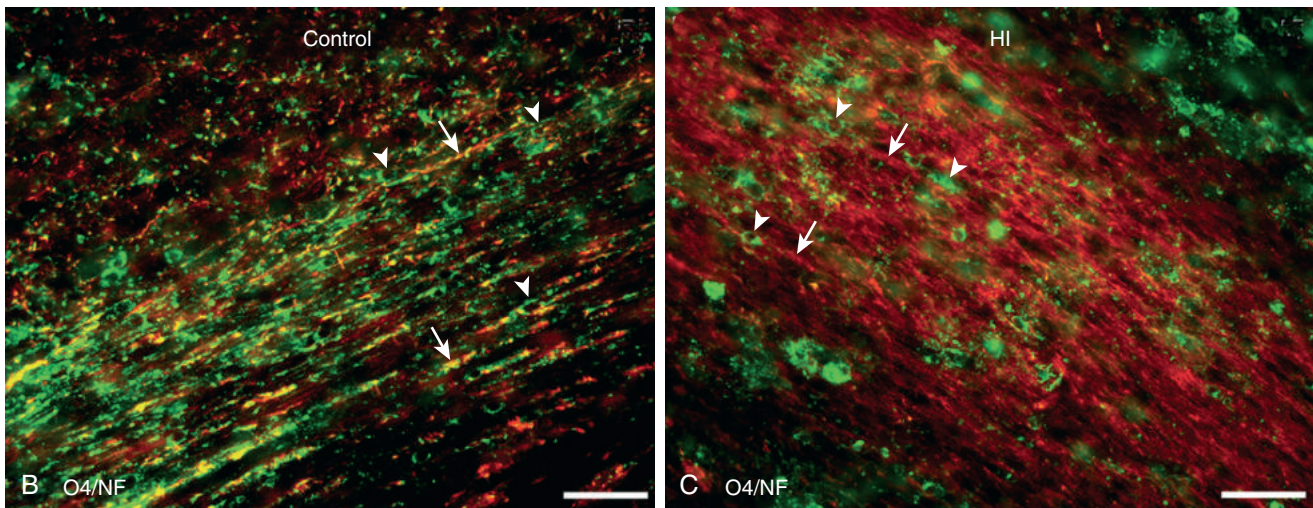
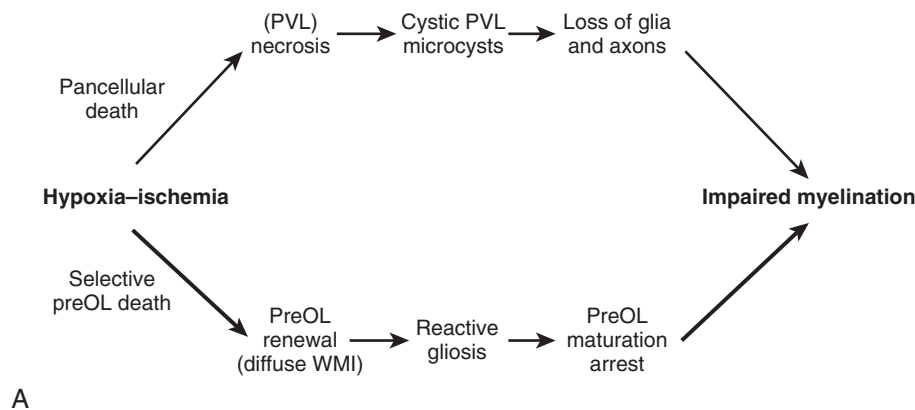
Pathogenesis of Chronic Myelination Failure and Potential Therapeutic Strategies

Myelination disturbances are related to aberrant regeneration and repair responses to acute death of preOLs. The preterm developing brain contains a population of early preOLs that serve as an apparent

reservoir of cells that can mount repair responses after WMI. In response to preOL death, early preOLs rapidly proliferate and partially differentiate to regenerate preOLs that are killed in early WMI (Segovia et al., 2008; Buser et al., 2012). Thus the preterm human white matter is capable of mounting a robust repair response to injury. Despite this inherent plasticity, the injured white matter displays robust astrogliosis, which diffusely extends beyond the core of injury as a functional “glial scar” that blocks the normal maturation of preOLs to myelinating cells (Buser et al., 2012). Hence the pathogenesis of chronic diffuse WMI leads to a disruption of the normal progression of myelination through a series of dysmaturation events that result in regeneration of preOLs that accumulate in chronic lesions but fail to differentiate to myelinating

oligodendrocytes (Fig. 60.8). By contrast, focal necrotic WMI results in a loss of both glia and axons that causes an irreversible block on myelination (see Fig. 60.8).

The mechanism by which this glial scar contributes to myelination failure is an active area of investigation. It appears likely that pronounced disturbances in the composition of the extracellular matrix result in the generation of inhibitory molecules that block preOL maturation. One widely studied molecule is hyaluronic acid, which is generated by reactive astrocytes within the glial scar and is digested by hyaluronidases that are upregulated in response to WMI (Back et al., 2005a; Preston et al., 2013; Hagen et al., 2014). These hyaluronidases generate bioactive fragments of hyaluronic acid that block preOL maturation. Preclinical studies



• **Fig. 60.8** Pathogenesis of Myelination Failure in Chronic Diffuse White Matter Injury. (A) Distinctly different pathogenetic mechanisms mediate abnormal myelination in focal necrotic lesions (periventricular leukomalacia [PVL]; *upper pathway*) versus lesions with diffuse white matter injury (WMI; *lower pathway*). When it is most severe, hypoxia-ischemia (HI) triggers white matter necrosis (*upper pathway*) with pancellular degeneration that depletes the white matter of glia and axons. Severe necrosis results in cystic PVL, whereas milder necrosis results in microcysts. Milder HI (*lower pathway*) selectively triggers early oligodendrocyte progenitor (preOL) death, but preOLs are rapidly regenerated in chronic lesions enriched in reactive astrocytes that contribute to a block in preOL differentiation to myelinating oligodendrocytes. Myelination failure in diffuse WMI thus results from preOL arrest rather than axonal degeneration, as occurs with white matter necrosis. Note that the lower pathway is the dominant one for many contemporary preterm survivors, whereas the minor upper pathway reflects the declining burden of white matter necrosis. (B) Typical appearance of normal early myelination in neonatal rodents. Axons are visualized in red and early myelination of axons is in green. (C) Arrested maturation of preOLs in a chronic white matter lesion where numerous preOLs (green) are seen, but the axons (red) are diffusely unmyelinated. Scale bars 100 μ m. HI, Hypoxia-ischemia; PreOL, oligodendrocyte progenitor; PVL, periventricular leukomalacia; WMI, white matter injury.

demonstrated that functional remyelination was promoted by a broad-spectrum hyaluronidase inhibitor (Preston et al., 2013). The downstream signaling involved in chronic WMI appears to include Wnt pathway members as well as epidermal growth factor receptor-mediated pathways (Fancy et al., 2011; Scafidi et al., 2014). It is currently unknown whether this chronic injury environment will have a negative influence on stem cell-based therapies to promote myelination (Goldman et al., 2008; Webber et al., 2009).

Since multiple signaling pathways appear to be involved in the pathogenesis of chronic WMI and myelination failure, one potential therapeutic strategy is to target multiple signaling pathways via the pleiotropic growth factor erythropoietin (Epo), which is widely used to stimulate neonatal erythropoiesis. Epo is currently under evaluation as a protective agent for WMI, because of its demonstrated actions to promote angiogenesis, neurogenesis, and gliogenesis during normal brain maturation (Juul et al., 2015). A randomized controlled trial of repeated intravenous administration of recombinant high-dose Epo to preterm neonates in the first 2 days of life was found to be safe and was associated with enhanced white matter maturation as defined by diffusion tensor imaging (Fauchere et al., 2015; O’Gorman et al., 2015), but 2-year neurodevelopmental outcomes were not improved (Natalucci et al., 2016). The Preterm Erythropoietin Neuroprotection (PENUT) trial, which randomized neonates born between 24 and 27% weeks’ gestation to high-dose Epo therapy for 2 weeks followed by maintenance Epo therapy until 32 completed weeks, is ongoing (Juul et al., 2015). Potential benefit for neonatal encephalopathy in term infants was demonstrated in a recent double-blinded, placebo-controlled trial of Epo administered with therapeutic hypothermia. A significant reduction in MRI-defined brain injury was observed in the perinatal period as was improved motor function at 1 year of age (Wu et al., 2016). Several phase III trials to test the efficacy of Epo as a neurotherapeutic for hypoxic ischemic encephalopathy (HIE) are ongoing or planned.

Preterm Cerebral Gray Matter Injury

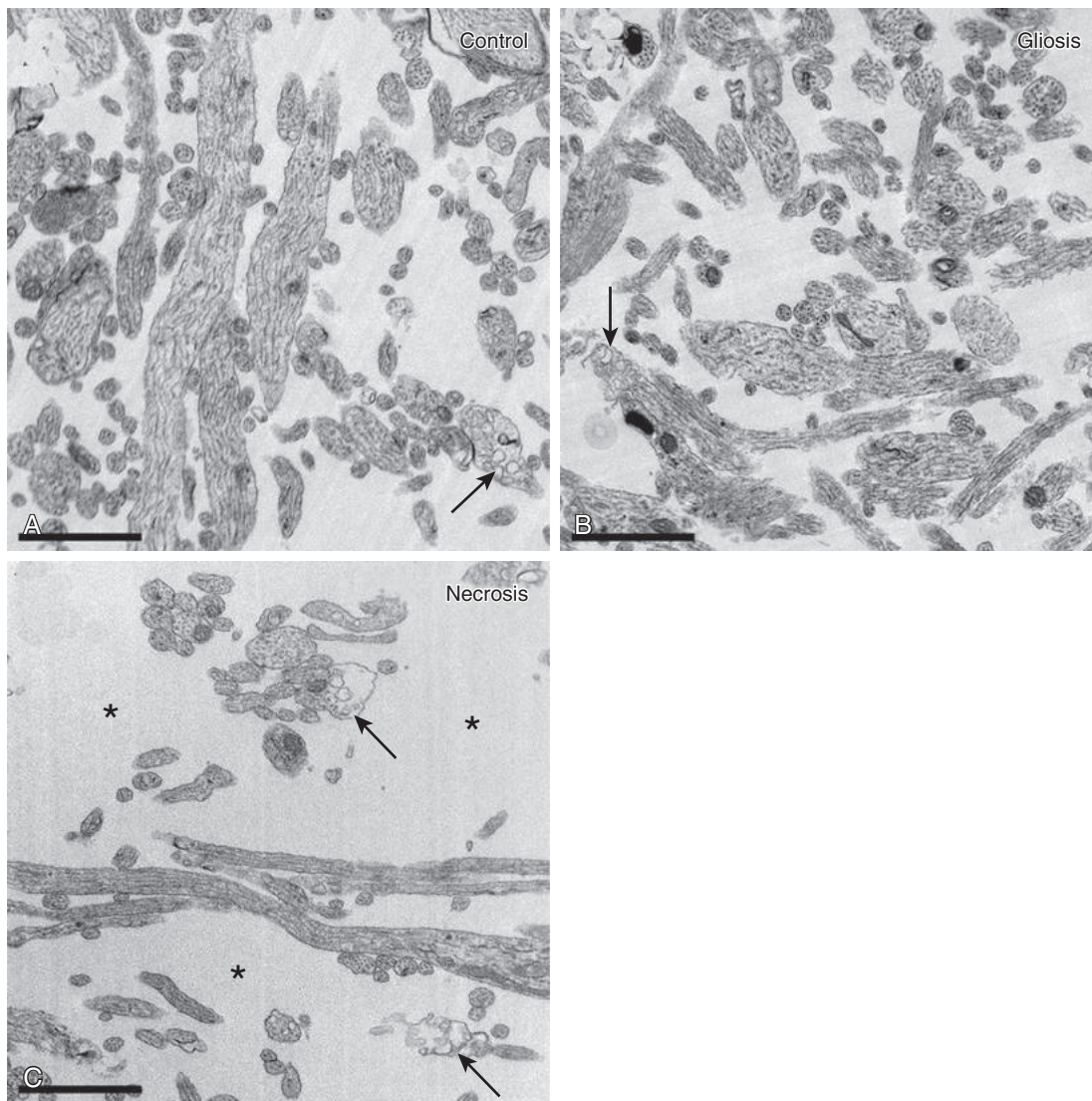
Several neuroimaging studies have identified significant reductions in the growth of human cortical and subcortical gray matter structures, including the basal ganglia, thalamus, hippocampus, and cerebellum (Srinivasan et al., 2007; Tam et al., 2009; Keunen et al., 2012; Nossin-Manor et al., 2012; Vinall et al., 2013a). Consistent with the findings of these structural studies, preterm newborns at term age also exhibit reduced functional connectivity between the cortex and thalamus on functional connectivity MRI (Smyser et al., 2010). Children and adults born preterm with normal neurocognitive function nevertheless express altered cortical activation and functional connectivity during language and visual processing (Gozzo et al., 2009; Narberhaus et al., 2009; Schafer et al., 2009; Doesburg et al., 2011, 2013). Thalamocortical connections are also disrupted in preterm newborns with WMI, resulting in visual dysfunction (Counsell et al., 2007). Altered functional connectivity in children and adolescents born preterm is thus a significant risk factor for adverse neurocognitive outcomes (Gozzo et al., 2009; Schafer et al., 2009; Mullen et al., 2011).

The relative contributions of destructive versus dysmaturational processes to these common structural, functional, and neurobehavioral abnormalities are under active investigation. Two complementary potential mechanisms may explain impaired cerebral growth in preterm survivors. The first may involve primary degeneration of neurons in multiple cortical and subcortical gray matter structures or secondary neuronal degeneration related to axonal injury in foci of white matter necrosis. Subplate neurons,

for example, were proposed to be particularly vulnerable to HI in neonatal rodents. Subplate neurons are a transient population required to establish cortical circuitry through guidance cues for thalamocortical connections. They were reduced in a number of human autopsy cases with diagnosed necrotic WMI (Kinney et al., 2012). These findings are consistent with the link between impaired thalamocortical connectivity and cognitive delays in preterm children (Ball et al., 2015). Similarly, in rodents, significant subplate neuron loss appears to occur only in association with more severe cortical neuronal degeneration (McQuillen et al., 2003; Okusa et al., 2014). Human autopsy studies also found neuronal loss in the cortex, basal ganglia, thalamus, and cerebellum in association with necrotic WMI and axonal degeneration (Pierson et al., 2007; Andiman et al., 2010; Nagasunder et al., 2011; Kinney et al., 2012). The apparent mechanism relates to retrograde degeneration of neurons in response to axonal injury in the white matter. As discussed earlier (see Fig. 60.4A–C), axonal injury is a prominent feature of WMI where necrosis is present (Kinney and Back, 1998; Haynes et al., 2008). Significant axonal degeneration in diffuse nonnecrotic WMI (Fig. 60.9) has not been observed in either human or experimental models (Buser et al., 2012; Riddle et al., 2012). Hence primary or secondary neuronal degeneration appears to occur in the setting of more severe cerebral injury, where cystic necrotic WMI would also be common.

However, as noted earlier, white matter necrotic lesions are a minor component of diffuse WMI in experimental models and in contemporary cohorts of human WMI cases (Riddle et al., 2011; Buser et al., 2012). In human autopsy cases with early diffuse WMI and preOL degeneration, neither the preterm gray matter nor the preterm white matter displayed evidence of significant oxidative stress to degenerating neurons or axons (Fig. 60.10; Back et al., 2005b). Significant early neuronal loss was not observed in association with human nonnecrotic diffuse WMI (Back, 2005; Pierson, 2007). Diffuse WMI thus triggers selective preOL degeneration but appears to spare axons and migrating neurons in the white matter.

These observations support the theory that significant neuronal loss may be uncommon in contemporary cohorts of preterm survivors. Consistent with these observations, these infants have more extensive gray matter abnormalities than “injuries” identified by MRI signal abnormalities. To define the underlying cellular responses that accompany impaired cerebral gray matter growth, we have a model of global preterm ischemia in preterm fetal sheep. This model closely reproduces the spectrum of WMI and gray matter injury in humans (Back et al., 2012). In fact, diffuse WMI in preterm fetal sheep resulted in reduced growth of the cerebral cortex (Dean et al., 2013) and caudate nucleus (McClendon et al., 2014) without loss of neurons. These experimental findings are consistent with observations of microstructural dysmaturation of the cerebral cortex in preterm neonates with restricted postnatal growth (Vinall et al., 2013a). Reduced cerebral growth was accompanied by a significant *reduction* in the complexity of the dendritic arbors of the major populations of cortical and caudate projection neurons (Fig. 60.11). Importantly, during normal fetal sheep and human development, significant cerebral cortical growth is accompanied by a pronounced *increase* in the dendritic arbor of projection neurons. These morphologic disturbances in dendritic maturation on dysmature projection neurons were accompanied by significant reductions in the density of dendritic spines, which are the major sites where synaptic transmission occurs. Indeed, electrophysiologic studies found significant persistent abnormalities in excitatory synaptic activity at 1 month after preterm HI

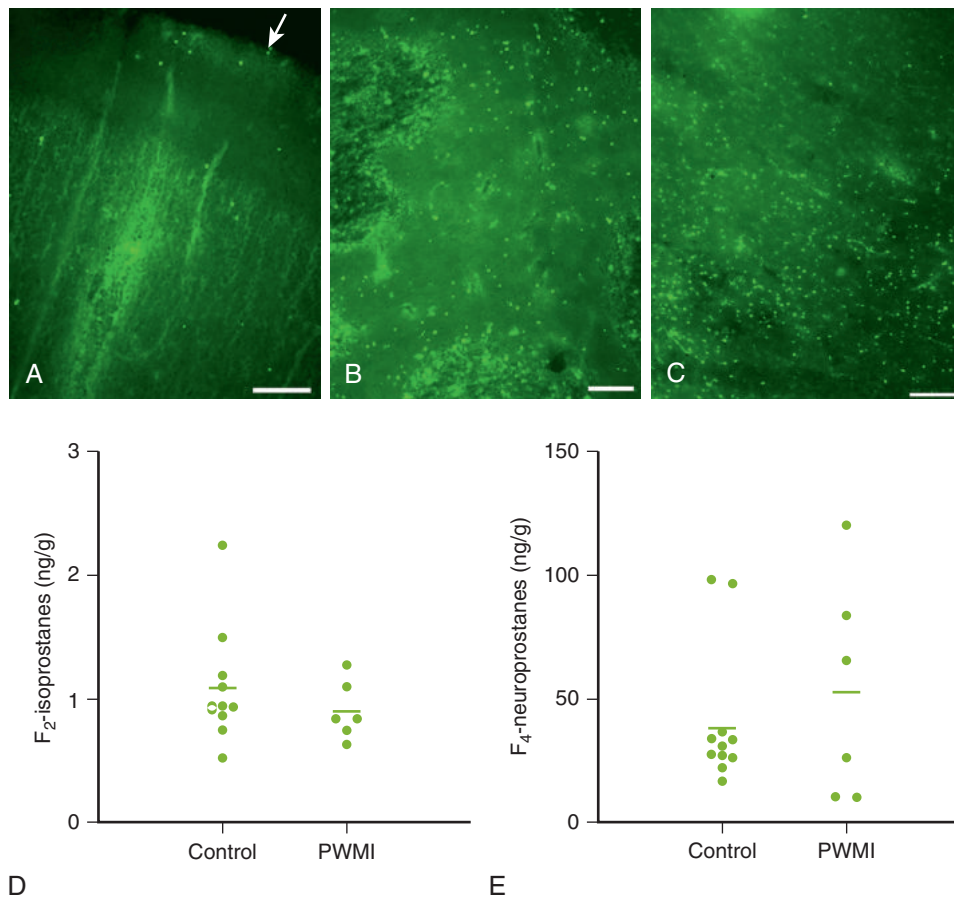


• **Fig. 60.9** Axon Degeneration Is Uncommon in Chronic Diffuse White Matter Injury. White matter injury resulted from hypoxia–ischemia in preterm fetal sheep. Injured axons were identified by electron microscopy, since optimal markers of axon degeneration have not been developed for light microscopy. (A) Normal preterm white matter contained numerous axons with normal axoplasm and intact microtubules. A swollen degenerating axon (arrow) was rarely seen in control white matter. (B) Regions of diffuse white matter injury were very similar to normal controls in the paucity of degenerating axons. (C) By contrast, regions of focal white matter necrosis (i.e., periventricular leukomalacia) contained large areas devoid of axons (asterisks) as well as numerous degenerating axons (arrows). Scale bars 2 μ m. (From Riddle A, Maire J, Gong X, et al. Differential susceptibility to axonopathy in necrotic and non-necrotic perinatal white matter injury. *Stroke*. 2013;43:178–184.)

(McClendon et al., 2014). The reduced synaptic activity of these projection neurons is thus consistent with the notion that dysmaturation of the dendritic arbor of projection neurons is accompanied by disturbances in neuronal function. Hence these findings support the observation that milder forms of cerebral injury nevertheless result in impaired cerebral growth that involves disturbances in neuronal maturation that affect large distinct populations of neurons in multiple cortical and subcortical gray matter structures.

Since disturbances in maturation of preterm neurons occur at a critical window in the establishment of human neuronal connections, even transient neuronal dysmaturation may have persistent global effects on the subsequent development of CNS circuitry.

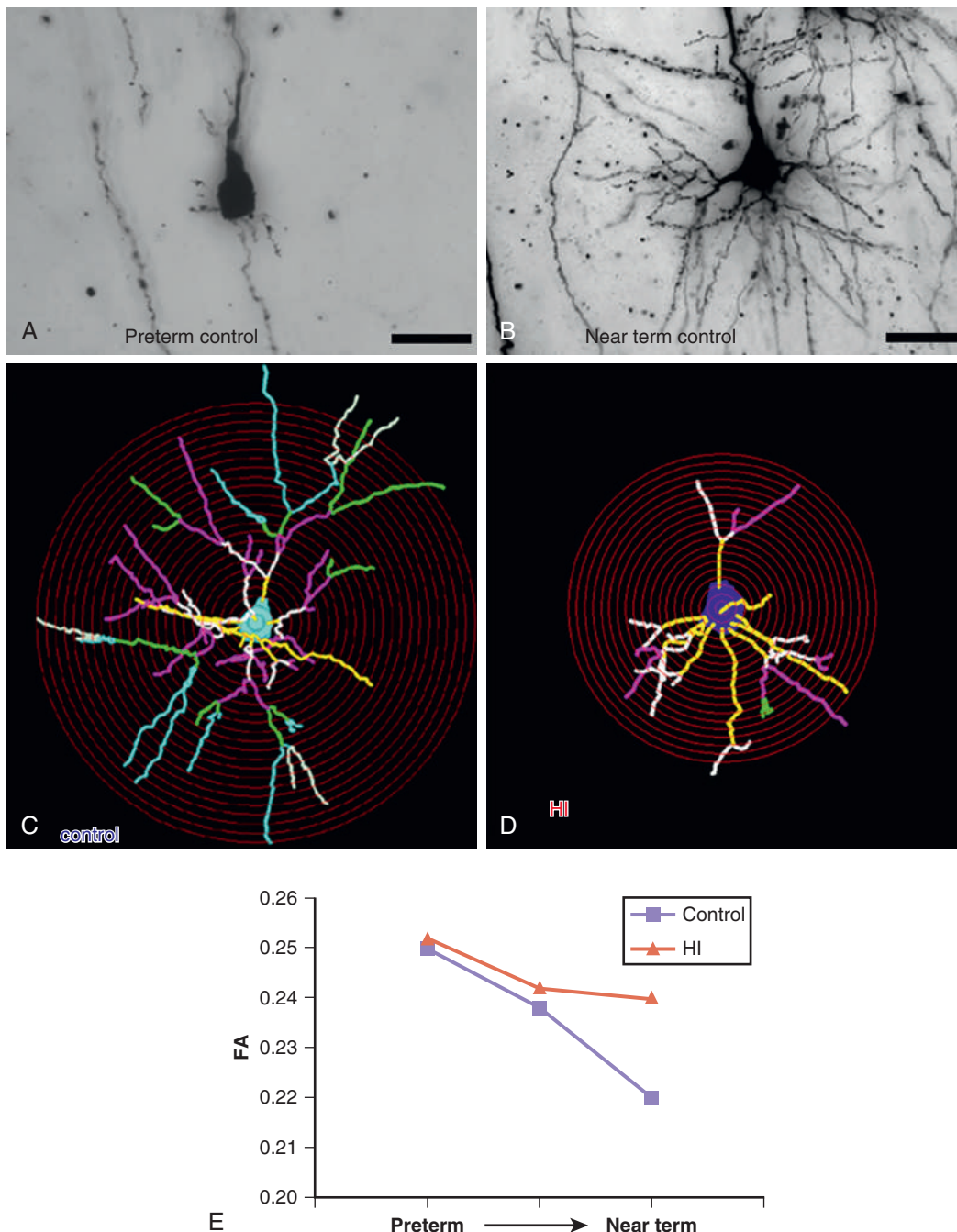
Consistent with this notion, recent neuroimaging studies have evaluated whether reduced cerebral growth is accompanied by disturbances in the microstructure of the developing cerebral cortex. During normal development, the cerebral cortex displays a normal progressive loss of cortical fractional anisotropy. This loss of anisotropy is related to the progressive maturation of cortical projection neurons (Dean et al., 2013), which are the major population of neurons in the cortex. Immature cortical projection neurons display an “anisotropic morphology” related to their simplified dendritic arbor, which causes asymmetric diffusion of water along their dendrites. As neuronal maturation progresses, the dendritic arbor assumes an “isotropic morphology” in which



• **Fig. 60.10** In contrast to oligodendrocyte progenitors, neurons and axons are markedly more resistant to oxidative injury in gray and white matter. (A) Typical paucity of cell death in the overlying cerebral cortex from a preterm infant with diffuse white matter injury at autopsy. Cell death is visualized in (A–C) by TUNEL staining, which detects DNA fragmentation of apoptotic and necrotic cells. The arrow marks the pial surface. (B, C) The periventricular white matter from the same case showed extensive oligodendrocyte progenitor degeneration. The individual degenerating cells appear as *bright green dots*. (D, E) When compared with controls, no significant oxidative injury to the cerebral cortex was observed in autopsy brains where significant preterm white matter injury was observed. Oxidative injury was measured with two very stable markers optimized for preterm human autopsy brains: F2-isoprostanes are a general marker of oxidative injury to cells and F4-neuroprostanes specifically detect injury to neurons and axons. Scale bars: (A, C) 200 μ m; (B) 100 μ m. PWMI, Preterm white matter injury. (Adapted from Back SA, et al. Selective vulnerability of preterm white matter to oxidative damage defined by F2-isoprostanes. *Ann Neurol*. 2005;58:108-120.)

water diffusion along dendritic processes is similar in all directions. Thus dysmature cortical projection neurons have a simplified dendritic arbor that results in greater “anisotropic morphology.” This reduced dendritic arbor complexity accounted for the greater anisotropy that was observed experimentally in chronic white matter lesions relative to the reduced anisotropy in normal white matter (see Fig. 60.11E). In the setting of reduced cortical growth, the gray matter of human preterm survivors was found to display increased anisotropy, consistent with greater dysmaturity (Ball et al., 2013; Vinall et al., 2013a). Disturbances in cortical fractional anisotropy were associated with impaired somatic growth (weight, length, and head circumference), even after coexisting brain injuries

on MRI (e.g., WMI) and other aspects of systemic illness (e.g., infection) had been accounted for (Vinall et al., 2013a). Improved microstructural white matter development and less IVH are linked to higher postnatal docosahexaenoic acid levels, suggesting the potential of nutrition strategies to promote optimal brain maturation (Tam et al., 2016). Hence multiple factors, including nutritional status and exposure to cerebral ischemia, may contribute to the pathogenesis of neuronal dysmaturity in preterm survivors. Further studies are needed to define how disturbances in neuronal maturation contribute to the widespread disturbances in cerebral connectivity that appear to underlie the enduring neurocognitive and behavioral deficits observed in preterm survivors.



• **Fig. 60.11** After hypoxic-ischemic white matter injury, the preterm brain is enriched in immature neurons that do not degenerate but are highly susceptible to impaired maturation that manifests itself as a less complex dendritic arbor. (A) A typical pyramidal projection neuron from the preterm cerebral cortex of a control fetal sheep that was visualized with a Golgi silver stain. Note the paucity of processes and the simplified appearance of this typical preterm neuron. (B) Neurons undergo a dramatic increase in complexity of the dendritic arbor in near term animals. (C, D) Four weeks after preterm ischemia, cortical projection neurons display disrupted maturation. A typical control neuron (C) displays highly arborized dendrites, whereas a brief preterm exposure to hypoxia-ischemia resulted in neurons with a significantly more simplified dendritic arbor (D). The reduced complexity of the dendrites can be appreciated from the overlay of the red concentric Scholl rings, which illustrate that the processes of the dysmature neurons intersect less frequently with the rings. The yellow, white, pink, green, and blue lines represent first-order, second-order, third-order, fourth-order, and fifth-order branches respectively from the soma. Note the overall reduction in the size and complexity of the branching pattern of the ischemic neurons in (D). (E) Reductions in cortical growth also manifest themselves as disturbances in cortical fractional anisotropy (FA), a magnetic resonance imaging measure of maturation and complexity of the white matter microstructure. Note the normal progressive decline in FA in controls (blue) between preterm and near term cortical development. In response to ischemia, higher cortical anisotropy (more restricted water diffusion) was observed in response to ischemia (red) relative to controls (blue), which was related to the reduced complexity of the dendritic arbor of the ischemic neurons, for example, in (D) versus controls, for example, in (C). Scale bars: (A, B) 20 μ m. FA, Fractional anisotropy; HI, hypoxia-ischemia. (From Back SA, Miller SP. Brain injury in premature neonates: a primary cerebral dysmaturation disorder? *Ann Neurol*. 2014;75: 469–486.)

Summary

Advances in neonatal neurologic intensive care have been accompanied by considerable progress in the reduction in the incidence and overall severity of IVH in preterm neonates. These advances have contributed to similar reductions in the incidence of more severe cystic necrotic cerebral WMI and gray matter injury. Unexpectedly, despite these pronounced reductions in injury severity, neurodevelopmental morbidity persists at very high rates (Synnes et al., 2010). Preterm neonates are surviving with an evolving constellation of motor, cognitive, and behavioral disabilities that appear to be related to a broad spectrum of injury that ranges

from relatively uncommon severe forms of injury to more moderate injury that is accompanied by widespread disturbances in cellular maturation. This translates to large numbers of brain cells that fail to fully mature during a critical window in development of neural circuitry. These recently recognized forms of cerebral gray and white matter dysmaturation raise new diagnostic challenges and support the urgent need for new therapeutic strategies focused on regeneration and repair to reverse the processes that promote dysmaturation.

Suggested Readings

- Back SA, Miller SP. Brain injury in premature neonates: a primary cerebral dysmaturation disorder? *Ann Neurol*. 2014;75:469-486.
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Brain Injury in the Term Infant

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KEY POINTS

- Hypoxic–ischemic encephalopathy (HIE) is a major cause of neonatal brain injury and mortality worldwide.
- Magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) are useful imaging tools to identify brain injury and help predict long-term neurodevelopmental outcomes in neonates with hypoxic–ischemic encephalopathy.
- Cerebral arterial or venous thrombosis may be the cause of unexplained seizures.
- Infections acquired in utero can be detected after birth.
- Neonates with congenital heart disease (CHD) have a high incidence of brain abnormalities based on neuroimaging and are at increased risk for neurodevelopmental impairment.

Hypoxic–Ischemic Encephalopathy

Worldwide, hypoxic–ischemic encephalopathy (HIE) is the leading cause of neonatal brain injury (Wu et al., 2004) and neonatal mortality (Black et al., 2010), with neurodevelopmental impairments (NDIs) such as intellectual disability, cerebral palsy (CP), hydrocephalus, and seizures noted in approximately 50% of survivors. Intrapartum hypoxic events caused an estimated 717,000 deaths in 2010 (~1 in 5 of all neonatal deaths worldwide) (Liu et al., 2012). Motor disabilities and long-term NDIs including cognitive, neuropsychological, educational, and behavioral problems are common in surviving neonates with moderate-to-severe HIE (Marlow et al., 2005; van Handel et al., 2007). Brain injury secondary to hypoxia ischemia during labor has been noted in up to 14.5% of term born children with CP (Eunson, 2015). The emotional impact and costs associated with medical and rehabilitative care of infants with HIE are substantial (Wang et al., 2008). The Centers for Disease Control and Prevention estimated the lifetime costs for all people with CP born in 2000 to be US\$14.7 billion (~US\$1.2 million per person with CP) (Centers for Disease Control and Prevention, 2004).

Etiology

The etiology of HIE is multifactorial, but typically results from a serious hypoxic–ischemic event (acute or prolonged) occurring before or during labor or at delivery. Hypoxia leads to cardiac and vascular compromise, with subsequent diminished cerebral perfusion

and oxidative metabolism that may result in various degrees of hypoxic–ischemic brain injury. Underlying factors associated with these hypoxic–ischemic events include maternal problems associated with poor placental perfusion (e.g., maternal hypotension, preeclampsia, chronic vascular disease), primary placental perfusion problems (tight nuchal cord, prolapsed cord, true knot, abruptio placenta, or uterine rupture), and fetal oxygenation/perfusion problems (e.g., fetomaternal hemorrhage, fetal thrombosis). The etiology of HIE is often based on speculation and is challenging to determine definitively. Placental analysis has an important role in investigating potential factors that may contribute to fetal compromise and lead to HIE (fetal thrombotic vasculopathy, chronic villitis with obliterative fetal vasculopathy, chorioamnionitis with severe fetal vasculitis, or meconium-associated fetal vascular necrosis) (McDonald et al., 2004; Redline, 2005). Neonates with HIE often have placental abnormalities, particularly neonates without clinically recognized sentinel birth events. In these neonates, severe acidosis and inflammatory placental pathology are common (Chang et al., 2012). Intrapartum inflammatory factors, including maternal fever, chorioamnionitis, and prolonged rupture of membranes, may increase the risk for HIE (Blume et al., 2008).

Diagnosis

Birth History and Delivery Room Presentation

The diagnosis of HIE is made by careful birth history, neurologic examination, and laboratory studies. Birth histories consistent with HIE include descriptions of intrauterine distress (evidenced by fetal heart rate tracing abnormalities) (Murray et al., 2009), meconium passage, or a history of difficult labor and delivery (Box 61.1). Following delivery, newborns with HIE typically have respiratory failure requiring positive pressure ventilation, often needing endotracheal tube intubation with assisted ventilation. Newborns with HIE may also develop cardiac arrest requiring cardiopulmonary resuscitation and epinephrine. Low Apgar scores (<5) at 5 and 10 minutes of age are consistent with an acute peripartum or intrapartum event resulting in HIE. Neonates with HIE who sustained an intrapartum hypoxic event typically have a fetal umbilical artery pH of less than 7.0 or base deficit greater than or equal to 12–15 mmol/L or both (Neonatal encephalopathy and neurologic outcome, 2014).

Clinical Signs and Symptoms

The timing, degree, and duration of impaired cerebral perfusion and hypoxia impact the severity of brain injury in term newborns

• BOX 61.1 A Sentinel Hypoxic or Ischemic Event Occurring Immediately Before or During Labor and Delivery

A ruptured uterus
Severe abruptio placentae
Umbilical cord prolapse
Amniotic fluid embolus with coincident severe and prolonged maternal hypotension and hypoxemia
Maternal cardiovascular collapse
Fetal exsanguination from either vasa previa or massive fetomaternal hemorrhage
A ruptured uterus

From: Executive summary: Neonatal encephalopathy and neurologic outcome, second edition. Report of the American College of Obstetricians and Gynecologists' Task Force on Neonatal Encephalopathy. *Obstet Gynecol.* 2014;123(4):896–901.

TABLE 61.1 Clinical Staging of Hypoxic–Ischemic Encephalopathy

| | Stage 1 (Mild) | Stage 2 (Moderate) | Stage 3 (Severe) |
|------------------------------|---------------------|-----------------------|----------------------------|
| Consciousness | Hyperalert | Lethargic or obtunded | Stupor or coma |
| Activity | Normal | Decreased | Absent |
| Neuromuscular control | | | |
| a. muscle tone | Normal | Mild hypotonia | Flaccid |
| b. posture | Mild distal flexion | Strong distal flexion | Intermittent decerebration |
| c. stretch reflexes | Overactive | Overactive | Decreased or absent |
| Primitive reflexes | | | |
| a. suck | Weak | Weak or absent | Absent |
| b. Moro | Strong | Weak, incomplete | Absent |
| c. tonic neck | Slight | Strong | Absent |
| Autonomic function | | | |
| a. pupils | Dilated | Constricted | Variable, unequal |
| b. heart rate | Tachycardia | Bradycardia | Variable |
| Seizures | None | Common | Uncommon |

Modified from Sarnat, HB, Sarnat M. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. *Arch Neurol.* 1976;33:696–705.

with HIE. At birth, neonates with HIE are depressed and manifest clinical symptoms consistent with neurologic injury within the first few hours after delivery. The clinical presentation of affected neonates may evolve over a period of 72 hours and is often categorized using Sarnat staging, a classification scale originally based on the assessment of neonates over 36 weeks' gestation (Table 61.1) (Sarnat and Sarnat, 1976). Neonates presenting with a Sarnat stage 1 (mild encephalopathy) appear hyperalert with wide-open eyes, often with a “stunned look” or a blank stare, and dilated pupils. These neonates typically have normal tone but a heightened Moro

reflex reactive to tactile stimuli, bright light, or loud noises. Neither clinical nor subclinical electrographic seizures are typically noted in neonates with Sarnat stage 1 HIE. Neonates with Sarnat stage 2 (moderate encephalopathy) present lethargic with low tone, a weak suck, constricted pupils, a decreased Moro reflex, and often have clinical seizures. Severely brain-injured neonates, presenting with Sarnat stage 3 (severe encephalopathy), appear stuporous with flaccid tone, intermittent decerebrate posturing (rare), absent reflexes (suck, gag, and Moro), and poorly reactive pupils. Respiratory disturbances are also common with moderate and severe HIE, with periodic breathing and apnea present in most affected neonates (Sasidharan, 1992).

HIE is the most common cause of seizures in the neonatal period, a time period characterized by a developmental mismatch between excitatory and inhibitory states of various neurotransmitter systems, including glutamate receptors (*N*-methyl-D-aspartate, [NMDA]; α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, [AMPA]; and kainite receptors) and γ -aminobutyric acid (GABA) receptors (Shetty, 2015). Clinically apparent seizures may be noted in the first 24 hours after birth. Seizures are often subtle in presentation, manifested as abnormal eye movements (e.g., tonic, horizontal eye deviation with or without jerking, eyelid blinking), oral–buccal–lingual movements (e.g., sucking or lip smacking), or limb movements (e.g., swimming motions or bicycle pedaling). Apneic events and vital sign instability may also be presentations of seizure activity. Electrographic seizures without clinical correlate may also be present, and therefore the American Clinical Neurophysiology Society has recommended conventional continuous video-electroencephalogram (cEEG) through cooling and rewarming for neonates with moderate or severe encephalopathy. Alternatively, amplitude-integrated electroencephalography (aEEG) may be used as a screening device.

Serial examinations and monitoring are important to properly assess neonates with HIE since the clinical signs and symptoms often evolve over time. Neonates with mild HIE may develop a normal examination by 12 hours of age, whereas neonates with more severe HIE remain stuporous, often with respiratory failure and dilated pupils that are fixed or poorly reactive to light. Severely affected neonates may show some improvement in consciousness after 72 hours; however, most of them will have abnormal tone (hypotonia > hypertonia), pronounced feeding difficulties (e.g., diminished suck and gag reflexes, problems with swallowing), and varying degrees of limb weakness. In general, neonates who have a quick clinical recovery and a normal examination at 1 week of age typically have normal long-term outcomes.

Laboratory and Ancillary Studies

Laboratory tests and ancillary studies are helpful in assessing newborns with suspected HIE. Multisystem organ failure is common in babies with HIE, evidenced by metabolic and hematologic abnormalities, hepatic, renal, gastrointestinal, and cardiac dysfunction. While most neonates with brain injury from intrapartum fetal asphyxia sustain multiorgan injury, in cases of severe, acute, intrapartum asphyxia, neonates may present with evidence of brain injury without multiorgan dysfunction (Martin-Ancel et al., 1995; Phelan et al., 1998). A basic metabolic panel and an arterial blood should be obtained shortly after delivery since metabolic complications such as hypoglycemia, hypocalcemia, hyponatremia, hypoxemia, and acidosis are frequently seen. A serum lactate is often elevated following hypoxia or ischemia, reflecting anaerobic metabolism of glucose for energy in the setting of decreased tissue

oxygenation. A complete blood count will facilitate assessment of leukopenia or a leukocytosis, which may suggest infection, a hematocrit will assess for anemia (e.g., in cases of known or suspected abruption), and platelet counts will assess for thrombocytopenia, which may occur with disseminated intravascular coagulopathy (DIC). Coagulation studies to assess for DIC and to guide clinical management should be performed if clinically suspected. Infants with DIC have a prolonged prothrombin time and activated partial thromboplastin time, a decreased fibrinogen level, and increased fibrin degradation products, such as D-dimer. Liver function may be abnormal and can be assessed by checking serum alanine transferase, aspartate transferase, alkaline phosphatase, lactate dehydrogenase, total protein, serum albumin, bilirubin (total and direct), and an international normalized ratio (Shastri et al., 2012). Initial newborn creatinine levels may reflect maternal values, but a rising creatinine, especially in the setting of poor urine output, is consistent with acute kidney injury. Electrocardiography, echocardiogram, and cardiac enzymes (serum creatine kinase, creatine kinase-MB isoenzyme, and troponin I) can be obtained to assess cardiac injury and/or dysfunction, which is often present with HIE (Agrawal et al., 2012; Shastri et al., 2012). Neonates with HIE often have decreased heart rate variability on continuous electrocardiography (Matic et al., 2013; Vergales et al., 2014). Lumbar puncture should be performed if the history is not consistent with perinatal distress, to rule out conditions that may mimic HIE, such as meningitis. Other causes of neonatal encephalopathy, such as sepsis, metabolic disorders, arterial or venous stroke, cerebral dysgenesis, congenital neuromuscular disorders, and inborn errors of metabolism may also present similarly to HIE and should be considered in the differential diagnosis.

Electroencephalography

In neonates with moderate or severe HIE, monitoring for the presence of electrographic seizures is strongly recommended. cEEG is currently the gold standard for identifying neonatal seizures; however, aEEG is a commonly used monitoring tool for assessing cortical electrical activity trends and detecting electrographic seizures (Shah et al., 2014). In neonates with HIE monitored by cEEG, treatment of electrographic seizures is associated with decreased seizure burden, which may be important since an increased seizure burden is associated with increased brain injury on magnetic resonance imaging (MRI) and worse long-term outcomes (Shah et al., 2014; Srinivasakumar et al., 2015). An abnormal aEEG background pattern has been shown to correlate with an abnormal MRI in uncooled patients, but therapeutic hypothermia decreases this association (Padden et al., 2015).

Neuroimaging

Cranial ultrasonography (US) can be used as a first-line imaging tool in newborns with HIE since it can be performed at the bedside in these critically unstable neonates. While subtle signs of HIE may be difficult to detect with US, in cases of severe HIE, US may demonstrate increased focal or diffuse brain parenchyma echogenicity, slit-like ventricles, and obliteration of the extracerebral cerebrospinal fluid (CSF) spaces and the interhemispheric fissure (Dinan et al., 2014). US may also provide preliminary information about brain malformations or other potential causes of neonatal encephalopathy that may present as HIE. Although US is a valuable tool to assess neonates with suspected brain injury, MRI is the study of choice for neonates with HIE. In nondysmorphic encephalopathic newborns, MRI done in the first week after birth (after rewarming, day 4–5) can assess for injury patterns consistent with HIE and

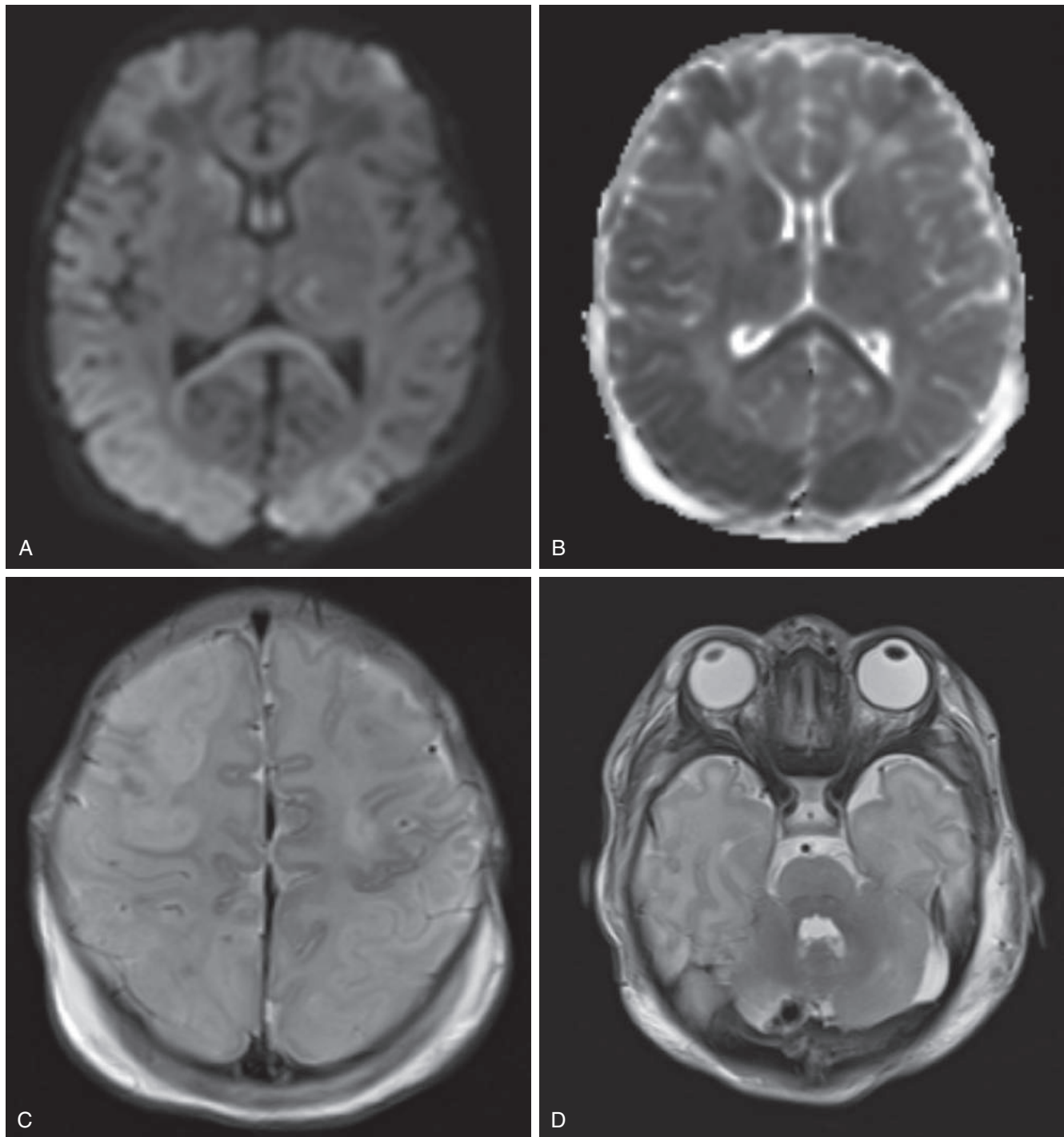
rule out other differential diagnoses, including neurogenetic, neurovascular, or inflammatory diseases requiring intervention (Miller et al., 2002). Using a combination of conventional imaging (T1- and T2-weighted images), diffusion-weighted imaging (DWI), diffusion tensor imaging, and MRI/magnetic resonance spectroscopy (MRS) can provide information on microstructure, connectivity, and brain metabolism and assist with determining the timing of brain injury in newborns with HIE (Barkovich et al., 2006; Neonatal encephalopathy and neurologic outcome, 2014). MRI performed in the newborn period has a high predictive value in neonates, with and without therapeutic hypothermia treatment, for subsequent neurologic impairment at 18 months of age (Rutherford et al., 2010a). DWI, which measures the random Brownian motion of water molecules within a voxel of tissue, can identify early ischemic brain neonatal tissue but may underestimate the final extent of basal ganglia and thalamic lesions (Rutherford et al., 2010b). As MRI techniques have continued to evolve, allowing for more insight into the extent and patterns of brain injury, computed tomography (CT) is less often used to assess newborns with suspected HIE. In addition to concerns for radiation exposure, CT lacks the sensitivity to evaluate the characteristics and extent of brain injury in term encephalopathic infants (Neonatal encephalopathy and neurologic outcome, 2014).

Near-Infrared Spectroscopy

Near-infrared spectroscopy (NIRS) allows noninvasive, continuous bedside monitoring of cerebral hemodynamics and oxygenation. In neonates with HIE treated with hypothermia, regional cerebral blood flow measured by MRI strongly correlates with mixed venous saturation values measured by NIRS (Spearman correlation coefficient $r = 0.88$; $P = .0085$) (Wintermark et al., 2014). In neonates with HIE treated with hypothermia, aEEG background patterns at 24 hours of age have a decreased positive predictive value, whereas the mean tissue oxygenation index (reflecting oxygen saturation in veins, capillaries, and arteries), measured by NIRS at 12 hours of age, correlates with outcomes, with significantly higher cerebral oxygenation levels seen in neonates who had a poor outcome compared to those with normal outcomes (Ancora et al., 2013). More studies are needed to establish the diagnostic and predictive value of NIRS in neonates with HIE treated with hypothermia.

Brain Injury Patterns

The neuropathology and pathogenesis of neonatal brain injury associated with HIE have been thoroughly described by Volpe, who recognizes four major neuropathologic varieties: (1) selective neuronal necrosis (most common), (2) parasagittal cerebral injury, (3) periventricular leukomalacia, and (4) focal ischemic brain necrosis (Volpe, 2008). Under the first category of selective neuronal necrosis, different injury patterns may occur depending on the insult severity and duration: a *diffuse pattern* secondary to a very severe and prolonged insult (all levels of neuroaxis affected), a *cerebral cortical–deep nuclear pattern* because of a moderate-to-severe and prolonged insult (involving cerebral neocortex, hippocampus, and basal ganglia thalamus), and a *deep nuclear–brainstem pattern* associated with a severe and abrupt insult (basal ganglia–thalamus–brainstem involvement). Diffuse cortical injury involving the basal ganglia and thalamus (Fig. 61.1) is more common in neonates who had an emergent cesarean delivery, an intense resuscitation, more severe encephalopathy, and an increased seizure burden and is associated with worse motor impairment and cognitive outcomes at 30 months of age (Miller et al., 2005). Parasagittal cerebral



• **Fig. 61.1** A Term Male Neonate With Severe Hypoxic–Ischemic Encephalopathy. (A) Axial diffusion-weighted image and corresponding (B) apparent diffusion coefficient map shows widespread, patchy cytotoxic edema involving both cerebral cortex, as well as deep nuclei and corpus callosum. (C) Axial T2-weighted image shows patchy cortical T2-weighted signal abnormality as well. (D) Dark signal in the lower medial right cerebellar hemisphere seen on axial T2-weighted image indicates intraparenchymal cerebellar hemorrhage. (Images courtesy of Dr. Francisco Perez and Dr. Teresa Chapman, Seattle Children’s Hospital, Seattle, WA.)

injury, also referred to as a *watershed predominant pattern*, is associated with partial prolonged intrapartum hypoxia and involves the parasagittal white matter with cortical gray matter involvement in severe insults. Parasagittal cerebral injury leads to cognitive deficits more often than functional motor deficits (Miller et al., 2005).

Mechanisms of Injury

Global ischemic events may cause decreased perfusion and injury to vulnerable watershed regions of the developing brain dependent on dual blood supply from the most distal branches of two large arteries

(e.g., anterior and middle cerebral arteries [MCAs]). Poor perfusion leads to a lack of oxygen and glucose, resulting in energy failure and loss of mitochondrial function, which is the central cellular problem underlying HIE. Without sufficient glucose and oxygen, adenosine triphosphate (ATP) production diminishes and energy-dependent membrane pumps fail, resulting in disrupted cellular ion gradients, neuronal membrane depolarization, neurotransmitter release (e.g., glutamate), increased cytosolic calcium levels, induction of destructive enzymes (e.g., phospholipases, proteases, and endonucleases), and free radical damage. Activated phospholipases (e.g., phospholipase A2) hydrolyze cellular membrane phospholipids and release free fatty acids such as arachidonic acid, which can increase glutamate release, uncouple oxidative phosphorylation, and inactivate membrane sodium/potassium-ATPase, resulting in cell damage. Proteases degrade cytoskeletal proteins, and cyclooxygenases stimulate arachidonic acid and prostaglandin production, which can perpetuate membrane peroxidation. Excitotoxic glutamate receptor-mediated injury can occur through overactivation of voltage-dependent and calcium-permeable NMDA receptors, causing neuron depolarization and intracellular calcium accumulation, which triggers apoptosis. Activation of AMPA glutamate receptors may result in necrotic death of mature oligodendrocytes, which are key myelin-producing cells (Leuchtmann et al., 2003).

If the newborn is resuscitated after a hypoxic-ischemic event, brain tissue reperfusion occurs, which may propagate a complex series of cellular events that evolve rapidly through amplifying signaling cascades that involve reactive oxygen species, reactive nitrogen species, cytokines, and caspases (cysteine-specific proteinases) that induce cell dysfunction and/or death. Cell death may occur by a number of processes such as necrosis (organelle swelling and vacuolation, cell membrane integrity loss, and random chromatin digestion), apoptosis (chromatin aggregation, nuclear and cytoplasmic condensation, formation of membrane-bound apoptotic bodies), and autophagy, a self-degradative process that involves proteolytic degradation of cytosolic components at the lysosome (large numbers of cytoplasmic vacuoles, partially condensed nuclear chromatin, and preservation of cellular integrity) (Northington et al., 2011).

Management of Hypoxic-Ischemic Encephalopathy

Initial management steps should focus on promoting adequate oxygenation, ventilation (i.e., securing an airway), and circulation. Serial monitoring of laboratory tests (e.g., blood gas, basic metabolic panel, ionized calcium, lactate, complete blood count, liver enzymes, and coagulation studies) and correction of metabolic derangements (e.g., hypoglycemia, hyperglycemia, and hypocalcemia) should be performed. Clinical and electrographic seizure activity should be assessed (e.g., with cEEG or aEEG) and controlled to limit further neurologic injury (Bjorkman et al., 2010; van Rooij et al., 2010). Inotropic agents may be required to correct hypotension, and echocardiography is useful to assess for evidence of myocardial dysfunction. Neonates with HIE often have renal injury manifesting with oliguria or anuria with elevated creatinine levels. Close monitoring of electrolytes and fluid restriction to limit edema is recommended. Hyponatremia may result from fluid overload from renal dysfunction and/or decreased urine output from syndrome of inappropriate secretion of antidiuretic hormone. Therapeutic hypothermia is standard-of-care for infants with moderate and severe HIE in developed countries and should be implemented within 6 hours after delivery, with timely referral to a center with a hypothermia program as needed. Passive cooling to $33^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$

can be done before active head or whole-body cooling, but active cooling during transport is recommended if possible. Cooling should be done at centers with available support from neurology (access to cEEG or aEEG), neuroradiology (access to MRI), and other services such as nephrology, gastrointestinal, cardiology, extracorporeal life support (ECLS), physical therapy, and nutrition services.

Prognosis

Accurate prediction of short-term and long-term outcomes in babies with HIE remains a challenge and requires further study, particularly in the setting of therapeutic hypothermia (Ahearne et al., 2016). An early normal neurologic examination (in uncooled neonates) is associated with favorable developmental outcomes at 2 years of age, whereas an early abnormal neurologic examination has limited long-term predictability. An abnormal examination noted at the time of discharge correlates significantly with adverse outcomes (Murray et al., 2010). Similarly, a normal MRI in the first week is associated with normal outcomes, while outcomes with watershed or basal ganglia injury on MRI are more uncertain. Deep gray matter injury on MRI correlates best with poor outcomes. MRS can also be helpful in prognosticating, as a low *N*-acetylaspartate (NAA) peak and high lactate peaks correspond to severe injury. Research to discover predictive HIE-associated biomarkers is ongoing.

Apgar Score

The Apgar score is subjective and cannot distinguish the severity of HIE. Apgar scores have limited utility in predicting long-term outcomes, as illustrated by the National Institute of Child Health and Human Development Neonatal Research Network whole-body cooling randomized controlled trial follow-up study, in which 1 in 5 babies with an Apgar score of 0 at 10 minutes survived to school age without moderate or severe disability (Natarajan et al., 2013).

Seizures

An increased seizure burden and excessive EEG discontinuity correlate with worse brain injury on MRI and are predictive of abnormal neurodevelopmental outcome in neonates treated with therapeutic hypothermia (Briatore et al., 2013; Dunne et al., 2017). Postnatal evaluation 3–6 hours after birth of term newborns with suspected HIE using aEEG appears to reliably predict neurodevelopmental outcome. Flat tracing (very low voltage, isoelectric tracing with activity below $5\ \mu\text{V}$), continuous extremely low voltage (around or below $5\ \mu\text{V}$), and burst-suppression (discontinuous background pattern with periods of inactivity intermixed with higher amplitude bursts) patterns are predictive of poor long-term outcomes (follow-up range of 12 months to 6 years) (Toet et al., 1999). During therapeutic hypothermia, from 48 hours of age, aEEG shows accurate prediction of long-term (18–24 months) outcomes (Cseko et al., 2013). Return of sleep-wake cycling on aEEG is a favorable prognostic indicator (Osredkar et al., 2005; Cseko et al., 2013). A normal EEG pattern at 6 hours of age has a 100% positive predictive value for a normal outcome at 2 years (Murray et al., 2009).

Magnetic Resonance Imaging and Spectroscopy

MRI and MRS are useful in predicting outcome in neonates with HIE (Goergen et al., 2014; Hayakawa et al., 2014; Nanavati et al., 2015). Ischemia patterns on MRI (done in the first week after birth) correlate with neurodevelopmental outcomes at 2 years of age; favorable outcomes are associated with watershed patterns, whereas central and diffuse patterns of ischemia are associated with unfavorable outcomes (Twomey et al., 2010). Diffusion tensor

imaging facilitates evaluation of the location, orientation, and integrity of white matter pathways by determining water molecule diffusion patterns in white matter tracts (Choudhri et al., 2014; Massaro et al., 2015). Diffusion tensor imaging abnormalities (at median of 8 days of age) of the corpus callosum and corticospinal tract in neonates with HIE treated with hypothermia predict poorer cognitive and motor performance, respectively, in early childhood (15 to 21 months of age). Higher basal ganglia and thalamic perfusion demonstrated at postnatal age of 4.5 days (range 2–7 days) with arterial spin labeling MRI, a noninvasive technique to evaluate brain perfusion, are associated with adverse outcomes at 9–18 months of age in neonates who had HIE (De Vis et al., 2015). Additional studies correlated with long-term outcomes are needed to clarify the utility of newer MRI techniques for diagnostic, prognostic, and therapeutic purposes.

Hypoxic-ischemic injury may lead to neuronal death with associated decreased NAA levels and impaired mitochondrial and oxidative metabolism associated with increased brain lactate levels. Based on metaanalysis of 32 studies, involving 860 infants with HIE, deep gray matter (thalamic or basal-ganglia) lactate/NAA is the most accurate quantitative magnetic resonance biomarker for predicting neurodevelopmental outcomes (at ≥ 12 months of age; Thayil et al., 2010).

Outcomes

Therapeutic hypothermia for neonates with moderate and severe HIE is now standard-of-care in developed countries, and this treatment has positively impacted neonatal outcomes (Jacobs et al., 2013). In term and late preterm infants with moderate-to-severe encephalopathy and evidence of intrapartum asphyxia, therapeutic hypothermia decreases the combined outcome of mortality or major neurodevelopmental disability to 18 months of age from 61% (409/666 control newborns) to 46% (312/678 treated newborns) (typical risk ratio 0.75, 95% confidence interval (CI) 0.68–0.83; typical risk difference -0.15 , 95% CI -0.20 to -0.10) (Gunn et al., 1998; Eicher et al., 2005; Gluckman et al., 2005; Shankaran et al., 2005; Azzopardi et al., 2009; Simbruner et al., 2010; Zhou et al., 2010; Jacobs et al., 2011). One out of 7 neonates with moderate-to-severe encephalopathy will benefit from therapeutic hypothermia (95% CI 5–10). Unfortunately, despite treatment with therapeutic hypothermia, 44% of neonates with moderate-to-severe encephalopathy will still die or have major long-term neurodevelopmental disabilities. Neonates with mild HIE usually have no long-term deficits, whereas those with severe HIE often die or are severely impaired with spastic quadriplegia, cortical visual impairment, and seizure disorders, despite cooling. Neonates with moderate HIE have outcomes ranging from normal to severely abnormal. A normal clinical examination at 7–10 days is encouraging for a normal long-term outcome, whereas a severely abnormal examination is concerning for a poor long-term prognosis. Early EEG at the end of the first week of age can further assist in predicting outcomes at 2 years of age (Biagioni et al., 2001).

Since prevention of HIE has not been successful, adjunctive treatments to therapeutic hypothermia are urgently needed to improve outcomes. At a global level, other interventions that might be used in low-income and middle-income countries are also needed, as therapeutic hypothermia is often not available or may even be harmful (Robertson et al., 2008). Hopefully, discovery of better biomarkers will help identify, provide insight into therapeutic options, and accurately predict outcomes in neonates with HIE.

Perinatal Arterial Ischemic Stroke

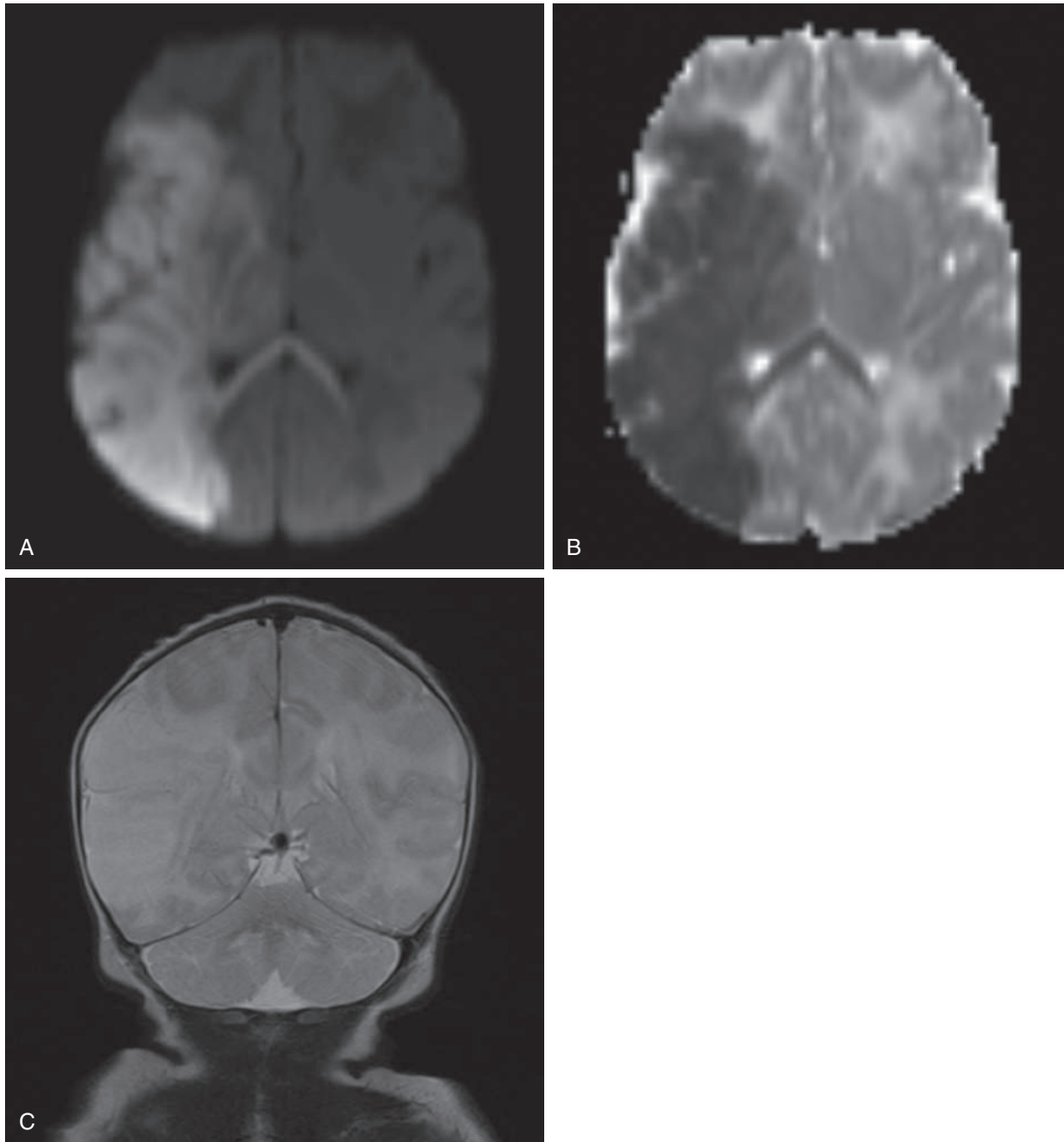
Perinatal arterial ischemic strokes (PAIS) are focal or multifocal arterial thrombosis or embolization occurring between 20 weeks' postmenstrual age and 28 days' postnatal life (van der Aa et al., 2014). The reported incidence varies between 1 in 1600 and 1 in 5000 live births (Lee et al., 2005; Laugesaa et al., 2007). The true incidence is likely to be higher given that most of the studies were retrospective and MRI was not routinely used. PAIS is more common in males and non-Hispanic black ethnicity and occurs most often in the left MCA distribution. Risk factors for PAIS include maternal primiparity, preeclampsia, prolonged rupture of membranes, chorioamnionitis, and cord anomalies (Harteman et al., 2012). Presence of more than one of these risks factors can increase the probability of PAIS to 1 in 200 (Lee et al., 2005). Complicated deliveries involving emergency cesarean section or instrumentation have also been associated with PAIS.

The pathogenesis of PAIS is not well understood. Physiologic changes in the mother during pregnancy may cause a hypercoagulable and prothrombotic state. Fetuses are also at increased risk for developing clots as physiologic polycythemia leads to hyperviscosity, and there is a depressed anticoagulant activity present. These factors, coupled with the placenta having areas of reduced blood flow, increase the proclivity for thrombotic generation on the fetal side of the placenta. These thrombi will travel via the umbilical vein and are poised to pass through the patent foramen ovale to enter the systemic and most importantly the cerebral arteries. Other fetal conditions leading to increased risk of PAIS include twin pregnancies, twin-to-twin transfusion, arteriovenous malformations, prolonged neck traction, and cardiac defects (Golomb et al., 2006; Benders et al., 2007). PAIS lesions are usually singular (70%) and isolated to the anterior circulation (71%), posterior circulation (7%), or, less commonly, both (20%) (Kirton et al., 2011). Strokes are most commonly left sided (51% all strokes, 73% of all anterior strokes), right sided (9%), or bilateral (20%) (Kirton et al., 2011).

Most infants with PAIS are asymptomatic at birth, but signs of acute illness are seen in 25% of infants with PAIS (Kirton et al., 2011). In the week following birth, most newborns with PAIS become symptomatic, with the most prevalent symptom being seizures (up to 70%–90%) (Hayward and Adappa, 2014). Diffuse neurologic signs are more common than focal signs, with abnormal tone and depressed level of consciousness more common than hemiparesis. Nonspecific symptoms include breathing and feeding difficulty.

Given that the most common presenting symptom is seizures, the work-up should begin with ruling out hypoglycemia, hypocalcemia, electrolyte disorders, infection, and metabolic syndromes. MRI is the gold standard test for the detection of PAIS. Sequences to be used include T1- and T2-weighted images, DWI, and MRI angiography. Fig. 61.2 demonstrates MRI of a term male with a PAIS involving the right MCA. Of these sequences, the most sensitive is DWI performed in the first week of life. The area of infarction appears as a zone of high intensity on DWI and low intensity on the apparent diffusion coefficient map. After the first week the ischemic tissue appears to normalize, even though the area continues to be ischemic, a process known as pseudonormalization. Reduced contrast between cortex and white matter can be seen in the first 48 hours on T2-weighted imaging. Lower signal intensity will be seen on T1-weighted images. As time from the injury passes, cortical highlighting develops.

EEG should be performed to help localize the origin of the seizures as well as management of the seizures. Cranial US is widely



• **Fig. 61.2** A 4-Day-Old Term Male Neonate With Right Middle Cerebral Artery Stroke. (A) Axial diffusion-weighted image shows abnormal bright signal throughout the right cerebral hemisphere in the middle cerebral artery territory. The corpus callosum is also involved. (B) Apparent diffusion coefficient map shows dark signal, confirming this is true cytotoxic edema. (C) Coronal T2-weighted image shows abnormal loss of gray-white matter differentiation, consistent with a subacute time frame (at least 6 hours) for the injury. A head magnetic resonance angiogram showed no definite stenotic lesion (not shown here). (Images courtesy of Dr. Randolph Otto and Dr. Teresa Chapman, Seattle Children's Hospital, Seattle, WA.)

available in most centers that do not have MRI capability. The sensitivity of US improves from 68% in the first few days to 87% in the first week (Cowan et al., 2005). Because strokes may occur in the posterior circulation, posterior fontanelle imaging is necessary. Unlike MRI, cranial US is operator dependent, and thus detection rates will vary between centers. Use of CT is limited and discouraged because of poor sensitivity and high radiation dose.

The optimal thrombosis evaluation is currently debated, and one review found the studies to be “contradictory or inconclusive due to lack of statistical power” (Kenet et al., 2010). Rates of possible thrombophilia as an underlying cause of PAIS vary in the literature up to as high as 68%. However, the International Pediatric Stroke Study found that only 19% of PAIS infants were diagnosed as having possible increased lipoprotein(a) level, methylene

tetrahydrofolate reductase mutations, elevated β_2 -glycoprotein level, factor V Leiden, prothrombin gene 20210A, low anti-thrombin III level, antiphospholipid antibodies, plasminogen activator inhibitor, or low protein S level (Kirton et al., 2011).

Therapy for PAIS is largely supportive. Anticonvulsant therapy has the best evidence for use, but most infants will not require long-term therapy, and many experts increasingly suggest early discontinuation (Kirton et al., 2011). Anticoagulation is currently debated in the literature, and the type of antithrombotic medication used varies from country to country. There is most consensus for use of anticoagulant therapy in infants with congenital heart disease (CHD) and PAIS, as they are at increased risk of ongoing clot formation (Monagle et al., 2008).

Neurodevelopmental outcomes depend on the size and location of the stroke. Mortality in PAIS is very low. PAIS occurring in the MCA territory may result in a hemiplegia rate of 50% (Chabrier et al., 2011). While most infants will be discharged seizure free, up to 50% will later develop epilepsy. Cognitive and language deficits are more common in children with hemiplegia or epilepsy following PAIS (Ricci et al., 2008). Visual function is also altered by PAIS. Behavioral problems may also be seen.

Cerebral Sinus Venous Thrombosis

Cerebral sinus venous thrombosis (CSVT) has a reported incidence of 0.6–12 per 100,000 live births (deVeber et al., 2001; Berfelo et al., 2010). The wide range of reported incidence is likely due to variable awareness among clinicians and therefore variable use of neuroimaging to detect CSVT. Impaired venous drainage from CSVT causes increased venous pressure that can result in increased capillary hydrostatic pressure. This elevated pressure leads to vasogenic edema and hemorrhagic infarction in the distribution of the cerebral sinus venous. Maternal risk factors for CSVT include preeclampsia, chorioamnionitis, and gestational diabetes. Complicated delivery, meconium aspiration, and the need to be intubated have also been associated with CSVT. Similar to PAIS, CHD is a major risk factor. Postnatal conditions such as meningitis, sepsis, dehydration, and ECLS are associated with CSVT. Presenting signs and symptoms are subtle and nonspecific: seizure is the most common symptom, but respiratory distress, lethargy, apnea, and poor feeding can also be present with CSVT.

Neuroimaging is crucial to the diagnosis of CSVT. The superior sagittal sinus has the highest rate of thrombus, followed by straight and transverse sinuses. Most neonates have multiple sinuses involved. MRI is the gold standard, especially when the MRI venography (MRV) sequence is performed (Fig. 61.3). MRI serves two purposes: (1) to document the sinus involved and (2) to identify associated lesions. CSVT can sometimes be detected by T1- and T2-weighted images. Restricted diffusion on DWI will be present in areas affected by CSVT. MRV allows the clinician to see the venous system without the use of contrast.

US can be used to diagnose CSVT; however, it has a high false-negative rate and requires operator experience in the use of Doppler to measure venous sinus flow (Miller et al., 2012). US is able to detect intraventricular and thalamic hemorrhages with ease, and CSVT should be ruled out in term neonates with unexplained intraventricular hemorrhage (Wu et al., 2003). CT has a high false-negative rate and, given the high radiation, is generally not preferred (Nwosu et al., 2008). As with PAIS, thrombophilia evaluation should be performed and should include protein-based assays as well as genetic testing. While the genetic testing can be done acutely, the protein-based studies are most frequently

performed at around 6 months of age because of the large volume of blood required for testing and also because consumption of factors during acute thrombosis may lead to inaccurate results.

Treatment of CSVT should target the inciting factor: for instance, treating dehydration, meningitis, or CHD. Thrombus progression is seen in 25% of neonates, and no complications have been seen with anticoagulation for infants with associated intracranial hemorrhages (Moharir et al., 2010). Therefore most practitioners will treat CSVT with unfractionated heparin or low-molecular-weight heparin in neonates without hemorrhage. Those with hemorrhage that show progression of the CSVT at 5–7 days should have anticoagulation therapy started. Treatment duration is between 6 and 12 weeks.

Few studies of CSVT have been performed, and follow-up data are limited. Early mortality figures showed death rates between 2% and 5%; however, more recent studies with improved detection rates of CSVT show mortality rates between 19% and 25% (Wasay et al., 2008). Of the survivors, 60%–80% develop motor impairment, including CP, cognitive delay, and epilepsy.

Neonatal Hypoglycemia

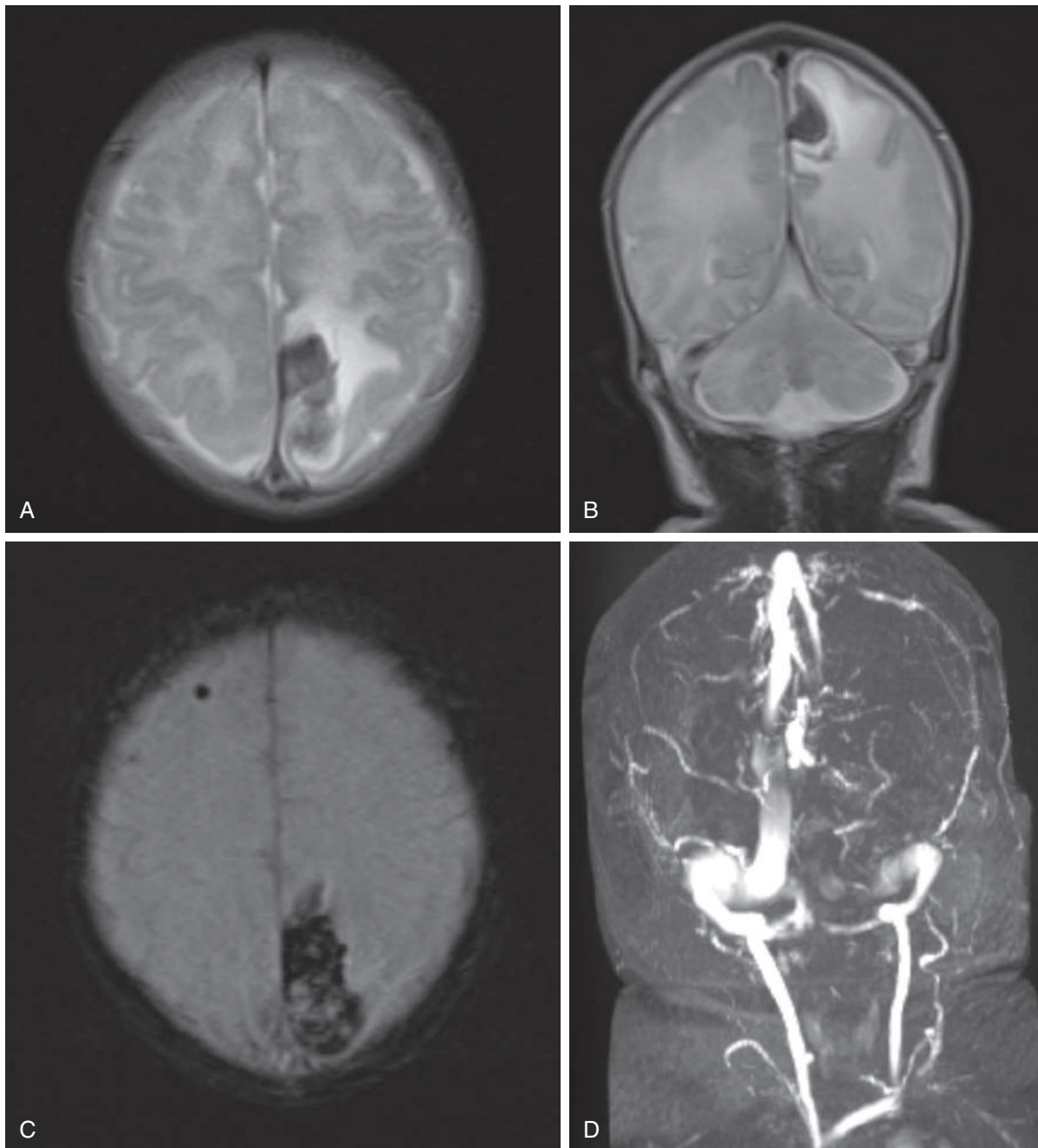
The exact level or duration of hypoglycemia that will cause injury is unknown. The literature uses variable definitions for hypoglycemia that further complicate the picture. Blood glucose values in the fetus are 70% of maternal levels and rapidly fall in the first hour after birth to as low as 25 mg/dL. The American Academy of Pediatrics guidelines for asymptomatic hypoglycemia has a cutoff of 45 mg/dL (Committee on Fetus and Newborn and Adamkin, 2011). However, the Pediatric Endocrine Society has recently published that glucose levels should be kept at greater than 50 mg/dL for the first 48 hours after birth, then at greater than 60 mg/dL after 48 hours (Thornton et al., 2015). Symptomatic hypoglycemia can manifest as cyanosis, tremors, apnea, seizures, change in consciousness, irritability, and feeding problems. Neonatal risk factors for hypoglycemia include small for gestational age, maternal diabetes, perinatal asphyxia, respiratory distress, sepsis, and congenital metabolic disorders.

The proposed mechanism of hypoglycemia-induced injury is by increased excitatory neurotransmitter levels that lead to excessive NMDA receptor activation. This activation induces increased intracellular sodium and calcium concentrations. Increased calcium influx into cells causes activation of phosphatidases and proteases, which alters mitochondrial function and generates free radicals. ATP production is hampered, which leads to apoptosis and neuronal necrosis.

Hypoglycemia may lead to brain swelling, necrosis, and white matter demyelination, especially in areas rich with NMDA receptors. Brain regions affected include the parieto-occipital cortex, corpus callosum, basal ganglia, thalamus, and posterior limb of the internal capsule. Long-term sequelae associated with hypoglycemia include visual impairment, epilepsy, and cognitive deficits. However, a recent study showed no difference in neurocognitive outcomes at 2 years of age between hypoglycemic and nonhypoglycemic neonates (McKinlay et al., 2015). The degree of injury is likely directly related to depth and duration of hypoglycemia and the presence of any comorbidities, especially HIE.

Central Nervous System Infections

Maternal infections during pregnancy and labor can affect the fetal/neonatal brain. There is a fourfold increased risk of CP in



• **Fig. 61.3** A 15-Day-Old, Former 34 Weeks' Gestation, Preterm Male Neonate With Hypoxic–Ischemic Encephalopathy and Dural Venous Sinus Thrombus. (A) Axial and (B) coronal T2-weighted images show abnormal dark signal in the medial left parietal lobe parenchyma, with surrounding high signal. (C) Axial susceptibility weighted imaging shows dark signal and blooming artifact, consistent with blood products. (D) Coronal maximum intensity projection image from a magnetic resonance venogram shows diminished flow in the lower sagittal sinus and left transverse sinus consistent with thrombus. Findings are consistent with hemorrhaging venous infarct and surrounding vasogenic edema. (Images courtesy of Dr. Jason Wright and Dr. Teresa Chapman, Seattle Children's Hospital, Seattle, WA.)

pregnancies complicated by chorioamnionitis. Pregnancies with chorioamnionitis are more likely to have additional neonatal complications such as HIE, meconium aspiration leading to pulmonary hypertension and hypoxia, sepsis, and neonatal meningitis. Pulmonary hypertension as a result of pneumonia can cause CP in 30% of term neonates as a result of synergistic injury from hypoxia and sepsis.

Meningitis

Neonates are at greater risk of meningitis than other age groups because of inefficiency of the alternative complement pathway, deficient migration and phagocytosis of neutrophils, and decreased T-cell and B-cell activity, leaving them at risk for infections with encapsulated bacteria. Group B streptococcus (GBS) is the leading

cause of meningoencephalitis in the term newborn period, followed by *Escherichia coli* and *Listeria monocytogenes*. The incidence of bacterial meningitis is 0.3 per 1000 live births. Herpes simplex virus (HSV) incidence varies from 1 per 5000 to 1 per 26,000 live births. Emerging pathogens include enterovirus, human parechovirus-3, and *Enterobacter sakazakii*.

Symptoms in the first 48 hours of age include temperature instability, apnea of bradycardia, hypotension, feeding difficulty, hepatic dysfunction, and irritability alternating with lethargy. In contrast, neurologic symptoms are more commonly seen after 48 hours of age. These include stupor, irritability, seizures, bulging anterior fontanelle, gaze deviation, cranial nerve palsies, and extensor posturing. Unlike children and adults, nuchal rigidity is not common. Any neonates with signs of sepsis or nonspecific symptoms in which meningitis might be a cause should have a lumbar puncture to examine the CSF. Many experts suggest ordering a HSV polymerase chain reaction (PCR) on all neonates with suspected meningitis. Up to one-third of infants who have negative blood cultures had positive CSF cultures, suggesting that cases of meningitis may be missed if lumbar punctures are not performed (Stoll et al., 2004). The classic findings of decreased CSF glucose, elevated CSF protein, and pleocytosis are seen more often with Gram-negative meningitis, late Gram-positive meningitis, and viral meningitis, especially caused by HSV. No single CSF parameter exists that can reliably exclude the presence of meningitis in a neonate (Garges et al., 2006).

The choice of an antibiotic regimen should be based on the likely pathogen, the local patterns of antibacterial drug sensitivities, and the policies of the hospital. Cefotaxime, in addition to ampicillin, is the usual empiric coverage used due to penetration of the blood-brain barrier by cephalosporins, but some experts suggest that ampicillin plus an aminoglycoside is a better choice because of synergy. Treatment duration is usually 14–21 days, and most experts suggest a repeat lumbar puncture 2–3 days into treatment. Corticosteroids have been shown to reduce long-term sequelae, particularly hearing loss, in older infants with *Haemophilus influenzae* type B meningitis and *Streptococcus pneumoniae* infection; however, use of corticosteroids is not recommended for neonates with meningitis (Chaudhuri, 2004). Acyclovir is the empiric treatment of choice for suspected viral meningitis.

Complications associated with meningitis include cerebral edema, hydrocephalus, hemorrhage, abscess formation, infarction, and ventriculitis. Cerebral edema occurs from direct cytotoxic cell injury, vasogenic changes, and inappropriate antidiuretic hormone secretion. Obstruction to CSF drainage can lead to hydrocephalus in up to 24% of infants. Hemorrhage and infarction can be caused by venous or arterial thrombosis. Cerebral abscess occurs in as many as 13% of neonates with meningitis and should be considered with new seizures, signs of elevated intracranial pressure (ICP), or new focal neurologic signs, and brain imaging with contrast is essential for making the definitive diagnosis (Pong and Bradley, 1999). Ventriculitis occurs in as many as 20% of neonates with meningitis and results in sequestration of infection to areas that are poorly accessible to systemic antimicrobial drugs (Unhanand et al., 1993). Inflammation of the ependymal lining of ventricles often obstructs CSF flow. Failure to respond to appropriate antibiotic therapy and signs of elevated ICP may suggest the diagnosis of ventriculitis, in which case intraventricular administration of antibiotics may be necessary.

Survivors of neonatal meningitis are at significant risk for moderate-to-severe disability, including problems with language,

motor function, hearing, vision, cognition, and epilepsy. As many as 20% of children identified as normal at 5-year follow-up may have significant educational difficulties lasting into late adolescence. Poor prognostic indicators include low birth weight, prematurity, significant leukopenia or neutropenia, high levels of protein in the CSF, delayed sterilization of the CSF, and coma. Seizures lasting longer than 72 hours and the need for inotropes predict moderate-to-severe disability or death with 88% sensitivity and 99% specificity (Klinger et al., 2000). In a prospective sample of more than 1500 neonates surviving to the age of 5 years, the prevalence of motor disabilities (including CP) was 8.1%, learning disability occurred in 7.5%, seizures in 7.3%, and hearing problems in 25.8% (Bedford et al., 2001). No problems were reported in 65% of infants who survived GBS meningitis and in 41.5% of those who survived *E. coli* meningitis.

Herpes Simplex Virus

The majority of HSV infections result from intrapartum transmission, with occasional postnatal exposure occurring through oropharyngeal shedding or cutaneous shedding of virus by parents or hospital contacts. Most neonates will present in the second postnatal week. Neonates may present with pallor, irritability, high-pitched cry, respiratory distress, fever, jaundice, seizures, hepatic dysfunction, and DIC (Kimberlin, 2004). Work-up should include HSV PCR of CSF, cultures of fluid from vesicles, and swabs of the nasopharynx, conjunctiva, and rectum. Central nervous system (CNS) infection with HSV can lead to necrotizing lesions, which result in microcephaly, porencephalic cysts, and CP. Mortality among neonates with HSV infection of the CNS is 15%. The two HSV serotypes (HSV-1 and HSV-2) carry the same risk of mortality. However, HSV-2 is more commonly associated with morbidity, including CP, intellectual disability, seizures, microcephaly, and ophthalmic defects (Kimberlin, 2004). HSV-1 infection tends to be more diffuse in neonates, instead of the medial temporal and insular involvement seen with children and adults. Although the use of acyclovir has reduced the morbidity and mortality associated with HSV infection, neurologic sequelae are likely in 50% of neonates with HSV meningitis (Kimberlin, 2004).

Toxoplasmosis

The prevalence of toxoplasmosis is 1 per 1000 live births and is caused by *Toxoplasma gondii* (Pickering, 2012). This parasite can pass the placenta from an infected mother to infect the fetus. Pregnant women are cautioned not to be exposed to cat feces and to cook meat until it is well done. Granulomatous necrosis, hydrocephalus, and calcifications in the basal ganglia and periventricular regions result from CNS infection with *T. gondii* and can be present on prenatal US. In the absence of hydrocephalus, microcephaly is also reported. Neonates can present with enlarged liver or spleen, eye drainage, feeding difficulties, intrauterine growth restriction, seizures, or tiny red spots or bruising. Congenital toxoplasmosis treatment consists of pyrimethamine, sulfadiazine, and leucovorin for up to 1 year (Petersen, 2007). Despite adequate treatment, 30% of infants with toxoplasmosis will have seizures, motor abnormalities, or blindness.

Cytomegalovirus

Cytomegalovirus (CMV) is the most common congenital viral infection, with an incidence of 0.6–0.75 of all deliveries in the

United States (Swanson and Schleiss, 2013). Transmission to the fetus can occur in primary infections of the mother or reactivation of a latent infection at any gestational age. Postnatal infection rarely causes significant illness in term newborns and is not associated with long-term disability. Congenital infections may result in intrauterine growth restriction, thrombocytopenia, hydrops, jaundice, hepatosplenomegaly, microcephaly, periventricular calcification, seizures, and sensorineural hearing loss. About 40%–58% of newborns who are symptomatic at birth go on to develop sequelae, including sensorineural hearing loss, intellectual disability, seizure disorder, CP, visual deficits, or developmental delay (Boppana et al., 1992; Dollard et al., 2007).

Diagnosis in the neonate can be made by PCR, culture, or antigen testing (pp65 antigen) of urine, blood, or saliva within the first 3 weeks of life (Bhatia et al., 2010). Antibody titers cannot reliably indicate the diagnosis, as maternal CMV immunoglobulin G crosses the placenta, and neonates mount weak immunoglobulin M responses. Complete blood count and liver function tests may reveal pancytopenia and hepatitis, and coagulation studies may be abnormal. Renal function is checked as a baseline before beginning treatment with ganciclovir. Audiologic assessment should be performed on all infants with congenital CMV infection, as sensorineural hearing loss may be absent at birth, and frequent evaluations are required throughout childhood to evaluate for the possibility of hearing deterioration (Dahle et al., 2000).

Sensorineural hearing loss can be prevented by early treatment with ganciclovir. One randomized study indicated that 84% of ganciclovir recipients either had improved hearing or maintained normal hearing between baseline and 6 months. In contrast, only 59% of control patients had improved or stable hearing (Kimberlin et al., 2003). Results were even more encouraging when the study and control groups were compared for subsequent maintenance of normal hearing, as none of the ganciclovir recipients had a worsening in hearing between baseline and 6-month follow-up, compared with 41% of control patients. The intravenous ganciclovir therapy group had fewer developmental delays at 6 and 12 months compared with untreated infants (Oliver et al., 2009).

Zika Virus

The World Health Organization declared the rapidly spreading epidemic of Zika virus (ZIKV) a “Public Health Emergency of International Concern” on February 1, 2016, based on emerging evidence that the virus might cause severe fetal brain injury, specifically, severe microcephaly (Gulland 2016a, 2016b). ZIKV is an arbovirus (mosquito-borne) member of the Flaviviridae family, which includes dengue, West Nile virus, chikungunya, and yellow fever. Recently, sexual transmission has also been documented. In adults infected with ZIKV, only 20% of individuals become symptomatic, experiencing a self-limited infection characterized by fever, rash, conjunctivitis, muscle/joint pain, and fatigue, although an estimated 1 of 200–300 infected individuals develop Guillain-Barré syndrome. In 2015, a ZIKV epidemic in Northeastern Brazil was linked with a precipitous increase in microcephaly, intracranial calcifications, and ocular abnormalities. The microcephaly appeared to be much more severe than typical microcephaly caused by other viral infections (CMV, rubella) or genetic syndromes. The affected fetal brain was often over 4 standard deviations below mean, with loss of gyri, marked hydrocephalus, and areas of brain necrosis. Vertical transmission has now been confirmed, with increased miscarriage as well as affected fetuses from all trimesters increasingly documented in endemic areas.

While many different cell lines can be infected with ZIKV, neural progenitor cells (NPCs) are a direct target of ZIKV (Tang et al., 2016). ZIKV-infected NPCs had increased cell death, downregulated proliferation, and altered neurosphere production (Garcez et al., 2016; Li et al., 2016; Tang et al., 2016). Normal brain development is highly dependent on NPC differentiation, migration, and maturation, which is impaired by ZIKV, leading to microcephaly (Li et al., 2016). Children born to ZIKV-infected mothers have shown a wide range of abnormalities, ranging from mild effects to severe microcephaly, almost agyric brains, parenchymal calcifications, hydrocephalus, and cerebellar hypoplasia (Culjat et al., 2016). It is unknown if infection after birth can lead to similar problems or if in utero infection is required to cause long-term problems. Immunization and treatments are being investigated, but for now delay of pregnancy, use of condoms, control of the *Aedes* mosquito population, and prevention of mosquito bites are the only strategies available.

Inborn Errors of Metabolism

Chronic encephalopathy results from the majority of inborn errors of metabolism. The pattern of injury is dependent on which area of the brain is most affected (gray or white matter or both, subcortical or cortical gray matter nuclei). The mechanism of action is not fully understood but involves disrupted astrocyte function, excitotoxicity, and energy failure (Gropman, 2012). A few prototypical inborn errors of metabolism will be discussed.

Phenylketonuria

Phenylketonuria (PKU) is an autosomal recessive disorder caused by a deficiency in phenylalanine hydroxylase, required to convert phenylalanine to tyrosine, which is essential to make dopamine. Dopamine is essential for prefrontal pyramidal neurons involved with working memory and inhibitory control. Prefrontal white matter pathology on structural MRI scans and diffusion tensor imaging can be seen in adults with PKU (Anderson and Leuzzi, 2010). Lifelong restriction of phenylalanine is necessary, as precipitous drops in intellectual quotient following termination of the diet occur in adults. Maternal PKU that is not well controlled is teratogenic and may result in growth retardation, microcephaly, significant developmental delays, and birth defects in offspring, regardless of whether the fetus has PKU (Levy, 2003).

Urea Cycle Disorders

Urea cycle disorders are autosomal recessive disorders with the exception of ornithine transcarbamylase deficiency, which is X-linked. Neonates with absent urea cycle enzyme activity typically present with hyperammonemic coma within the first week after birth. Outcomes can be severe, with high morbidity and mortality approaching 50% (Batshaw and Monahan, 1987). Acute hyperammonemia selectively affects the white matter of the brain and initially may be seen as reversible changes involving the deep sulci of the insular and perioral region watershed territories. Hypoperfusion associated with urea cycle disorders may play a role in causing brain injury.

Methylmalonic Acidemia

Methylmalonic acidemia (MMA) is a deficiency of the adenosylcobalamin-dependent enzyme methylmalonyl-coenzyme A mutase characterized by accumulation of methylmalonic acid.

It has an autosomal recessive inheritance. MMA typically presents in either a newborn who was healthy for the first days to weeks of life or in infants having a history of poor feeding, vomiting, progressive lethargy, floppiness, and muscular weakness. Diagnosis is by urine organic acids that demonstrate large amounts of methylmalonic acid, methylcitrate, propionic acid, and 3-hydroxy propionic acid. Plasma amino acids typically show elevation of glycine but may be normal. MRI scans typically demonstrate involvement of basal ganglia and white matter, with the globus pallidus being selectively affected (Gao et al., 2009).

Congenital Heart Disease

As survival of infants with CHD has improved, there has been more focus on assessment of long-term morbidities, particularly neurodevelopmental outcomes. Children with severe cyanotic CHD have a high incidence of brain abnormalities at birth, with further brain injury commonly diagnosed by MRI after surgical correction (McQuillen et al., 2007, 2010; McQuillen and Miller 2010; Miller et al., 2007; Dimitropoulos et al., 2013). These children are at increased risk of significant NDI, with approximately 50% of survivors affected (Martinez-Biarge et al., 2013). Whether abnormal cerebral circulation in utero results in altered brain development or impaired hemodynamics in the perioperative period leads to hypoxic brain injury needs further clarification. Doppler US scans at term and fetal brain MRI/MRS at 36–38 weeks' gestation have demonstrated that most neonates with CHD (all types) have abnormal neurodevelopmental features compared to control neonates but that abnormalities are more pronounced in CHD lesions associated with decreased oxygenated blood supply to the brain (Masoller et al., 2016). The prevalence of brain lesions has been shown to differ depending on the type of CHD, varying from 34% in cases of transposition of the great arteries to 49% in cases of left-sided heart lesions (Khalil et al., 2014). While identified genetic syndromes, present in up to 30% of children with CHD (Down syndrome and 22q11 deletion being the most common), are independent risk factors for NDI, infants with CHD have a high prevalence of brain lesions on neuroimaging even in the absence of chromosomal or genetic abnormalities and independent of the surgical risk (Marino et al., 2012). Although the brain abnormalities detected by neuroimaging in infants with CHD may increase their risk of NDI, further investigation is needed to establish causality between specific brain lesions and types of NDI in relation to specific types of CHD.

Other potential risk factors for NDI and/or biomarkers of brain injury in infants with CHD include seizures and stroke. Seizures in the newborn and in the perioperative period are common in patients with CHD. Unfortunately, seizures are often subclinical, so they may not be recognized or treated (Gunn et al., 2012; Naim et al., 2015). In the Boston Circulatory Arrest Study, which involved 171 infants with D-transposition of the great arteries who had cEEG monitoring in the first 48 hours postoperatively, seizures were detected in 20% of patients, with clinical seizures being detected in only 6% of patients (Helmers et al., 1997). Neuropsychological follow-up of 139 of these patients at 16 years of age demonstrated that a postoperative seizure was the medical variable most associated with poor outcomes (Bellinger et al., 2011). In preschool survivors who had cardiac surgery during infancy, postoperative electrographic seizures are associated with NDI, characterized by executive function impairments and a higher prevalence of deficits in social interactions and repetitive/restricted behaviors (Gaynor et al., 2013).

Similar to seizures, stroke in the newborn and in the perioperative period is common and may be clinically silent in patients with CHD. Before and after repair, stroke is seen on MRI in 10%–30% of infants with CHD (Martinez-Biarge et al., 2013). Infants with cyanotic CHD undergoing palliative surgery are most frequently affected by arterial ischemic stroke during the periprocedural period, with NDI seen in the majority of survivors (Asakai et al., 2015). Because of the increased prevalence of seizures and stroke in infants with CHD, diligent perioperative screening and long-term follow-up studies are needed to further clarify risk factors associated with CHD, with the hope that improved understanding will lead to the development of effective treatment strategies.

Bilirubin-Induced Neurologic Dysfunction and Kernicterus

Hyperbilirubinemia is very common in the newborn period and is usually not associated with negative effects in most babies. While a direct linear relationship between total serum/plasma bilirubin (TB) levels and developmental delay has not established, elevated TB levels have been associated with bilirubin neurotoxicity and long-term NDI (Maimburg et al., 2010). Increased unconjugated bilirubin levels may lead to increased unbound or “free” bilirubin, which strongly binds myelin-rich membranes, making neurons a main target of bilirubin toxicity, with bilirubin-induced brain injury primarily affecting only a subgroup of neurons in selected areas of the basal ganglia, brainstem, and cerebellum (Watchko and Tiribelli, 2013). Elevated bilirubin levels may induce nonneuronal cells such as astrocytes and microglia to produce proinflammatory cytokines that may contribute to bilirubin-induced brain injury (Brites, 2011).

Neonatal hyperbilirubinemia may lead to acute bilirubin encephalopathy, a condition characterized by lethargy and abnormal behavior, evolving to frank neonatal encephalopathy, opisthotonus, and seizures (Wusthoff and Loe, 2015). Severe hyperbilirubinemia may lead to bilirubin neurotoxicity associated with development of kernicterus, an irreversible, chronic neurologic condition characterized by choreoathetoid CP, impaired upward gaze, and sensorineural hearing loss (Kaplan et al., 2011). The estimated incidence of acute bilirubin encephalopathy/kernicterus in North America and Europe is between 0.4 and 2.7 cases per 100,000 live births among term and late-preterm neonates (born at ≥ 35 weeks' gestation) (McGillivray and Evans, 2012) but is estimated to be much higher in low-income and middle-income countries (Olusanya et al., 2014). Milder degrees of neonatal hyperbilirubinemia than those resulting in kernicterus may result in bilirubin-induced neurologic dysfunction (BIND), a severe and irreversible syndrome that manifests after the neonatal period as developmental delay, cognitive impairment, disordered executive function, and behavioral and psychiatric disorders (Johnson and Bhutani, 2011; Wusthoff and Loe, 2015). Bilirubin-associated brain injury seen in infants with BIND may occur in the basal ganglia, central and peripheral auditory and visual pathways, hippocampus, diencephalon, subthalamic nuclei, midbrain, and cerebellum.

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Neuroprotection Strategies for the Newborn

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KEY POINTS

- Neonatal brain injury may result from an interruption of normal development (predominant in preterm infants) or from damage to existing tissues (predominant in term infants).
- Current approaches to neuroprotection in preterm infants include prenatal administration of steroids, administration of magnesium sulfate, delayed cord clamping, and administration of caffeine.
- Therapeutic hypothermia is the only proven therapy for term infants with intrapartum-related complications.
- New neuroprotective approaches, including use of erythropoietin, melatonin, and stem cell therapy, are currently in clinical trials. Ideal neuroprotection will likely include multiple targeted approaches at different times.

The two most common causes of death of newborns globally are prematurity and intrapartum-related complications. The *Lancet* has repeatedly published data about the global, regional, and national causes of child death, and together these two problems consistently account for close to 50% of neonatal deaths worldwide (Black et al., 2010; Liu et al., 2012, 2015). For neonates who survive after premature delivery and/or intrapartum-related complications, the burden of disease is also enormous (Global Burden of Disease Pediatrics Collaboration, 2016). The sequelae of these problems differ with the resources available to help the child, but even in the most affluent countries, with all available treatments used, death or neurodevelopmental impairment (NDI) occurs in approximately 50% of affected infants. Children with neonatal stroke, cyanotic heart disease, pulmonary hypertension, inborn errors of metabolism, kernicterus, and fetal alcohol exposure, among other problems, are also at high risk of NDI and could potentially benefit from neuroprotection strategies. Impairment may include intellectual deficiencies, motor deficiencies, deafness, blindness, and an increased risk of problems such as autism spectrum disorder. As the field of neonatology has accomplished marvels in improving survival in recent decades, the new focus must be on helping these survivors to thrive, to live full and healthy lives without impairment. To accomplish this audacious goal, there must be new developments in neuroprotective strategies.

Mechanisms of Disease: Identification of Targets for Neuroprotection

Neonatal brain injury can occur from clastic injury (injury to existing tissues) or from an interruption or disruption of normal developmental processes. Clastic injury is most often seen in term infants, while in preterm infants, interruption of normal development is most often an important component of long-term injury.

Clastic Lesions

In experimental studies, cerebral hypoxia–ischemia of sufficient severity to deplete tissue energy reserves (primary insult) is often followed by transient but complete restoration of glucose utilization, adenosine triphosphate, and phosphocreatine on reperfusion/reoxygenation. Thereafter a secondary decrease in the levels of high-energy phosphates occurs in parallel with a decrease in tissue glucose metabolism and development of cell injury. Similarly, infants with neonatal encephalopathy (also termed *neonatal ischemic encephalopathy*) show characteristic abnormalities in cerebral energy metabolism, which is frequently normal soon after birth but shows a progressive decline in the ratio of the concentration of phosphocreatine to inorganic phosphate some hours later (Azzopardi et al., 1989). Infants displaying this phenomenon develop severe neurodevelopmental impairment or die, and there is a close relationship between the magnitude of the late decline in the ratio of the concentration of phosphocreatine to inorganic phosphate, reduced brain growth, and the severity of NDI.

These findings suggest that most of the injury associated with hypoxia–ischemia evolves over time after rather than during the insult. Hypothermia following hypoxia–ischemia takes advantage of this window and reduces secondary energy failure and brain injury (Thoresen et al., 1995). However, the mechanisms involved in secondary brain injury are incompletely understood, and such information is critical for development of the next generation of therapies to be combined with hypothermia in term infants with neonatal encephalopathy.

The deficit in high-energy phosphates induced by hypoxia–ischemia leads to a primary failure to maintain transmembrane ionic gradients, release of neuroactive compounds into the extracellular compartments, accumulation of intracellular Ca^{2+} , and the

activation of a series of mechanisms that if sustained will lead to immediate cell death. If the individual is resuscitated, these acute alterations are completely or partly reversed, but the complex process has been started in which multiple interrelated factors may produce secondary brain injury. The precise mechanisms of damage are incompletely understood, but some components of the process have been elucidated (Thornton et al., 2012). Excitatory amino acids, mitochondrial impairment (loss of mitochondrial function, altered biogenesis and fusion/fission cycle, release of factors initiating the apoptotic cascade), intracellular calcium regulation, generation of reactive oxygen species, including nitric oxide, cell death mechanisms (including different types of cell death, such as apoptosis, necroptosis, ferroptosis, parthanatos, and autophagy), changes in the availability of trophic factors, and the immunoinflammatory system are all implicated in the process. In addition, experimental evidence and some recent epidemiologic studies support the hypothesis that perinatal systemic inflammation could play a key sensitizing role to hypoxic-ischemic/excitotoxic brain insults, a process that could make hypothermia less efficient (Hagberg et al., 2015).

Interruption/Disruption of Normal Development

Until a few years ago the prevailing dogma was that, in preterm neonates, immature oligodendrocyte precursors, which are highly sensitive to oxidative stress and excitotoxicity, were dying (clastic damage), leading to white matter cysts (Volpe et al., 2011). More recent neuropathology studies have shown that these cells do not die in significant numbers but rather are largely unable to mature completely and therefore cannot contribute to axon myelination despite their presence in the white matter (Verney et al., 2012). These nonclastic white matter abnormalities most likely contribute significantly to the diffuse white matter injury described on magnetic resonance imaging (MRI) in numerous preterm infants (Rutherford et al., 2010; Kidokoro et al., 2013).

MRI studies performed in preterm infants show two key features supporting the hypothesis that changes in gray matter structures are also implicated in the pathomechanisms of encephalopathy of prematurity: (1) the connectivity between gray structures is significantly reduced, especially thalamocortical connections, and (2) the microstructure of cortical structures is reduced, although the cellular and molecular correlates are still largely unknown (Lodygensky et al., 2010). On the basis of experimental evidence, different hypotheses can be suggested: (1) reduced number of neurons and in particular of interneurons that are lately produced and could be affected by preterm birth; (2) reduced arborization of neurons; and (3) reduced spine/synapse density (Penn et al., 2015). Further studies are necessary to determine key cellular mechanisms and underlying molecular pathways so as to identify potential targets.

Epidemiologic, neuropathologic, and experimental studies have identified systemic inflammation and related neuroinflammation as a key mechanism involved in white matter maldevelopment and potentially in gray matter maldevelopment (Hagberg et al., 2015). Microglia, the resident brain macrophage, seem to play a key role in neuroinflammation and therefore appear as an appealing target for neuroprotection. However, microglia have multiple roles. They can be deleterious to the developing brain when they acquire a proinflammatory phenotype, but they can also play prerenal roles by removing debris and releasing proplastic factors. In addition, microglia play an important role in normal neurite pruning and synaptic elimination during brain development (Tay et al., 2016).

Brain damage associated with perinatal adverse events had, until recently, been considered a fixed disease. However, different lines of evidence suggest that this might not always be true (Fleiss and Gressens, 2012). According to emerging evidence, in addition to the developmental disruption associated with the initial insult to the immature brain, injury processes can persist for months to years. These “tertiary mechanisms” of damage likely include persistent inflammation and epigenetic changes and may prevent endogenous repair and regeneration. These processes, such as ongoing inflammation, may sensitize patients to further injury or could predispose them to develop age-related cognitive dysfunction. Treatment of tertiary mechanisms of damage might be possible by various means, including preventing the repressive effects of microglia and astrocyte overactivation, recapitulating developmentally permissive epigenetic conditions, and using cell therapies to stimulate repair and regeneration. Recognition of tertiary mechanisms of damage might be the first step in a complex translational task to tailor safe and effective therapies that can be used to treat the already developmentally disrupted brain long after an insult.

Therapeutic Approaches

Current Therapies for Preterm Infants: Prenatal Therapies

Steroids

Steroids given 24 hours to 7 days before delivery have long been known to reduce the risk of death or morbidity in preterm singleton pregnancies of 28 to 32 weeks' gestation with a risk reduction of more than 40% (Mwansa-Kambafwile et al., 2010; Chawla et al., 2013). The benefits include decreased early death, respiratory distress syndrome, intraventricular hemorrhage (IVH), and necrotizing enterocolitis. Despite this known benefit, change in clinical practice was slow, with use increasing from 8% in 1985 to 52% in 1995, 75% in 2000, and 85% in 2015. Recent studies have shown that prenatal administration of steroids also benefits preterm multiples and infants as premature as 22 weeks' gestation (Boghossian et al., 2016; Wei et al., 2016). Repeated courses can be given for threatened preterm birth, although the safety of more than two courses is not established, with some evidence that fetal head growth might be affected with multiple repeated courses (Whitelaw and Thoresen, 2000; Bonanno et al., 2007).

Magnesium Sulfate

Magnesium sulfate therapy is a well-studied prenatal therapy with potential to reduce cerebral palsy (CP) and improve gross motor function following preterm birth. In vitro, magnesium alleviates excitotoxic damage by binding to the magnesium site on the *N*-methyl-D-aspartate glutamate channel (Zeevalk and Nicklas, 1992). There is also evidence that magnesium can reduce secondary inflammation, decrease free radical formation, stabilize cell membranes, inhibit free radical production, and improve cardiovascular stability (Galinsky et al., 2014).

There have been five prospective randomized controlled trials to determine possible benefits of magnesium for neuroprotection. The magnesium dose in these studies differed, and the use of an infusion following a bolus dose was also inconsistent between studies. The Cochrane Collaboration published a metaanalysis of the 6145 women included in these studies in 2009 (Doyle et al., 2009). It found no difference in infant mortality but a 31% decrease in the overall incidence of CP in the magnesium-treated group,

with a relative risk (RR) of 0.68 (95% confidence interval [CI] 0.54–0.87). There was also a 39% decrease in the risk of substantial motor dysfunction among those infants who received magnesium (RR 0.61, 95% CI 0.44–0.85). There were no differences in blindness, deafness, intellectual impairment, IVH, white matter injury, low Apgar score at 5 minutes, neonatal seizures, hypotonia, need for respiratory support, bronchopulmonary dysplasia at 28 days or 36 weeks, or length of hospital stay. There were no maternal differences in mortality or obstetric complications. To avoid one child developing cerebral palsy, 63 women (95% CI 43–155) must be treated (Doyle, 2012; Jacquemyn et al., 2015).

Delayed Cord Clamping

In 2012 the American Academy of Pediatrics and the American College of Obstetricians and Gynecologists issued a committee opinion recommending delayed umbilical cord clamping (DCC) (>30 seconds) in preterm neonates for a potential 50% reduction in IVH (Committee on Obstetric Practice, American College of Obstetricians and Gynecologists, 2012). This opinion was informed by several randomized controlled trials demonstrating increases in neonatal hematocrit, decreases in IVH, and improved physiologic stability with less need for pressor support. In follow-up studies of infants who underwent DCC versus immediate clamping, long-term benefits were noted at 18 to 22 months and 4 years of age with improved gross and fine motor scores and in social domains (Garofalo and Abenhaim, 2012; Andersson et al., 2015; Brocato et al., 2016; Rabe et al., 2016).

The optimal period of delay before clamping the umbilical cord in preterm infants is still undetermined. A prospective cohort study of infants of less than 32 weeks' gestational age compared DCC at 30–45 seconds with DCC at 60–75 seconds. Infants with the longer delay had higher hematocrits at less than 2 hours (49.2% vs 47.4%, $P = .02$), and reductions in the rates of delivery room intubation (11% vs 22%, $P = .004$), hypothermia on admission (1% vs 5%, $P = .01$), surfactant therapy (13% vs 28%, $P = .001$), intubation in the first 24 hours (20% vs 34%, $P = .004$), and any intubation (27% vs 40%, $P = .007$), and they received fewer red blood cell transfusions (20% vs 33%, $P = .008$) (Song et al., 2015). These results suggest that for preterm infants, a more prolonged placental transfusion after delivery may be beneficial.

Umbilical cord milking also holds appeal since if it provides benefits similar to those of DCC, it would allow more immediate resuscitation of critically ill infants. A recent metaanalysis of six studies (292 preterm infants underwent umbilical cord milking and 295 underwent immediate cord clamping) concluded that, similarly to DCC, umbilical cord milking also improved initial hematocrit, decreased the incidence of transfusion, with a pooled risk ratio of 0.74 (95% CI 0.61–0.90; $P = .002$), and decreased the risk of necrotizing enterocolitis, IVH, and death (Dang et al., 2015).

Caffeine

In landmark work studying the effects of caffeine on preterm outcomes in 2006 individuals, Schmidt et al. (2006) showed that in addition to reducing apnea and the need for mechanical ventilation, treatment of infants with birth weights of 500–1250 g decreased the outcome of death or survival with one or more of the following impairments: cerebral palsy, cognitive deficit, blindness, and deafness. A follow-up study that captured data from 1640 of the original 2006 babies enrolled in the Caffeine for Apnea of Prematurity (CAP) trial showed no sustained benefit, although there was a trend toward improved motor function: of the 833 caffeine-treated children,

176 (21.1%) died or survived with one or more impairments, compared with 200 of the 807 placebo-treated children (24.8%) (odds ratio adjusted for center 0.82, 95% CI 0.6521.03, $P = .09$). Further analysis has shown a lower rate of developmental coordination disorder in caffeine-treated infants compared with placebo-treated infants (Schmidt et al., 2006, 2007, 2012; Doyle et al., 2014).

Kangaroo Care

Skin-to-skin contact between a mother and a premature infant, often called *kangaroo care*, has been shown to have multiple short-term and long-term benefits. The benefits include increased physiologic stability, decreased maternal anxiety or depression, improved breastfeeding, increased pain tolerance, improved growth, and decreased mortality (Johnston et al., 2008; Athanasopoulou and Fox, 2014; Bera et al., 2014a, 2014b; Conde-Agudelo and Diaz-Rossello, 2014; Boundy et al., 2016). Scher et al. (2009) showed that skin-to-skin contact for 1.5 hours per day, 4 days per week for 8 weeks accelerated electroencephalogram (EEG) signs of brain maturation.

There are emerging long-term findings that the effects of kangaroo care can be sustained, improving attention and quality of movements (Silva et al., 2016) and enhancing child cognitive development and executive functions for up to 10 years (Feldman et al., 2014).

Therapies on the Horizon for Preterm Infants

Melatonin

Melatonin (*N*-acetyl-5-methoxytryptamine), a neurohormone derived from the amino acid tryptophan and secreted by the pineal gland, is a strong antioxidant capable of scavenging free radicals and stimulating several antioxidative enzymes, including glutathione, glutathione reductase, peroxidase, and superoxide dismutase (Reiter et al., 2000). Melatonin can directly stimulate cellular membrane G protein-coupled high-affinity melatonin receptors (melatonin receptors 1 and 2) that activate numerous second messenger cascades, which differ in cell-, tissue-, and species-specific ways. Melatonin can also induce receptor-independent intracellular activities by targeting calcium-binding proteins, cytoskeletal and scaffold proteins, and components of mitochondrial signaling (Luchetti et al., 2010). Melatonin's safety profile and antioxidant, antiinflammatory, and antiapoptotic properties have made it an attractive neuroprotective candidate for treating neonates with neonatal encephalopathy (Alonso-Alconada et al., 2013; Biran et al., 2014).

Melatonin is neuroprotective in several animal models relevant to preterm brain injury, including a neonatal mouse model of excitotoxic white matter damage (with or without systemic inflammation), a hypoxia-ischemia model of neonatal brain damage, and inflammation-induced white matter damage in fetal sheep (Ramanantsoa et al., 2013). On the basis of these preclinical data, a pharmacokinetic study was done in preterm neonates to determine the melatonin dosage that would best mimic fetal exposure to maternal secretion of melatonin in the third-trimester fetus (Merchant et al., 2013). On the basis of this pharmacokinetic study and targeting the same melatonin concentration, a randomized clinical trial with melatonin supplementation as a potential neuroprotective agent has been initiated, and the primary outcome based on MRI assessment at corrected term age should be available soon. This study is based on the hypothesis that preterm infants, who are not yet able to produce their own melatonin, might demonstrate improved neurodevelopment when exposed to appropriate in utero levels.

Melatonin also holds promise as a prenatal neuroprotectant that could be administered to pregnant women since it appears safe, crosses the placenta (Okatani et al., 1998), and crosses the blood–brain barrier (on the basis of adult rat studies) (Vitte et al., 1988). Further research is needed to clarify the mechanisms by which the pleiotropic neurohormone melatonin may regulate neuronal cell survival, brain tissue homeostasis, and neuroprotection in neonates with neonatal encephalopathy.

Erythropoietin Neuroprotection

Erythropoietin (Epo) was first discovered for its erythropoietic effects and is most commonly used to treat anemia in individuals with chronic renal failure. Epo functions by binding to homologous cell surface Epo receptors, which are prevalent on nascent red blood cells. Epo receptors are also expressed by multiple cell types in the central nervous system (Juul et al., 1998; Bernaudin et al., 1999; Mu et al., 2005), including neuronal progenitor cells (Wang et al., 2004), subsets of mature neurons (Wallach et al., 2009), astrocytes (Sugawa et al., 2002), oligodendrocytes (Sugawa et al., 2002; Genc et al., 2006; Iwai et al., 2010), microglia (Chong et al., 2003), and endothelial cells (Wang et al., 2004). Epo is produced in the brain, primarily by astrocytes, and is thought to be an important trophic factor during brain development as well as an endogenous protective factor. Brain Epo is upregulated after prolonged hypoxia, so elevated Epo levels at birth likely reflect chronic intrauterine hypoxia and may be a useful biomarker to predict increased complications of prematurity such as necrotizing enterocolitis (Holm et al., 2016). When Epo is administered exogenously for the purposes of neuroprotection, high doses are needed to penetrate the blood–brain barrier, unless the blood–brain barrier is damaged (Juul et al., 2004; Statler et al., 2007). Epo does not cross the placenta, so it cannot be given prenatally for neuroprotective purposes. Epo has antiinflammatory, antiexcitotoxic, antioxidant, and antiapoptotic effects (Sun et al., 2005; Juul et al., 2009; Rees et al., 2010) and promotes neurogenesis, oligodendrogenesis, and angiogenesis, which are essential for repair of injury and normal neurodevelopment (Shingo et al., 2001; Wang et al., 2004; Kumral et al., 2005; Iwai et al., 2007; Osredkar et al., 2010; Gonzalez et al., 2013; Wassink et al., 2017). Some of these effects may be mediated by Epo stimulation of growth factors required for normal brain growth such as brain-derived neurotrophic factor and glial cell–derived neurotrophic factor (Dzietko et al., 2004; Wang et al., 2004). Epo effects are dose dependent, and multiple doses are more effective than single doses (Kellert et al., 2007; Gonzalez et al., 2007, 2009). Epo reduces neuronal loss and learning impairment following brain injury (Demers et al., 2005; Larphaveesarp et al., 2016). It also decreases white matter injury in animal models (Savino et al., 2006; Vitellaro-Zuccarello et al., 2008; Li et al., 2009; Iwai et al., 2010; Rees et al., 2010; Zhang et al., 2010; Yamada et al., 2011). Although Epo is most often administered immediately after injury in preclinical studies, investigators have shown that even when administration is initiated as late as 72 hours or 1 week after injury, there is evidence of improved behavioral outcomes, enhanced neurogenesis, increased axonal sprouting, and reduced white matter injury (Iwai et al., 2010; Reitmeir et al., 2011; Jantzie et al., 2016; Larphaveesarp et al., 2016).

In addition to cell-specific effects in the brain, Epo increases iron utilization as erythropoiesis is increased. Iron is highly reactive and normally sequestered by transport proteins. Unbound iron produces free radicals and subsequent oxidative injury. Preterm infants have measurable free iron, the level of which increases after

transfusions of red blood cells or during metabolic instability such as sepsis (Buonocore et al., 2003; Ozment and Turi, 2009). Epo may contribute to neuroprotection in preterm infants by decreasing free iron levels and thereby ferroptosis.

Translational Trials of Neonatal Erythropoietin Neuroprotection for Preterm Infants

Two preliminary reports of preterm infants treated prospectively have shown a benefit:

1. Preterm infants weighing 500–1250 g treated with either Epo (400 U/kg three times per week, $n = 29$) or darbepoetin (10 U/kg per dose once per week, $n = 27$) from birth to 35 weeks' postmenstrual age had an average cumulative cognitive score 8–10 points higher than placebo-treated controls, with erythropoietin-treated infants earning scores of 97.9 ± 14 and darbepoetin-treated infants scoring 96.2 ± 7.3 compared with 88 ± 14 for controls ($n = 24$). Epo recipients also performed statistically better than controls on object permanence testing (Ohls et al., 2014). The combined scores for NDI or death were significantly better in the erythropoietin-treated group and the darbepoetin-treated group, with combined scores of 15.5% compared with 48.2% in the control group, and improved intellectual outcomes were sustained at 3.5–4 years of age, although the preterm infants still performed less well than age-matched term infants (Ohls et al., 2016).
2. Follow-up of extremely low birth weight infants who received Epo at 500–2500 U/kg for three doses in a phase I/II trial (Juul et al., 2008) showed that Epo treatment correlated with improvement of cognitive ($R = 0.22$, $P < .05$) and motor ($R = 0.15$, $P < .05$) scores (McAdams et al., 2013).

A randomized, double-masked phase II trial of Epo neuroprotection for preterm infants has been completed (NCT00413946). Four hundred and forty-eight infants (gestational age 26–31 weeks) were randomized to receive Epo (3000 U/kg, $n = 228$) or saline ($n = 220$) administered at 3, 12–18, and 36–42 hours after birth. The mean gestational age at enrollment was 29.3 weeks, with birth weight of approximately 1220 g. The dose and dosing regimen were found to be safe, with no increase in retinopathy of prematurity or any other complication of prematurity (Fauchère et al., 2015), and a subset of those treated with Epo were found to have improved white matter integrity on near term MRI (Leuchter et al., 2014; O'Gorman et al., 2015). However, long-term follow-up of 81% of enrolled infants at 2 years' corrected age was disappointing, showing no benefit in the primary outcome of Mental Development Index of the Bayley Scales of Infant and Toddler Development second edition or in secondary outcomes of motor development, cerebral palsy, hearing or visual impairment, or growth parameters (Natalucci et al., 2016). The authors note that the study was underpowered to detect a significant difference because the incidence of adverse outcomes was lower than expected when the study was being planned. It is also possible that a longer course of Epo is required for neuroprotection of preterm infants during the prolonged period of potential vulnerability in the initial hospitalization.

Risks of Intervention

In adults, complications of prolonged Epo treatment include polycythemia, seizures, hypertension, stroke, myocardial infarction, congestive heart failure, tumor progression, and shortened time to death. None of these adverse effects have been reported in Epo-treated neonates in more than 3000 patients enrolled in randomized controlled trials (Juul, 2012; Wang et al., 2015;

Natalucci et al., 2016). Epo trials in term and preterm neonates for the purposes of testing its erythropoietic effect have shown it to be a safe drug for use in this population. In particular, an increased risk of retinopathy of prematurity for preterm infants treated with Epo has not been substantiated in prospective randomized controlled trials (Ohlsson and Aher, 2014; Fauchère et al., 2015; Ohls et al., 2015). Accumulating data using neuroprotective doses (1000–3000 U/kg per dose) also demonstrate safety (Fauchère et al., 2015). A Cochrane review of Epo neuroprotection of preterm infants concluded that there is long-term neurodevelopmental benefit from early Epo use, with no increase in morbidity (Wang et al., 2015).

The Preterm Erythropoietin Neuroprotection Trial (PENUT; IND 12656, [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01378273) NCT01378273) is a phase III randomized, placebo-controlled, double-blind study of Epo neuroprotection in infants born between 24 and 28 weeks' gestation. Nine hundred and forty patients will be enrolled at multiple sites across the United States and will be evaluated at 22–26 months' corrected age. Enrolled patients will receive the study drug from less than 24 hours of age to 32 completed weeks' postmenstrual age. This study is adequately powered to provide definitive results regarding the neuroprotective potential of Epo in this population. The primary outcome is death or severe NDI at 22–26 months' corrected age, with a secondary outcome of death, severe NDI, or moderate NDI. Patients will be evaluated by the third edition of the Bayley Scales of Infant and Toddler Development and a standardized neurologic examination. Results should be available in 2018 (Juul et al., 2015).

Future Outlook on Neuroprotection for Preterm Brain Injury

There are three concepts we would like to bring to the reader's attention that we believe are likely to inform the next generation of therapies moving into clinical trials. These are applicable to both term and preterm infants: cell-specific targeting, harnessing endogenous protection (specifically, regulating microglial phenotype), and tertiary-phase injury processes. In addition, for infants born preterm, the concept of paucity of protectors, unblocking the maturation blockade of oligodendrocytes, and normalizing changes to the gray matter must be addressed.

Cell-Specific Targeting

Improvements in techniques for separating cells into pure populations have fostered cell type–specific analyses in injury models to understand injury and repair (Williamson et al., 2011; Smith et al., 2014; Schang et al., 2014b). These studies have revealed that the same pathway can participate in opposing ways during normal development or during injury in adjacent cell types. A striking example of this is found in the Wnt pathway. Specifically, decreasing Wnt pathway signaling is crucial for normal oligodendrocyte maturation, with increased signaling noted in oligodendrocytes in areas of white matter injury (Fancy et al., 2009). In contrast, in microglia, high Wnt pathway activity is required to prevent these innate immune cells from becoming activated to a proinflammatory, oligodendrocyte-damaging phenotype (Van Steenwinckel et al., 2016). This cell specificity can be harnessed by targeting nanoparticles to facilitate the cell-specific delivery of neurotherapeutics (Kannan et al., 2014). One of the first and most robust examples of this approach was the use of a polyamidoamine dendrimer to deliver *N*-acetyl-D-cysteine to reduce brain injury in a rabbit model

of CP (Kannan et al., 2012). The dose of *N*-acetyl-D-cysteine required for equivalent neuroprotection was reduced 10-fold by use of cell-targeting technology when compared with intraperitoneal injection.

Microglial Activation States

Our understanding of the importance of neuroinflammation in almost every kind of brain injury and disease has lead to an explosion of research into the activation state of microglia (Vexler et al., 2006; Ziebell and Morganti-Kossmann, 2010; Drake et al., 2011; Hagberg et al., 2012; Heneka et al., 2015). Microglia are unique yolk sac–derived resident innate immune cells of the brain with self-renewing potential and numerous critical roles in normal brain development and injury (Ransohoff and Perry, 2009; Hart et al., 2012; Tay et al., 2016). The activation state of microglia is dependent on extracellular cues, and they can morph into phenotypes that when simplified can be described as proinflammatory M1–like, immunoregulatory M2a–like, and antiinflammatory M2b–like (Chhor et al., 2013). This understanding that microglia can be protective or regenerative (Kitamura et al., 2009; Hamelin et al., 2016) is further complicated by observations that these functions are temporally and spatially regulated in an injury-specific or disease-specific manner (Hart et al., 2012; Hu et al., 2012; Wang et al., 2013). This increasingly nuanced understanding of how microglia participate in injury and normal development (Schmid et al., 2009; Nagamoto-Combs and Combs, 2010; Tarassishin et al., 2011; Butovsky et al., 2014; Tang et al., 2014) means that the route of administration and the timing must be considered when one is attempting to modulate microglial function to maximize the protective/regenerative effectiveness of microglia (Hickman et al., 2008; Hu et al., 2012). This growing knowledge of microglial biology will be further facilitated by the aforementioned cell isolation techniques. Infusions of microglia or their bone marrow–derived myeloid cell “cousins” monocytes and macrophages are starting to be considered as potential therapies for brain injury because of their similarities to stem cells, including the secretion of trophic factors, migratory ability, and their long life span (Cartier et al., 2014). Infusions of these cells, either genetically or chemically prepolarized to a repair-regenerate phenotype, are neuroprotective in models of multiple sclerosis (Takahashi et al., 2007; Mikita et al., 2011; Beutner et al., 2013).

Tertiary-Phase Injury Processes

There is increasing clinical and experimental evidence to support the presence of persistent, active mechanisms that prevent regeneration and/or exacerbate brain damage following perinatal brain injury (Dammann, 2007; Fleiss and Gressens, 2012); these mechanisms are termed *tertiary-phase injury processes*. Most notably, it appears that neuroinflammatory processes persist, including microglial activation (Bilbo et al., 2008; Van Steenwinckel et al., 2013). From a clinical perspective, therapies that can be applied long after injury to improve neurologic condition are highly appealing. Together with improved understanding of cell specificity and repair-regenerative microglial phenotypes, research into tertiary-phase injury will hopefully unlock the potential for neurologic improvements for the many hundreds of thousands of people with perinatally acquired brain injury. Early clinical trials using stem cells to treat perinatal brain injury have targeted the tertiary phase (patients 1–12 years of age) because of safety issues of treating patients in the acute phase. The potential for pharmacologic therapies to improve outcomes when they are administered

weeks or months after injury is being demonstrated in animal models (Saraceno et al., 2010; Zhang et al., 2011; Byrnes et al., 2012).

Paucity of Endogenous Protectors

The fetus is supplied via the placenta with many factors, including progesterone, melatonin, thyroid hormones, and estrogen. A lack of these factors because of early birth is hypothesized to contribute to NDI in preterm infants and may be an exacerbating factor in injury to term infants (Dammann and Leviton, 1999; Dammann and O'Shea, 2008; Hirst et al., 2008). Each of these substances has been shown to be neuroprotective in animal models of perinatal injury (Gibson et al., 2008; Brown et al., 2009; Schang et al., 2014b). The hypothesis of a paucity of neuroprotectors is several decades old, and clinical trials for several of the prime candidates have produced negative or inconclusive findings (Schang et al., 2014a; Schuit et al., 2015; Norman et al., 2016). However, it is hoped that other endogenous protectors may also have potential, such as neuregulins (Dammann et al., 2008; Taveggia et al., 2008), allopregnanolone (Yawno et al., 2007; Fleiss et al., 2012), and esterol (Trajkovic et al., 2006).

Targeting Oligodendrocyte Maturation

As the quality of obstetric care has improved, the pathophysiology of injury to infants born preterm has changed. After preterm birth, it is known that oligodendrocytes are a cell type that is particularly vulnerable to injury, particularly between 22 and 32 weeks' gestation. However, it is not the death of oligodendrocytes but a maturation blockade that is responsible for the characteristic hypomyelination observed in these infants (Billiards et al., 2008; Favrais et al., 2011; Verney et al., 2012; Schang et al., 2014a). Stimulating maturation of oligodendrocytes is also an avenue of therapeutic design for multiple sclerosis (Franklin and Kotter, 2008; Fancy et al., 2010). In multiple sclerosis lesions, oligodendrocytes proliferate locally and then mature in a relapsing and remitting progression. Over time it is the failure of oligodendrocyte maturation and remyelination that leads to the accumulation of lesions that impair function. It is unknown if therapies "borrowed" from the field of multiple sclerosis, where oligodendrocytes proliferate in the context of injury and then fail to mature, will be beneficial to preterm infants with encephalopathy of prematurity, where oligodendrocytes born in the normal developmental milieu undergo developmental arrest (Franklin and Ffrench-Constant, 2008; Fancy et al., 2011a). Drugs designed for treatment of multiple sclerosis that target oligodendrocyte maturation that may also benefit infants with perinatal injury include XAV939, rapamycin, olesoxime, benztropine, and activin A (Fancy et al., 2009; Fancy et al., 2011b; Miron et al., 2013; Kremer et al., 2015).

Targeting Gray Matter Damage

Encephalopathy of prematurity has historically been considered a white matter injury, but the changing spectrum of injury has allowed us to identify subtle changes in gray matter (Boardman et al., 2006; Ball et al., 2011, 2013; Zubiaurre-Elorza et al., 2011). The exact nature of gray matter changes is still not fully understood but appears to involve changes in interneuron number and/or distribution (Salmaso et al., 2014) and changes in synapse density

(Dean et al., 2011a, 2011b; McClendon et al., 2014). There are no drugs to our knowledge to directly target these processes, only attempts to normalize factors leading to preterm birth and downstream neuroinflammatory processes.

Current Therapies for Term Infants

Therapeutic Hypothermia

Therapeutic hypothermia (TH) (72 hours of cooling to $33.5 \pm 0.5^\circ\text{C}$ followed by slow rewarming, 0.5°C per hour to normothermia) is now the standard of care to treat neonates with moderate-to-severe hypoxic-ischemic encephalopathy (HIE). Numerous randomized controlled trials have investigated the benefit of therapeutic hypothermia for improving outcomes of newborns with HIE, and multiple metaanalyses are available for review.

A Cochrane metaanalysis by Jacobs et al. (2013) included 11 randomized controlled trials, comprising 1505 term and late preterm infants with moderate-to-severe HIE. This review concluded that TH resulted in less death and better neurodevelopmental outcomes for survivors. Eight of the 11 studies (1344 infants) demonstrated that TH decreased the combined outcome of death or major neurodevelopmental disability at 18 months of age (46%, 312/678, vs. 61%, 409/666, in controls) (typical relative risk [RR] 0.75, 95% confidence interval [CI] 0.68–0.83; typical risk difference [RD] -0.15 , 95% CI -0.20 to -0.10). The number needed to treat to benefit one newborn is 7 (95% CI 5–10).

Secondary outcomes of the Cochrane review included mortality, major neurodevelopmental disability, adverse effects of cooling, and additional indicators of neurodevelopmental outcome (e.g., severity of EEG abnormality, seizures, MRI findings). Eleven studies (1468 infants) supported decreased mortality with TH (25%, 186/736, vs 34%, 250/732, in controls, RR 0.75, 95% CI 0.64–0.88), for a number needed to treat of 11. Eight studies (917 infants) demonstrated that TH decreases neurodevelopmental disability in surviving infants (26%, 130/495, vs 39%, 166/422, in controls, RR 0.77, 95% CI 0.63–0.94), for a number needed to treat of 8. On the basis of the available randomized controlled trials, TH appears to increase survival without increasing major disability in survivors.

Reported adverse effects of TH include sinus bradycardia, thrombocytopenia, fat necrosis, disseminated intravascular coagulopathy, and rarely pulmonary hypertension (Gunn et al., 1998; Eicher et al., 2005; Gluckman et al., 2005; Shankaran et al., 2005; Azzopardi et al., 2009; Simbruner et al., 2010; Zhou et al., 2010; Jacobs et al., 2011). TH has not been associated with a significant increase in the rates of major cardiac arrhythmia and hypotension or in the need for inotropic agents.

Long-term outcome data are currently available from 379 of the 1505 infants (25.2%) included in the Cochrane metaanalysis. One hundred and nine of 208 (91.3%) participants in the US National Institute of Child Health and Human Development study were assessed at the ages of 6–7 years (Shankaran et al., 2012). The primary outcome of death or an intelligence quotient (IQ) below 70 remained lower in the TH group (46 of 97 children, 47%) than in the control group (58 of 93 children, 62%) but was no longer significant (RR in the TH group 0.78, 95% CI 0.61–1.01). The death rate remained significantly lower in the TH group (28% vs 44% of controls, $P = .04$) as did the rate of death or severe disability (41% vs 60% of controls, $P = .03$). There was no

significant difference in the rates of moderate or severe disability (35% of the TH group vs 38% of controls, $P = .87$) or CP (17% of the TH group vs 29% of controls, $P = .14$). On the basis of this study, it appears TH increases survival without increasing the rates of major disability, an IQ score below 70, or cerebral palsy in surviving children.

One hundred and twenty-seven of 325 infants (39%) enrolled in the European Total Body Hypothermia for Neonatal Encephalopathy (TOBY) trial were assessed at 6–7 years of age. At 18 months, children in the TOBY trial who had received TH had reduced risks of cerebral palsy and better Mental Developmental Index and Psychomotor Developmental Index scores based on the Bayley Scales of Infant and Toddler Development, second edition (Azzopardi et al., 2009). At 6–7 years of age children who had received TH (75 of 145, 52%) had significantly higher survival with an IQ of 85 or higher compared with children not treated with TH (52 of 132, 39%, RR 1.31, $P = .04$) (Azzopardi et al., 2014). While the percentages of children who died in the TH group (29%) and the control group (30%) were similar, in survivors, normal neurologic outcomes were more common in the TH group (65 of 145, 45%) than in the control group (37 of 132, 28%); RR 1.60. The risk of CP was significantly lower in surviving children from the TH group (21%) than in surviving children from the control group (36%). Children from the TH group also had less moderate or severe disability (22%) than children from the control group (37%).

Follow-up of children from the Cool Cap trial also demonstrated that 18-month neurodevelopmental assessments are predictive of long-term functional outcomes. Guillet et al. (2012) reported outcomes on 62 (32 cooled, 30 controls) of 135 (46%) surviving 7–8-year-old children enrolled in the Cool Cap trial. With use of a WeeFIM instrument to qualitatively measure self-care, mobility, and cognitive function, disability status at 18 months was strongly associated with WeeFIM ratings at 7–8 years of age ($P < .001$), supporting a sustained treatment effect of TH for neonatal encephalopathy. Unfortunately, this follow-up study had insufficient power to determine whether treatment with TH affected long-term outcome.

Cooling Duration and Depth

Even when treated with TH, close to 50% of neonates with moderate-to-severe HIE die or experience significant NDI. In an attempt to optimize this therapy, a clinical trial was conducted to determine whether a longer duration of TH or cooling to a lower temperature might show further benefit (Shankaran et al., 2014). The trial was stopped with only 364 neonates enrolled (of 726 planned) because of safety and futility concerns. Analysis of the predefined secondary outcomes, safety and neonatal intensive care unit (NICU) deaths, was done by marginal comparisons of 72 hours' versus 120 hours' duration and 33.5°C versus 32.0°C (predefined secondary outcomes). Both longer and deeper cooling were associated with increased mortality. Death rates increased from 7% (7 of 95 neonates) to 16% (15 of 96 neonates) when cooling at 33.5°C was extended from 72 to 120 hours. Similarly, death rates increased from 14% (13 of 90 neonates) to 17% (14 of 83 neonates) when cooling at 32°C was extended from 72 hours to 120 hours. The adjusted RR for NICU deaths for the 120-hour group versus the 72-hour group was 1.37 (95% CI 0.92–2.04) and for the 32.0°C group versus the 33.5°C group was 1.24 (95% CI 0.69–2.25). Future analyses will determine the primary outcome of death or disability at 18–22 months.

Therapies on the Horizon for Term Infants

Adjunctive Neuroprotective Treatments Plus Therapeutic Hypothermia

Erythropoietin

As noted earlier, Epo is a glycoprotein originally identified for its role in erythropoiesis but which has remarkable neuroprotective and reparative effects in the central nervous system (reviewed in Rangarajan and Juul, 2014; Juul and Pet, 2015). In the setting of hypoxia–ischemia, Epo receptor expression is rapidly upregulated (Sola et al., 2005; Jantzie et al., 2013). If Epo is available to bind to the upregulated receptor, cell survival is promoted, but in the absence of Epo, the pathway of programmed cell death predominates (Jantzie et al., 2013, 2015). This creates an important rationale for exogenous Epo administration, given that upregulation of Epo may take several hours, whereas brain injury can occur after brief but catastrophic insults that are insufficient to stimulate an increase in endogenous Epo synthesis (Traudt et al., 2013).

Preclinical studies of Epo neuroprotection following hypoxic–ischemic brain injury show robust histologic and functional benefit (Kumral et al., 2004; Achterberg et al., 2005; Chang et al., 2005; Demers et al., 2005; Gonzalez et al., 2007; McPherson et al., 2007; Iwai et al., 2010; Sargin et al., 2010; Pet and Juul, 2014; Rangarajan and Juul, 2014; Juul and Pet, 2015). Epo has both early and late beneficial effects. Early benefits include antiapoptotic and antiinflammatory effects (Digicaylioglu and Lipton, 2001; Sun et al., 2005; Kellert et al., 2007; Juul et al., 2009; Xiong et al., 2011), while later effects include increased neurogenesis, plasticity, and tissue remodeling after hypoxia–ischemia (Iwai et al., 2007; Ransome and Turnley, 2007; Yang et al., 2007; Wang et al., 2008; Reitmeir et al., 2011). In nonhuman primates, Epo reduces the combined outcome of death or CP and improves neurologic function in animals undergoing hyperthermia therapy for HIE (Traudt et al., 2013). Phase I and phase II clinical trials have raised no safety concerns and suggest that infants with HIE treated with multiple doses of Epo during the first week of life have better neurologic outcomes (Zhu et al., 2009; Elmahdy et al., 2010). The Neonatal Erythropoietin in Asphyxiated Term Newborns (NEAT) and Neonatal Erythropoietin And Therapeutic Hypothermia Outcomes in Newborn Brain Injury (NEATO) trials have demonstrated safety, feasibility (Wu et al., 2012), and beneficial outcomes as measured by early MRI, biomarkers, and 6-month and 2-year outcomes (even among infants with significant brain injury seen on MRI) (Rogers et al., 2014; Wu et al., 2016). Epo is commercially available and safe in infants. Phase III trials are now in the early stages of execution internationally, including the Preventing Adverse Outcomes of Neonatal Hypoxic Ischaemic Encephalopathy with Erythropoietin trial (Australia) and the High-Dose Erythropoietin for Asphyxia and Encephalopathy trial (United States).

Xenon

Xenon, a noble gas that crosses the placenta and the blood–brain barrier, binds to *N*-methyl-D-aspartate glutamate receptors to inhibit function, thus decreasing neuronal apoptosis (Franks et al., 1998; Ma et al., 2007). Significant benefit was demonstrated in preclinical studies of HIE (Lobo et al., 2013), so the multicenter Total Body Hypothermia Plus Xenon (TOBY-Xe) trial was conducted in the United Kingdom. Ninety-two infants (36–43 weeks) were enrolled, 46 of whom were randomly assigned to cooling only, and 46 were randomly assigned to xenon treatment plus cooling (Azzopardi et al., 2015). The primary outcomes were assessment of reduced thalamic lactate to *N*-acetylaspartate ratios measured with magnetic

resonance spectroscopy and preserved fractional anisotropy in the posterior limb of the internal capsule determined by MRI within 15 days of birth. Lactate to *N*-acetylaspartate ratios have been demonstrated to be a good predictive imaging biomarker of neurodevelopmental outcomes (Thayyil et al., 2010). Changes in fractional anisotropy, a measure of brain connectivity derived from the diffusion tensor imaging that assesses the degree of regional anisotropic diffusion, correlate well with subsequent outcomes in neonates with HIE (Tusor et al., 2012). The TOBY-Xe trial was underpowered to detect changes in the lactate to *N*-acetylaspartate ratios but adequately powered to detect changes in fractional anisotropy. Although no serious adverse events were recorded, no significant magnetic resonance differences were detected between groups. On the basis of the magnetic resonance results of 37 infants in the cooling-only group and 41 in the cooling plus xenon group, early TH plus treatment with 30% xenon for 24 hours begun more than 6 hours after birth is not likely to improve clinical outcomes compared with TH alone for newborns with neonatal encephalopathy. Multiple factors that may impact inhaled xenon treatment outcomes, including the timing, dose, and duration of treatment, need further study. Study results of the CoolXenon3 Study (ClinicalTrials.gov NCT02071394) combining TH with 18 hours of xenon inhalation in cooled infants with HIE are pending.

Argon

In addition to xenon, another inert gas, argon, has shown promise as an adjunct to hypothermia in a piglet model of neonatal encephalopathy (Broad et al., 2016), building on a wealth of in vitro and small animal model of injury data supporting its neuroprotective qualities (Ulbrich and Goebel, 2015). Argon is more abundant than xenon and as such is cheaper and more practical to use clinically, not requiring a complex rebreathing/scavenger setup. As yet there are no clinical trials using argon to treat any neurologic injury.

Melatonin

Neuroprotective benefits of melatonin have been demonstrated in an HIE piglet model, in which intravenously administered melatonin plus TH significantly improved cerebral energy metabolism on the basis of proton magnetic resonance spectroscopy studies, reduced apoptosis in deep brain structures, and decreased microglial activation in the cortex at 48 hours after injury (Robertson et al., 2013). In uncooled term human newborns with neonatal encephalopathy ($n = 10$), oral melatonin (eight doses of 10 mg each separated by 2-hour intervals) administration within the first 6 hours after delivery reduced the serum malondialdehyde, a lipid peroxidation product, and nitrite/nitrate levels at 12 and 24 hours compared with those in untreated, uncooled controls ($n = 10$), suggesting a role for melatonin in reducing oxidative damage (Fulia et al., 2001). In a recent prospective trial by Aly et al. (2015) involving 45 term newborns, 30 with HIE and 15 healthy controls, compared with TH alone ($n = 15$), melatonin treatment (10 mg/kg daily for five enteral doses) plus TH ($n = 15$) was associated with decreased seizures per EEG and decreased white matter abnormalities on MRI after 2 weeks of age and improved survival without neurologic or developmental abnormalities at 6 months of age ($P < .001$).

Stem Cells

There have been several decades in which stem cells have promised to revolutionize the treatment of diseases, from Alzheimer disease to CP (Diukman and Golbus, 1992; van Bekkum, 1998; Mattson,

2000). In the field of perinatal brain injury, these hopes have been confounded by research issues that include a lack of consistent use of cell type (caused by no thorough comparison of cell types ever being made), no standardization for the preparation of cells, and issues in interpreting species-specific effects of cells. There is still a great deal that needs to be understood in the field; for a comprehensive review, see Fleiss et al. (2014). Despite these unresolved questions there are several ongoing clinical trials (Table 62.1). Of the trials thus far completed, only one robustly designed study (double blind, randomized, and placebo controlled) has reported outcomes (Min et al., 2013). This study combined Epo treatment and umbilical cord blood therapy and reported improvements in motor and functional scores at 6 months of age for combined Epo treatment and umbilical cord blood therapy compared with Epo treatment only and no treatment. This study was small (30 patients per arm) and did not have an umbilical cord blood-only group. No effect of Epo was observed, and the study authors suggest the main treatment effect was due to umbilical cord blood treatment, not Epo. In addition, only patients in whom stem cells had been administered were treated with the immunosuppressant cyclosporine. Given the importance of persisting inflammation in the neuropathologic processes of CP (see Tertiary Phase Injury Processes; Fleiss and Gressens, 2012), this is an important confounder. Several additional trials with robust design are under way.

The following sections describe the main cell types proposed for neurotherapeutic use and provide information on the ongoing clinical trials in the field of perinatal brain injury. The great majority of clinical trials are performed with umbilical cord blood–derived stem cells or bone marrow–derived stem cells (Table 62.1).

Umbilical Cord Stem Cells. Umbilical cord blood contains various stem cell populations, including mesenchymal stem cells (see later), endothelial progenitor cells, and umbilical cord blood mononuclear cells (UCB-MNCs). UCB-MNCs differentiate in vitro into virtually all types of mature cell, including neural cells. Mononuclear cells (MNCs) are also found in Wharton jelly, and together with UCB-MNCs these MNCs have a greater proliferative potential than MNCs derived from the bone marrow. The accessibility, low antigenicity, and absence of ethical issues for cell harvesting make these cells attractive therapeutic options.

Mesenchymal Stem Cells. Mesenchymal stem cells (MSCs) can be derived from fetal and adult tissues, including first-trimester fetal blood, liver and bone marrow, placenta, adult bone marrow, and umbilical cord blood. MSCs can differentiate into all forms of mesenchymal tissue (e.g., bone, cartilage, fat) and neurons, although MSCs derived from fetal sources retain a greater potential for plasticity compared with adult-derived MSCs. The attributes of MSCs that make them attractive therapeutics include their low immunogenicity, as they do not express major histocompatibility complex class II, and their antiinflammatory and immunosuppressive properties.

Embryonic Stem Cells. Embryonic stem cells (ESCs) are derived from the inner mass of the blastocyst and can self-renew indefinitely, being able to maintain an identical phenotype following cell division. ESCs are pluripotent (able to generate cells from the ectoderm, mesoderm, and endoderm) and represent an almost unlimited supply of cells for transplant. However, there are concerns about teratoma formation after transplant, as they are obviously not autologous, and their use has considerable ethical concerns.

Induced Pluripotent Stem Cells. Induced pluripotent stem cells (iPSCs) are created by causing terminally differentiated somatic cells to revert to pluripotency by chemical or genetic reprogramming.

TABLE 62.1 Clinical Trials Using Stem Cell Therapy in Patients Following Perinatal Brain Injury

| Title | Trial Duration | Intervention | Age at Inclusion | Enrollment |
|--|------------------------|---|-----------------------|-----------------|
| A Safety and Feasibility Study of Autologous Cord Blood and Human Placental Derived Stem Cells in Neonates With Severe Hypoxic-Ischemic Encephalopathy | Jan. 2016 to Jun. 2019 | Autologous human placental-derived stem cells administered in conjunction with autologous cord blood in neonates with severe hypoxic-ischemic encephalopathy (diagnosed as per standard hypothermia inclusion criteria). Safety only | Less than 6 h | 20 (estimated) |
| Efficacy of Stem Cell Transplantation Compared to Rehabilitation Treatment of Children With Cerebral Palsy (Palsy) | Oct. 2013 to Dec. 2015 | Mesenchymal stem cells derived from umbilical cord, four doses versus rehabilitation only versus no therapy no rehabilitation controls | 1–14 years | 300 (estimated) |
| Is Autologous Umbilical Cord Blood Reinfusion Beneficial in Children With Cerebral Palsy: A Randomized, Blinded, Placebo-Controlled, Crossover Study | Jun. 2010 to Jul. 2016 | Two injections, one of autologous human cord blood–derived stem cells and one vehicle control administered 12 months apart. Randomization of order of injections. Two-year follow-up | 12 months to 6 years | 120 |
| Evaluation the Side Effects of Bone Marrow Derived CD133 Cells Transplantation in Cerebral Palsy Patients | Oct. 2011 to May 2012 | Intrathecal injection of bone marrow–derived CD133 cells. Safety only | 4–12 years | 12 |
| The Safety of Multiple Intrathecal Injection of Bone Marrow Derived CD133 Cells in Patients With Cerebral Palsy | Apr. 2012 to Apr. 2014 | Intrathecal injection of bone marrow–derived CD133 cells versus patients not receiving stem cells | 4–12 years | 8 |
| Effects of the Infusion of Autologous Noncryopreserved CD34+ Cells in Newborns With Asphyxia | Jan. 2012 to Apr. 2013 | Intravenous infusion of autologous stem cells within the first 48 h after birth versus control group of patients who meet the inclusion criteria (Apgar score 0–3 at 1 min, metabolic acidosis, hypoxia, multiple organ failure) but who do not wish to have the intervention | 37–42 weeks | 20 |
| Safety and Efficacy of Bone Marrow MNC for the Treatment of Cerebral Palsy in Subjects Below 15 Years | Mar. 2011 to Aug. 2014 | Intrathecal injection of 1 million autologous mononuclear cells | 3–15 years | 100 (estimated) |
| Allogeneic Umbilical Cord Blood and Erythropoietin Combination Therapy for Cerebral Palsy | May 2010 to Apr. 2011 | Allogeneic umbilical cord blood infusion under nonmyeloablative immunosuppression, plus erythropoietin twice weekly for 4 weeks (two initial doses of 500 IU/kg intravenously, followed by six doses of 250 IU/kg subcutaneously) and rehabilitation versus erythropoietin plus rehabilitation versus rehabilitation only | 10 months to 10 years | 105 |
| Safety and Effectiveness of Cord Blood Stem Cell Infusion for The Treatment of Cerebral Palsy in Children | Jan. 2010 to Feb. 2014 | Mononuclear cell–enriched cord blood unit prepared for infusion versus placebo | 1–12 years | 20 |
| Intrathecal Autologous Stem Cells for Children With Hypoxic/Ischemic Brain Injury | Jul. 2009 to Apr. 2010 | Patients will be stimulated with granulocyte colony stimulating factor five times, then bone marrow will be harvested and patients will be later infused with 8–10 mL of stem cells (CD34 ⁺) by the intrathecal route versus baseline motor scores | 1–8 years | 18 |

BBS, Berg Balance Scale; DTI, diffusion tensor imaging; ¹⁸F-FDG PET, [¹⁸F] fluorodeoxyglucose positron emission tomography; IQ, intelligence quotient; MNC, mononuclear cells; MRI, magnetic resonance imaging.

References (Min et al., 2013; Mancias-Gueria, 2014; Zali et al., 2015).

| Status | Study Design | Outcome Measures | Sponsor and Trial Identifier |
|-------------------------------------|--|--|--|
| Not yet recruiting | End point classification: safety Intervention model: single group assignment Masking: open label Primary purpose: treatment | Primary: infusion reaction within 30 days Secondary: neurologic improvement (MRI, DTI, and Sarnat testing) | New York Medical College, United States NCT02434965 |
| Ongoing | Allocation: randomized End point classification: safety/efficacy Intervention model: parallel assignment Masking: single blind (outcomes assessor) Primary purpose: treatment | Primary: Gross Motor Function Measure-88 and Gross Motor Function Measure-66 at 12 months Secondary: biochemical analysis of glutamic species at 12 months | General Hospital of Chinese Armed Police Forces NCT01929434 |
| Completed and results pending | Allocation: randomized End point classification: efficacy Intervention model: crossover assignment Masking: double blind (subject, caregiver, investigator, outcomes assessor) Primary purpose: treatment | Primary: standardized measures of neurodevelopmental function at 2 years Secondary: at 2 years, (1) quality of life, (2) MRI changes, (3) blood transcriptomics | Duke University Medical Center, United States NCT01147653 |
| Completed (Zali et al., 2015) | End point classification: safety Intervention model: single group assignment Masking: open label Primary purpose: treatment | Primary: infusion reaction within 30 days Secondary: neurologic scores (speech and motion) | Royan Institute, Iran NCT01404663 |
| Ongoing | Allocation: randomized End point classification: safety/efficacy Intervention model: parallel assignment Masking: open label Primary purpose: treatment | Primary: infusion reaction within 48 hours and motor and sensory score at six months Secondary: neurologic score (Gross Motor Function Measure-66), balance (BBS), and incidence of spasm | Royan Institute, Iran NCT01763255 |
| Completed and results not available | Allocation: nonrandomized End point classification: safety/efficacy Intervention model: parallel assignment Masking: open label Primary purpose: prevention | Primary: Amiel-Tison neurologic assessment at 1 week and 1 year | Hospital Universitario Gonzalez Monterrey, Mexico NCT01506258 |
| Ongoing | End point classification: safety/efficacy Intervention model: single group assignment Masking: open label | Primary: at 6 months, Ashworth scale and overall motor control using Oxford scale Secondary: at 6 months, IQ using Binet-Kamat scale, social behavior testing, and reduction in deformity | Chaitanya Hospital, Pune, India NCT01832454 |
| Completed (Min et al., 2013) | Allocation: randomized End point classification: safety/efficacy Intervention model: parallel assignment Masking: double blind (subject, caregiver, investigator, outcomes assessor) Primary purpose: treatment | Primary: Gross Motor Performance Measure at 1, 3, and 6 months compared with the baseline Secondary: Korean version of Bayley Scales of Infant Development-II Mental Scales, Quality of Upper Extremity Skills Test, and Pediatric Evaluation of Disability Inventory at 0, 1, 3, and 6 months. Brain glucose metabolism using ¹⁸ F-FDG PET at 2 weeks. MRI at 6 months. Adverse reactions by 6 months | Sung Kwang Medical Foundation, South Korea NCT01193660 |
| Ongoing | Allocation: randomized End point classification: safety/efficacy Intervention model: crossover assignment Masking: double blind (subject, caregiver, investigator, outcomes assessor) Primary purpose: treatment | Primary: safety via repeated follow-up over 1 year with clinical and laboratory evaluations Secondary: standardized Gross Motor Function Measure evaluation | Georgia Regents University, United States NCT01072370 |
| Completed (Mancia-Gueria, 2014) | End point classification: efficacy Intervention model: single group assignment Masking: open label Primary purpose: treatment | Primary: Battelle Developmental Inventory at 30 days Secondary: Battelle Developmental Inventory at 180 days | Hospital Universitario Gonzalez Monterrey, Mexico NCT01019733 |

Reprogramming techniques were initially limited to genetic manipulations using integrating viruses. Newer techniques using chemical reprogramming are a significant improvement as it is thought that these cells will have a reduced risk of aberrant proliferative ability after transplant. A benefit of these iPSCs is autologous transfer, but reprogramming rates are low, and great technical skills are required.

Neural Stem Cells. Neural stem cells (NSCs) are derived from the embryonic or fetal brain but are also found in the adult subventricular zone and dentate gyrus. NSCs can be derived from iPSC to overcome issues related to nonautologous transplants. NSCs can differentiate into neurons, oligodendrocytes, and astrocytes, but the same risk of teratoma formation exists for NSC as ESC and iPSCs.

Amniotic Fluid–Derived Stem Cells. Amniotic fluid contains stem cells with a phenotypic profile between that of ESCs and that of MSCs. Amniotic fluid–derived stem cells are multipotent and nontumorigenic. They need to be harvested by amniocentesis, limiting their use.

Clinical Management Strategies

Along with seizures, neonates with neonatal encephalopathy frequently demonstrate cardiovascular instability evidenced by hypotension, metabolic acidosis, and pulmonary hypertension. Unlike the more standardized TH guidelines, clinical approaches regarding antiepileptic use, fluid resuscitation, pressor support (including hydrocortisone treatment), the use of base replacement (e.g., sodium bicarbonate), ventilation strategies, oxygen saturation targets, inhaled nitric oxide use, and blood transfusion parameters may all influence outcomes in the setting of TH.

The use of medications to provide sedation and prevent shivering in cooled newborns with neonatal encephalopathy must also be further studied. The current use of morphine during TH is not evidence based and may not be ideal because of its side effect profile (e.g., respiratory depression, urinary retention, constipation) and because it does not specifically prevent shivering. Dexmedetomidine (DEX) and clonidine, both α_2 -adrenergic receptor agonists, are promising alternative sedatives because they specifically prevent shivering without suppressing respirations. DEX reduces inflammation (Taniguchi et al., 2008; Yang et al., 2008), does not produce abnormal brain histologic features (neonatal rats) (McAdams et al., 2015), and produces neuroprotection in animal models of HIE (Laudenbach et al., 2002; Paris et al., 2006; Sato et al., 2010). Currently, trials to assess the pharmacokinetics and safety of DEX (Cool DEX study, NCT02529202) and clonidine (NCT02252848) in newborns with neonatal encephalopathy during TH are under way.

Future Outlook on Term Brain Injury Neuroprotection

As highlighted in the initial sections of this chapter, cell death plays a significant role in brain injury in the term infant, and as such, more therapies are specifically tailored to target these processes. The research areas discussed in the following sections have not yet been studied in clinical trials for perinatal brain injury but may be of future use following validation in this population.

Remote Ischemic Postconditioning

Remote ischemic postconditioning is a promising therapeutic intervention whereby brief episodes of ischemia/reperfusion of a

limb abrogate damage in another organ (such as the brain) that has experienced hypoxia–ischemia. Remote ischemic postconditioning has been shown to protect the adult and neonatal brain in rodent models of hypoxia–ischemia when applied immediately at reperfusion or delayed by up to 24 hours (Drunalini Perera et al., 2014) and when applied immediately in a piglet model of neonatal encephalopathy induced with hypoxia–ischemia (Ezzati et al., 2016). The molecular mechanism of action underpinning protection by remote ischemic postconditioning is still under debate but likely involves soluble factors released from the postconditioned tissues. There are 12 registered clinical trials (ClinicalTrials.gov; limb+ischemic+postconditioning) in adults using a modified cuff, on the arm akin to the cuffs used for blood pressure measurement, for indications including liver and heart transplants and stroke. However, in the piglet model, inguinal cuffs to occlude the femoral artery of both legs were necessary, and issues need to be overcome for the design, manufacture, and training in the use of such equipment in a clinical setting for perinatal brain injury.

Targeting Inflammation

Extensive preclinical and clinical data suggest that the presence of inflammation sensitizes the term brain to injury leading to neonatal encephalopathy (Fleiss et al., 2015). Marked improvements in obstetric care have reduced the burden of neonatal encephalopathy in term infants, but the rate remains at 8.5 per 1000, and the standard therapy of hypothermia reduces poor outcome in only one-eighth of infants. Stratification of patients by inflammatory status is increasingly considered a potential way to identify responders/nonresponders to hypothermia and/or as a key to identify patients who might benefit from alternative therapies. Increasing interest in biomarker discovery (Beckstrom et al., 2011; Blaise, 2013) and our burgeoning ability to implement crib side testing will facilitate patient stratification for this. No specific differences in the inflammatory response of term infants that would necessitate specific antiinflammatory agents to be designed have been identified, but this is an understudied area. More than likely, we will witness the repurposing of inflammatory therapies, especially in concert with cell-specific delivery to reduce off-target toxic effects of drugs that have failed in traditional testing.

Targeting Autophagy

Autophagy is the orderly recycling of intracellular components and is critical for normal cellular function. In times of injury or stress, autophagy can be protective, by providing alternative sources of energy and by eliminating toxins or pathogens, but it can also promote cell death (for an extensive review, see Descloux et al., 2015). As such, understanding and modulating autophagy are avenues of interest for neurotherapeutic design. There is a lack of specific pharmacologic tools to modulate autophagy, but genetic studies in mice have proven the neuroprotective capacity of modulating autophagy (Koike et al., 2008; Ginot et al., 2014), including in the immature brain (Xie et al., 2016). Furthermore, the levels of markers for autophagy are increased in human postmortem analysis of both injured premature brains (Vontell et al., 2015) and injured term brains (Descloux et al., 2015). Of particular interest is basic research into mitophagy, recycling of mitochondria, as mitochondrial dysfunction is a keystone of cell death in perinatal brain injury (Hagberg et al., 2014; Thornton and Hagberg, 2015). Ideal neuroprotection will likely include multiple targeted approaches at different times.

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63

Neonatal Neuroimaging

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KEY POINTS

- Cranial ultrasonography is useful for evaluating ventricular size and hemorrhage in preterm neonates during their neonatal intensive care unit (NICU) stay.
- Magnetic resonance imaging (MRI) is the most sensitive imaging modality for many of the forms of injury found in both term and preterm neonates, including watershed injury, basal ganglia injury, white matter injury, and stroke.
- Computed tomography (CT) should only be used when MRI is not available.
- The MRI findings following brain injury are dynamic, with diffusion imaging being most sensitive 2–4 days after injury, and T₁-weighted and T₂-weighted imaging being most sensitive thereafter.
- Neuroimaging studies of both term and preterm neonates have prognostic value.

CONTROVERSY BOX

Is Brain Magnetic Resonance Imaging Near Term Equivalent Age Helpful in Extremely Preterm Babies?

Pros: An MRI study obtained near the time of hospital discharge (term equivalent age) has better predictive value for subsequent neurodevelopmental outcome than any other clinical or imaging metric, particularly when systematic MRI scoring systems are employed. The use of MRI to help identify those infants at high risk for neurodevelopmental impairment may allow targeting of therapy services to those infants who would benefit most from them, and early initiation of appropriate therapy services may improve outcomes. MRI at term may also be used as a quality neurologic outcome metric for brain injury that is difficult to detect on ultrasound, such as white matter and cerebellar injury, and brain growth measures, thereby allowing the neonatal unit to track the true incidences of injury and impaired brain growth in their patient population.

Cons: There are no studies proving that obtaining an MRI at the time of hospital discharge in very preterm infants leads to improved neurodevelopmental outcomes. Furthermore, the imprecise relationship between MRI findings and outcome may cause uncertainty in prognosis, leading to increased anxiety for parents.

Additional comments: Discussing long-term prognosis for very preterm infants with their parents prior to discharge from the NICU should be standard practice. This discussion should be informed by clinical markers such as gestational age, clinical history, and neurologic examination as well as MRI (when appropriate). We suggest that term MRI studies be obtained on those infants at highest risk for adverse outcome based on gestational age and/or clinical course. In this context, it is important that clinicians be trained in the relationship between gestational age, clinical course, MRI, and outcome. Concerns regarding parental anxiety are based on anecdotal evidence but highlight the importance of the nature of this communication to empower and enable families rather than cause concern.

In this chapter, we focus primarily on the three main techniques for assessing brain structure and injury in infants—cranial ultrasonography (CUS), computed tomography (CT), and magnetic resonance imaging (MRI). We review the fundamentals of the methods and describe their application to the preterm and term-born populations. We also review methods that are under development and may prove clinically useful in the future.

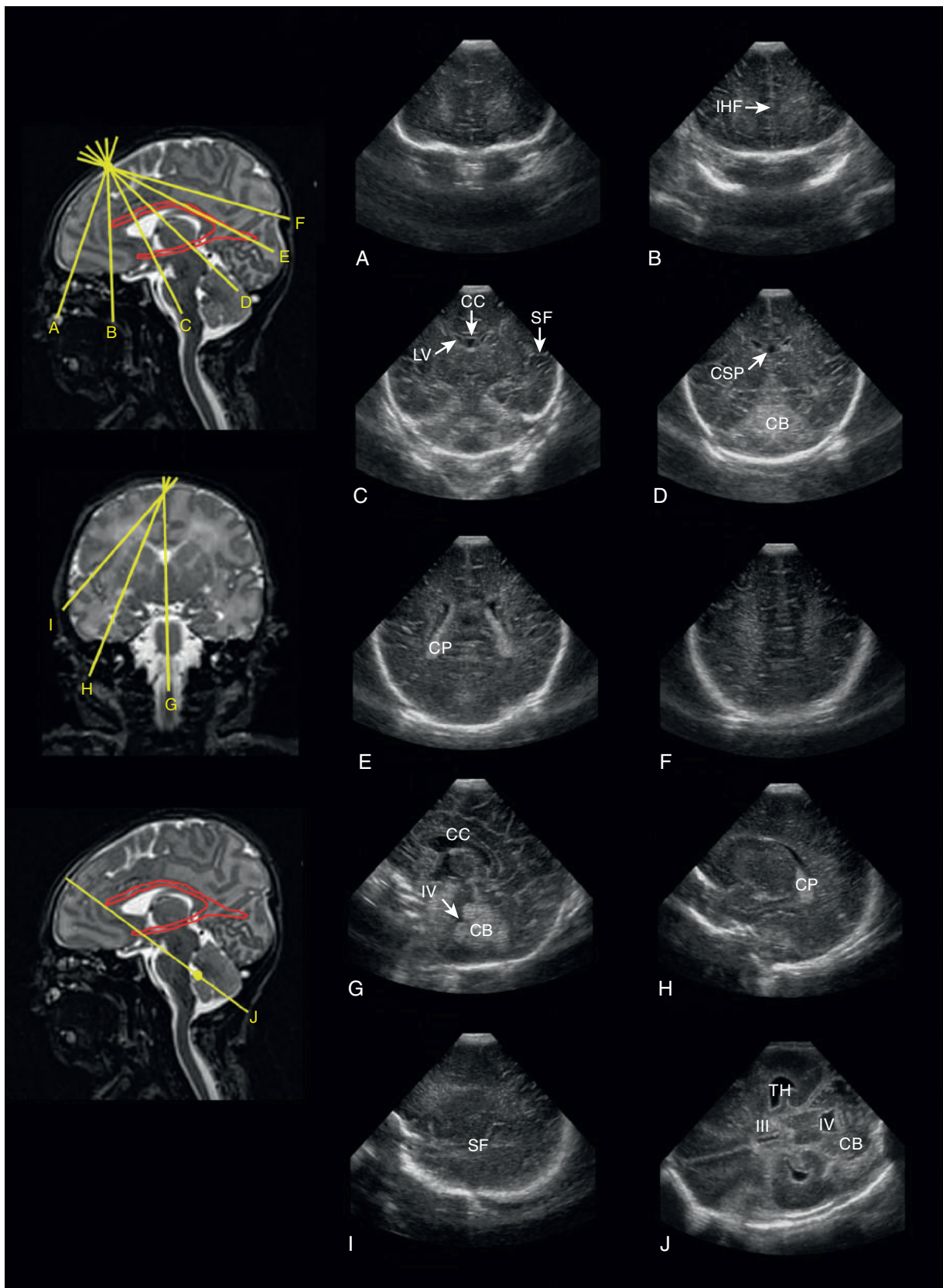
Imaging Modalities

Cranial Ultrasonography

CUS is based on the reflection of ultrasound from tissue. Since ultrasound does not penetrate bone well, CUS is limited to infants with open fontanels (though ultrasound can sometimes be obtained through the squamous portion of the temporal bone). CUS studies are mainly done through the anterior fontanel, often with inclusion of images of the posterior fossa taken through the mastoid foramen (Fig. 63.1). Since image quality falls off with distance from the ultrasound probe, images of the posterior fossa obtained through the mastoid foramen have richer detail than those obtained through the anterior fontanel. Image quality also worsens somewhat as the fontanels gradually close, and obtaining CUS images of the brain is no longer feasible once the fontanel closes completely at the approximate age of 6 months.

Tissue interfaces, such as that between cerebrospinal fluid (CSF) and brain parenchyma, give a strong echo return. Consequently, CUS excels at providing an outline of the ventricular system. Hemorrhage is also readily visible and appears bright because of the strong echo from the loosely packed red blood cells. This combination of properties makes CUS very useful for evaluating posthemorrhagic hydrocephalus as well as cystic changes in the periventricular white matter in preterm neonates (vide infra). In comparison, the echo return from brain parenchyma is considerably smaller, with parenchyma appearing relatively dark compared with hemorrhage or the ventricular outline. Furthermore, there is little contrast between injured and uninjured tissue. As a result, areas of *nonhemorrhagic* brain injury, such as stroke, are difficult to detect with CUS, though areas of nonhemorrhagic injury may sometimes appear brighter than surrounding tissue.

It is important that clinicians caring for preterm neonates personally review the images from every CUS study on their patients, just as neonatologists look at every chest X-ray and neurologists every MRI study. One potential hurdle to learning to evaluate CUS studies is its unique imaging planes. A standard CUS image set obtained through the anterior fontanel typically starts with



• **Fig. 63.1** Head Ultrasound Studies and Their Corresponding Imaging Planes. The imaging planes (yellow) are shown on a midline sagittal magnetic resonance image upon which an outline of a lateral ventricle is drawn in red. (A–I) The ultrasound images were taken through the anterior fontanel. (J) The image was taken through the mastoid foramen. CB, Cerebellum; CC, corpus callosum; CP, choroid plexus; CSP, cavum septum pellucidum; III, third ventricle; IV, fourth ventricle; IHF, interhemispheric fissure; LV, lateral ventricles; SF, Sylvian fissure; TH, temporal horn of the lateral ventricle.

slices that are very close to coronal in the anterior part of the brain, with the images becoming progressively more oblique, and closer to axial, for images from the posterior parts of the brain (see Fig. 63.1A–F). Images are then obtained with the ultrasound probe oriented to obtain a sagittal midline image (see Fig. 63.1G), as well as parasagittal images of the hemispheres and lateral ventricles (see Fig. 63.1H–I). Images obtained through the mastoid window are also oblique and may extend superiorly/anteriorly to include the third ventricle in very preterm neonates, for whom the mastoid window is still wide open (see Fig. 63.1J). Another effect of the necessity of imaging around rather than through bone is that the cerebral convexities cannot be seen using CUS.

In addition to structural information, ultrasound also has the capacity to provide measurements related to blood flow. This ability results because the frequency of the ultrasound signal used for imaging undergoes a Doppler shift when reflected by moving structures such as the cells in flowing blood. Thus Doppler ultrasonography has proven useful for evaluating the patency of both arteries and veins. This Doppler shift is readily converted to units of velocity (m/s), but deriving a blood flow value (mL/min per g of tissue) from a velocity value is not straightforward, as factors such as the diameter of the vessel, laminar blood flow, and the angle of the ultrasound beam relative to the blood vessel must be taken into account. In practice, the Doppler measurements are usually expressed as the resistive index, which is a unitless number calculated as the difference between systolic and diastolic flow velocities divided by the systolic flow velocity. Notably, resistive index is not affected by changes in the angle of the probe relative to the blood vessel. A related index is the pulsatility index, which is calculated in the same fashion as the resistive index except that the denominator is the mean flow velocity rather than systolic flow velocity. These measurements are typically taken from the anterior cerebral artery as it wraps around the genu of the corpus callosum and/or the middle cerebral artery as it turns in a superior–inferior direction. These vessel segments are chosen because the beam from the ultrasound probe, positioned at the anterior fontanel, is parallel to the arteries at these points, providing more consistent measurements. A comparison of CUS with other imaging modalities is provided in Table 63.1.

Computed Tomography

CT scanning has been used to study infants since its invention in the 1970s and was the imaging modality used 40 years ago to

develop the Papile classification of intracranial hemorrhage for preterm neonates (Papile et al., 1978). It is similar to CUS in that it excels at showing hemorrhage and the ventricular outline. In addition, like CUS, it does not provide particularly good tissue contrast for nonhemorrhagic injury. However, unlike CUS, it provides a clear view of the cerebral convexities. It provides a reasonable view of the posterior fossa, but image quality in this area is often degraded by the effects of surrounding bone. CT scanning is falling out of favor as an imaging modality for infants because of concerns regarding its use of ionizing radiation. In the rapidly developing brain, irradiation may cause injury that affects subsequent IQ (Ron et al., 1982; Hall et al., 2004). Furthermore, there is concern about an increased incidence of subsequent head and neck cancers (Karlsson et al., 1998; Brenner et al., 2003; Boice, 2015; Krille et al., 2015).

Magnetic Resonance Imaging

In comparison with CUS, MRI has the disadvantage that infants must be moved from the intensive care unit to the radiology department for study, though this may change as MR scanning systems that are suitable for housing within the neonatal intensive care unit (NICU) are developed. Furthermore, the infant is relatively inaccessible while in the MRI scanner in the event of a medical emergency. The major advantage of MRI relative to CUS and CT is that it provides unmatched structural detail, high sensitivity to parenchymal injury, and high sensitivity to brain malformations (Raybaud et al., 1996; Cowan et al., 2005; de Vries and Volpe, 2013). From a practical standpoint, imaging infants requires adaptations of the scanning process. For example, optimum signal-to-noise ratio, and hence better image quality, is achieved when the radiofrequency coil used for imaging is size-matched to the infant head, although it remains common practice to use a head coil designed for adult imaging for imaging infants. In addition, image contrast is different for newborns as compared with older children (see below) so that pulse sequence timing parameters must be optimized for the infant brain to obtain images with optimum contrast-to-noise ratio. Finally, infants are considerably less cooperative in holding still during the scanning process than older patients. As a result, it is standard practice in some centers to sedate infants to minimize movement. However, a variety of approaches have been developed to mitigate subject motion (Glover and Pauly, 1992; Pipe, 1999; Mathur et al., 2008; Tamhane and Arfanakis, 2009; Olesen et al., 2010; Gholipour et al., 2011; Tisdall et al., 2012; Ooi et al., 2013; Gumus et al., 2015). Thus sedation

TABLE 63.1 Comparison of Imaging Modalities

| Modality | Advantages | Disadvantages |
|----------------------------------|---|---|
| Cranial ultrasound (CUS) | <ul style="list-style-type: none"> • Bedside test • Inexpensive • Excellent for evaluating ventricular size and hemorrhage • Provides an indication of vessel patency (Doppler) | <ul style="list-style-type: none"> • Unable to image cerebral convexities • Challenging for nonradiologist to interpret • Poor tissue contrast for nonhemorrhagic injury • Misses subtle brain malformations such as heterotopias |
| Computed tomography (CT) | <ul style="list-style-type: none"> • Readily available in most medical centers • Relatively inexpensive • Shows hemorrhage and the ventricular outline well | <ul style="list-style-type: none"> • Uses ionizing radiation • Poor tissue contrast for nonhemorrhagic injury • Requires the infant to be transported to the scanner |
| Magnetic resonance imaging (MRI) | <ul style="list-style-type: none"> • Offers a rich variety of contrast types (structural and functional) • Provides unparalleled image detail • Shows parenchymal injury well • Shows malformations and heterotopias well | <ul style="list-style-type: none"> • Expensive • Requires the infant to be transported to the scanner. • It can be challenging to monitor infants while in the scanner. • Scan times are longer than for CT or CUS. |

for scanning should no longer be standard practice, though may still be necessary in some cases.

T₁-Weighted and T₂-Weighted Imaging

MRI differs from CUS and CT in that it offers a wide variety of contrast types. Most MRI is based on the detection of signal from the hydrogen nuclei of water ($^1\text{H}_2\text{O}$), which are present at a concentration of approximately 110 mol/L in tissue. For “conventional” (T_1 -weighted and T_2 -weighted) imaging, image contrast is based on the T_1 or T_2 relaxation time constants of water ^1H . These time constants vary with the local chemical environment. For example, ^1H in CSF water has a relatively large T_2 time constant, and hence CSF appears bright on T_2 -weighted images compared with other tissues.

White matter contrast on MRI studies changes dramatically between birth and 1 year of life. Unmyelinated white matter appears dark on T_1 -weighted images and bright on T_2 -weighted images. With myelination, the T_1 and T_2 time constants change such that myelinated white matter has the opposite signal properties—bright on T_1 -weighted images and dark on T_2 -weighted images. This change in contrast can be employed to detect myelination (Ganzetti et al., 2014), which takes place at different rates in different brain areas. For example, primary motor cortex and visual cortex develop earlier than most other brain areas, and this corresponds to the relatively early myelination of their associated white matter tracts. In a term-born neonate, the myelinated corticospinal tract and optic radiations appear bright on T_1 -weighted images against a background of darker-appearing, unmyelinated white matter (Fig. 63.2). Since the majority of white matter in newborns is unmyelinated, and the signal characteristics of gray matter do not change appreciably during development, gray–white contrast is inverted in neonates compared with older infants and children. As myelination proceeds, white matter signal intensity gradually changes (Almli et al., 2007). Between the ages of 6 and 9 months, white and gray matter signal intensities are similar on both T_1 -weighted and T_2 -weighted images, making it difficult to obtain good gray–white image contrast (see Fig. 63.2). Thus while MRI may still be useful in patients at this age, it is not particularly sensitive for detecting subtle cortical malformations and heterotopias. By the age of 1 year, gray–white contrast is fully inverted and is similar to that of older children and adults.

One final consideration for T_1 -weighted and T_2 -weighted imaging is the detection of hemorrhage. While both CT and CUS also detect hemorrhage, MRI can provide both an indication of the age of the injury (Table 63.2) and the presence of associated nonhemorrhagic parenchymal injury. MR angiography can also sometimes be used to identify associated vascular occlusion. A detailed description of

the clinical use of MRI for detection of hemorrhage is provided near the end of this chapter.

Diffusion Imaging

The contrast in diffusion images is based on water motion, with water displacements on the order of 2–10 μm being measured. The method was originally used for measuring diffusion in liquids and was subsequently adapted to MRI in the mid-1980s (LeBihan et al., 1986). When the method is applied to tissue, the parameter describing water displacements is referred to as the *apparent* diffusion coefficient (ADC, with units of mm^2/s) in recognition of the fact that water motion in tissue is influenced by a variety of factors in addition to Brownian motion, such as active transport and barriers to water movement. Diffusion imaging quickly became a mainstay of clinical imaging when it was discovered that it provides an early marker of stroke (Moseley et al., 1990). It is now known that diffusion images are sensitive to brain injury because water ADC values decrease within minutes following a variety of injuries in addition to stroke, including seizure (Zhong et al., 1993, 1995; Righini et al., 1994; Prichard et al., 1995), spreading depression (Latour et al., 1994; Busch et al., 1995; Rother et al., 1996; Takano et al., 1996), excitotoxic injury (Benveniste et al., 1992; Verheul et al., 1993), and trauma (Ford et al., 1994).

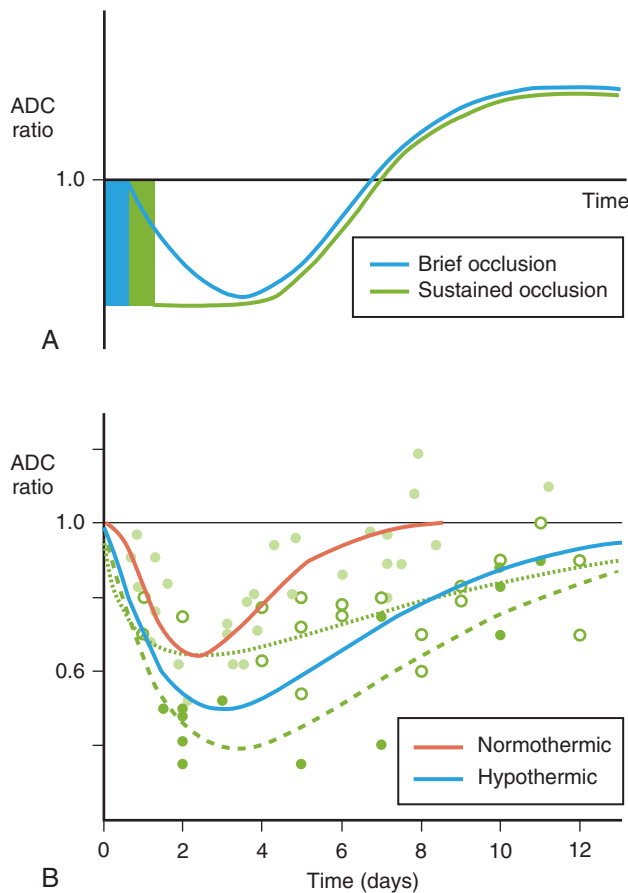
The ADC changes following injury are dynamic, both on a time scale of hours and a time scale of days. In rodent studies (Li et al., 2000), water ADC values in an area of stroke fall within minutes of occlusion of the middle cerebral artery. If the occlusion is maintained for 90 minutes or more, the ADC values remain low for 3–4 days and then gradually rise to greater than normal values by 5–6 days (Fig. 63.3A). This process is known as *pseudonormalization* because ADC values pass through normal 4–6 days after injury. Notably, the time course of ADC changes is different for briefer injury. If blood flow is restored after 30 minutes of occlusion, ADC values return to normal within minutes of restoring blood flow (see Fig. 63.3A). This is followed by a secondary ADC decline within the first 24 hours and a subsequent increase with pseudonormalization at 4–6 days. It has been suggested that this short-term return of ADC values to normal followed by a secondary decline may be related to secondary energy failure, but this has not been proven. However, this more complex time course following briefer injury may explain the presence of “diffusion negative” injury. In adults with stroke, diffusion MRI within the first day shows nearly all strokes, failing to show the injury in only approximately 6% of cases (Oppenheim et al., 2000). The failures may be due to a small fraction of stroke patients in whom blood flow spontaneously returns shortly after injury. In term-born infants, the incidence of diffusion negative injury is higher, perhaps on the order of 30% (McKinstry et al., 2002b). This is probably related to the different mechanism of injury in this population. One can imagine a neonate in utero who undergoes a period of hypoxic–ischemic injury (e.g., caused by placental abruption). This period of injury may be relatively brief if the neonate is rescued by being delivered and resuscitated. In this case, ADC values in injured tissue may transiently return to normal before undergoing a secondary decline followed by pseudonormalization. For neonates with somewhat longer injury, there is no short-term return of ADC values to normal. ADC values are low at delivery and stay low until pseudonormalization takes place. The time course of ADC changes in term-born neonates with injury is shown in Fig. 63.3B. Note also that ADC values tend to fall lower and for longer in neonates treated with therapeutic hypothermia (Bednarek et al., 2012). Overall, diffusion imaging is most sensitive to injury

TABLE 63.2 Magnetic Resonance Imaging Signal Changes After Parenchymal Hemorrhage

| Age of Hemorrhage | SIGNAL INTENSITY | |
|----------------------|------------------|-----------------|
| | T_1 -Weighted | T_2 -Weighted |
| 1–3 days | Isointense | Low |
| 3–10 days | High | Low |
| 10–21 days | High | High |
| 3–6 weeks | High | High |
| 6 weeks to 10 months | Isointense | Low |



• **Fig. 63.2** Sagittal (Left Column) and Axial (Right Column) Images From Subjects at Varying Ages. The images in the first row are from a newborn. Note that myelination of the corticospinal tract is visible at this age as an increase in signal intensity on the T₁-weighted image (*white arrow*). The images in the second row are from an 8-month-old child. Note the relatively poor gray-white contrast at this age as the signal intensity of white matter changes with myelination changes. Myelination of the optic radiations is visible on the T₂-weighted image as reduced signal intensity (*black arrows*). The images in the bottom row are from a 14-month-old child. Note that gray-white contrast is now fully reversed as a result of myelination.



• **Fig. 63.3** Time Course of Apparent Diffusion Coefficient Change Following Brain Injury. (A) The time course of apparent diffusion coefficient (ADC) change following brain injury. Blue represents 30-minute occlusion. Green represents 90-minute occlusion. The data are a composite from animal studies. (B) The time course of ADC change following brain injury in term-born human neonates. Red represents normothermic neonates. Blue shows neonates treated with therapeutic hypothermia. ADC ratios rather than absolute ADC values are used in the ordinate of both graphs because ADC values vary regionally in neonates and the areas of injury vary neonate to neonate. The ratio represents injured tissue over normal tissue, so values less than 1 indicate a reduction in ADC values. The dotted green line represents data from neonates treated with therapeutic hypothermia who had mild to moderate injury based on follow up MRI studies. The dashed green line represents data from those neonates with severe injury. ([A] Adapted from Kinstry RC, Miller JH, Snyder AZ, et al. A prospective, longitudinal diffusion tensor imaging study of brain injury in newborns. *Neurology*. 2002;59:824–833. [B] Adapted from Bednarek N, Mathur A, Inder T, Wilkinson J, Neil J, Shimony J. Impact of therapeutic hypothermia on MRI diffusion changes in neonatal encephalopathy. *Neurology*. 2012;78:1420–1427.)

2–4 days following injury but may show injury during the first day if the injury is severe. T_1 -weighted and T_2 -weighted imaging, on the other hand, usually show injury after 4–5 days. This timing should be taken into account when ordering/interpreting MRI studies to evaluate injury in neonates.

Another interesting aspect of diffusion is diffusion anisotropy. In white matter, water ADC values are greater parallel to axons than perpendicular to them. This is because water moving parallel to axons can move freely within myelin layers without crossing lipid membranes. Water moving perpendicular to axons must pass through myelin layers or go around them, which reduces their displacements.

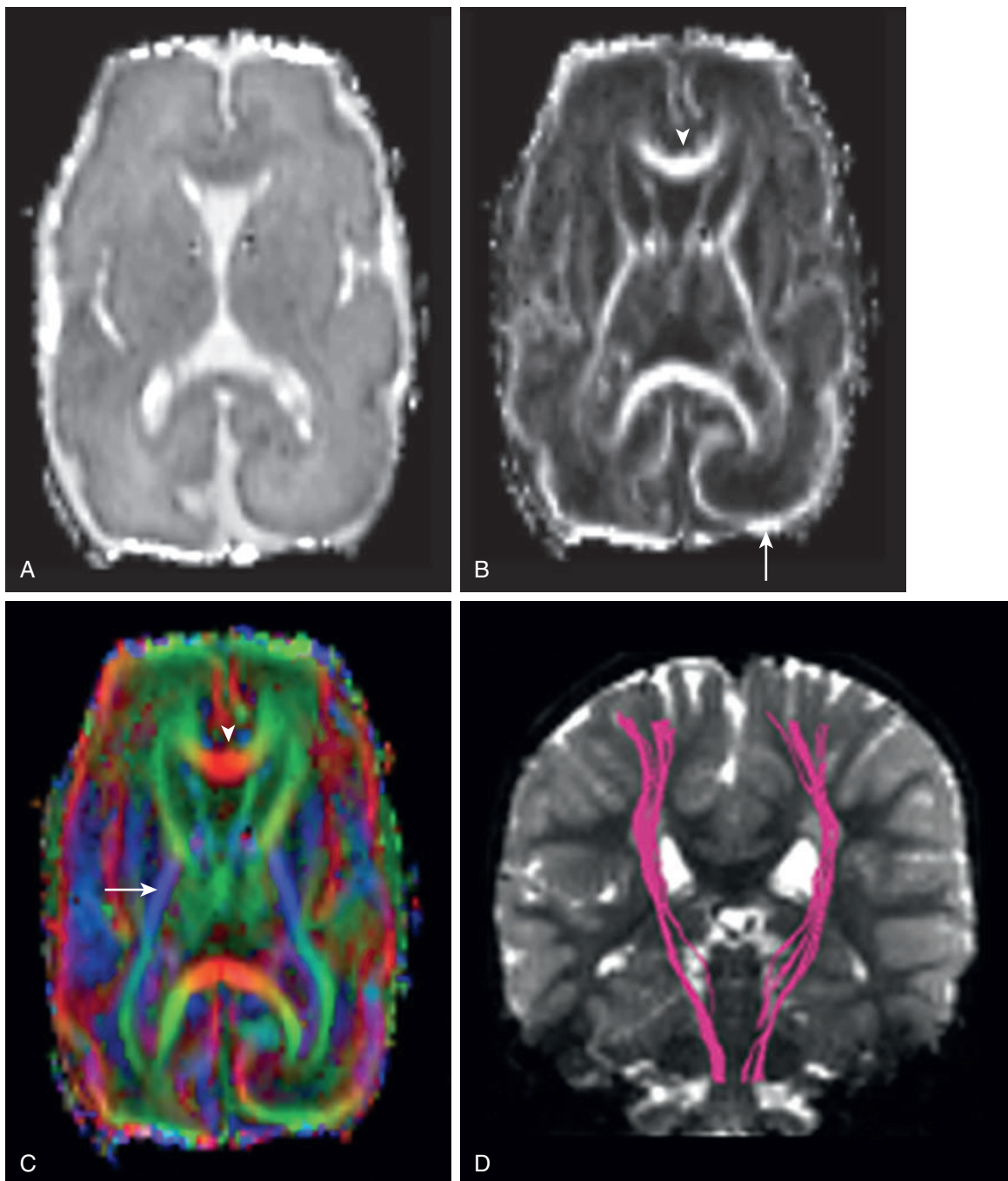
This spatial variation in water ADC values is referred to as anisotropy, and diffusion for which ADC is the same in all directions is called isotropic. When diffusion images are obtained, diffusion is measured several times, along different axes, for each individual image slice. For each element, or voxel, in the image, these measurements can be combined to provide a spatial representation of water displacements. This representation can be expressed mathematically as a tensor, and hence the name “diffusion tensor imaging.” While these tensors can be displayed as ellipsoids (Neil, 2008), ellipsoid representations are cumbersome to use in clinical practice. As a result, summary parameters from these ellipsoids are shown (Fig. 63.4).

Measurement of diffusion anisotropy is applied most often to the evaluation of white matter. During development, anisotropy values increase dramatically with the addition of myelin (Huppi et al., 1998a; Neil et al., 1998), though unmyelinated white matter has a degree of diffusion anisotropy by virtue of the parallel arrangement of tubular axons. In many research studies, high anisotropy is associated with healthy white matter, and lower values are associated with injury or other abnormalities. Diffusion anisotropy measures can also be applied to gray matter. While cortical gray matter in adults has very low anisotropy values, this is not the case early in development, especially prior to term equivalent age. The developing cortical plate has a radial organization to its microstructure caused by the presence of radial glia and the apical dendrites of pyramidal cells (McKinstry et al., 2002a; Kroenke et al., 2005). This microstructure leads to high anisotropy values for the cortical plate for preterm infants in whom water ADC values are greater parallel to radial glia and apical dendrites than perpendicular to them. As the cortical plate matures, this radial organization is disrupted by the addition of basal dendrites to pyramidal cells, involution of radial glia, and myelination of intracortical white matter. As a result, anisotropy values fall steadily, reaching low values by term equivalent age and remaining low thereafter. Note that the pattern of anisotropy change over time is opposite for white and gray matter. Anisotropy values for the developing cortical plate are high and decrease as the cortex matures; anisotropy values for white matter initially are low and increase as white matter myelinates. The well-known regional variation in the rate of cortical development is also reflected in regional variation in the rate of change of cortical water anisotropy values (Kroenke et al., 2009).

While diffusion anisotropy is not commonly used in clinical practice, it can be helpful for white matter tractography (Basser et al., 2000). In this usage, the orientation of greatest water ADC values is determined for each white matter voxel. Since this is parallel to the direction of the axons in that voxel, this orientation can be used to follow fibers from voxel to voxel, thereby following a particular white matter tract (see Fig. 63.4D). This is starting to be used clinically for neurosurgical patients for whom identification of white matter tracts can help determine the optimum surgical approach (Potgieser et al., 2014).

Angiography

MR angiography is an important imaging modality for the evaluation of newborns. While a variety of approaches have been used to delineate vessels, contrast is usually based on macroscopic water motion. For example, it is possible to reduce the signal from static, extravascular water within an imaging slice so that the strongest remaining signal arises from intravascular water that flows into the slice during image acquisition. This can then be used to generate an angiogram. As noted below, angiography is particularly useful for assessing vascular occlusion in perinatal arterial stroke (Lequin et al., 2009) and for detecting sinovenous thrombosis (Berfelo et al., 2010).

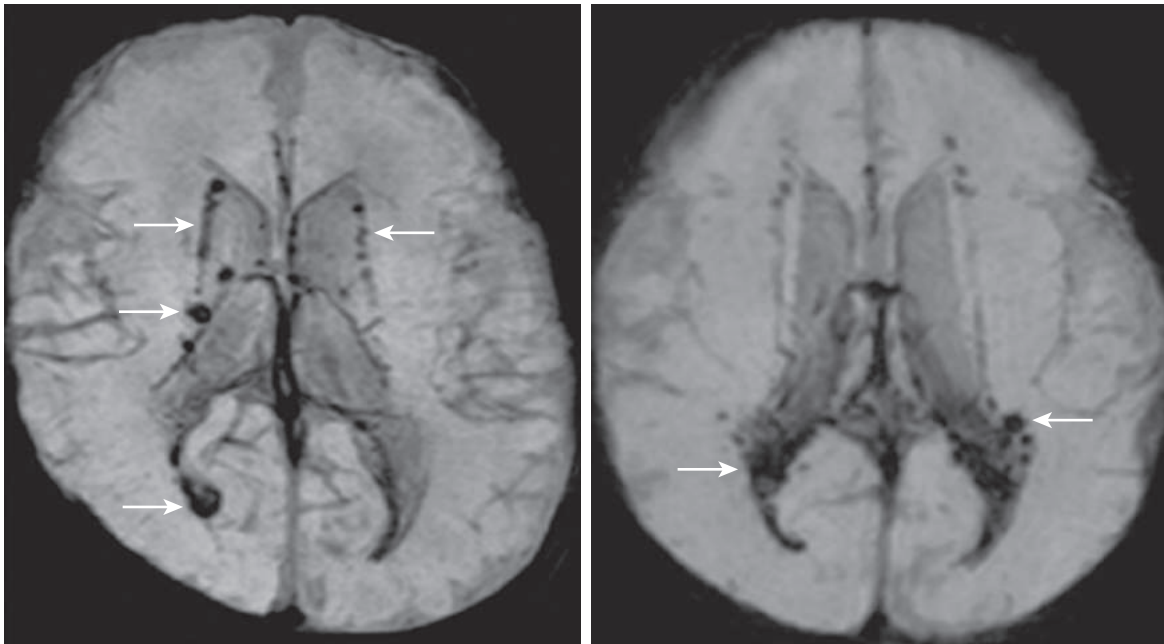


• **Fig. 63.4** Diffusion Magnetic Resonance Imaging Data From Preterm (A–C) and Term (D) Neonates. (A) A diffusion map in which image intensity corresponds to water diffusion coefficient. (B) An anisotropy map in which image intensity corresponds to the degree of anisotropy. The *arrow* indicates an area of the developing cortical plate that has high anisotropy. The *arrowhead* indicates the genu of the corpus callosum. (C) A color map showing the preferred direction of water displacements: red = medial–lateral, green = anterior–posterior, and blue = superior–inferior. Note the medial–lateral crossing fibers of the corpus callosum (*arrowhead*) and superior–inferior corticospinal fibers of the posterior limb of the internal capsule (*arrow*). (D) Diffusion tractography of the corticospinal tracts (purple) in a term neonate.

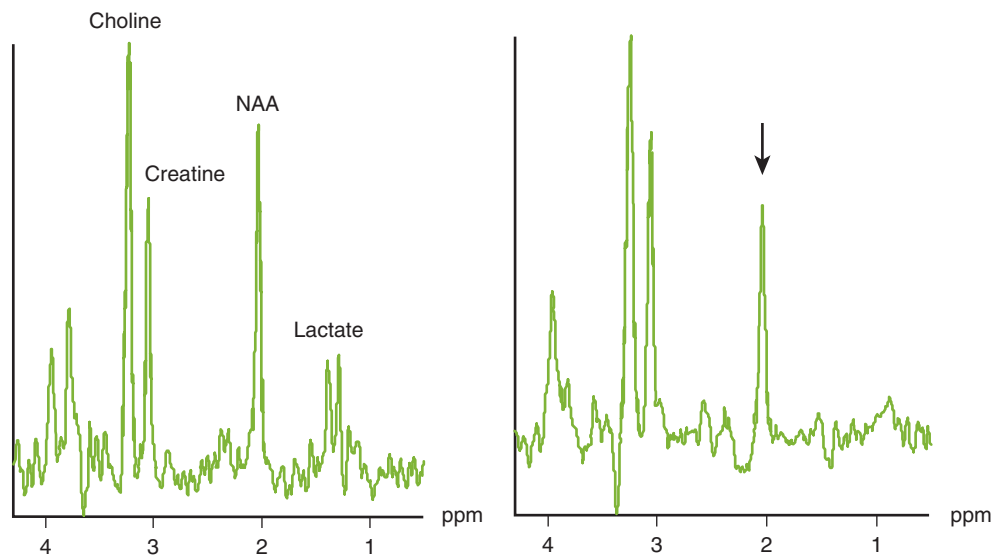
Susceptibility-Weighted Imaging

Another form of contrast available in MR images is based on differences in local magnetic susceptibility and is sometimes known as T_2^* -weighted imaging. Susceptibility effects are caused by the juxtaposition of regions of different magnetic properties or susceptibilities. In the head, such areas are found at air–tissue

interfaces such as the sinuses, where they can cause unwanted image distortions, and near hemorrhages, where the reduced iron in deoxyhemoglobin produces strong susceptibility effects. Susceptibility-weighted images are extremely sensitive to hemorrhage, which appears dark (Fig. 63.5). Because magnetic field distortions caused by susceptibility effects extend beyond the susceptibility



• **Fig. 63.5** Axial, Susceptibility-Weighted Images From a Former Preterm Neonate. Note the areas of signal dropout caused by periventricular hemorrhage (arrows).



• **Fig. 63.6** ^1H Magnetic Resonance Spectra Obtained From a Human Brain at 3.0 Tesla. The spectrum on the left shows a relatively large lactate doublet in an asphyxiated term neonate. The spectrum on the right, obtained 9 days later, shows resolution of the lactate doublet and a reduction in the NAA resonance (arrow) relative to the choline and creatine resonances caused by neuronal loss. NAA, *N*-acetyl-containing compounds; ppm, parts per million.

boundary, small hemorrhages appear as relatively large areas of low signal on susceptibility-weighted images, making them very conspicuous and also making them appear larger than they actually are.

Magnetic Resonance Spectroscopy

As noted above, the concentration of water ^1H in brain is on the order of 110 mol/L, which provides abundant signal for the imaging modalities described above. MR spectroscopy involves the detection of ^1H in brain metabolites, such as lactate, which are present in concentrations on the order of 10^{-2} mol/L. This factor of 10^4

difference in concentration makes MR spectroscopy more challenging than conventional imaging. For example, it is not feasible to obtain a high-resolution image of brain lactate concentration. Instead, an MR spectrum is obtained from a single region of interest (single voxel spectroscopy), or a grid of spectra is obtained from a thick slice of brain (often known as chemical shift imaging). For typical ^1H spectroscopy in clinical use, the resonance peaks visible are choline, creatine, *N*-acetyl-containing compounds (NAA), and lactate (Fig. 63.6). The reasons that these resonances are more conspicuous than others are related to their chemistry and the fact that these metabolites are present in relatively higher concentrations.

Choline serves as a component of membranes and is also a constituent of the neurotransmitter acetylcholine. Creatine, when phosphorylated, stores energy in the form of phosphate bonds. (Phosphocreatine levels reflect the cellular energy state, but their detection requires phosphorous ^{31}P spectroscopy, as creatine and phosphocreatine are indistinguishable using ^1H spectroscopy.) The precise role of NAA in brain metabolism is unclear, but it is widely believed that NAA is found primarily in neurons and not glia, and a reduction in NAA level is often taken to reflect a reduction in the number of neurons present in a given region. Lactate is an intermediary of energy metabolism, and its levels may be increased under a variety of circumstances, including lack of oxygen and anaerobic glycolysis. Lactate levels may also be increased by the presence of inflammatory cells, which often utilize anaerobic glycolysis. Note that the ^1H signal from the methyl group of lactate is a doublet (a pair of peaks) because of its chemistry. In theory, it is possible to quantify metabolite levels (in mM) by comparing resonance amplitudes of metabolites with the water resonance amplitude in the same brain region. This quantitation is rarely employed in clinical practice, and resonance amplitudes are more often expressed as ratios. For example, the NAA/choline or NAA/creatine ratio may provide an estimate of the fraction of cells in a given area that are neurons. Finally, nuclei in addition to ^1H are detectable by MR spectroscopy. They include ^{31}P , sodium (^{23}Na), an isotope of carbon (^{13}C), and even the other two isotopes of hydrogen (^2H or nonradioactive deuterium and ^3H or radioactive tritium). While detection of these other nuclei is of significant scientific/research interest, particularly detection of hyperpolarized ^{13}C for noninvasive assessment of metabolism, non- ^1H spectroscopy has not yet found its way into clinical use.

Preterm Neonates

In recent decades, survival rates for very preterm neonates (born at less than 30 weeks' gestation) have improved dramatically due to advances in perinatal and neonatal care. In contrast to this improvement in mortality, long-term neurodevelopmental outcomes have not improved and remain problematic, with significant associated costs to individuals, families, and society (Anderson and Doyle, 2008; Hintz et al., 2011). In recent years, significant investigation has been undertaken to correlate perinatal, medical, and physical examination findings with long-term neurodevelopmental outcomes in an attempt to identify the neonates at greatest risk. High-grade brain injury (grade III or IV intraventricular hemorrhage [IVH], posthemorrhagic hydrocephalus, large cerebellar hemorrhage, and/or cystic periventricular leukomalacia [c-PVL]) is among the major risk factors for adverse neurodevelopmental outcome. This section will explore the neuroimaging characteristics of these forms of brain injury on CUS and MRI alongside a brief description of their neuropathologic characteristics.

Intraventricular Hemorrhage

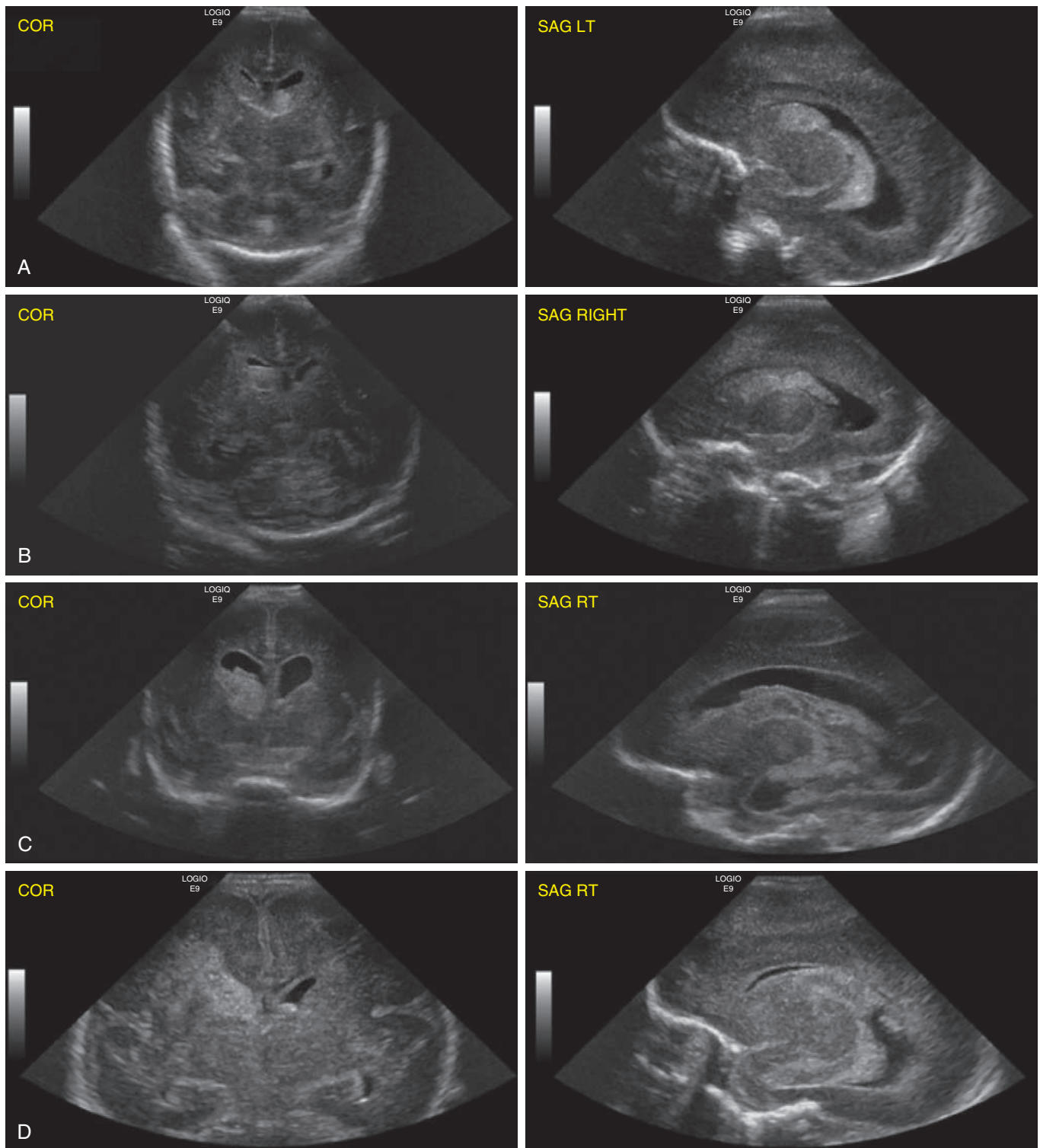
Germinal matrix-IVH is the most common variety of neonatal intracranial hemorrhage and is characteristic of the preterm neonate. IVH has traditionally been graded as grade I–IV, as first reported by Papile and colleagues (Papile et al., 1978). This system is based on the presence and amount of blood in the germinal matrix and the lateral ventricles (Fig. 63.7). Grade I represents hemorrhage confined to the subependymal germinal matrix, grade II is hemorrhage within the lateral ventricles without ventricular dilatation, grade III is hemorrhage with ventricular dilatation and/or

hemorrhage occupying more than 50% of the ventricle, and grade IV requires parenchymal hemorrhage, typically abutting the superolateral aspect of the body and atrium of the lateral ventricle. Although grade IV IVH is now referred to as periventricular hemorrhagic infarction rather than IVH per se, most reports continue to classify the cranial ultrasound findings according to this earlier established classification system.

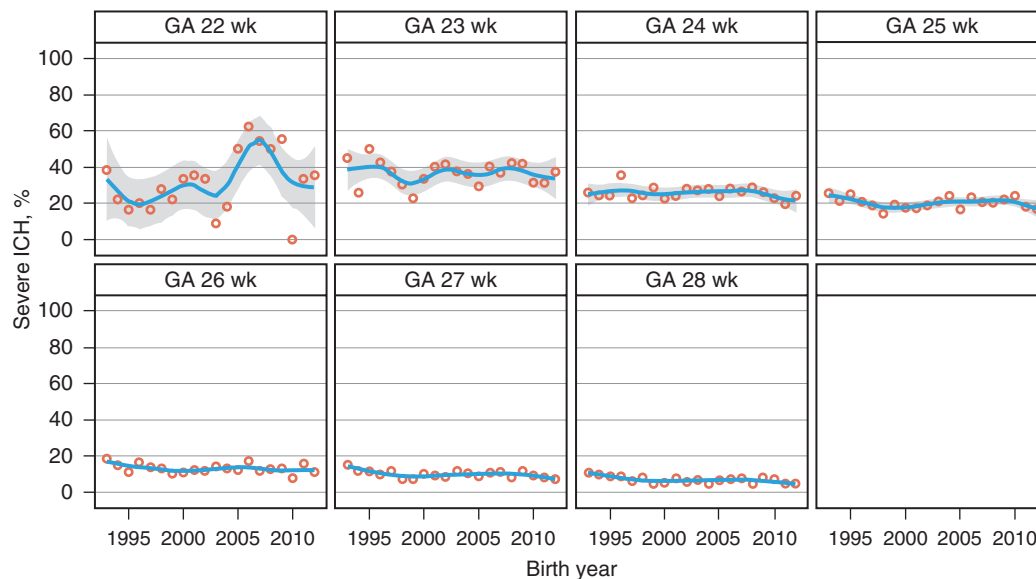
The incidence of IVH in preterm neonates remains high. Incidences derived from neonates studied in the late 1980s through the 2000s have documented that the incidence of IVH has remained unchanged at a level of approximately 25%. This is clearly documented in recent data summarizing the risk of IVH between 1993 and 2012 (Stoll et al., 2015). These data suggest that there may be a small decline in the risk of IVH for neonates with gestational ages of 26–28 weeks, with no change in the most immature neonates born at 22–25 weeks' gestation (Fig. 63.8). It has long been known that there is a higher risk for all forms of IVH in the most immature preterm neonates, with neonates less than 750 g having a risk of any IVH that is approximately threefold higher than for a preterm neonate over 1250 g (42% vs 14%) and a 10-fold higher risk of grade III–IV IVH (20% vs 2.1%) (Wilson-Costello et al., 2005). The other perinatal risk factors for IVH are summarized in Fig. 63.9.

CUS is the cornerstone of diagnosis for IVH in the preterm neonate and provides accurate and useful information for grading. At times, it may be difficult to distinguish a germinal matrix only (grade I) from a small intraventricular (grade II) IVH. Under these circumstances, a view of the lateral ventricles through the posterior fossa may be of assistance because blood tends to settle in the occipital horns of the lateral ventricles (neonates are usually lying face up during the CUS study), which are well seen with this view. MRI can also show the presence of IVH with similar accuracy, particularly in the acute phase, although it is not practical for serial examinations of preterm neonates. However, if MRI is first performed at term equivalent age, a small grade I hemorrhage may no longer be visible as a hemorrhage on conventional T_1 -weighted and T_2 -weighted images but may still be inferred from the presence of a germinal matrix cyst at the site of the original hemorrhage and/or an area of low signal intensity on susceptibility-weighted images reflecting remnants of blood products in the area.

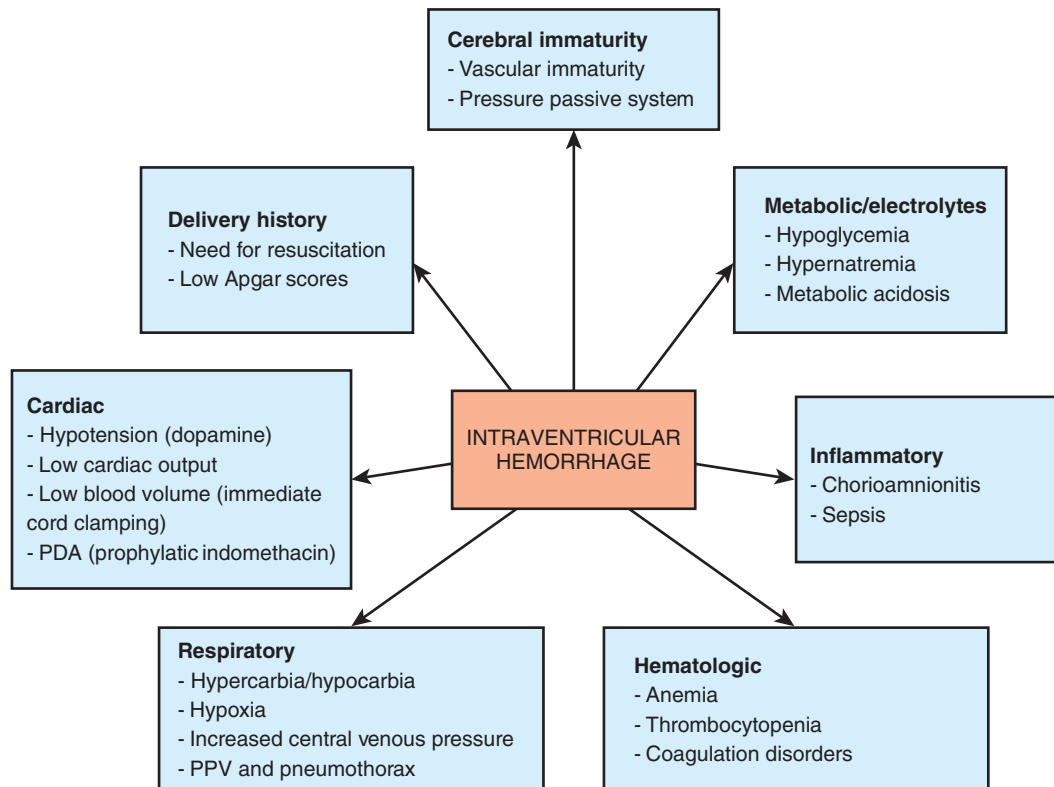
Information on the typical timing of IVH can inform the decision of when to undertake CUS in the preterm neonate early in the hospital course. In a cumulative series of 105 neonates with IVH studied by CUS from the first hours of life, approximately 50% had onset of hemorrhage on the first postnatal day, an additional 25% on the second day, and an additional 15% on the third day (Volpe, 2001). In a single study of 1105 neonates weighing 2000 g or less at birth, approximately 40% of the 265 who developed IVH did so within the first 5 hours of life (Paneth et al., 1993). The likelihood of onset of hemorrhage on the first postnatal day varied inversely with birth weight; in one series, 62% of hemorrhages in neonates between 500 g and 700 g birth weight occurred in the first 18 hours (Perlman and Volpe, 1986). In general, if screening were to be confined to a single postnatal day in the first days of life, a scan on the fourth postnatal day would be expected to detect approximately 90% of all hemorrhages. However, progression of the lesions occurs in approximately 20%–40% of the affected infants, with maximal extent of the lesion attained usually within 3–5 days of the initial diagnosis (Volpe, 2001). Thus a second scan after approximately 5 days is necessary to identify the maximal extent of hemorrhage.



• **Fig. 63.7** Grading of the Severity of Germinal Matrix–Intraventricular Hemorrhage on Coronal and Parasagittal Cranial Ultrasonography. (A) Grade I: germinal matrix hemorrhage. (B) Grade II: intraventricular hemorrhage (IVH) (filling < 50% of the ventricular volume). (C) Grade III: IVH with ventricular dilatation. (D) Grade V: large IVH with associated parenchymal echogenicity (hemorrhagic infarct). COR, Coronal; SAG RT, right parasagitta. (Courtesy of Walsh B, Inder T, Volpe JJ. IVH. In: Polin R, Abman SH, Rowitch D, Benitz WE [eds], *Fetal and Neonatal Physiology*, 5th ed. Elsevier, 2016, chapter 134, pp. 1333–1349.)



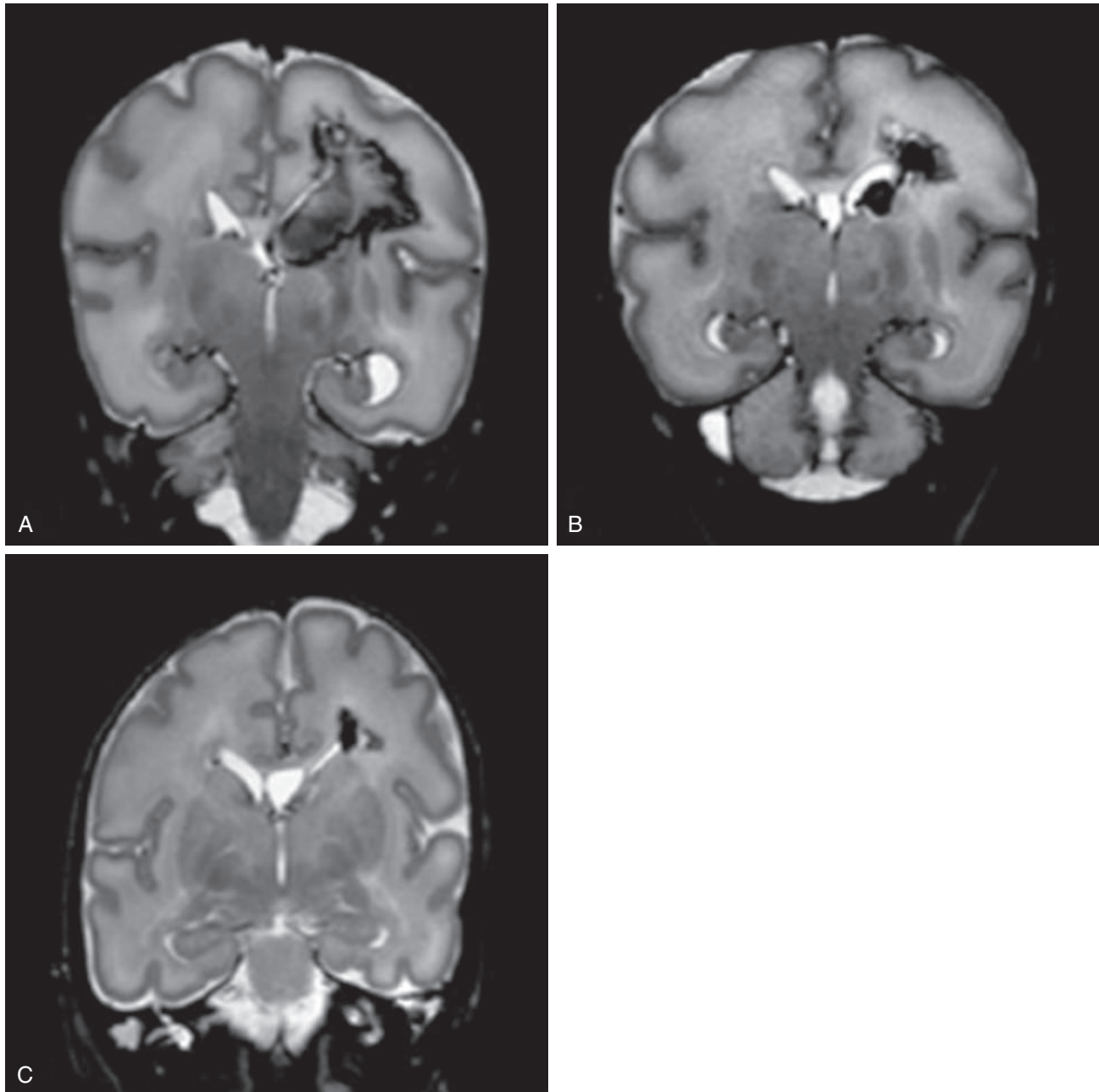
• **Fig. 63.8** Severe Intracranial Hemorrhage by Birth Year and Gestation for Neonates Born at 22–28 Weeks' Gestation 1993–2012. Circles show the percentage of neonates born each year who were evaluated by cranial ultrasonography and diagnosed with grade III–IV intraventricular hemorrhage, a smoothed curve shows the trend, and shading indicates the 95% confidence interval for the curve. The year–gestational age interaction was significant ($P = .03$). Relative risks for the change per year were adjusted for study center, maternal race/ethnicity, neonate gestational age, small for gestational age, and sex. GA, Gestational age. (Data from Stoll BJ, Hansen NI, Bell EF, et al.; Eunice Kennedy Shriver National Institute of Child and Human Development Neonatal Research Network. Trends in care practices, morbidity, and mortality of extremely preterm neonates, 1993–2012. *JAMA*. 2015;314:1039–1051.)



• **Fig. 63.9** Risk Factors for Intraventricular Hemorrhage. PDA, Patent ductus arteriosus; PPV, positive pressure ventilation.

Approximately 30%–50% of neonates with a grade III–IV IVH develop posthemorrhagic ventricular dilatation (PHVD), and 20%–40% of neonates will consequently need a permanent ventriculoperitoneal shunt (Adams-Chapman et al., 2008; de Vries et al., 2013b). Thus preterm neonates with blood in the lateral ventricles (grade II–IV IVH) should be followed with serial CUS studies to monitor ventricular size. While qualitative evaluation of ventricular size can be useful, it is more informative to evaluate ventricular size in a quantitative fashion using established normative

values (Davies et al., 2000). Several quantitative measurements have been developed, including ventricular index, ventricular height, anterior horn width, and thalamo–occipital distance. No one measurement has proven to be superior to the others (Fig. 63.10) (Brouwer et al., 2010). However, the occipital horn area is the first and the frontal horn the last area to enlarge after IVH (Allan et al., 1982). Thus measurement of the thalamo–occipital dimension of the lateral ventricle (Brouwer et al., 2010) via the posterior fontanel is potentially the earliest indicator of ventriculomegaly.



• **Fig. 63.10** T₂-Weighted Coronal Magnetic Resonance Images From Three Preterm Neonates With a Periventricular Hemorrhagic Infarction. (A) A large periventricular hemorrhagic infarction (PVHI) is communicating with the lateral ventricle. (B) A smaller PVHI appears to be separate. (C) A small frontal PVHI without associated intraventricular hemorrhage. (Adapted from de Vries LS, Benders MJ, Groenendaal F. Progress in neonatal neurology with a focus on neuroimaging in the preterm infant. *Neuropediatrics*. 2015;46:234–241.)

At present, there are no standard recommendations for the frequency of CUS studies in this situation. Our practice has been to obtain CUS studies twice per week until ventricular size is stable for 1 week and then every 1–2 weeks thereafter.

In addition to helping identify infants in need of CSF drainage procedures, CUS is also useful for evaluating the effect of CSF drainage using preintervention and postintervention imaging and guiding the frequency and volume of CSF drainage necessary to reduce ventricular size. For example, when using lumbar puncture to treat ventriculomegaly infants with PHVD, the volume of CSF to drain varies greatly between infants (10 mL/kg is typical), with some infants requiring removal of large volumes of CSF to improve ventricular size (Hunt et al., 2003). In this case, CUS studies before and after the procedure give an indication of its effectiveness.

It is worth noting that the ventricular size at which to intervene with a CSF drainage procedure, as well as the drainage procedure to be used, remains an area of active research (Mazzola et al., 2014). Furthermore, the role of aggressive CSF drainage in improving outcomes remains unproven, although there are studies suggesting that more aggressive drainage may be helpful. For example, there are retrospective data suggesting that earlier intervention may be associated with a reduced need for a ventriculoperitoneal shunt and improved neurodevelopmental outcomes (de Vries et al., 2002; Brouwer et al., 2008). In addition, greater ventriculomegaly was associated with worse neurodevelopmental outcome in a study of 173 preterm neonates with grade III–IV IVH (Fig. 63.11) (Srinivasakumar et al., 2013). Overall, more data are needed to settle this issue. A multicenter randomized trial of early versus late ventricular intervention (ELVIS, number ISRCTN43171322, or ClinicalTrials.gov NCT00875758) has completed recruitment, and the results should shed further light on this question.

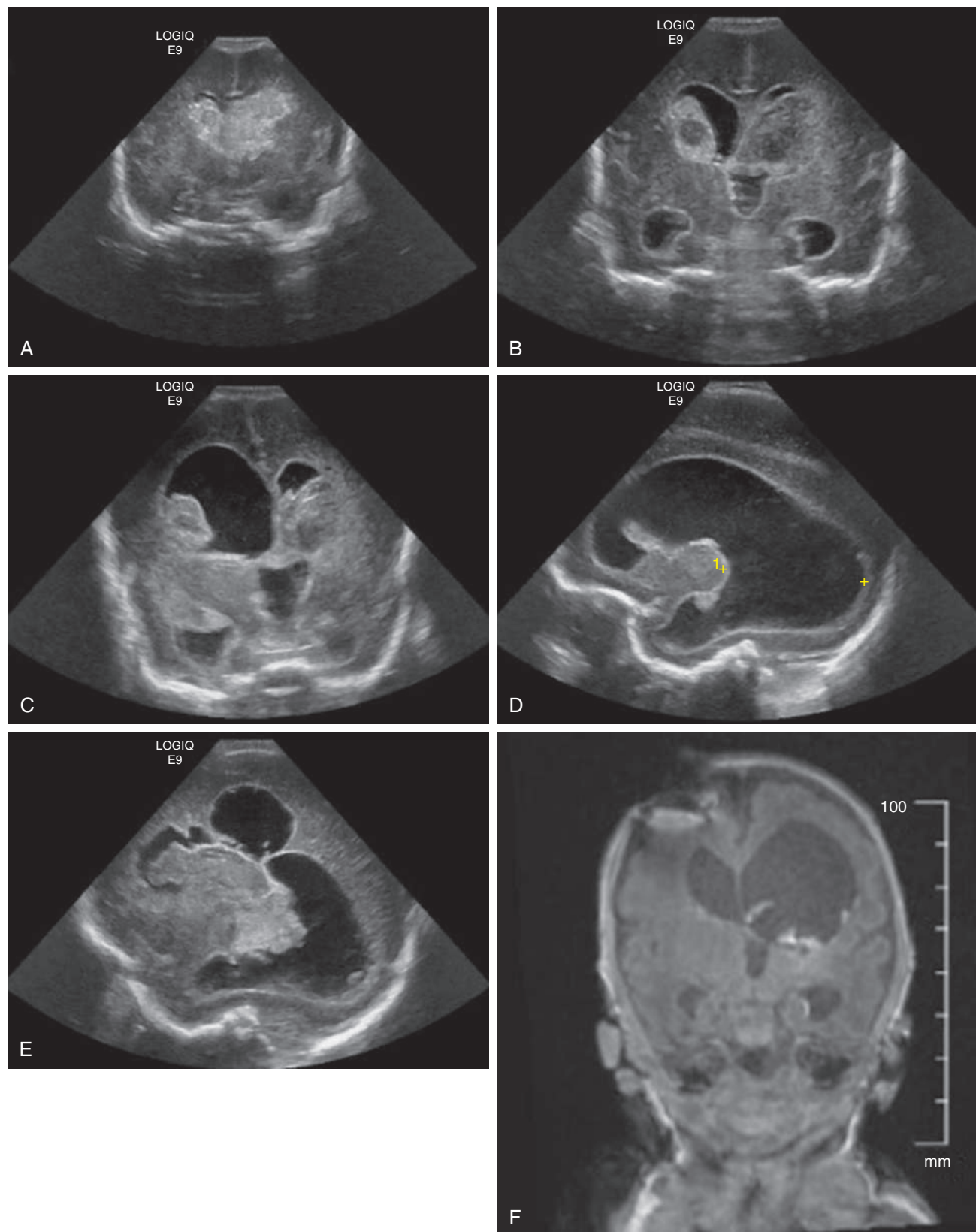
From a practical standpoint, it is important to make a clear clinical distinction between ventriculomegaly resulting from periventricular cerebral atrophy and ventriculomegaly resulting from hydrocephalus with attendant impairment of CSF dynamics for the formulation of appropriate management decisions. In general, the development of ventriculomegaly resulting from atrophy occurs slowly, over several weeks, and is not associated with the development of increased intracranial pressure (perhaps with a bulging fontanel) or rapid head growth and evolves to a state of stable ventricular size. In hydrocephalus, ventricular size may be unstable, decreasing if the condition is transient or increasing if the condition is progressive. The typical evolution of PHVD in a very preterm neonate (24 weeks' gestation) with grade IV IVH is shown in Fig. 63.12. Note the steady progression in ventricular dilatation, which is relieved by subgaleal shunt. The MRI at term equivalent demonstrates the asymmetry from left intraparenchymal volume loss and ex vacuo expansion.

The use of MRI to detect IVH early in the hospital course of a very preterm neonate is impractical for a variety of reasons, and there is no consensus regarding its use in this role. Nevertheless, MRI may be helpful for evaluating infants with PHVD by showing parenchymal details of periventricular hemorrhagic infarction that are useful for prediction of later neurodevelopmental outcome. Grade IV IVH can range from a small focal hemorrhage to an extensive hemorrhage involving most of both cerebral hemispheres (Jary et al., 2012). MRI can be used to more accurately determine the extent of a grade IV hemorrhagic lesion, which is a strong determinant of outcome (de Vries et al., 2015). With or without MRI, it is important for the clinician to appreciate the extent of a grade IV IVH (Fig. 63.13), ideally by reviewing the images, to accurately counsel the baby's family.

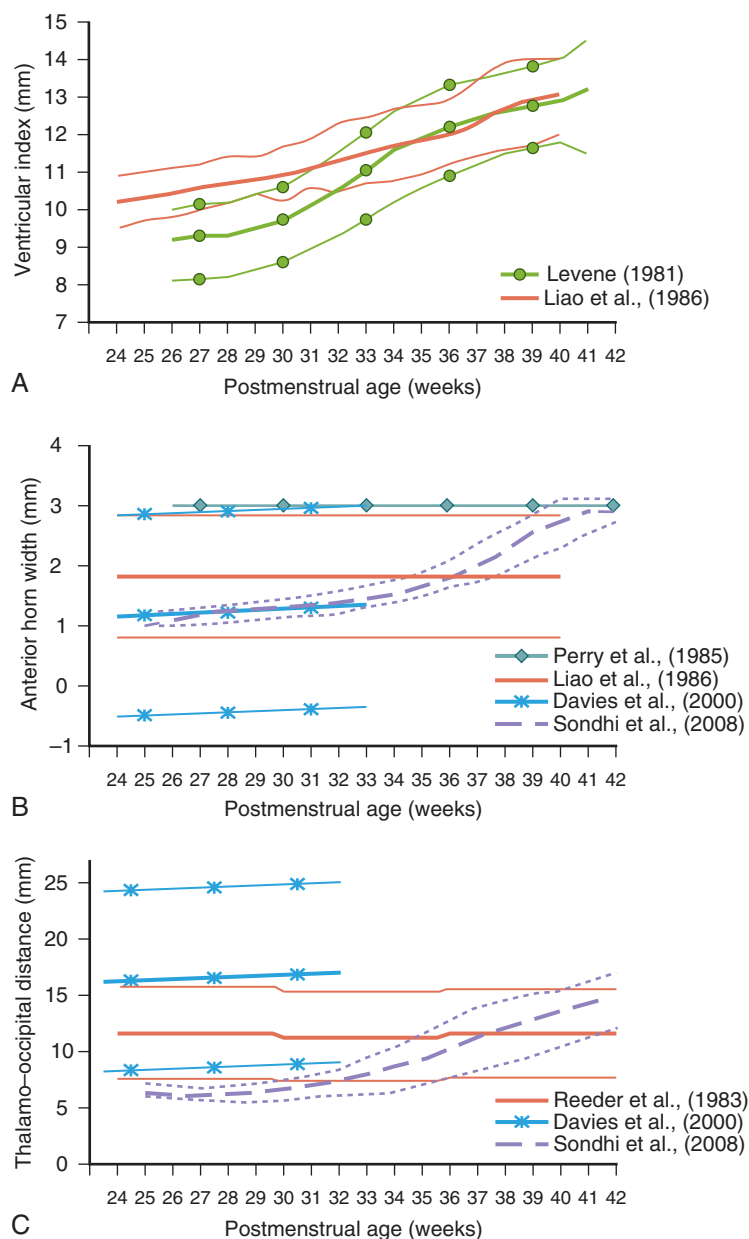
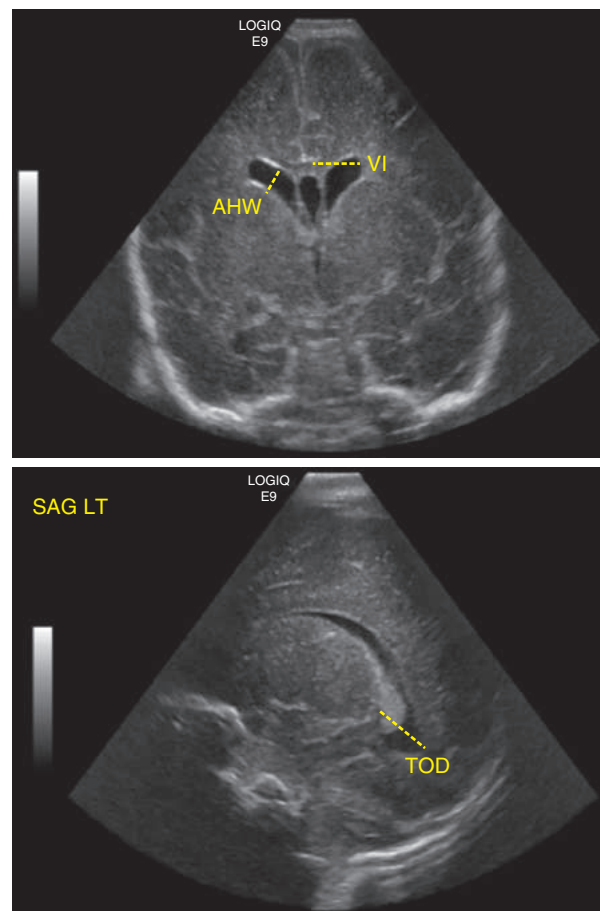
White Matter Injury

Extensive cystic white matter injury (Fig. 63.14), or c-PVL, is now a relatively uncommon problem in very and extremely preterm neonates, with subtle white matter injury now recognized more commonly, particularly on MRI, in this population (Hamrick et al., 2004; Kidokoro et al., 2014). CUS is relatively limited for showing these more subtle white matter lesions (Inder et al., 2003), but nonhemorrhagic injury can sometimes be detected. It has been suggested that any abnormality in the cerebral white matter of echo density at least as echogenic as the choroid plexus and persisting for at least 7 days is significant. Furthermore, the presence of white matter inhomogeneity or a "patchy" appearance on CUS should also alert the clinician to potential white matter abnormalities (Fig. 63.15) (van Wezel-Meijler et al., 2011). These lesions can be hemorrhagic or ischemic in origin, and a combination of diffusion-weighted and susceptibility-weighted MRI can help distinguish between the two (Niwa et al., 2011). They also can be detected by MRI both early and at term equivalent age, tending to be more abundant on the early MRI. Thus early MRI shows the full extent of the white matter lesions, while MRI at term equivalent age identifies the remaining lesions and shows early glial scarring and associated white matter volume loss (Fig. 63.16). Another, more confluent MRI white matter signal abnormality, known as diffuse excessive high signal intensity, has also been described. It is seen on T₂-weighted images at term equivalent age (Counsell et al., 2003) and is a common finding (Dyet et al., 2006). While these white matter signal intensity changes are associated with increased ADC values on diffusion imaging, the qualitative identification of signal changes on T₂-weighted images is rather subjective, and recent studies have failed to identify a relationship between these signal changes and outcome at either 18 or 30 months of age (Kidokoro et al., 2011; Skiold et al., 2012).

As noted above, extensive c-PVL, which is readily visible with both CUS and MRI, has declined over the last decades and now has an incidence of less than 1% in some cohort studies (van Haastert et al., 2011). There is also more localized variant c-PVL. This variant is also relatively uncommon but is more difficult to detect, requiring repeated cranial ultrasound examinations for at least 4–6 weeks for its identification. The cysts are typically located in the frontoparietal white matter adjacent to the lateral border of the lateral ventricles and extend to the occipital white matter in more severe cases. They are usually only visible for a few weeks and may have fully resolved by term equivalent, but their appearance is predictive of subsequent cerebral palsy (CP) (de Vries et al., 2004). Their "resolution" is probably due to coalescence of the cysts with the adjacent lateral ventricle. As a result, their manifestation on MRI at term equivalent may be subtle, consisting of mild to moderate ventricular dilatation and an irregularly contoured ventricular wall. Detection of these transient cystic changes by CUS can be challenging. The American Academy of Neurology recommended in 2002 that all preterm neonates (<30 weeks' gestation) undergo one CUS at 7–14 days for identification of large hemorrhages and a second CUS at 36–40 weeks to identify cystic lesions or ventriculomegaly for prediction of long-term outcome (Ment et al., 2002). However, transient periventricular cystic change may be missed using this protocol. Furthermore, in a study of 1473 extremely low birth weight infants (<1000 g) with only two CUS studies done at mean ages of 6 and 47 days, 29% of those with normal CUS studies had CP or a Bayley Mental Developmental Index less than 70 at 18–22 months of age (Laptook et al., 2005). Thus the finding of two normal CUS studies does



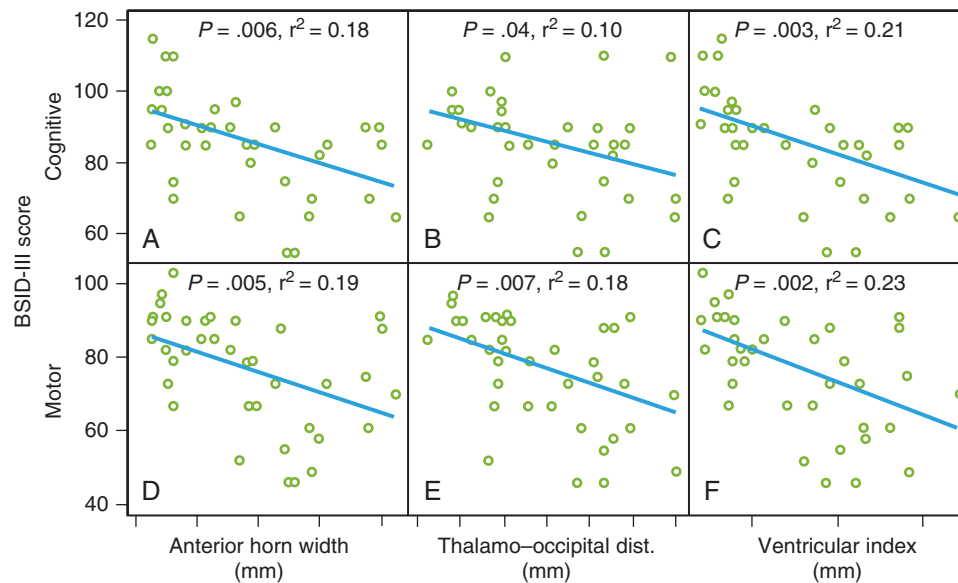
• **Fig. 63.11** Grade IV Intraventricular Hemorrhage With Complicating Periventricular Hemorrhagic Infarction in a Preterm Neonate Born at 24 Weeks' Gestation. (A) Cranial ultrasonography (CUS) on day 2 of life shows a large left-sided intraventricular hemorrhage (IVH) with intraparenchymal echodensity consistent with a periventricular hemorrhagic infarction (PVHI). (B) CUS on day 12 of life shows a significant increase in ventricular size. (C) Coronal and (D) sagittal CUS studies on day 20 of life show significant ventricular dilatation with a thalamo-occipital diameter of 33 mm (measured between the yellow crosses on the thalamus, labeled 1, and the occipital horn of the lateral ventricle) one day before a subgaleal shunt was inserted. (E) Sagittal CUS on day 40 of life showing a left porencephalic cyst resulting from the PVHI. Note that ventricular size is reduced following insertion of the subgaleal shunt. (F) Magnetic resonance imaging at day 84 of life (36 weeks' postmenstrual age) demonstrating asymmetric ventriculomegaly caused by ex vacuo loss of volume in the left hemisphere. There is also left thalamic injury with hemosiderin present. There is an imaging artifact caused by magnetic susceptibility effects over the right hemisphere surface from the subgaleal shunt. Note that the gyral development remains immature for 36 weeks' postmenstrual age.



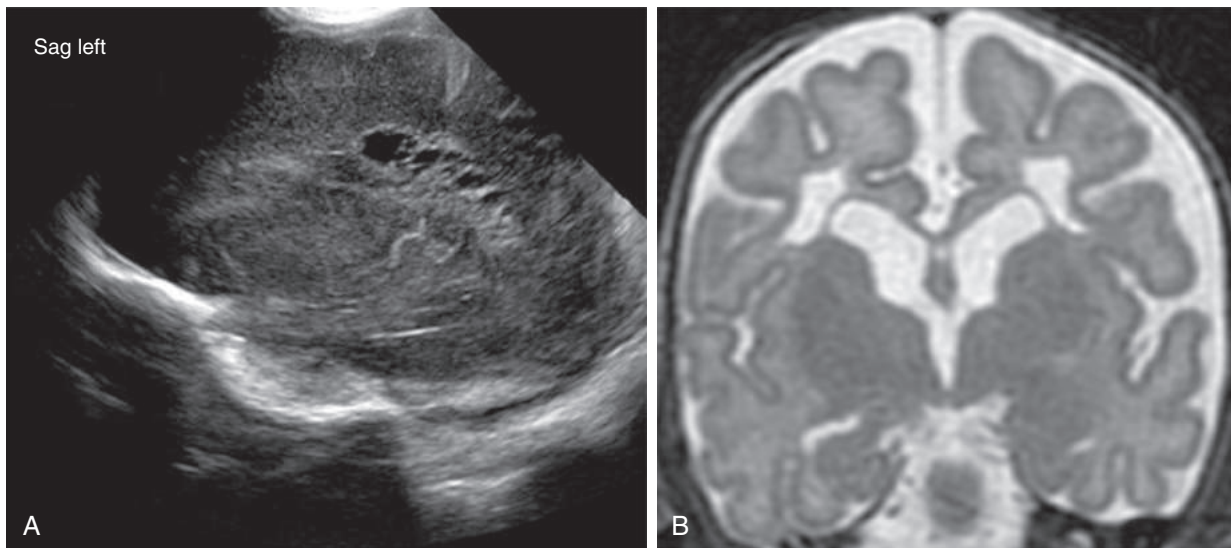
• **Fig. 63.12** Overview of the reference curves for the (A) ventricular index, (B) anterior horn width, and (C) thalamo-occipital distance according to Davies et al., (2000). Regression lines with 95% confidence intervals are from (Levene, 1981) and (Liao et al., 1986). Also adapted from (Brouwer et al., 2012). AHW, Anterior horn width; TOD, thalamo-occipital distance; VI, ventricular distance. (Data from Brouwer AJ, Brouwer MJ, Groenendaal F, Benders MJ, Whitelaw A, de Vries LS. European perspective on the diagnosis and treatment of posthaemorrhagic ventricular dilatation. *Arch Dis Child Fetal Neonatal Ed.* 2012;97:F50–55; Davies MW, Swaminathan M, Chuang SL, Betheras FR. Reference ranges for the linear dimensions of the intracranial ventricles in preterm neonates. *Arch Dis Child Fetal Neonatal Ed.* 2000;82:F218–223; Levene MI. Measurement of the growth of the lateral ventricles in preterm infants with real-time ultrasound. *Arch Dis Child.* 1981;56:900–904; Liao MF, Chaou WT, Tsao LY, Nishida H, Sakanoue M. Ultrasound measurement of the ventricular size in newborn infants. *Brain Dev.* 1986;8:262–268.)

not have a strong positive predictive value for normal neurodevelopmental outcome. Some centers obtain more frequent CUS studies of very preterm neonates in order to better delineate the nature and progression of intracranial lesions with the hopes of improving prognostic ability (Table 63.3) (Wezel-Meijler and de Vries, 2014). While the relative lack of sensitivity of CUS for injury leading to

neurodevelopmental impairment may be due, to some degree, to missing transient abnormalities, its inability to detect more subtle forms of brain injury, such as diffuse PVL at any time, undoubtedly also contributes. The prevalence of the various types of white matter abnormalities for preterm and term control neonates at term equivalent age is shown in Table 63.4.



• **Fig. 63.13** Bayley Scales of Infant Development Scores for Motor and Cognition at 18–24 Months in 173 Preterm Neonates With Grade III–IV Intraventricular Hemorrhage. *BSID-III*, Bayley Scales of Infant Development. (Adapted from Srinivasakumar P, Limbrick D, Munro R, et al. Posthemorrhagic ventricular dilatation—impact on early neurodevelopmental outcome. *Am J Perinatol*. 2013;30:207–214.)

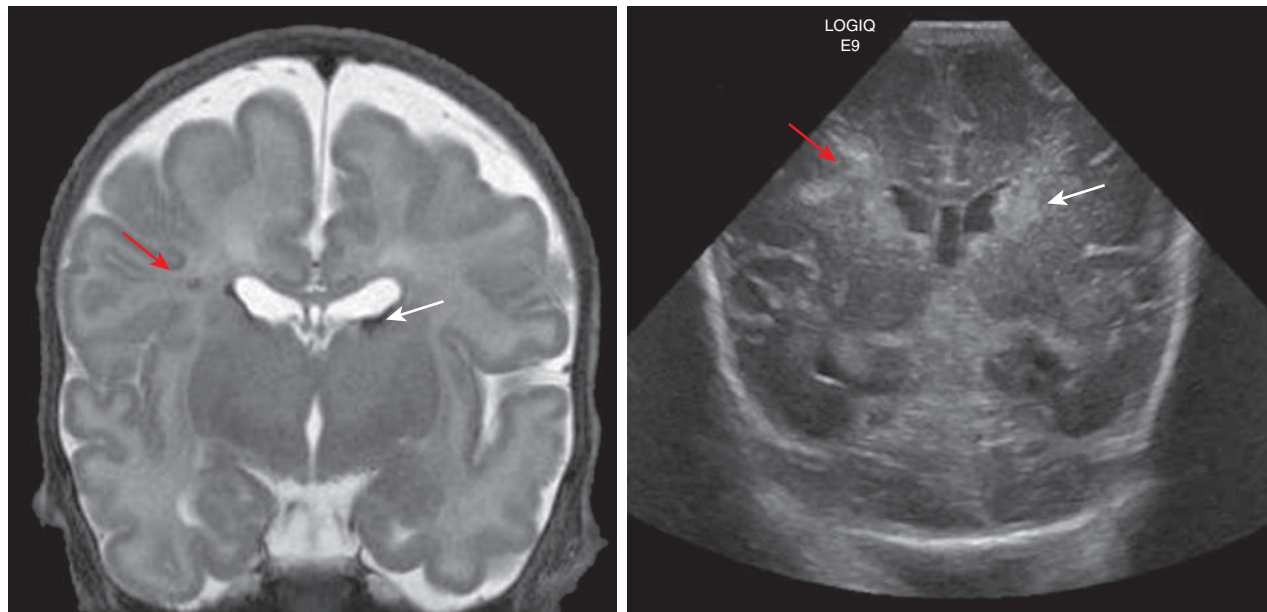


• **Fig. 63.14** Cystic periventricular leukomalacia visible on (A) cranial ultrasound at 28 days of age and (B) magnetic resonance imaging at term equivalent age. Note also the more widespread loss of white matter volume, immature gyral folding, and loss of thalamic volume on the magnetic resonance imaging study.

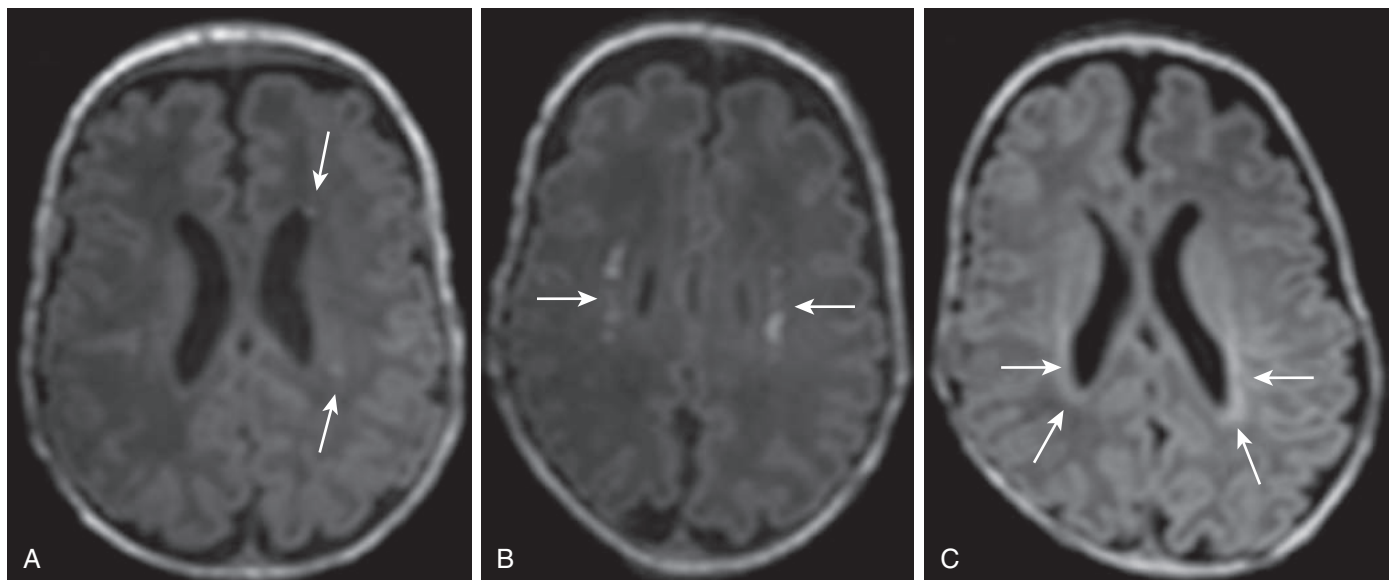
Cerebellar Hemorrhage

Cerebellar hemorrhage (CBH) is increasingly recognized in the very preterm neonate. This is probably the result of two key factors—the improved survival of extremely preterm neonates (24–28 weeks' gestation) who are at greatest risk for CBH and more routine use of the mastoid window in CUS evaluations (see Fig. 63.1) (Limperopoulos et al., 2005; Steggerda et al., 2009; Ecury-Goossens et al., 2010). The cerebellum undergoes rapid and complex development during the preterm period. From 24–40 weeks' gestation, the cerebellar volume, as assessed with three-dimensional volumetric ultrasound, increases fivefold, and the surface area of the cerebellar

cortex increases more than 30-fold (Volpe, 2009b). Based on CUS, the reported incidence of CBH ranges from 2%–9% depending on the gestational age of the population studied. When MRI is performed as well, the incidence is much higher, ranging from 15%–20% (Limperopoulos et al., 2005; Steggerda et al., 2013). This may be because CUS is only sensitive to hemorrhages that are more than 4–5 mm in size. Smaller, punctate CBHs are far more common but can only be diagnosed with MRI, particularly susceptibility-weighted images (Parodi et al., 2015; Plaisier et al., 2015). Of note, larger CBHs tend to be associated with supratentorial lesions such as IVH and evolve to atrophy of the affected cerebellar hemisphere on MRI at term equivalent age.



• **Fig. 63.15** White matter echo density in a 28-week preterm neonate comparing cranial ultrasound on day 5 of life (left) with linear hemorrhagic lesions in the periventricular white matter (red arrow) and thalamus (white arrow) on term equivalent magnetic resonance imaging.



• **Fig. 63.16** White matter lesions (arrows) for (A) focal small punctate lesions, (B) multiple focal punctate lesions, and (C) linear hyperintensity in the periventricular white matter.

Predicting Outcome With Imaging at Term Equivalent Age

Clinicians and researchers continue to possess limited ability to definitively predict and meaningfully improve neurodevelopmental outcomes. While both CUS and MRI have value in this regard, neither is perfect (Table 63.5) (de Vries et al., 2013a). As noted above, CUS is strongly predictive of subsequent cerebral palsy, in the right hands (de Vries et al., 2004), but normal CUS studies are not good predictors of normal neurodevelopmental outcome. MRI is significantly more sensitive to subtle injuries to white matter and cerebellum. Below, we briefly outline the literature on MRI at term equivalent age and outcome for preterm neonates.

Structural Magnetic Resonance Imaging

There is an increasing amount of literature available on the association of white matter abnormalities with structural MRI and neurodevelopmental outcome. White matter abnormalities have been associated not only with motor difficulties (Spittle et al., 2011) but also with impairments in executive functioning (Edgin et al., 2008; Woodward et al., 2011), verbal and visuospatial working memory (Clark and Woodward, 2010), language skills (Foster-Cohen et al., 2010; Reidy et al., 2013), and learning and attention (Murray et al., 2014; Omizzolo et al., 2014). Cortical gray matter abnormalities can also be detected with structural MRI, but gray matter tissue signal abnormalities in preterm neonates are less

common than white matter signal abnormalities, and gray matter injury is more typically manifest as alterations of cortical folding and enlarged extracerebral spaces. In a study of 167 very preterm neonates, gray matter abnormalities were found in half of the neonates and consisted of abnormal/immature cortical folding patterns and/or enlarged subarachnoid space (Woodward et al., 2006). These abnormalities were associated with an increased risk of severe cognitive delay, psychomotor delay, and CP at age 2 but to a lesser extent than white matter abnormalities (Woodward et al., 2006). In contrast, a study of 76 preterm neonates in which

a similar scoring system for gray matter abnormality was used found no association between gray matter abnormality and outcome at age 9 years (Iwata et al., 2012). It is worth noting that alterations of cortical folding and enlarged extracerebral spaces involve both gray and white matter. Cortical folding probably represents an interaction between cortical gray matter and underlying white matter, whereas enlarged extracerebral spaces may reflect an overall reduction in cerebral volume involving multiple brain tissue types.

The literature on cerebellar abnormalities detected by structural MRI and outcome in preterm neonates is relatively sparse, but cerebellar injury is associated with adverse neurodevelopmental outcome. Neonates with isolated cerebellar lesions have a range of neurodevelopmental deficits, including severe motor disabilities, abnormalities of expressive and receptive language, and cognitive deficits (Limperopoulos et al., 2007). They additionally experience a higher incidence of autism and behavioral dysfunction (Brossard-Racine et al., 2015). Isolated cerebellar injury has also been associated with impairment of regional volumetric growth in the contralateral cerebrum, with corresponding deficits of language, motor, and social-behavioral function (Bolduc et al., 2011; Limperopoulos et al., 2014).

While structural MRI is often helpful for predicting neurodevelopmental outcome of preterm neonates, a relatively high proportion of neonates without any evidence of brain injury (IVH, PVL, or CBH) have abnormal outcomes. For example, in one study the mean mental developmental index of preterm neonates with normal structural MRI was 87, with a 6% incidence of cerebral palsy (Kidokoro et al., 2014). In a metaanalysis of the prognostic accuracy of abnormalities on term MRI for predicting long-term outcome in preterm neonates (Van't Hooft et al., 2015), the sensitivity and specificity for predicting CP were 77% and 79%, respectively. The corresponding values for predicting cognitive impairment were 66% and 61%. It is important to note that the analysis was done

TABLE 63.3 Recommendations for Cranial Ultrasonography by Gestational Age at Birth

| | GESTATIONAL AGE AT BIRTH (WEEKS) | | | |
|--|----------------------------------|--------------------|---------|---------|
| | 23–26 | 27–29 | 30–32 | 33–35 |
| Postnatal age at which cranial ultrasonography should be performed | days 1, 2, and 3 | day 1 | day 1 | day 1 |
| | 1 week | 1 week | 1 week | 1 week |
| | 2 weeks | 2 weeks | | |
| | weekly to 31 weeks | weekly to 31 weeks | 3 weeks | 3 weeks |
| | alternating weeks to 36 weeks | at 36 weeks | | |
| | term | term | term | term |

TABLE 63.4 Nature and Prevalence of White Matter Abnormalities in the Very Preterm Neonate^a

| Variables | Score 0 | Score 1 | Score 2 | Score 3 | Score 4 |
|---------------------------------|-----------------------------------|---|--|----------------------------------|----------------------------|
| CEREBRAL WHITE MATTER | | | | | |
| Cystic lesions | Nil 94/100 | Focal unilateral 2/0 | Focal bilateral 1/0 | Extensive unilateral 2/0 | Extensive bilateral 1/0 |
| Focal signal abnormality | Nil 80/90 | Focal punctate 13/10 | Extensive punctate 5/0 | Linear 2/0 | |
| Myelination delay | PLIC and corona radiata 67/100 | Only PLIC 27/0 | Minimal – no PLIC 6/0 | | |
| Thinning of the corpus callosum | Nil 41/82 | Partial (genu/body <1.3 mm or splenium <2.0 mm) 55/18 | Global (genu/body <1.3 mm and splenium <2.0 mm) 4/0 | | |
| Dilated lateral ventricles | Both sides VD <7.5 mm 27/77 | One side 7.5 mm < VD < 10 mm 20/18 | Both sides 7.5 mm < VD < 10 mm or one side VD > 10 mm 43/5 | Both sides VD > 10 mm 10/0 | |
| Volume reduction | cBPD ≥77 mm 22/86 | 77 mm > cBPW ≥ 72 mm 31/9 | 72 mm > cBPW ≥ 67 mm 41/5 | 67 mm > cBPW 6/0 | |

^aData are shown as percent preterm/percent term control.

cBPD, Corrected biparietal diameter; cBPW, corrected biparietal width; PLIC, posterior limb of internal capsule; VD, ventricular diameter.

TABLE 63.5 Comparison of Cranial Ultrasonography and Magnetic Resonance Imaging Used for the Prediction of Motor Outcome at 18–30 Months

| | | Number | Age at Follow-Up (months) | Sensitivity | Specificity | PPV | NPV |
|--|------------|--------|---------------------------|-------------|-------------|------|------|
| Valkama et al., (55) (severe IVH/PVL/FI) | CUS | 51 | 18 | 0.67 | 0.85 | 0.57 | 0.89 |
| | MRI | 50 | | 0.82 | 0.97 | 0.90 | 0.95 |
| Woodward et al., (57) (severe IVH/PVL, moderate–severe WMI) | CUS | 167 | 24 | 0.18 | 0.95 | 0.23 | 0.91 |
| | MRI | | | 0.65 | 0.85 | 0.31 | 0.95 |
| de Vries et al., (44) (CUS only) (severe IVH/c-PVL/FI) | CUS | 1460 | 24 | 0.76 | 0.95 | 0.48 | 0.99 |
| de Vries et al., (25) (sequential CUS/MRI-TEA) | CUS | 1691 | 24 | 0.57 | 0.98 | 0.44 | 0.99 |
| | MRI | 77 | | 0.92 | 0.55 | 0.73 | 0.90 |
| de Vries et al., (25) (combined serial CUS, MRI-TEA) (severe IVH/c-PVL/FI) | CUS MRI | 77 | 24 | 0.79 | 0.94 | 0.96 | 0.69 |
| Mirmiran et al., (56) (moderate–severe WMI; focal par. injury) | CUS | 61 | 30 | 0.43 | 0.82 | 0.33 | 0.87 |
| | MRI | | | 0.86 | 0.89 | 0.60 | 0.97 |
| Munck et al., (60) ^a | CUS | 180 | 24 | 0.54 | 0.95 | 0.47 | 0.96 |
| | MRI | | | 0.85 | 0.78 | 0.23 | 0.98 |
| Leijser et al., (7) ^b | CUS | 32 | 24 | 0.75 | 0.86 | 0.43 | 0.96 |
| | MRI | | | 1.00 | 0.86 | 0.43 | 1.00 |
| Skiold et al., (40) (MRI only) (moderate–severe WMI) | MRI | 107 | 30 | 0.60 | 0.96 | 0.50 | 0.98 |

^aMajor abnormalities: IVH grades III–IV, hemorrhage of the brain parenchyma, white matter cysts, abnormal T1 or T2 signals in cortex, basal ganglia, thalamus, cerebellum or internal capsule, abnormality of the corpus callosum, an extracerebral space width of 6 mm or more, and ventriculitis.

^bSevere CUS: multicystic PVL and/or focal echodensities within the white matter; severely abnormal MRI: extensive SI changes with hemorrhagic or (pre)cystic lesions in the periventricular white matter, with periventricular and/or subcortical extension.

cPVL, Cystic periventricular leukomalacia; CUS, cranial ultrasonography; FI, focal infarction; IVH, intraventricular hemorrhage; MRI, magnetic resonance imaging; NPV, negative predictive value; PPV, positive predictive value; PVL, periventricular leukomalacia; SI, signal intensity; TEA, term equivalent age; WMI, white matter injury.

on a small number of studies, with only two or three studies included in each category of outcome, underscoring the serious need for more research in this area. Nevertheless, the relative lack of sensitivity of structural MRI for identifying brain injury in the preterm neonate has led to a search for more sensitive approaches, including volumetric studies, diffusion imaging analysis, and functional connectivity MRI, in hopes of finding more sensitive and accurate predictors of outcome.

As above, the finding of enlarged extracerebral spaces on term equivalent MRI may reflect a small brain caused by volume loss and/or poor growth as a consequence of injury and undernutrition during the period from preterm birth to term age. For research studies, brain volume can be quantified with more specificity: i.e., after segmenting brain into tissue classes such as cortical gray matter, white matter, deep nuclear gray matter, brainstem, and cerebellum. Widespread alterations in cerebral volumes have been described for preterm neonates imaged at term age (Huppi et al., 1998b), and a number of studies have related volume changes with neurodevelopmental outcome. At short-term follow-up (< 2 years), neurodevelopmental disability was associated with reduced cortical and deep nuclear gray matter volumes, increased CSF volume (Inder et al., 2005; Young et al., 2015), reduced white matter volume (Peterson et al., 2003), reduced hippocampal volume (Beauchamp et al., 2008; Thompson et al., 2008), reduced total cerebral tissue volume (Woodward et al., 2005), and reduced cerebellar volume (Van Kooij et al., 2012a). At 5 years, a relationship was found between reduced cerebellar volume and poorer executive function and motor skills (Lind et al., 2010). Finally, smaller infant

hippocampal volumes were associated with lower verbal memory scores at 7 years of age (Thompson et al., 2013). Volumetric analysis of infant brain MR images requires specialized computer software and, often, user intervention to ensure that segmentations are done correctly, which hampers the use of these measures in routine clinical practice. Simpler, one-dimensional measures from structural MR images are highly correlated with volumes and could potentially be useful in a clinical setting (Nguyen The Tich et al., 2009). For example, biparietal diameter, which correlates with overall brain volume, was predictive of cognitive and motor outcomes in 2-year-old subjects after adjustment for perinatal variables and social risk (Tich et al., 2011).

Diffusion Magnetic Resonance Imaging

Diffusion MRI is unique in that it provides aspects of both structural and functional information. The rapid reduction in brain water ADC associated with acute brain injury reflects an alteration in brain “function” in the sense that this reduction takes place within minutes of injury, a much shorter time frame than that of the structural changes that are subsequently detectable by histology. For the discussion here, we focus more on the microstructural information available through diffusion imaging. This information is most commonly applied to white matter and is encoded as diffusion anisotropy. Traditionally, higher anisotropy is taken to reflect “healthier” or more “ordered” white matter.

During early brain development, overall water diffusion coefficients decrease steadily, most likely as a reflection of the reduction

in brain water content that accompanies maturation. Diffusion anisotropy values in white matter, on the other hand, increase during development in association with myelination (Huppi et al., 1998a; Neil et al., 1998). As noted above, the most common white matter injury in preterm neonates is characterized by diffuse white matter changes. This diffuse injury is defined histologically (Volpe, 2009a), but diffusion imaging provides supporting evidence for its presence in the form of globally reduced white matter diffusion anisotropy values in preterm neonates (Huppi et al., 2001).

Abnormalities of diffusion have been correlated with neurodevelopmental outcome as well. For infants evaluated at less than 2 years old, high diffusion coefficient values for white matter (Kaukola et al., 2010) and cerebellum (Brouwer et al., 2014) were associated with worse motor outcomes. Low white matter anisotropy values, particularly in the posterior limb of the internal capsule and corpus callosum, were also associated with poor motor outcomes (Arzoumanian et al., 2003; Drobyshevsky et al., 2007; Rose et al., 2009; van Kooij et al., 2012b; Chau et al., 2013; De Bruine et al., 2013). In longitudinal studies of preterm children at ages 4 through 7 years, high ADC values in the right orbitofrontal area have been associated with social-emotional problems (Rogers et al., 2012), and high ADC values in regions of the occipital pole and cerebellum were associated with impairment of motor and executive function (Thompson et al., 2014). In addition, low anisotropy values in the posterior limb of the internal capsule were associated with poor motor outcome at age 4 years (Rose et al., 2007).

In the studies outlined above, low white matter anisotropy was associated with impaired outcome, but this relationship is not universal. In a study of preterm children evaluated at age 2 years, lower anisotropy in the right inferior temporal lobe, but higher anisotropy in the left inferior temporal lobe, was associated with lower motor scores. In another study, lower anisotropy in the left cingulum bundle was associated with better social-emotional competence (Rogers et al., 2016). These results are consistent with diffusion studies on older subjects with autism and other developmental impairments (Cheon et al., 2011). The microstructural alterations underlying these opposing results are not fully understood. It has been hypothesized that reduced axonal branching or fewer fiber tracts crossing the tract of interest may lead to higher anisotropy in injured white matter areas.

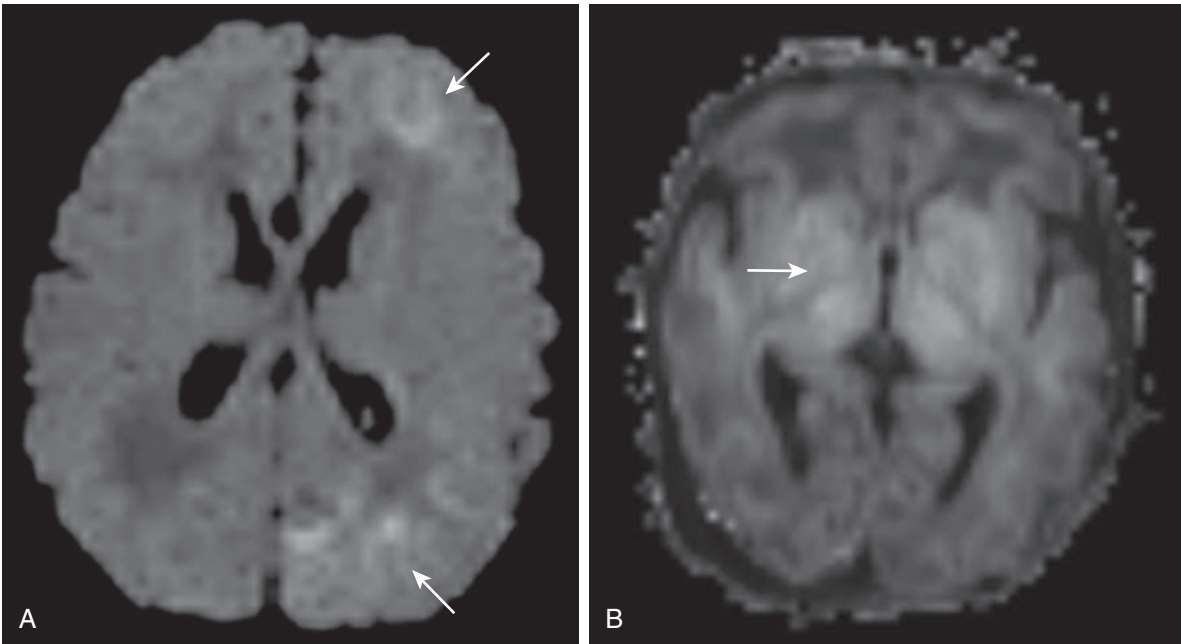
Term Neonates

Neonatal Encephalopathy

Among the clinical indications for neuroimaging studies from term neonates, the presence of neonatal encephalopathy is the most common. Neonatal encephalopathy has been defined as “a clinically defined syndrome of disturbed neurologic function in the earliest days of life in the term infant, manifested by difficulty with initiating and maintaining respiration, depression of tone and reflexes, subnormal level of consciousness, and often by seizures” (Nelson and Leviton, 1991). It affects 2–6 per 1000 live births, has a 15%–20% mortality rate, and 25% of survivors are left with severe disability. It is important to bear in mind that not all neonatal encephalopathy is caused by intrapartum ischemia (Kurinczuk et al., 2010). It has been estimated that on the order of 40% is due to other causes such as infection, stroke, metabolic disorder, and genetic disorders (Shah et al., 2006). The common patterns of brain injury in the encephalopathic term neonate include diffuse global injury, deep nuclear gray matter injury, brainstem injury, watershed injury, periventricular white matter injury, and focal

infarction. Of these, deep nuclear gray matter injury (25%–75% of cases) and watershed injury (15%–45% of cases) are most common (Barkovich et al., 1998; Task Force on Neonatal Encephalopathy, 2014). Deep nuclear gray matter injury involves the deep gray nuclei and perirolandic cortex, extending further into the cortex when severe. The watershed pattern involves injury at the watershed areas between the anterior and middle cerebral arteries anteriorly and the middle and posterior cerebral arteries posteriorly. Watershed injury may be unilateral or bilateral and affect the anterior watershed, the posterior watershed, or both. It primarily involves white matter but can extend into the cortical gray matter in severe cases. An example of each category of injury is shown in Fig. 63.17. From a clinical standpoint, the two forms of injury tend to be associated with different clinical scenarios. The watershed pattern of injury often follows normal labor with minimal resuscitation, relatively good Apgar scores, and an umbilical cord pH of greater than 7.00. During the postnatal period, the watershed pattern of injury is typically associated with proximal limb weakness, truncal hypotonia, and a relatively high incidence of seizures. Later in life, this injury pattern is associated with predominantly cognitive impairments with fewer functional motor deficits (Miller et al., 2005). The deep nuclear gray matter pattern of injury, on the other hand, often follows a sentinel event (e.g., cord prolapse, placental abruption, or uterine rupture) with low Apgar scores and an umbilical cord pH of less than 7.00. These neonates are more profoundly encephalopathic and may have evidence of multiorgan injury involving the heart, kidneys, and liver. During the postnatal period they are often hypotonic and feed poorly. Subsequent neurologic impairment tends to be more severe in these patients and depends on the extent of injury, as has been outlined by Martinez-Biarge et al. (2010). Furthermore, signal abnormality in the posterior limb of the internal capsule on MR imaging has a strong association with subsequent motor deficit in these neonates (Martinez-Biarge et al., 2011). It is important to bear in mind that while the patterns described here are usually associated with hypoxic-ischemic injury, there are mimics of these patterns associated with other causes such as meningitis (Hernandez et al., 2011) and metabolic disorders (Johnston and Hoon, 2000). Thus the presence of either of these patterns on MRI may be consistent with a hypoxic-ischemic insult but does not prove that one occurred. Thus the clinician should be wary.

In addition to the findings derived from conventional and diffusion imaging, spectroscopy can also provide information regarding prognosis. A rise in lactate and fall in NAA are the most significant changes observed, with lactate being detected within 24 hours following injury and NAA beginning to decrease after 48 hours (Barkovich et al., 1999). Elevated lactate levels are seen for months after injury and thus do not always indicate acute injury, although the persistence of lactate signal signifies a worse prognosis (Miller et al., 2002). In a metaanalysis of 32 studies grouping 806 newborns with neonatal encephalopathy, the lactate/NAA ratio in the deep gray matter had strong prognostic accuracy for disability, with a pooled sensitivity of 82% and a specificity of 92% (Thayyil et al., 2010). Although this measure was useful for predicting death or profound disability, more detailed anatomic imaging may assist in refining prognostic information. A study of term neonates who underwent conventional diffusion MRI and spectroscopy measurements in the basal ganglia at a median of day 4 of life showed that the addition of quantitative measures ADC, lactate/NAA, or both improved the predictive power for conventional imaging for adverse neurodevelopmental outcome (for lactate/NAA, area under the curve [AUC] = .85 and $P = .006$;



• **Fig. 63.17** Common Patterns of Cerebral Injury. These are diffusion images obtained 2–4 days after injury in which areas of injury appear bright (arrow). (A) A watershed injury, predominantly in the anterior and posterior watershed areas of the left hemisphere. (B) A basal ganglia/thalamic injury (arrow).

TABLE 63.6 Neuroimaging in 1421 Infants From the Vermont Oxford Neonatal Encephalopathy Registry

| | Ultrasonography | Computed Tomography | Magnetic Resonance Imaging |
|--|---------------------------|---------------------------|----------------------------|
| Number of infants | 729 (51%) 42% of total | 477 (34%) 28% of total | 1074 (75%) 63% of total |
| Mean (SD) of age (days) at first examination | 3.1 (4.4) | 3.2 (3.5) | 7.3 (8.7) |
| Abnormal | 232 (32%) | 271 (57%) | 717 (67%) |
| Hemorrhage | | | |
| IVH/SE | 56 (8%) | 59 (12%) | 79 (7%) |
| Extraaxial | 24 (3%) | 165 (35%) | 212 (20%) |
| Parenchymal | 37 (5%) | 57 (12%) | 105 (10%) |
| Deep nuclear gray matter injury | 70 (10%) | 50 (10%) | 309 (29%) |
| White matter injury | 16 (2%) | 15 (3%) | 271 (25%) |

IVH, Intraventricular hemorrhage; SD, standard deviation; SE, subependymal hemorrhage.

for ADC values from the basal ganglia, AUC = .93 and $P < .001$) (Alderliesten et al., 2011).

Different imaging modalities—CUS, CT, and MRI—provide different information for patients with neonatal encephalopathy. Table 63.6 shows data from the Vermont Oxford Neonatal Encephalopathy Registry on the clinical application of these modalities (Task Force on Neonatal Encephalopathy, 2014). Note that all three modalities remain in fairly wide use. Note also that the mean time to obtaining the imaging study varies by modality, with CUS and CT being performed at a mean age of 3 days as compared with 7 days for MRI. This probably reflects the more demanding logistics of moving a neonate to the MRI suite for study. However, MRI outperforms both CUS and CT for detection of abnormalities, detecting injury in 67% of cases as opposed to

32% and 57%, respectively. Importantly, the additional injuries detected by MRI are clinically relevant. While CUS and CT show sensitivity mainly for intraventricular and extraaxial hemorrhage, MRI is much more sensitive to injury to deep nuclear gray matter and white matter (e.g., watershed injury). For neonates with neonatal encephalopathy, the American College of Obstetrics and Gynecology (with the endorsement of the American Academy of Pediatrics) suggests that information regarding the likely timing of injury is best obtained with early imaging (during the first 24–96 hours of life) with follow-up imaging to define the full nature of the abnormalities, optimally at 10 days of life (but with an acceptable window between 7 and 21 days of life) (American College of Obstetrics and Gynecology, 2014). This recommendation is supported by the finding that the extent of injury may change in up

to 20% of neonates on images obtained between days 3–4 and those obtained later (>7 days), particularly in neonates with hypoglycemia and moderate–severe lesions in the deep nuclear gray matter (Chakkarapani et al., 2016). However, from a practical standpoint, often only a single MRI study can be obtained. In that circumstance, we recommend that the single study be obtained later than 1 week after the initial insult and as late as feasible. Finally, it may be useful to obtain an MRI study earlier than days 3–4 in some instances, for example, to confirm the absence of injury in neonates for whom early rewarming is being considered (often in association with a normal examination and electroencephalography). Another instance is to confirm the severity of injury in profoundly encephalopathic neonates for whom redirection of care is under consideration. In this case, diffusion MRI will very likely show the injury from its onset, and MR spectroscopy (lactate/NAA ratio) will be informative.

Sinovenous Thrombosis

Neuroimaging is necessary for detecting sinovenous thrombosis as well as following patient response to therapy. Sinovenous thrombosis is less common than neonatal encephalopathy and has an incidence of 2–12 per 100,000. Infants usually present with seizures and/or encephalopathy. Risk factors for sinovenous thrombosis include hypoxic–ischemic encephalopathy, complicated delivery, complicated pregnancy, dehydration, prematurity, congenital heart disease, sepsis, and prothrombotic abnormalities (Moharir et al., 2011). From an imaging standpoint, sinovenous thrombosis is often initially detected by CUS or MRI as IVH with or without associated thalamic hemorrhage (Fig. 63.18). The presence of a clot in a sinus may sometimes be visible as an area of high signal intensity (bright) on T₁-weighted imaging. When these findings are present, MR venography is very helpful for delineating the thrombosis, and involvement of multiple sinuses and veins is relatively common. Identifying the extent of thrombosis is important because it is a treatable condition, and it is common to use anticoagulant therapy (cautiously, in the presence of significant hemorrhagic injury). MR venography provides a means of evaluating the response to anticoagulant therapy, and follow-up imaging is typically obtained some weeks following the initiation of therapy.

Stroke

Perinatal stroke has been defined as focal ischemic brain injury secondary to vascular occlusion (Kirton and deVeber, 2009). It has an incidence of approximately 1 per 5000 live births, making it much more common than sinovenous thrombosis. As described in detail above, the appearance of stroke evolves over time. It is initially most readily detected by diffusion MR and is subsequently visible on T₁-weighted and T₂-weighted images (Fig. 63.19). In the case of nonhemorrhagic stroke, MRI is considerably more sensitive than CUS or CT, making MRI the preferred imaging modality. Because focal neurologic signs are relatively rare in newborns with stroke, it is not uncommon for perinatal strokes to go undiagnosed until later in the first year or so of life when neurologic deficits (typically hemiparesis) become obvious. In a study of 248 neonates with arterial ischemic stroke, part of the International Pediatric Stroke Study (Kirton et al., 2011), 72% presented with seizures and 63% with nonfocal neurologic signs. Once stroke is detected, consideration should be given to MR angiography to more fully define the anatomy of the injury. In the International Pediatric Stroke Study (Kirton et al., 2011),

infarcts preferentially involved the anterior circulation and the left hemisphere and were multifocal in 30% of neonates. The causes of neonatal stroke are often obscure, with cardiac and prothrombotic abnormalities identified in less than 20% of the newborns. Research on neurologic outcome following stroke is relatively sparse (Lehman and Rivkin, 2014), although clinical experience suggests that neonates have a much greater capacity for recovery than adults with similar injuries. For example, in neonates with middle cerebral artery occlusion, hemiparesis is only present in 26% of children by age 2 years, although this number increases to 50%–70% when there is corticospinal tract involvement on MRI (Husson et al., 2010).

Vein of Galen Malformation

Vein of Galen malformation is the most common arteriovenous malformation of the newborn, and the majority are identified during the neonatal period. The malformation is associated with dilatation of the vein of Galen and straight sinus extending to the torcula (Fig. 63.20). Newborns often present with hydrocephalus caused by compression of the cerebral aqueduct or high-output cardiac failure. Seizures are also not uncommon. Prognosis depends upon the size of the malformation, age at diagnosis, and successful neurosurgical outcome.

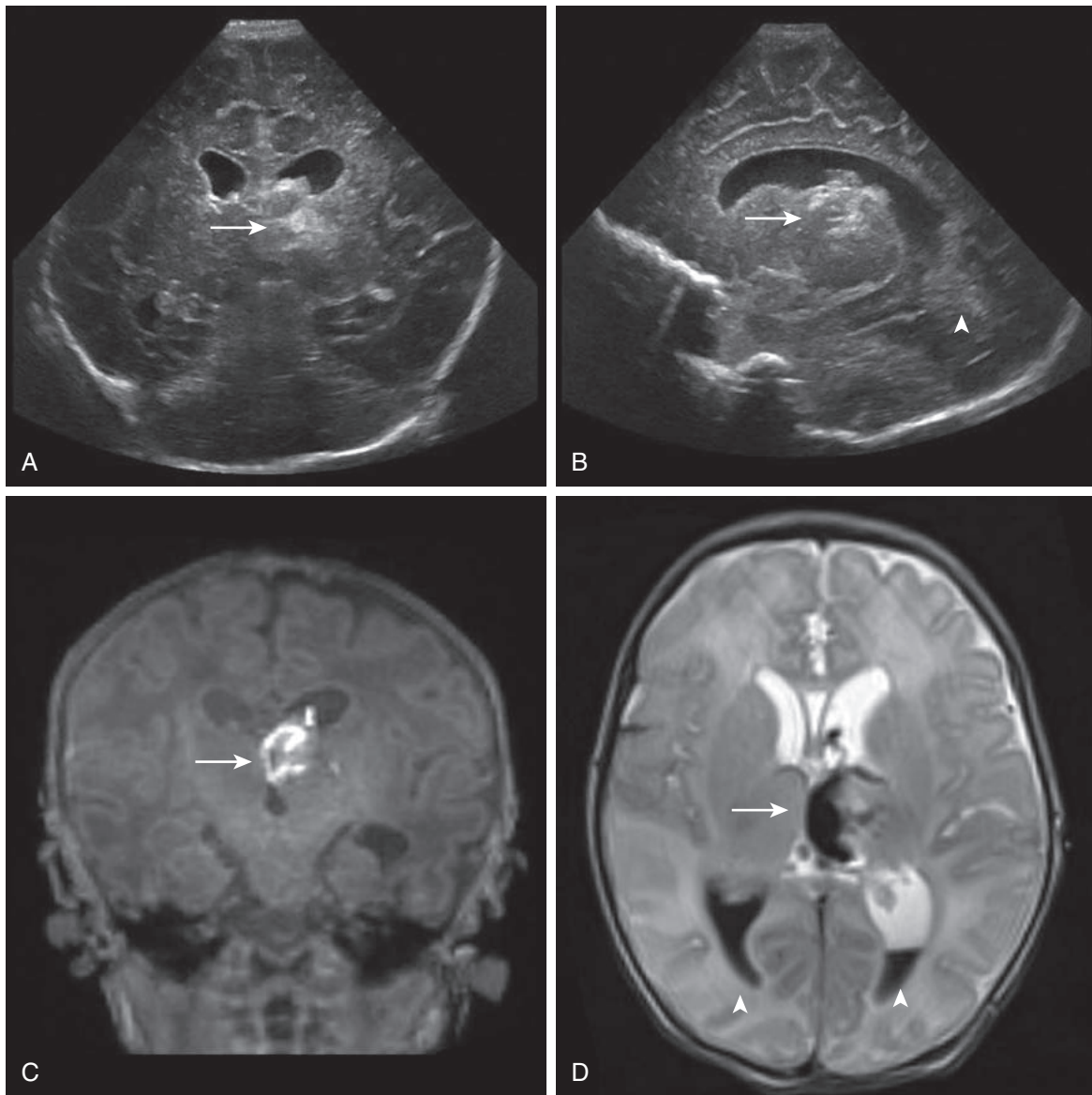
Infection

The imaging appearance of infection is remarkably diverse, varying not only with the infectious agent but also having different appearances with the same infectious agent (Table 63.7). For example, the brain injury associated with bacterial meningitis includes watershed injury, arterial occlusion with stroke, capillary thrombosis with smaller areas of injury, and sinovenous thrombosis, all of which have a characteristic appearance on MRI. As noted above, bacterial meningitis may very closely mimic hypoxic–ischemic injury (Hernandez et al., 2011). The characteristic abnormalities associated with congenital cytomegalovirus infection are shown in Fig. 63.21. The marked predilection for parechovirus (which is postnatally acquired) for white matter is evident in Fig. 63.22.

Other Intracranial Hemorrhages

Aside from the IVHs described in detail above for preterm neonates, the other major, clinically important types of neonatal intracranial hemorrhage are: (1) epidural hemorrhage, (2) subdural hemorrhage, including posterior fossa subdural hemorrhages, (3) primary subarachnoid hemorrhage, and (4) other forms of intraparenchymal hemorrhages (other than cerebellar). The approximate incidence, anatomic site of blood, relative frequency in preterm versus term born neonates, and the usual clinical gravity of these hemorrhages, including CBH and IVH, are noted in Table 63.8.

The incidence of intracranial hemorrhage has been challenging to define, as most studies have focused on symptomatic newborns, and some hemorrhages are asymptomatic. In one small study of symptomatic newborns, the estimated incidence was 4.9 per 10,000 live births (Hanigan et al., 1995). The largest epidemiologic data relate to the Californian Perinatal Database, which includes maternal and neonatal hospital discharge records on 600,000 infants (2500–4000 g) born to nulliparous women. In this study, the incidence of symptomatic intracranial hemorrhage associated with spontaneous delivery was 1 per 1900 births, vacuum extraction delivery was 1 per 860 births, and forceps delivery was 1 per 664

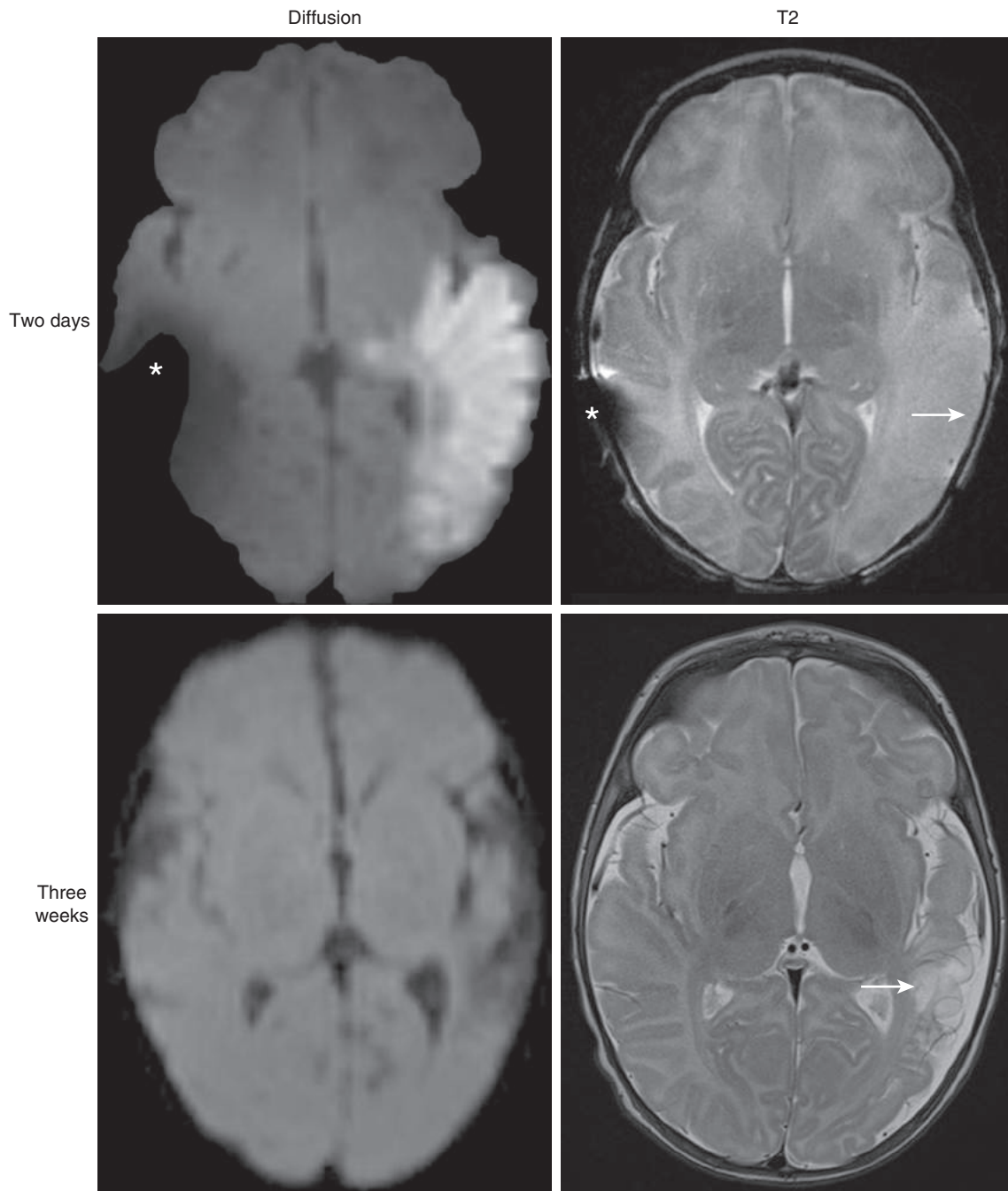


• **Fig. 63.18** Images From a Term Newborn With Sinus Thrombosis. (A) A coronal cranial ultrasonography (CUS) image with a bright-appearing thalamic hemorrhage (arrow). (B) A parasagittal view of the left lateral ventricle. Note the thalamic hemorrhage (arrow) and blood in the occipital horn of the ventricle (arrowhead). (C) The coronal T₁-weighted magnetic resonance (MR) image corresponding to the CUS image in A. The thalamic hemorrhage appears bright (arrow). (D) An axial T₂-weighted MR image in which hemorrhage appears dark. Note the thalamic hemorrhage (arrow) and blood in the lateral ventricles (arrowheads).

births (Towner et al., 1999). In contrast, more recent studies utilizing MRI in asymptomatic newborns in the first month of life have revealed a much higher frequency of intracranial hemorrhage. A large prospective study found an 8% prevalence of subdural hemorrhage in this population (Whitby et al., 2004; Rooks et al., 2008). A second study of 88 asymptomatic neonates born via vaginal delivery who underwent MRI between the ages of 1 and 5 weeks demonstrated 17 term neonates with intracranial hemorrhage for a study prevalence of 26% (Looney et al., 2007). Such findings suggest that asymptomatic intracranial hemorrhage in term newborns is more frequent than previously thought.

With these limitations regarding the incidence of intracranial hemorrhage in mind, Table 63.8 provides a summary of the

location, incidence, and usual clinical outcomes of the main types of hemorrhage. Note that subdural hemorrhage is more frequent in the term neonate than in the preterm neonate and is frequently asymptomatic but can be clinically serious if large. Primary subarachnoid hemorrhage is more frequent in the preterm neonate than in the term neonate and is fairly common but is almost always clinically benign. Cerebellar hemorrhage is more frequent in the preterm neonate than in the term neonate and can have developmental consequences as outlined above. As also outlined above, IVH, almost exclusively a lesion of the preterm neonate, affects developmental outcome. IVH recently has been more commonly recognized in the term-born infant, particularly in relation to sinovenous thrombosis and/or hypoxic-ischemic

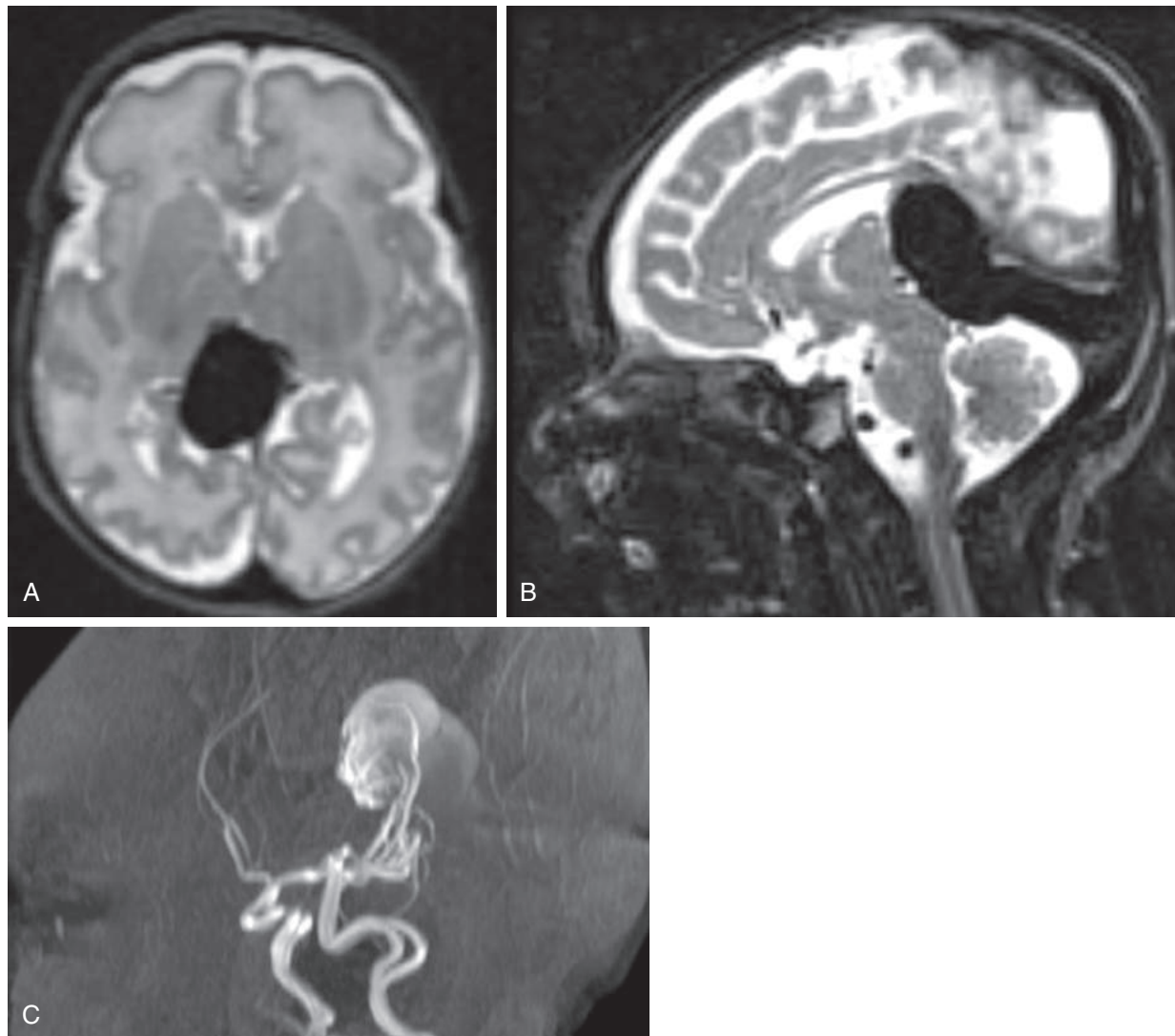


• **Fig. 63.19** Images from a term newborn who presented with episodes of rhythmic jerking of the right upper and lower extremities with rightward eye deviation at approximately 24 hours of age. Note the area of stroke on the diffusion image at age 2 days (top left), which appears bright (representing a low apparent diffusion coefficient value). Note also that the injury is visible on the T_2 -weighted image (top right), in this case as loss of the cortical ribbon (*arrow*). The *asterisk* on each image denotes an area of magnetic susceptibility artifact. The lower row images were obtained at age 3 weeks. Note that the injury is no longer detectable on diffusion imaging (bottom left) but is now an area of encephalomalacia on the T_2 -weighted image (bottom right; *arrow*).

cerebral injury. Other forms of intraparenchymal hemorrhage, more frequent in the term neonate than in the preterm neonate, are uncommon.

The three primary brain imaging modalities—CUS, CT, and MRI—have different sensitivities for detecting hemorrhage. As noted above, CUS is the method of choice for evaluating IVH

in preterm neonates because of its suitability for serial imaging of critically ill neonates. However, CUS is not the modality of choice for all forms of hemorrhage. In a study analyzing 4171 term-born infants, imaging was performed for 2006 patients with CUS, 933 patients with CT, and 2690 patients with MRI. Although cranial ultrasound identified IVH well, it lacked the



• **Fig. 63.20** Images From a Term Newborn With Vein of Galen Malformation. (A, B) Note the large flow void on the T₂-weighted images. (C) The corresponding angiogram. (Images courtesy of Dr. Bob McKinstry.)

sensitivity of MRI and CT for identifying other types of hemorrhage and intracranial injury and was particularly limited for the detection of extraaxial hemorrhage (subdural, subarachnoid, and extradural) (Pfister et al., 2012; Barnette et al., 2014). CT was recommended in the 2002 American Academy of Neurology practice parameters for neonates with birth trauma and a low hematocrit or coagulopathy (Ment et al., 2002) on the basis of data from two small studies reporting on CT diagnoses of intracranial hemorrhages leading to interventions (Odita and Hebi, 1996; Perrin et al., 1997). Although the authors were unable to determine the impact of the imaging findings on the neonates who needed surgical intervention, only 9 of 933 neonates with CT examinations underwent any central nervous system surgery. Given the risks of radiation exposure associated with CT imaging, we suggest using MRI, when available, to detect extraaxial hemorrhage. The use of MRI has the added benefit of better sensitivity for detecting parenchymal injury than CT. Development of more rapid MRI sequences to allow for shorter studies to detect cerebral hemorrhage should enhance physician comfort with this as a first-line technique.

Subdural Hemorrhage

MRI is more effective than CT in the delineation of posterior fossa subdural hemorrhage (Barkovich, 2005). Detection of subdural hematoma by ultrasound scanning, although reported, generally is difficult. Moreover, even when these hematomas are detected, the extent and distribution of supratentorial lesions are usually demonstrated far better by MRI or CT and infratentorial lesions are detected better by MRI. In addition, the vast majority of subdural hematomas are infratentorial, where ultrasound has even greater challenges in accurate diagnosis (Fig. 63.23).

Subarachnoid Hemorrhage

The diagnosis of primary subarachnoid hemorrhage is usually made by MRI or CT and, on rare occasions, by ultrasound (Barnette et al., 2014). On CT, distinction between the normal, slightly increased attenuation in the regions of the falx and major venous sinuses and the increased attenuation caused by subarachnoid hemorrhage may be difficult. Sometimes, the possibility of primary

**TABLE
63.7****Magnetic Resonance Imaging Findings for Neonatal Infections**

| Infection | Imaging Findings | Infection | Imaging Findings |
|------------------------------|--|---------------------------------------|--|
| Bacterial meningitis | Basal ganglia injury Watershed injury Sinovenous thrombosis Infarct (may be multifocal) Abscess Extraaxial empyema Ventriculomegaly | Varicella (congenital) | Diffuse cerebral necrosis Cerebellar hypoplasia Cortical malformations, including pachygyria |
| Cytomegalovirus (congenital) | Calcifications (periventricular and cortical) Cortical malformations (lissencephaly, polymicrogyria, heterotopias) Ventriculomegaly Cerebellar hypoplasia Periventricular leukomalacia Porencephaly | Herpes simplex (congenital) | Microcephaly with severe volume loss |
| Toxoplasmosis (congenital) | Diffuse cerebral necrosis Porencephaly Hydranencephaly Diffuse cerebral calcifications Periventricular necrosis Hydrocephalus | Herpes simplex (peripartum/postnatal) | Early Multifocal injury Signal abnormality may be limited to the temporal lobes, cerebellum, or brainstem (may be hemorrhagic) Basal ganglia injury Watershed injury Late Multicystic encephalomalacia White and gray matter volume loss Calcification |
| Rubella (congenital) | Periventricular and basal ganglia calcification Ventriculomegaly Multifocal white matter lesions Leukoencephalopathy Subcortical cysts Cortical malformations are uncommon. | Zika (congenital) | Cortical malformations (lissencephaly, heterotopias, polymicrogyria) Parenchymal calcifications Ventriculomegaly Dysgenesis of the corpus callosum Cerebellar hypoplasia Brainstem hypoplasia |
| | | Parechovirus (neonatal) | Diffuse white matter injury with cortical sparing |

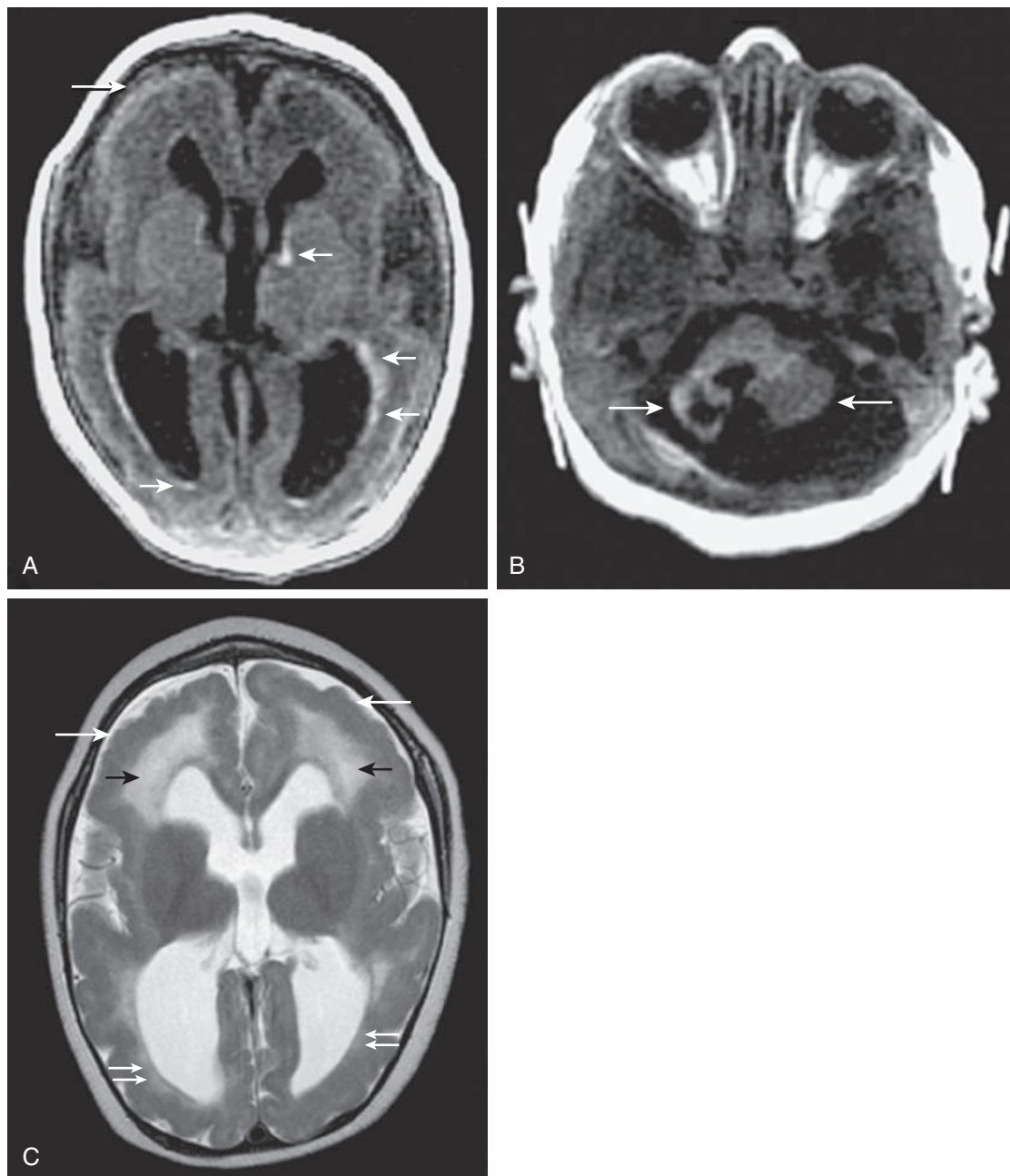
**TABLE
63.8****Intracranial Hemorrhage**

| Hemorrhage | Incidence % | Site of Blood | Full Term or Preterm | Usual Clinical Outcome |
|-----------------------|------------------|---|----------------------|------------------------|
| Extradural (Epidural) | Very rare | Between skull and outside of dura | FT > PT | Variable |
| Subdural | 5–25 | Between dura and arachnoid | FT > PT | Benign |
| Subarachnoid | 1–2 FT 10 PT | Between arachnoid and pia | PT > FT | Benign |
| Cerebellar | 0.1 FT 5 PT | Cerebellar hemispheres and/or vermis | PT > FT | Serious |
| Intraventricular | 0.2 FT 15 PT | Within ventricles or including periventricular hemorrhagic infarction | PT > FT | Serious |
| Parenchymal | 0.1 FT 2–4 PT | Cerebral parenchyma | FT > PT | Variable |

FT, Full term; PT, preterm.

subarachnoid hemorrhage is raised initially by the findings of an elevated number of red blood cells and an elevated protein content in the CSF, usually obtained for another purpose (e.g., to rule out meningitis). Exclusion of the relatively common (e.g., extension from subdural, cerebellar, or IVH) and uncommon (e.g., tumor, vascular lesions) causes of blood in the subarachnoid space is best done by MRI.

Ultrasonography is insensitive in detecting subarachnoid hemorrhage because of the normal increase in echogenicity around the periphery of the brain (Shackelford and Volpe, 1985). A large subarachnoid hemorrhage occasionally distends the Sylvian fissure and thus becomes detectable, but care must be taken not to confuse a Sylvian fissure distended with blood from the wide fissure seen consistently in preterm neonates and resulting from the normal



• **Fig. 63.21** A 16-day-old neonate born after 31 weeks' gestation with congenital cytomegalovirus infection identified in utero. (A) An axial T₁-weighted magnetic resonance imaging (MRI) scan shows an increased signal in the periventricular regions (*short arrows*), consistent with calcification, and diffuse polymicrogyria (*long arrow*). (B) Note the striking cerebellar hypoplasia (*arrows*). (C) At 6 months of age, the axial T₂-weighted MRI scan shows diffuse frontal polymicrogyria (*long arrows*), abnormal high signal intensity in cerebral white matter (*short black arrows*), and marked paucity of parieto-occipital cerebral white matter (*double white arrows*). (Courtesy of Dr. Omar Khwaja.)

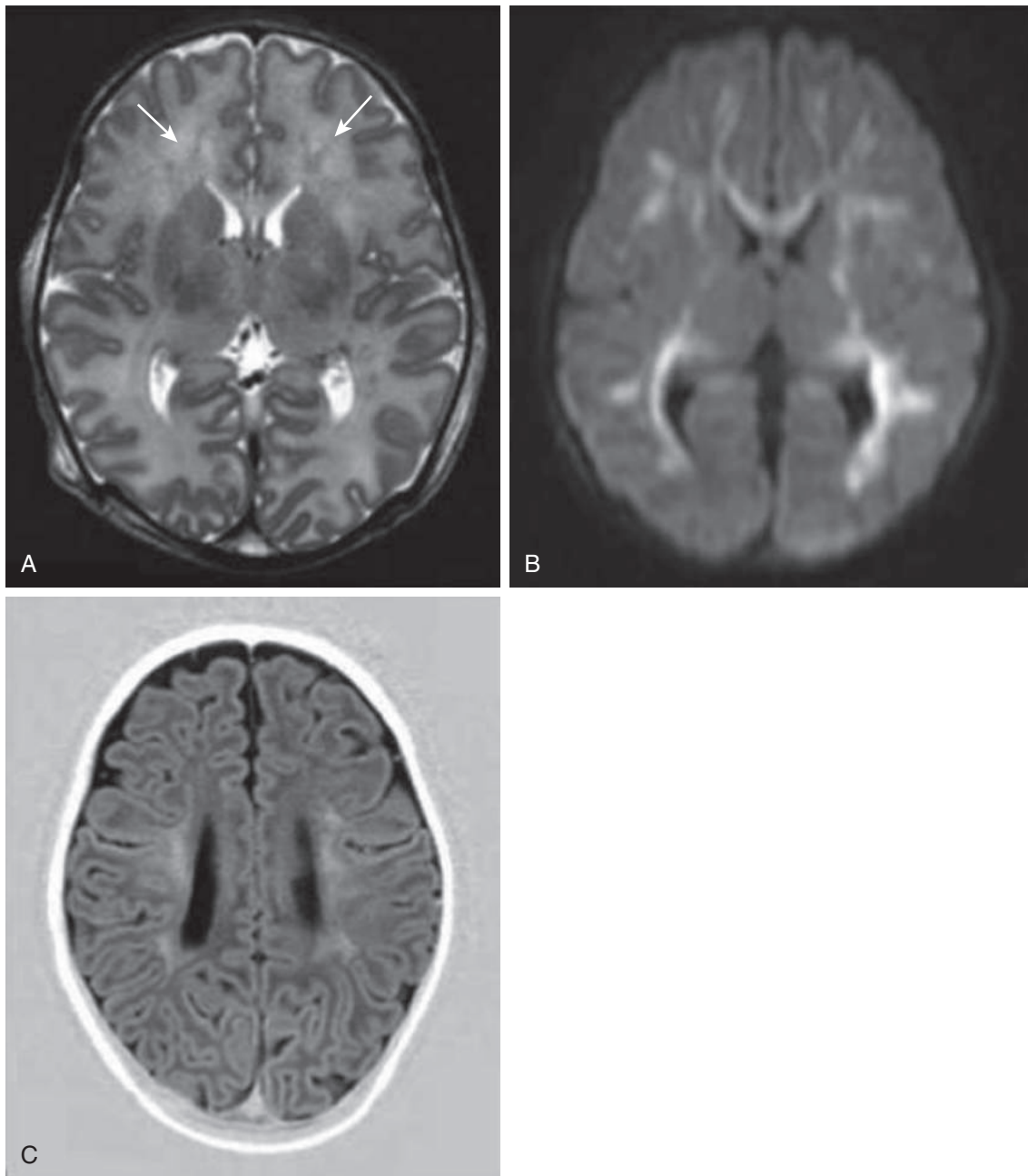
separation of the frontal operculum and superior temporal region until late in gestation ([Chamnanvanakij and Perlman, 1999](#)).

Methods Not Yet in Clinical Use

Quantitative Interpretation of Magnetic Resonance Imaging Studies

MRI studies are almost universally subjected to qualitative interpretation in the clinical setting. However, a number of quantitative

scoring systems have been devised (see [Table 63.4](#)), though there is no universally accepted, or gold standard, scoring system. Most scoring systems rely upon features of conventional T₁-weighted and T₂-weighted images, with a tendency for some scoring systems to focus mainly on loss of brain volume, while others focus primarily on signal abnormality. Since brain injury during the neonatal period may lead to both poor brain growth (small volume) and injury to remaining tissue (signal abnormality on MRI studies), it is useful to take both into account in a scoring system. To complicate matters further, some scoring systems focus on relatively confined



• **Fig. 63.22** Parechovirus Infection in a Neonate. T₂-weighted (A) and diffusion (B) magnetic resonance images obtained 6 days after the onset of human parechovirus infection in a neonate. (C) A T₁-weighted image obtained at 3 months. Note the multiple punctate white matter lesions (A, arrows) and diffusion abnormality (B, areas of low apparent diffusion coefficient appear bright) in the periventricular white matter and involving the optic radiation and the internal capsule. Note also the areas of high signal intensity in the periventricular white matter present at 3 months of age, suggesting gliosis (C). (Adapted from Verboon-Macielek MA, Groenendaal F, Hahn CD, et al. Human parechovirus causes encephalitis with white matter injury in neonates. *Ann Neurol*. 2008;64:266–273.)

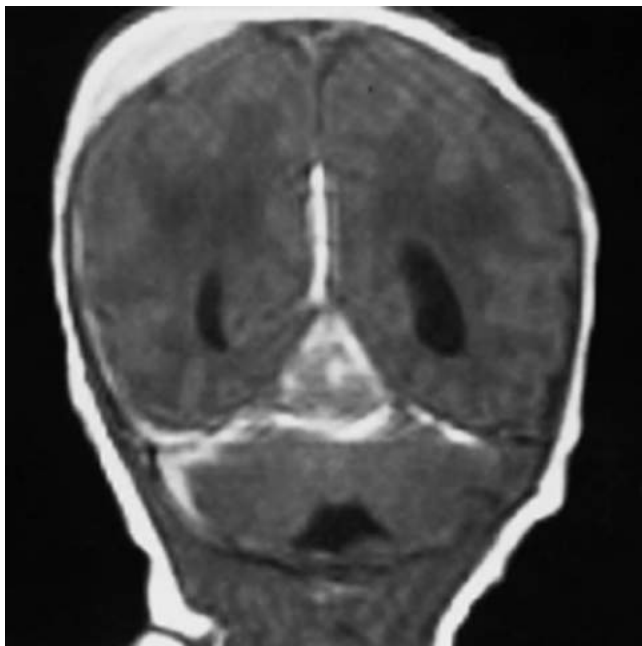
areas of the brain, such as white matter, without evaluation of cortical gray matter, deep nuclear gray matter, and/or the posterior fossa. The broadest scoring system would take both loss of volume and signal abnormality into account for the entire brain and cerebellum (Kidokoro et al., 2013).

Cortical Cartography

In addition to the scoring systems noted above, cortical cartography provides a means by which to quantify MRI studies. Cortical

cartography involves analysis of the cortical surface. With this approach, a surface is generated from conventional images and a number of summary parameters are measured to capture features of the cerebral topography. One of the more common of these is cortical surface area, which increases dramatically during the immediate postnatal period in preterm neonates. A second parameter is the gyrification index, a ratio of surface areas: the numerator is the cortical surface area, and the denominator is the cerebral hull area, which can be imagined as the surface area of cling wrap if it were wrapped around the brain (Van Essen, 2005). For a completely

smooth, or lissencephalic, brain, the gyrification index would be 1. As the number and depth of cortical folds increase the gyrification index increases. As would be expected, the gyrification index increases during normal brain development (Shimony et al., 2016). As for cortical surface area, the gyrification index is affected by preterm birth and is lower for preterm neonates at term equivalent age compared with control neonates in a region-specific fashion (Engelhardt et al., 2015). Gyrification index values have also been related to neurodevelopmental outcome (Dubois et al., 2008a). A third index derived from cortical cartography is sulcal depth, which is the distance between the cerebral hull and the bottom of each sulcus (Van Essen, 2005). As with the other parameters, sulcal depth increases with brain development (Dubois et al., 2008b; Zubiaurre-Elorza et al., 2009; Shimony et al., 2016), and abnormalities of sulcal depth have been described for preterm neonates (Engelhardt et al., 2015).



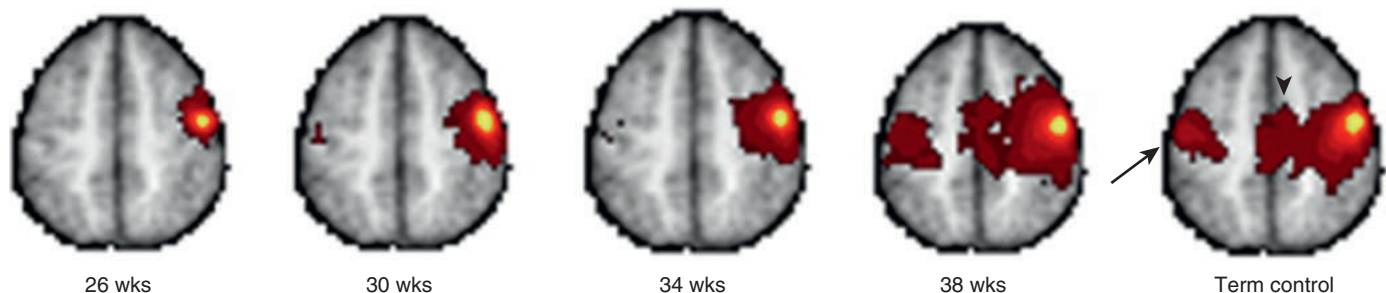
• **Fig. 63.23** Tentorial Subdural Hemorrhage With Blood Layering Along Both Leaves of the Tentorium and Posterior Falx. (Adapted from Castillo M, Fordham LA. MR of neurologically symptomatic newborns after vacuum extraction delivery. *AJNR Am J Neuroradiol.* 1995;16:816–818.)

Functional Magnetic Resonance Imaging

As described above for susceptibility-weighted imaging, the signal in an MRI study can be made sensitive to the presence of reduced iron in deoxyhemoglobin. In regions of hemorrhage, the effects are striking. However, more subtle effects can also be detected in the case of changes to intravascular deoxyhemoglobin concentration. For example, when neuronal firing rates increase in an area of brain because of activation by a task, local blood flow increases while local oxygen utilization changes very little. As a result, local deoxyhemoglobin levels fall, and this reduction is detectable as a small (on the order of a few percent) increase in the signal intensity on susceptibility-weighted images. This contrast forms the basis of functional MRI, in which susceptibility-weighted images are obtained before, during, and after a subject performs a task. The MR signal in these studies is referred to as blood oxygenation level dependent, or BOLD, signal. Areas of neural activation can be detected as areas of increased BOLD signal intensity during performance of the task. One might be forgiven for thinking that this particular approach is not very useful for studying neonates, who are not particularly adept at performing tasks on demand, but there is a variant of this method that can be used to identify neural networks in the resting, or even sleeping, subject. While the precise neurophysiologic basis of this method is not yet completely understood, it is probably related to spontaneous, gradual changes in local neuronal firing rates. These changes take place over tens of seconds or minutes and are associated with matching changes in local BOLD signal. If two brain regions are connected, these gradual changes in firing rate, and hence changes in BOLD signal intensity, take place synchronously. As a result, one can identify brain regions that are connected by searching for areas that have synchronous spontaneous fluctuations in BOLD signal. Conversely, areas that have an inhibitory connection show anticorrelated fluctuations in BOLD signal. This method can be used in preterm neonates to monitor the emergence of neural networks (Fig. 63.24). While functional connectivity MRI is not in routine clinical use and requires specialized data acquisition and software, studies have shown widespread alterations in neural networks in preterm neonates at term equivalent age despite normal conventional images (Smyser et al., 2016).

Near-Infrared Spectroscopy

Near-infrared spectroscopy (NIRS) is analogous to the pulse oximetry that is commonly used in clinical practice to monitor



• **Fig. 63.24** Resting State Functional Magnetic Resonance Imaging Data From Neonates at Various Postmenstrual Ages. A seed point was placed in the motor cortex (bright yellow dot). The connections between motor cortex, contralateral motor cortex (arrow) and supplementary motor cortex (arrowhead) emerge at approximately 38 weeks' gestation. (Adapted from Smyser CD, Inder TE, Shimony JS, et al. Longitudinal analysis of neural network development in preterm infants. *Cereb Cortex.* 2010;20:2852–2862.)

arterial oxygen saturation. Both pulse oximetry and NIRS use similar wavelengths of light. For pulse oximetry, the signal is processed to isolate the signal from arterial blood to measure arterial oxygen saturation. Localization is not particularly crucial in this case and is achieved by passing the light through a digit using a pair of optodes. Commercially available NIRS devices work very similarly to pulse oximetry. Since the signal is not usually processed to evaluate arterial blood, NIRS typically provides a relative measure of mixed venous tissue oxygenation. For research applications such as functional activation, localization to various brain regions may be achieved using optode arrays (Gregg et al., 2010; Liao et al., 2010). The parameters available from NIRS include oxyhemoglobin and deoxyhemoglobin levels, cerebral oxygen saturation, and the fraction of tissue oxygen extraction (this last parameter is calculated

in conjunction with arterial oxygen saturation levels obtained with pulse oximetry). While NIRS is not widely used in clinical practice, it has been used to study a variety of conditions, including infants with apnea and bradycardia (Petrova and Mehta, 2006), different modes of ventilation (Schwaberg et al., 2015; Guerin et al., 2016), IVH (Vesoulis et al., 2016), and extracorporeal membrane oxygenation (Liem et al., 1995). In a study of asphyxiated neonates, higher cerebral oxygen saturation and lower fractional cerebral tissue oxygen extraction after 24 hours were associated with poor neurodevelopmental outcome, suggesting secondary energy failure in these neonates (Toet et al., 2006).

Complete references used in this text can be found online at www.expertconsult.com

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Neonatal Neuromuscular Disorders

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KEY POINTS

- Evaluation of neonatal hypotonia includes neuromuscular conditions, and the diagnostic work-up should be approached in a stepwise manner.
- A normal creatine phosphokinase (CPK) does not completely rule out muscle disease.
- Electromyography (EMG) is useful in the diagnostic evaluation of hypotonia and weakness.
- The search for a genetic diagnosis is crucial in patients with neuromuscular disease.

Neonatal Neuromuscular Disorders

Neuromuscular disorders comprise diseases of the muscle (congenital myopathies and muscular dystrophies), neuromuscular junction (myasthenia gravis and congenital myasthenic syndromes), nerves (neuropathies), and anterior horn motor neurons (spinal muscular atrophies). They present in the neonatal period as floppy infant syndrome with or without contractures. Respiratory insufficiency and swallowing difficulties can be in the forefront of the clinical picture and are frequently associated with significant hypotonia and weakness.

This chapter reviews our current knowledge of neuromuscular disorders with neonatal onset and their clinical details alongside pathologic, genetic, and radiologic aspects as applicable. Finally, an approach to the diagnostic evaluation of neonates when a neuromuscular disorder is suspected is discussed.

Primary Muscle Disorders

Disorders of the muscle present in the early neonatal period with hypotonia or weakness. Primary muscle disorders can be subdivided into two categories: (1) muscular dystrophies and (2) congenital myopathies. Muscular dystrophies apparent in the early neonatal period or first few months of life are collectively referred to as “congenital muscular dystrophies” (CMDs). There are histopathologic differences between CMDs and congenital myopathies; CMDs demonstrate dystrophic changes on muscle biopsy, with disruption of the muscle fiber and its architecture, while congenital myopathies may have rather subtle changes such as fiber-type disproportion with preservation of the muscle fiber architecture.

Historically, classification of these disorders has been derived by histopathology. More recently, discovery of genetic defects has

led to increasing heterogeneity in the presentation of disorders. With this, newer schemas classify based on pathology and genetic findings.

Congenital Muscular Dystrophies

CMDs comprise a heterogeneous group of disorders characterized by a dystrophic process to the muscle on histopathology. With recent advancements in genetics, we are learning that many of the genetic mutations associated with CMDs may also present as congenital myopathies. Previously, CMDs were classified by the presence or absence of merosin on histopathology. Merosin, an extracellular matrix protein, was originally described as absent in one-third to one-half of cases of CMD (Dubowitz, 1994; Tome et al., 1994; Philpot et al., 1995) and can be associated with structural brain abnormalities (Philpot et al., 1999; Mercuri et al., 2001; Muntoni and Voit, 2004). Over the past decade, the advancement of genetics has allowed distinct clinical forms to be recognized, fostering a better understanding of this group of disorders.

Classification schemas alongside diagnostic approaches have been proposed that take into account this expansion of knowledge (Muntoni and Voit, 2004; Mercuri and Muntoni, 2012; Bonnemann et al., 2014). Given the numerous genes discovered and the ongoing expansion of causative genes, the CMDs have most recently been separated into seven subtypes of disorders: α -dystroglycanopathies, laminin α_2 -related dystrophy, *LMNA*-related dystrophy, collagen VI-related dystrophy, *SEPN1*-related myopathy, *RYR1*-related myopathies, and CMD without a genetic diagnosis (Bonnemann et al., 2014). *SEPN1* and *RYR1*, which are typically considered myopathies, are included in this classification scheme secondary to the varying phenotype at presentation.

α -Dystroglycanopathies

α -Dystroglycanopathies exhibit hypoglycosylation of α -dystroglycan (DG) on the cellular membrane of the muscle. The phenotype is extremely variable, with mild to severe clinical outcomes and subtypes that have significant brain involvement. More severe presentations include those of Walker–Warburg syndrome, muscle–eye–brain (MEB) disease, and Fukuyama congenital muscular dystrophy. The milder end of the spectrum includes individuals presenting in adulthood with limb girdle muscular dystrophies without brain or eye involvement.

DG is encoded by a single gene, *DAG1*, and is cleaved into two proteins, α -DG and β -DG. α -DG is an extracellular peripheral membrane glycoprotein that anchors to the cell membrane by

binding to a transmembrane protein, β -DG. α -DG also binds with high affinity to the extracellular matrix component laminin (Endo, 2015). Dystroglycanopathies are secondary to defective DG glycosylation because of mutations in a number of genes.

The number of genes involved in dystroglycanopathies have continued to increase and, to date, include at least seven genes; the first six are more frequently recognized (Kobayashi et al., 1998; Brockington et al., 2001; Yoshida et al., 2001; Beltran-Valero de Bernabe et al., 2002; Longman et al., 2003; van Reeuwijk et al., 2005a, 2005b; Roscioli et al., 2012; Willer et al., 2012; Buysse et al., 2013):

1. Protein *O*-mannosyl transferase 1 (*POMT1*; online Mendelian inheritance in man [OMIM] 607423)
2. Protein *O*-mannosyl transferase 2 (*POMT2*; OMIM 607439)
3. Protein *O*-mannose 1,2-acetylglucosaminyltransferase 1 (*POMGnT1*; OMIM 606822)
4. Fukutin (*FCMD*; OMIM 607440)
5. Fukutin-related protein (*FKRP*; OMIM 606596)
6. *LARGE* (OMIM 603590)
7. Isoprenoid synthase domain-containing protein (*ISPD*; OMIM 614631)

Studies on large populations of patients have shown that the reported mutations do not detect many of the patients with a clinical examination suggestive of an α -dystroglycanopathy, suggesting that there remain unknown genes (Mercuri et al., 2009). Clinical signs of patients include relative hypertrophy of the lower extremities, with wasting of the upper extremities and brain involvement. As genetic mutations have been found, the heterogeneity present with phenotypes has become increasingly apparent (Mercuri et al., 2006, 2009; Clement et al., 2008).

Below, three classic forms of α -dystroglycanopathies are discussed: Walker–Warburg syndrome, MEB disease, and Fukuyama congenital muscular dystrophy.

Walker–Warburg Syndrome

Walker–Warburg syndrome (WWS) is the most severe form of CMD with central nervous system (CNS) involvement. Neonatal hypotonia and severe weakness are accompanied by encephalopathy and poor vision. Ocular defects include retinal dysgenesis, microphthalmia, and anterior chamber malformations (Dobyns et al., 1989). Brain malformations include type II lissencephaly, which results in a cobblestone microgyric appearance to the cortex as well as dysmyelination of the white matter. Cerebellar malformations are also frequently present (Dobyns et al., 1989).

Infants may be thought to have CNS malformations alone; however, creatine phosphokinase (CPK) is markedly elevated. Initially, WWS was associated primarily with *POMT1* but has since been associated with the other dystroglycanopathy genes.

Muscle–Eye–Brain Disease

MEB disease typically presents at birth or in the first few months of life with hypotonia and weakness and, when compared with WWS, will have a milder phenotype of ocular abnormalities; these may not be determined until the first few years of life (Santavuori et al., 1989). Brain magnetic resonance imaging (MRI) reveals disorders of neuronal migration due to type II lissencephaly, such as pachygyria or polymicrogyria. Other associated imaging findings include cerebellar hypoplasia and white matter changes (Fig. 64.1). Initially, the *POMGnT1* gene was thought to be causative (Yoshida et al., 2001), but with time, all other reported dystroglycanopathies

have been associated with MEB as well (Mercuri et al., 2006; Godfrey et al., 2007; Clement et al., 2008).

Fukuyama Congenital Muscular Dystrophy

Fukuyama congenital muscular dystrophy, similar to other α -dystroglycanopathies presenting in the neonatal period, has clinical signs of hypotonia as well as weakness, poor feeding, and poor suck. Joint contractures may be present. CNS abnormalities are present, with cobblestone lissencephaly, pachygyria, or polymicrogyria and white matter changes. As compared to WWS and MEB, Fukuyama congenital muscular dystrophy often has more severe muscle involvement with lesser ocular manifestations. *FKTN*, with its gene product fukutin, is the only reported cause (Kobayashi et al., 1998). Fukuyama congenital muscular dystrophy has been classically described in Japanese populations (Fukuyama and Ohsawa, 1984); however, founder effects in *FKTN* have been reported in the Ashkenazi Jewish and Korean populations (Chang et al., 2009; Lim et al., 2010), with case reports in various other populations.

Other α -Dystroglycanopathies

A high suspicion for α -dystroglycanopathies should arise in any neonate with structural CNS abnormalities that involve cobblestone lissencephaly or pachygyria/polymicrogyria or when associated with ocular findings or clinical weakness. CPK will be significantly elevated in nearly all cases.

LAMA2-Related Muscular Dystrophy

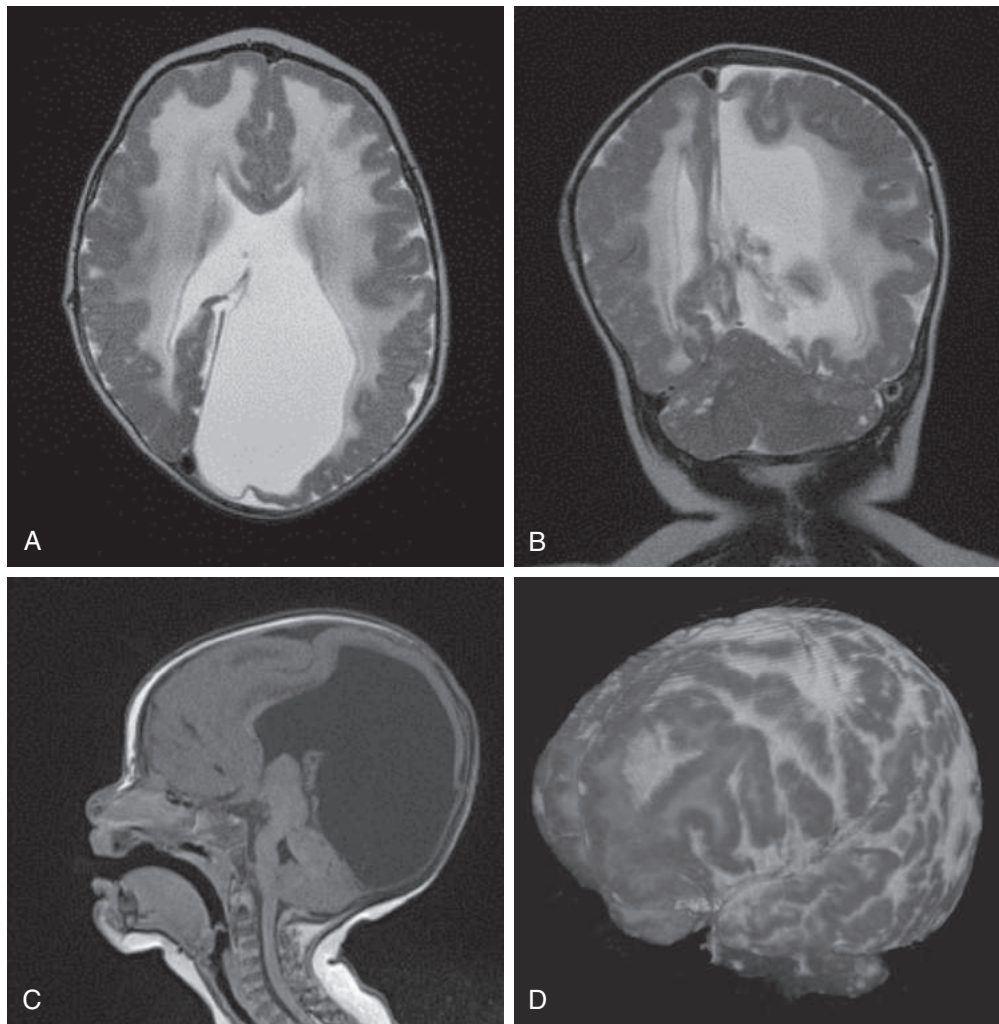
LAMA2-related muscular dystrophy is a cause of severe hypotonia in infancy and has been previously referred to as merosin-deficient CMD. It is secondary to a defect in *LAMA2*, which encodes for laminin α_2 , thus resulting in the newer and favored terminology of *LAMA2*-related muscular dystrophy (Hara et al., 2011). Laminin α_2 is a constituent of merosin, which is a heteromer of α_2 , β_1 , and γ_1 subunits; absence of laminin α_2 thus results in a deficiency of merosin. The term *merosin-deficient congenital muscular dystrophy* has fallen out of favor as it is not specific to the disease etiology and can also be histologically true of some dystroglycanopathies by an indirect effect.

Clinically, *LAMA2*-related muscular dystrophy may present in the neonate with severe hypotonia and weakness or within the first 6 months of life. Contractures may be present at birth, as well as a weak cry and respiratory failure. Neonates with *LAMA2* mutations typically have muscle involvement without significant encephalopathy; however, white matter abnormalities develop in nearly all patients (Ibrahim Abdulla et al., 2007; Kumar et al., 2014).

Suspicion for *LAMA2* mutations should exist in neonates with significant hypotonia and/or contractures in the setting of respiratory failure, without significant encephalopathy or ocular abnormalities. CPK levels in infancy and early childhood are elevated, often four to five times above normal limits (Oliveira et al., 2008). On histopathologic examination, muscle biopsy will demonstrate decreased or absent binding to laminin α_2 . Care for patients with *LAMA2* mutations is supportive in nature.

Congenital Myotonic Dystrophy

The congenital form of myotonic dystrophy presents differently from the childhood or classical adult phenotype. Neonates with



• **Fig. 64.1** Brain Magnetic Resonance Imaging Changes Associated With Dystroglycanopathies. Images from a patient with *POMGnT1* mutation. T2 axial (A) and coronal (B) images demonstrating cobblestone lissencephaly, white matter changes, and parietal lobe porencephaly. T2 sagittal FLAIR (C) demonstrating porencephaly and kinked brainstem. (D) Three-dimensional rendering of cobblestone lissencephaly. (Courtesy of Dr. Francisco Perez, MD PhD.)

congenital myotonic dystrophy are often noted in utero to have complications of polyhydramnios and reduced fetal movement and may deliver prematurely. At birth, signs of hypotonia and weakness are present. Talipes equinovarus, or bilateral fixed ankle contractures, are present. Facial features include facial diplegia with a “carp” mouth appearance and bilateral ptosis. Clinical examination demonstrates hyporeflexia or areflexia and diffuse weakness more severe in distal muscles than proximal ones. Grip and percussion myotonia are not present at this age and develop in childhood. Difficulty in management of secretions is common, as is weak suck with feeding difficulties, often necessitating nasogastric tube feeds. Often, neonates may present in respiratory failure or develop it in the first weeks of life. This is secondary to muscle weakness in the inspiratory and expiratory respiratory muscles, resulting in shallow breaths. In utero, this may result in pulmonary hypoplasia. These factors lead to high mortality in the neonatal intensive care unit (NICU), which is estimated at 25%–40% of cases (Reardon et al., 1993; Campbell et al., 2004; Wallgren-Pettersson et al., 2004a). The duration and severity of the respiratory muscle weakness and pulmonary hypoplasia are

key determinants of outcome. Prolonged mechanical ventilation, defined as greater than 4 weeks in duration, is a negative prognostic factor in these neonates (Rutherford et al., 1989).

The diagnosis of congenital myotonic dystrophy should be considered when there is a positive family history. The disease is transmitted by mother and shows anticipation. A detailed history and examination of the mother often reveals characteristic facial features associated with classical myotonic dystrophy, including frontal balding, ptosis, facial diplegia, temporal wasting, or cataracts. In addition, examination of the mother typically reveals grip myotonia. These symptoms are often subtle, such that the mother is unaware of her diagnosis.

Electrographic or clinical myotonia is not present in the neonatal period. The diagnosis is often suspected on the clinical basis and by examination of the mother and then confirmed by genetic studies. The *DMPK* gene, located at chromosome 19q13.3, contains a cytosine–thymine–guanine (CTG) trinucleotide repeat in the 3′ noncoding region (Mahadevan et al., 1992). Unaffected individuals have between 5 and 27 repeats, while patients with a classical presentation have 50–1000 repeats. Anticipation is present with

this area, as the expansion increases and becomes more unstable in later generations, resulting in earlier and more severe presentation. Neonates with congenital presentation have greater than 1000 CTG repeats, with disease severity associated with number of repeats (Tsiflidis et al., 1992).

Neonates who survive the neonatal period typically require ongoing respiratory support and can survive into adulthood with close respiratory and cardiac monitoring and with therapy; however, cognitive impairment is frequent and can be severe (Echenne et al., 2008).

Other Forms of Muscular Dystrophies

Other forms of muscular dystrophies, such as Duchenne muscular dystrophy, present in early childhood with onset of weakness and calf pseudohypertrophy. Newborns who later develop Duchenne muscular dystrophy are found to have very high CPK levels in the neonatal period and in the absence of any weakness. *LAMA2* deficiency, which can present in the neonatal period, can be first noted in childhood to early adulthood with varying severity as a limb girdle muscular dystrophy.

The *LMNA* gene, which is associated with Emery–Dreifuss syndrome in older children or adults, has been found in neonates and children with CMD (Mercuri et al., 2004; D’Amico et al., 2005; Quijano-Roy et al., 2008). *LMNA* encodes for lamin A/C, which is a nuclear envelope protein. The syndrome is classically described as reduced fetal movements, severe hypotonia, and weakness with a “dropped head” appearance because of involvement of the neck muscles (Quijano-Roy et al., 2008).

Type VI Collagen-Related Disorders

Collagenopathies affecting type VI collagen may present in the neonatal period or early infancy with CMD with the eponym Ullrich muscular dystrophy and a severe phenotype or with a milder phenotype, Bethlem myopathy. Genes identified with this disorder are *COL6A1*, *COL6A2*, and *COL6A3*. The prevalence of collagen type IV disorders is not completely known; however, it is thought to be most common in the North American population and second to Fukuyama muscular dystrophy in Japan (Bonnemant, 2011).

Clinically, neonates with the Ullrich muscular dystrophy phenotype will have significant hyperlaxity, especially of the distal joints, transient kyphosis, and prominent calcanei. Joint contractures and clubfoot may be present. Skin is pliable and hyperkeratosis pilari is present by infancy.

Congenital Myopathies

The term *congenital myopathies* refers to muscle disorders that present in neonates or early infancy without dystrophic changes on muscle biopsy. Congenital myopathies tend to have a slowly progressive or nonprogressive course. In fact, some patients with congenital myopathies may improve with age. The group of congenital myopathies were described initially based on histopathologic findings on muscle biopsy. As genetic knowledge has expanded, our understanding of congenital myopathies has changed; we have learned that specific gene mutations may present as different histopathologic phenotypes (Sewry et al., 2008). Congenital myopathies may present in the neonatal period but also throughout childhood. Histology can demonstrate nemaline myopathy, central core disease, myotubular myopathy, and minicore disease.

The typical neonate presents with floppiness, weakness, and facial hypotonia and can have contractures. There is a risk for respiratory failure because of weak respiratory muscles including the diaphragm. When compared with muscular dystrophies, CPK may be normal or mildly elevated. Electromyography (EMG) may be normal or show mild myopathic changes. Thus muscle biopsy is often the next step and, in addition to genetic testing, may lead to a definitive diagnosis.

Nemaline Myopathy

Nemaline myopathy derives its name from “nema” the Greek for thread. The muscle biopsy shows threadlike rods. The rods stain red on Gomori trichrome, giving its characteristic appearance. Nemaline myopathy can present throughout the life span with varying severity. Two basic presentations may occur neonatally. Newborns may present with significant hypotonia with weakness including bulbar involvement. The facial and axial muscles are involved. Neonates may require respiratory support because of weak respiratory muscles, frequent suctioning, and nutritional support with either feeding tubes or gastrostomy tube. In more severe forms, the pregnancy history is often notable for reduced fetal movements and polyhydramnios, and the neonate has arthrogryposis at birth with severe respiratory failure and feeding difficulties in addition to immobility (Romero et al., 2013). The term *fetal akinesia* syndrome is used in patients with nemaline rod myopathy who have this more severe form, and dysmorphic features may also be present.

Those patients affected with fetal akinesia presentation or the severe congenital form have a poor prognosis; often, no improvement is seen in respiratory function or weakness over the first several months of life. Those with the milder presentation show ongoing improvement and, with time, some will learn to ambulate. However, many may still require respiratory assistance because of nocturnal hypoventilation and may have failure to thrive or scoliosis.

Eight genes have been connected to nemaline myopathy, seven of which may present in the neonatal period: nebulin (*NEB*), skeletal muscle α -actin (*ACTA1*), slow α -tropomyosin (*TPM3*), β -tropomyosin (*TPM2*), slow troponin T (*TNNT1*), cofilin 2 (*CFL2*), and Kelch-like family member 40 (*KLHL40*) (Romero et al., 2013). Nebulin accounts for up to 50% of cases, and a range of phenotypes are reported (Wallgren-Pettersson et al., 2004b).

Core Myopathies

Central core and multiminicore diseases together comprise the “core myopathies” and are the most common myopathies across all age ranges (Jungbluth et al., 2011). Histopathologically, the two are grouped together as “core” disorders, given the pattern on Gomori trichrome stain. Because of focal decreased oxidative activity from the muscle disease, there is absence of oxidative stain on muscle biopsy. Based on this visual appearance, core diseases are classified as central core, multicore, minicore, or multiminicore.

Clinically, patients with central core disease may present across the life span and neonatally are distinct, with proximal greater than distal weakness and variable bulbar involvement (Malicdan and Nishino, 1993; Jungbluth et al., 2011). Clinical features at birth may include scoliosis, contractures, and the need for respiratory support; in the most severe neonatal cases, fetal akinesia syndrome may result (Romero et al., 2003). Serum CPK is often normal or mildly elevated.

The most common gene associated with central core disease is the *RYR1* gene; this encodes the ryanodine receptor, which is a calcium channel on the sarcoplasmic reticulum (Ferreiro et al., 2002; Jungbluth et al., 2002). The ryanodine receptor and mutations in *RYR1* may also result in malignant hyperthermia. Because of phenotypic variability, it remains uncertain which patients will have this susceptibility, thus necessitating caution in all affected individuals. Mutations in the *RYR1* gene may also result in phenotypes consistent with multiminicore myopathy on histology or even nemaline myopathy (Snoeck et al., 2015).

Multiminicore disease is rare in the neonatal period and, when present, is notable for marked axial weakness, myopathic facies, and respiratory failure. A portion of older patients may present with ophthalmoplegia, which is apparent in approximately 10% of neonates with multiminicore disease. Patients with a prenatal onset of multiminicore disease may present with arthrogryposis multiplex congenita because of decreased fetal movement secondary to muscle weakness.

Two genes are frequently associated with multiminicore disease. *SEPN1*, which encodes selenoprotein 1, accounts for the majority of patients (Ferreiro et al., 2002a). Selenoprotein 1 is also associated with rigid spine disease, suggesting that the two disorders are allelic. The next most common is the *RYR1* gene (Ferreiro et al., 2002b), which is also associated with central core disease, as noted above.

Myotubular (Centronuclear) Myopathy

Centronuclear myopathy, also referred to as myotubular myopathy, is a rare neonatal cause of weakness. The name comes from the histopathologic appearance of numerous centrally located nuclei in the muscle fibers (Heckmatt et al., 1985; Jungbluth et al., 2008). Presentation in neonates is notable for severe hypotonia with bulbar involvement, including extraocular muscle weakness and myopathic facies. Respiratory compromise with need for ventilation may be present (Heckmatt et al., 1985). Given the severity of involvement, congenital myotonic dystrophy is often considered as well, and thus a good family history and examination of the mother may aid in diagnosis. Neonates may be macrosomic or have undescended testes, which may aid in diagnosis. The *MTM1* gene, encoding for myotubulin, is located on the X chromosome at Xq28, thus resulting in the male predilection for this disease. However, secondary to abnormal X-inactivation, females may be affected in the setting of this mutation (Dahl et al., 1995).

Autosomal recessive mutations may also result in disease, with similar clinical features such as bulbar involvement, severe facial weakness with ptosis, and ophthalmoplegia (Wallgren-Pettersson et al., 1995). Mutations in *BIN1* and *DNM2*, encoding for

amphiphysin-2 and dynamin-2, respectively, may also result in disease, as can autosomal recessive mutations in *RYR1* (Jungbluth et al., 2007, 2008; Snoeck et al., 2015).

Outcomes from this disorder are highly dependent on the degree of respiratory support required, and clear prognostic factors are not established, thus necessitating individual consideration. Neonates with the X-linked form tend to have a more difficult course and may not survive the neonatal period; however, phenotypic variability is present (Barth and Dubowitz, 1998).

Motor Neuron Disorders

Motor neuron disorders comprise a group of genetic disorders that share involvement of the anterior horn motor neurons. This group is dominated by 5q spinal muscular atrophy (SMA), the most common inherited motor neuron disorder. A substantial number of other rare forms of motor neuron disease are characterized; the more common ones will be mentioned below.

5q Spinal Muscular Atrophy

5q SMA is an autosomal recessive disorder with an incidence of approximately 1 in 10,000 live births (Ogino et al., 2002). Onset and severity fall along a wide spectrum, from very severe cases with intrauterine onset, to mild cases with onset in adult years and mild disability. Given the wide variability, SMA is classified into three major types depending on severity and age of onset. Some authors recognize, in addition to the three main types, one additional type: SMA type 0. The majority of patients have a homozygous deletion of the *SMN1* gene. The great variability in clinical phenotype is secondary mainly to the number of *SMN2* copies that each individual carries. The *SMN2* gene is almost identical to *SMN1* and produces principally a truncated, nonfunctional protein as well as a small amount of functional protein. More *SMN2* copies correlate with the milder phenotype (Table 64.1). All patients undergo a course that includes a decline phase followed by a plateau phase. The decline phase occurs in utero for SMA type 0 newborns affected at birth. For infants whose onset is after birth, a period of normal development is followed by a decline phase that usually lasts for weeks to months.

SMA type 0 neonates have onset of weakness in utero and often are noted to have arthrogryposis. Respiratory distress at birth is the norm and facial weakness can be present in addition to profound hypotonia and limb muscle weakness. Most of these patients die in the first weeks of life. SMA type 1 or Werdnig–Hoffman disease presents between birth and 6 months of age. Some of these neonates become symptomatic soon after birth, while others become weak after a few months of more or less normal development. Typical

TABLE 64.1 Spinal Muscular Atrophy Types

| Spinal Muscular Atrophy (SMA) Type | <i>SMN2</i> Gene Copy Number | Age of Onset | Maximal Motor Milestone Reached | Natural Course |
|------------------------------------|------------------------------|-------------------|---------------------------------|---|
| SMA type 0 | 1 | In utero | None | Fatal in the first month of life |
| SMA type 1 | 2 | Birth to 6 months | None | Fatal in the first 2 years of life |
| SMA type 2 | ≥3 | 6–18 months | Sit independently | Severe morbidity and often shortened life span |
| SMA type 3 | ≥3 | >18 months | Walk independently | Moderate morbidity and possibly shortened life span |

patients have profound hypotonia and severe weakness. Weakness is more severe in the legs than arms. Proximal muscles are weaker than distal ones. Usually there are no antigravity movements of the more proximal limb muscles with some movements distally at the level of ankles/wrists or fingers/toes. Deep tendon reflexes are unobtainable, and the face is spared. In fact, these infants tend to have a very bright facial expression. Various degrees of respiratory insufficiency are present at the time of diagnosis. Worsening of respiratory function over time is the norm, with progression to full-time respiratory support frequent. Because of the disproportionate involvement of the intercostal muscles and relative sparing of the diaphragm, a “bell-shaped” chest conformation is noted. Tongue fasciculations are often present and should be sought for. Bulbar weakness is present in essentially all type 1 SMA patients, and it manifests with swallowing difficulties, choking, and poor feeding. Contractures are not part of the typical presentation of SMA, although they can develop after prolonged immobilization.

Diagnosis is usually straightforward. Once the typical clinical picture is recognized and CPK testing is normal or minimally elevated (up to 500 IU/L), genetic testing is the next step. Ninety-five percent of patients with SMA will have a homozygous deletion of the *SMN1* gene. The rest of the patients will be found to have a point mutation on one of the alleles, while the second allele is deleted. For the latter group, *SMN1* gene sequencing will be necessary to confirm the diagnosis. Many commercial laboratories offer, in addition to confirmatory SMA testing, a *SMN2* copy number that can be helpful in staging and prognosis. The majority of type 1 SMA patients have two copies of *SMN2*. Of note, CPK, while usually normal, can in rare cases be mildly elevated but at no higher than 500 IU/L. In the era of genetic testing, the role of muscle biopsy and EMG has diminished. When available, EMG can be a speedy way to confirm the diagnostic suspicion, especially if therapeutic decisions cannot wait until genetic testing returns. A muscle biopsy is reserved for atypical cases nowadays.

Management of patients with type 1 SMA remains supportive at this time (Iannaccone, 2007). However, clinical trials are ongoing, with approval of nusinersen by the FDA in December 2016, and other drugs may soon become commercially available. Agents that are currently in clinical trials include neuroprotective agents that target the *SMN2* gene with the purpose of increasing the production of the functional SMN protein as well as gene therapy (Arnold and Burghes, 2013). Preliminary results on nusinersen, which increases functional SMN protein and is more effective, is started early in the disease course. Hence, there is interest in including SMA in the newborn screen, given the importance of early treatment if diagnosed. Despite advances in management of these patients in the last two decades, type 1 SMA (especially with onset in the neonatal period) remains a devastating disease, often with a fatal course. Most type 1 SMA patients succumb within the first 2 years of life unless aggressive respiratory intervention is offered. We now care for many type 1 SMA children who have survived through aggressive respiratory and gastrointestinal management. Their quality of life remains a major concern. The choice of aggressive, life-maintaining therapies versus palliative care raises many ethical questions and debates and, until therapies that will modify the natural history of the disease are available, will continue to do so. The help of a neuromuscular specialist can be invaluable in order for parents to make informed decisions. If aggressive treatment is chosen, the two main areas requiring early support are respiratory and gastrointestinal function. Respiration is preferably supported via noninvasive means in the form of bilevel positive airway pressure (BiPAP), although, not infrequently, some of these infants require a tracheostomy and full-time ventilator

support. Infants with SMA type 1 have poor cough and Cough Assist has proven a very important tool in airway clearance. Consultation with a pediatric pulmonologist with experience in SMA management is necessary and should be initiated early on. Essentially all SMA type 1 patients with onset of symptoms in the first month of life will have significant swallowing difficulties, leading to recurrent aspiration and poor calorie intake. Gastrostomy tube placement and Nissen fundoplication performed early on are essential. Orthopedic complications such as scoliosis occur later. Noninvasive means of ventilation and a gastrostomy tube are often part of the palliative care plan as well.

Non-5q Spinal Muscular Atrophies

Non-5q SMAs comprise a genetically and phenotypically heterogeneous group of disorders that share motor neuron involvement. Different classifications are used, including mode of inheritance and pattern of muscle involvement. Some of these disorders are important entities for neonatologists, while others are not seen in newborns. The more important disorders that have neonatal presentation are addressed below.

Spinal Muscular Atrophy With Respiratory Distress

Spinal muscular atrophy with respiratory distress (SMARD) represents a group of motor neuron disorders that present at birth and share a number of distinguishing clinical features. These conditions are characterized by a rather sudden and severe respiratory insufficiency that leads to a requirement for ventilatory support as well as predominantly distal weakness and distal contractures. Respiratory insufficiency can be present at birth (suggesting an in utero onset) or develop in the first 6 months of life. Diaphragmatic weakness leading to diaphragmatic eventration is characteristic. These features differ from the relative diaphragmatic sparing and predominantly proximal weakness seen in 5q SMA. A number of genes have been identified so far. SMARD1, the better known form of SMARD, is an autosomal recessive disease caused by mutations in the *IGHMBP2* (immunoglobulin μ -binding protein 2) gene. SMARD2, an X-linked disease caused by mutations in the *LASIL* gene, has been described more recently (Butterfield et al., 2014). Both genes seem to play a role in ribosome biogenesis. Other genes are likely to be discovered. The management is supportive. Restrictive lung disease is the main cause of morbidity and mortality. Swallowing difficulties, aspirations, and poor caloric intake are also common, leading to gastrostomy tube placement. Cognitive development is believed to be normal. Palliative care is often offered for the more severe cases, while milder cases may benefit from aggressive respiratory and gastrointestinal management. Late-onset forms with a milder phenotype have also been described.

X-Linked Infantile Spinal Muscular Atrophy

X-linked infantile spinal muscular atrophy (XL-SMA) is a recently described motor neuron disease characterized by arthrogryposis, hypotonia, proximal weakness, facial weakness, and respiratory failure. XL-SMA has been found to be associated with hemizygous mutations in the *UBE1* gene (Ramser et al., 2008). The disease is usually fatal in the first 2 years of life. Management is supportive.

Pontocerebellar Hypoplasia Plus Spinal Muscular Atrophy

Pontocerebellar hypoplasia (PCH) is a heterogeneous group of inherited disorders that share hypoplasia or atrophy of the cerebellum and pons. Other CNS abnormalities are common. PCH type 1

has, as distinguishing features, muscle weakness and hypotonia caused by a motor neuron disease. So far mutations in three genes are identified as causing type 1 PCH: *VRK1*, *EXOSC3*, and *EXOSC8* (Renbaum et al., 2009; Wan et al., 2012; Boczonadi et al., 2014). All are inherited in an autosomal recessive pattern. Although the severity and age of onset varies, many of these patients present in the neonatal period with hypotonia, weakness, contractures, and respiratory distress as well as encephalopathy. Survival ranges from a few months to a few years. Management is supportive. Genetic diagnosis is important for family planning.

Transient Neonatal Myasthenia Gravis

Approximately 10%–15% of newborns born to mothers with autoimmune myasthenia gravis develop a transient form of myasthenia gravis. The disease results from passive transfer of antibodies and leads to fatigable weakness, including weakness of the respiratory and swallowing muscles. Supportive treatment is necessary for a brief duration of time (days to weeks), until the antibody titers decline and strength returns. Diagnosis is based on the presence of acetylcholine receptor (AChR) antibodies (rarely muscle-specific kinase antibodies) in newborn serum. More recently, it was recognized that some of the infants born to mothers with autoimmune myasthenia gravis have a less benign course with unexplained myopathic features, dysarthria, velopharyngeal insufficiency, and contractures. These patients may have feeding and respiratory difficulties in the neonatal period. Maternal AChR antibody status should be checked, even in the absence of symptoms, in order to prevent recurrence with future pregnancies (Hacohen et al., 2015).

Congenital Myasthenic Syndromes

Congenital myasthenic syndromes (CMS) include a growing number of heterogeneous disorders that are all characterized by the failure of neuromuscular transmission secondary to a genetic defect. A significant proportion of CMS cases present in the neonatal period or early infancy. Diagnosis is often delayed by years. The general clinical characteristics include fatigable muscle weakness involving the extraocular, bulbar, respiratory, and limb muscle systems in different combinations (Engel, 2012). Certain patterns are unusual enough to deserve special mention. *Dok7* CMS patients can present in the neonatal period with stridor due to bilateral vocal cord paralysis, respiratory distress, and feeding difficulties. Intubation and ventilator support are necessary for some patients (Jephson et al., 2010). Episodic respiratory deterioration with fatigue during feedings but otherwise little or no symptoms in between were noted in a patient with end-plate acetylcholinesterase deficiency (personal observation). Choline acetyltransferase mutations lead to hypotonia with marked bulbar symptoms and respiratory insufficiency in the neonatal period followed by life-threatening episodes of apnea later in infancy (Ohno et al., 2001). In a retrospective review of CMS cases presenting in early infancy, 8 out of 11 patients presented at birth in general with severe respiratory distress in addition to hypotonia, weakness, and contractures (Zafeiriou et al., 2004). Laboratory evaluation is usually unremarkable, with normal CPK. Muscle biopsy is either unremarkable or shows mild nonspecific findings. The importance of electrophysiologic studies, specifically repetitive nerve stimulation, is paramount in establishing the presence of a neuromuscular junction defect. Genetic testing is available commercially. Although a good number of CMS patients respond partially to pyridostigmine, patients with certain types of

CMS may worsen. Close observation is needed when pyridostigmine is administered, especially if the exact type of CMS is not known. Other medical treatments may be available, depending on the specific CMS identified. Respiratory support remains important though. Noninvasive ventilation is preferred as some patients improve with age. Nutritional support with a gastrostomy tube should be considered.

Peripheral Neuropathies

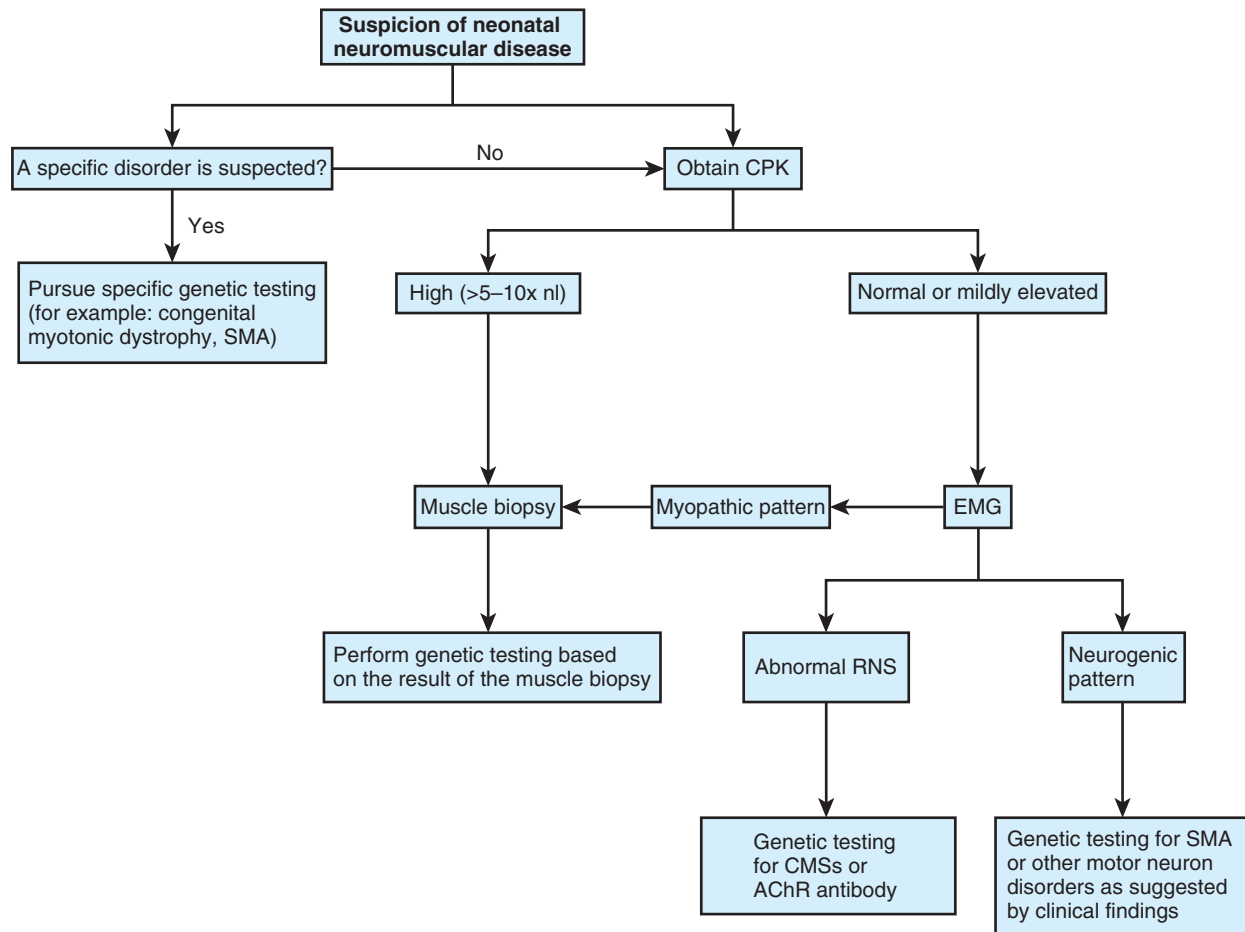
Inherited peripheral neuropathies are one of the least common causes of floppy infant syndrome in the neonatal period. The main presentations in the neonatal period are hypotonia and weakness, often with feet deformities. Respiratory difficulties may be present. Electrophysiologic studies will confirm the neuropathy and orient the genetic testing by subdividing them into axonal versus demyelinating (Baets et al., 2011). Management is supportive.

Approach to Neonatal Neuromuscular Disorders

The field of pediatric neuromuscular disorders has exploded following the genetic advances of the last two decades. While the old clinicopathologic classification remains useful, one needs to keep in mind that different genotypes can lead to the same phenotype, and the same genotype can present with different phenotypes. Many of these disorders have significant intrafamilial variability.

As in any area of medicine, a good history and physical examination, including a neurologic examination, are essential. Pregnancy evolution and complications such as polyhydramnios should be noted. A history of reduced fetal movements is important. Obstetric history may point to a hypoxic–ischemic injury as the etiology for hypotonia and weakness. Three generations of family history can identify other affected family members. One needs to keep in mind that many neuromuscular disorders may have significant intrafamilial variability. Looking for grip myotonia by shaking the mother's hand will help point the clinician toward CMD. This is something that should be done each time a newborn is evaluated for hypotonia. Physical and neurologic examination of the floppy newborn should include delineation of hypotonia (axial vs proximal vs distal vs diffuse) as well as weakness (proximal vs distal vs diffuse). The presence or absence of deep tendon reflexes, the resting position, and the amount of spontaneous movements are important.

Given the fact that the central causes of hypotonia are very common, signs and symptoms suggestive of CNS involvement should be sought. These include, but are not limited to, encephalopathy, seizures, dysmorphic features, history suggestive of hypoxic–ischemic injury, severe hypotonia in a setting of mild weakness, and metabolic derangements. A history of hypoxic–ischemic injury is not mutually exclusive for a neuromuscular condition, as many of these conditions place the newborn at risk for hypoxic–ischemic injury in and of themselves. Newborns with CMD seem to suffer superimposed hypoxic–ischemic injury relatively more frequently. Often the newborn that is floppy at birth undergoes brain and sometimes spine imaging. An MRI technique is usually preferred. In the majority of neuromuscular conditions, these studies are normal. Pontine and cerebellar hypoplasia will point in the direction of SMA with PCH. Some forms of CMD can often present with significant brain malformations, as mentioned above. The white matter changes described in merosin-deficient CMD are not apparent in the neonatal period. Brain and spine



• **Fig. 64.2** Diagnostic Approach to Neonatal Neuromuscular Disorders. *AChR*, Acetylcholine receptor; *CMSs*, congenital myasthenic syndromes; *CPK*, creatine phosphokinase; *EMG*, electromyography; *nl*, normal; *RNS*, repetitive nerve stimulation; *SMA*, spinal muscular atrophy.

imaging are most important in the evaluation of the central type of hypotonia.

In addition to the history and physical examination, the tools available to the clinician include:

1. CPK
2. EMG studies
3. Muscle biopsy
4. Genetic testing

Few gestalt diagnoses exist. CMD presents with typical facial features. If this is combined with maternal myopathic facial appearance and grip myotonia, one may go straight to genetic confirmation. The presentation of SMA in the neonatal period is another situation when gestalt diagnosis is possible for the experienced neonatologist. A proposed stepwise systematic algorithm for evaluation is presented in Fig. 64.2.

Creatine Phosphokinase

CPK is a rapid test that should be performed when a neuromuscular condition is first suspected. Significantly elevated values (more than 5 times normal) will point toward a muscle disorder, more likely a CMD. Normal or mildly elevated values can be seen in congenital myopathies and SMA.

Electromyography

Seen as a difficult test to perform in newborns, in the right hands, EMG can be of immense help. The main advantage of EMG is a rapid, on the spot, diagnosis of a neurogenic process versus myopathic process versus neuromuscular junction defect. When available, EMG should be offered early in the diagnostic process.

Muscle Biopsy

Despite advances in genetic diagnosis, muscle biopsy remains an important tool in the diagnosis of neuromuscular disorders. Its main utility consists in identification of particular types of congenital myopathy or CMD and, as a consequence, directing the genetic testing toward smaller panels of genes. As the pricing of genetic testing is decreasing, it is becoming feasible to start the work-up with genetic testing and employ muscle biopsy only if the first round of genetic tests fails to reveal a genetic abnormality.

Genetic Testing

The availability of genetic testing has increased exponentially in the last decade. A clinicopathologic diagnosis is no longer sufficient,

and every effort should be made for genetic confirmation. Single genes as well as panels of genes are now commercially available from multiple commercial laboratories.

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65

Neonatal Seizures

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KEY POINTS

- Neonatal seizures are common.
- Clinical assessment alone is insufficient for diagnosis, and ancillary tests are necessary.
- Seizures are often symptomatic of an underlying cause requiring investigation.
- Clinical decision making is divided between determining the etiology and treatment of seizures.

CONTROVERSY BOX

- The independent impact of electrographic-only seizures is uncertain. While increasing evidence suggests an effect on risk of the later development of epilepsy and neurodevelopmental outcomes, the aggressive treatment of electrographic seizures remains debated.
- First-line antiepileptic drugs (AEDs) have limited efficacy, resulting in varied provider practices and off-label use of further AEDs.
- Optimal length of treatment with AEDs after resolution of seizures varies by provider. Retrospective studies suggest shorter treatment length is sufficient.

Neonatal Seizures

Seizures in the neonate occur in 2 to 4 per 1000 live births and are a cause of neonatal morbidity and mortality (Lanska et al., 1995; Ronen et al., 1999; Saliba et al., 1999). Frequently, this onset is a neurologic emergency, requiring prompt and thorough diagnostic investigations and therapeutic interventions. Seizures in the newborn may be transient due to electrolyte abnormalities, the harbinger for underlying brain injury or abnormalities, or the initial presentation of an underlying medical disorder. The clinical appearance of seizures at this age differs from that seen in older infants and children. Seizures themselves may be subtle, or without clinical manifestations, and challenging to differentiate from other involuntary movements in the neonate.

Many dilemmas remain within diagnosis and management of neonatal seizures. Guidelines in recent years have been created to aid clinicians in how to diagnose neonatal seizures through ancillary diagnostic tests. There are ongoing efforts to determine how aggressively to treat seizures, which medications to utilize, how long to treat seizures, and the impact of neonatal seizures on neurodevelopmental outcome.

This chapter discusses the diagnosis, neurophysiologic criteria, etiologic considerations, treatment, and prognosis of neonatal

seizures. For the purposes of this chapter, the term *seizure* refers to an *epileptic event*: that is, those with electrographic correlate.

Diagnosis of Seizures

Neonatal seizures are often brief and subtle in appearance, thus raising diagnostic uncertainty when unusual movements are present. Frequently, clinical features suspicious for seizure result in initiation of treatment prior to ancillary testing. This, however, is not recommended. As newborns may have automatic or motor behaviors that mimic movements concerning for seizure, and encephalopathic newborns at high risk of seizures may develop paroxysmal movements that are nonepileptic alongside seizures, ancillary testing to aid in diagnosis is imperative to limit overtreatment as well as underrecognition of events. Medical providers of various backgrounds have been shown to have difficulty determining whether events represent seizure or not, based on clinical features alone. An observational study looking at healthcare providers' assessment of seizure identification of 20 video clips (11 were seizure, 9 were not) found that providers were correct on average 50% of the time when given history and clinical features alone (Malone et al., 2009). Additionally, identification of seizures by clinical criteria alone may underestimate the number of seizures present (Clancy et al., 1988; Murray et al., 2008).

With this in mind, the American Clinical Neurophysiology Society developed guidelines in 2011 on the use of continuous electroencephalography (EEG) monitoring in neonates (Shellhaas et al., 2011). While it is recognized that not all facilities will have the resources necessary to monitor, this guideline provides goals as to how to monitor for seizures in high-risk neonates or which neonates with suspicious events should be evaluated.

Many institutions incorporate amplitude-integrated EEG (aEEG) to aid in the detection of seizures. This tool uses two or four scalp EEG electrodes to obtain a raw tracing of information. This signal undergoes processing and time compression, creating a single channel tracing per hemisphere. The technical aspects of this approach are nicely reviewed elsewhere (El-Dib et al., 2009).

Continuous EEG and aEEG are discussed later in the Electrographic Seizure Criteria section.

Categorization of Seizures

Seizures in the neonate often are brief and subtle in nature and may present differently than seizures in older infants or children. Historically, neonatal seizures have been categorized by clinical semiology into five categories: focal clonic, multifocal clonic, tonic,

TABLE 65.1 Seizure Types

| Seizure Type | Characteristics | Nonepileptic Differential |
|--------------|---|---|
| Focal clonic | <ul style="list-style-type: none"> • Repetitive rhythmic jerking of an extremity or hemibody • There is a rapid phase and a slow phase of movement. • These movements cannot be suppressed by tactile restraint. | <ul style="list-style-type: none"> • Jittery movements, tremulousness • Nonepileptic movements tend to have equal phases of movement. |
| Focal tonic | <ul style="list-style-type: none"> • Stiffening of an extremity or hemibody | <ul style="list-style-type: none"> • Provoked bilateral stiffening • “Brainstem release phenomena” • Gastroesophageal reflux |
| Myoclonic | <ul style="list-style-type: none"> • Lightning fast jerk of a single or multiple extremities | <ul style="list-style-type: none"> • This can be epileptic or nonepileptic. |
| “Subtle” | <ul style="list-style-type: none"> • Tonic eye deviation • Head version • Chewing movements | <ul style="list-style-type: none"> • Bicycling movements • Chewing movements |

myoclonic, and subtle seizures (Volpe, 2008). Other classification schemata take into account the presence or absence of EEG correlates during clinical movements, separating movements into “epileptic” and “nonepileptic” movements (Table 65.1).

Clonic Seizures (Focal and Multifocal)

Rhythmic movements with a rapid flexor phase followed by a slower extension phase persisting despite flexion of the affected limb may represent a clonic seizure. This can be mistaken for nonepileptic phenomenon such as tremor or jitteriness in some infants. Distinguishing features between the entities include the rhythmicity of the event and its ability to be suppressed or altered by changes in positioning. Subtle but important distinguishing features include the rapidity of the flexor phase as compared with the extensor phase, as tremulousness or jitteriness tends to have symmetric flexor and extensor phases.

Focal clonic or hemiclonic seizures can be seen in neonates with injury localized to a specific site, such as a perinatal stroke or other cerebrovascular event (Clancy et al., 1985; Levy et al., 1985). Multifocal seizures, clonic seizures that arise at times from multiple locations, can be seen in neonates with multifocal or generalized brain abnormalities, such as hypoxic–ischemic encephalopathy.

Tonic Seizures

Tonic seizures are those with sustained flexion or extension of a muscle group. This can affect limbs but also the eye muscles with sustained eye deviation or the head, resulting in head version. Tonic seizures may be epileptic or nonepileptic, with bilateral tonic extension not having a correlate on EEG (Mizrahi and Kellaway, 1987). Nonepileptic tonic posturing may represent “brainstem release” in the setting of extensive cortical dysfunction. This cortical dysfunction may allow uninhibited subcortical expression to occur. Other movements that may be misidentified as tonic seizures include dystonic posturing and may coexist in neonates with seizures, especially in the setting of brain injury.

Myoclonic Seizures

Myoclonic movements are rapid, lightning fast jerks that can be focal, multifocal, or generalized in nature. Myoclonus can occur at multiple levels of the nervous system: cortical regions, brainstem,

and spinal cord. With this, myoclonus may be epileptic (myoclonic seizures) while others are not, requiring additional evaluation with neurophysiologic studies to determine the nature of the myoclonus. Myoclonus often represents underlying pathology and thus requires etiologic evaluation for metabolic, infectious, structural, and genetic causes (Scher, 1985).

In some instances, however, myoclonus is benign. In preterms, myoclonus can occur in the absence of nervous system abnormalities; however, consideration of metabolic or infectious causes is warranted when myoclonus is excessive. Healthy neonates may have exaggerated myoclonus in sleep, deemed *benign sleep myoclonus of the newborn* (Coulter and Allen, 1982).

Subtle Seizures

Subtle seizures are those with motor automatisms such as oral–buccal–lingual movements and are the most frequently seen subtype of neonatal seizures. They may be seen in conjunction with other seizure types, such as multifocal clonic or focal clonic seizures. Given their subtlety, these are notoriously difficult to determine clinically, where often one must first be cognizant of the neonate’s typical behaviors, movements, and autonomic findings and then detect unexplained alterations of these patterns. Repetitive movements such as bicycling, pedaling, as well as ocular, oral, or buccal–lingual movements are in this category. One should pay close attention to associated vital sign changes, such as otherwise unexplained fluctuations in heart rate, blood pressure, or oxygen saturation in association with this. As more benign movements may mimic these subtle seizure motor features, confirmation with EEG is recommended.

Nonepileptic Neonatal Movements

As noted previously, newborns are prone to a variety of movements that may raise suspicion for seizures but represent other neurologic or nonneurologic entities (Mizrahi and Kellaway, 1998). Given this clinical uncertainty, EEG with concurrent video is recommended to characterize the clinical event with neurophysiologic data (Shellhaas et al., 2011).

Tremulousness or Jitteriness

Tremor can be seen frequently in the neonate and can be mistaken for clonic activity by medical personnel. The phenomenology of

this movement is flexion and extension, with equal phases and amplitude of both, distinguishing this from clonic activity. Jitteriness is rhythmic tremors around a fixed axis. Repositioning of the affected limb may often decrease or extinguish the movement, as can flexion. Neonates may be alert or hyperalert, although this may be present in neonates with somnolence secondary to encephalopathy. Tremor may be asymmetric in nature and of varying amplitudes. Movements tend to be spontaneous but may occur in response to tactile stimulation.

Tremors as well as jittery movements can occur secondary to metabolic derangements such as hypoglycemia or hypocalcemia, as well as intracranial hemorrhage and growth restriction, or in hypothermia. Exposure to maternal medications such as selective serotonin reuptake inhibitors (SSRIs) or illicit substances such as cocaine and marijuana has also been associated with tremulousness. Nevertheless, these movements are common, with one study showing mild, moderate, or excessive jitteriness in 44% of healthy term infants (Parker et al., 1990).

Movements generally decrease with increasing postconceptional age, with a normal neurologic outcome (Parker et al., 1990).

Myoclonus Without Electrographic Correlate

Myoclonus is defined as a sequence of repetitive, often nonrhythmic movements, brief shock-like movements caused by sudden involuntary contraction or relaxation of one or more muscles (Sanger et al., 2010). Myoclonic movements may originate from multiple levels of the neuroaxis, as discussed previously, and may lack electrographic correlate. This nonepileptic myoclonus is more frequent in preterm newborns but also in term neonates and can be benign in some instances, while a harbinger for underlying abnormalities in others. Movements occur in neonates without encephalopathy and resolve with awakening. Benign neonatal sleep myoclonus was first described by Coulter and Allen (1982) and is considered a diagnosis of exclusion. Suspicion for this should arise when described in an otherwise healthy newborn with myoclonus that occurs in sleep and resolves with awakening and is typically arrhythmic and of varying amplitude. It may increase with attempts of physical restraint or be induced by rocking and occurs in all stages of sleep (Maurer et al., 2010). This typically resolves by 3 months of age, although some infants may have symptoms until 6 to 12 months of age (Maurer et al., 2010).

This is in contrast to myoclonus occurring in an infant with encephalopathy. In this case, myoclonus may be stimulus induced, with increased myoclonus to tactile or painful stimulation, and occurs in the setting of severe brain injury. Additional etiologic considerations in the neonate with myoclonus with encephalopathy include infection such as encephalitis or meningitis, intraventricular hemorrhage, periventricular leukomalacia, or metabolic disorders such as glycine encephalopathy (Scher, 1985). In these latter cases, the EEG background is typically abnormal and may have epileptiform discharges, although the myoclonus itself lacks epileptiform correlate (Scher, 1985). Certain medications may also produce nonepileptic myoclonus; midazolam-induced and lorazepam-induced myoclonus are reported in preterm or very low birth weight neonates (Lee et al., 1994; Magny et al., 1994; Sexson et al., 1995). If medication-induced myoclonus occurs, further use of the offending drug should be limited in the neonatal period where possible. As the response is thought to be developmental, use of benzodiazepines need not be avoided in infancy or childhood.

Dyskinesias

Dystonic and dyskinetic movements are frequent in the neonate and commonly may be mistaken for seizures. This group of movements is associated with the basal ganglia or the extrapyramidal pathways and can occur secondary to acute or chronic effects on these structures. Dystonia is the involuntary sustained or intermittent cocontraction of agonist and antagonist muscles resulting in abnormal posture (Sanger et al., 2010). In the neonate, it often represents intrapartum or antepartum injury with severe injury to the basal ganglia (Scher, 2008). Some inborn errors of metabolism may present with hypertonicity, opisthotonic posturing, or dystonia (e.g., maple syrup urine disease, monoamine neurotransmitter disorders) (Hyland, 1999; Strauss et al., 2006).

The dystonia or posturing seen reflects a functional subcortical disinhibition secondary to damage or malformation to the cortex (Sarnat, 1984). As dyskinetic and dystonic movements are frequent in encephalopathic neonates who may also have epileptic events, use of continuous video EEG is imperative to prevent misdiagnosis and inappropriate treatment of either diagnosis.

Neurophysiologic Diagnosis of Seizure

Neonatal seizures are challenging to identify clinically for a multitude of reasons, including challenges distinguishing from nonepileptic involuntary movements, overall subtlety, as discussed above, as well as the potential for seizures to electroclinically dissociate after treatment, becoming apparent only with the aid of EEG (Scher, 1994). Reports estimate that as many as 80% of seizures may be electrographic, without any clinical component (Clancy et al., 1988). The neurophysiologic definition of seizures, status epilepticus, electroclinical uncoupling, as well as the significance of interictal abnormalities are discussed below.

Because of the inability to accurately detect seizures clinically, it remains imperative to use ancillary studies to aid in the detection of seizures. Two modes of seizure detection will be briefly discussed: EEG and aEEG. There remains controversy regarding the degree to which one needs to treat electrographic-only seizures; however, there is a growing body of evidence to suggest the importance of treatment. This is discussed later in the Neurodevelopmental Outcomes section.

Electroencephalography

Conventional EEG, the gold standard for neonatal seizure detection, is an invaluable tool for seizure detection. Multiple electrodes are placed in accordance with the international 10–20 system, or with the modified neonatal montage, alongside a single electrocardiogram lead and respiratory belt. Interpretation of EEG requires mastery of the normal and abnormal patterns of term and preterm wakefulness and sleep. Aid from the EEG technologist or bedside nurse is imperative, to note potential events of concern and artifacts (such as nursing care, feeding, etc.).

Neurophysiologic Definitions

Seizures themselves have been described and characterized recently by the American Clinical Neurophysiology Society (Tsuchida et al., 2013). To be considered a seizure, rhythmic activity lasting 10 seconds with a minimum of 2μV peak-to-peak (pp) voltage that evolves in quality and subsequently resolves must be present. Events with these characteristics lasting less than 10 seconds are referred

to as *brief rhythmic discharges* (BRDs), and while they are not consistent with seizures, they pose an increased risk of developing seizures. Shorter-length studies may detect BRDs without electrographic seizures that have a high likelihood of coexisting, and thus continuous EEG monitoring is recommended to ensure appropriate recognition of seizures.

Status epilepticus, defined in older children or adults as a seizure lasting greater than 30 minutes or more than one seizure without a return to baseline in between, is not an appropriate definition in neonates, given the often comorbid encephalopathy and overall challenges in determining true return to baseline. With this, the definition of status epilepticus has been established as seizures constituting greater than 50% of an hour-long epoch, which is arbitrarily defined (Tsuchida et al., 2013).

Continuous monitoring of neonates with EEG is recommended in certain high-risk populations. These include instances where one may suspect neonatal seizures to occur, such as in acute brain injury secondary to perinatal asphyxia, in neonates with clinically suspected seizures, or when neonatal epilepsy is suspected. In some instances, it may be appropriate to continue monitoring after withdrawal of AEDs. Although seizures in the setting of acquired brain injury are not likely to recur shortly after resolution of seizures, those with neonatal epilepsy syndromes or cerebral malformations may deserve monitoring when AEDs are discontinued. It is recommended that neonates remain monitored for a 24-hour period once seizure-free.

Electroclinical Uncoupling

Administration of AEDs in the setting of electroclinical seizures can result in a phenomenon where electrographic seizures persist while the clinical manifestations resolve. This is termed *electroclinical uncoupling*. One study estimated that 25% of neonates had persistent electrographic seizures after receiving AEDs despite the resolution of clinical seizures (Scher, 1994).

This information suggests that seizures may be underrecognized without ancillary testing, resulting in the underdiagnosis and treatment of status epilepticus. The effects of undertreatment contribute to neurodevelopmental outcomes, as later discussed.

Amplitude-Integrated Electroencephalography

aEEG is a cerebral function monitor that has become available as a bedside tool more recently in the last decade in the United States. It utilizes 2 to 4 leads, typically placed over the central and parietal regions. Using sophisticated electronics, this raw signal is converted into a single channel that denotes the pp amplitude from one waveform to the next, which is then time compressed. Advantages of aEEG include bedside availability and interpretation, as well as reduced cost as compared with continuous EEG monitoring (Hellström-Westas et al., 2008). Because of the technical processing of this signal, there are limitations, including challenges in detection of brief or low-amplitude seizures or seizures that do not occur in the brain regions covered by aEEG (Shellhaas et al., 2007). During a seizure, there is a sudden and sustained increase of the lower and upper margins on aEEG, with raw EEG signal demonstrating a monomorphic waveform consistent with seizure (Hellström-Westas et al., 2008). aEEG may also be limited as it is prone to artifactual signals from movement, high-frequency oscillator ventilation, or extracorporeal membrane oxygenation (ECMO). In a recent systematic review of detection of neonatal seizure on aEEG (aEEG and a raw trace were examined simultaneously), a median sensitivity

of 76%, with a median specificity of 85%, was observed but with high variability when comparing studies (Rakshasbhuvankar et al., 2015). Consequently, it is still recommended that aEEG should be used as a screening tool where the gold standard of continuous EEG is limited but with placement of the latter when seizures are detected (Glass et al., 2013; Sanchez Fernandez and Loddenkemper, 2015).

Interictal Abnormalities

Beyond determination of seizures, EEG detects interictal findings that aid patient management. The presence of background abnormalities may be serially followed to determine the progression of an encephalopathy (Scher, 1994). While background findings may not be pathognomonic for particular etiologies, these findings in conjunction with the remainder of the diagnostic work-up (history, clinical examination, and laboratory and imaging findings) aid in the development of the overall prognosis for a patient.

Etiologies of Neonatal Seizures

Once it is determined that a neonate is having seizures, further management is divided into the treatment of seizures, discussed in a later section, as well as establishing the etiology of seizures. Neonatal seizures are not disease specific and can occur in a variety of conditions that may occur before, during, or after birth. Seizures may occur in the setting of electrolyte disturbances such as hypoglycemia, hypocalcemia, or hypomagnesemia and may respond to corrections of these. Seizures may harken underlying brain injury in the setting of hypoxic-ischemic encephalopathy antepartum injury, perinatal stroke, hemorrhage, trauma, or infection. Metabolic, genetic, or cerebral dysgenesis are additional etiologic considerations (Table 65.2).

Hypoxic-Ischemic Encephalopathy

Hypoxia-ischemia is considered the most common cause of neonatal seizures and is an important consideration in the neonate with encephalopathy and seizures. The American College of Obstetricians and Gynecologists initially published guidelines in 2004 proposing five criteria that suggest an intrapartum etiology: profound acidemia with a pH less than 7.0; Apgar score of 0 to 3 beyond 5 minutes of life; sudden sustained fetal bradycardia or absence of fetal heart rate variability; onset of multisystem disease within 72 hours; and supportive early imaging (American College of Obstetricians and Gynecologists, 2004). Given advances in knowledge over the last decade, this guideline was updated in 2014 and reflects the difficulties in determining whether an event was intrapartum and hypoxic-ischemic related or caused by another origin of neonatal encephalopathy (Executive Summary, 2014).

In the era of therapeutic hypothermia, identification of neonates who may benefit from this therapy, which is geared toward prevention of secondary energy failure and additional brain injury, includes neonates meeting laboratory criteria suggestive of hypoxic-ischemic injury, in addition to presence of moderate or severe encephalopathy (Gunn et al., 2005; Shankaran et al. 2005).

While hypoxic-ischemic encephalopathy is a common cause of neonatal seizures, it is important to recognize that injury may occur postnatally, and several antepartum and intrapartum factors carry a higher association with neonatal seizures. One study using logistic models found postnatal bleeding, placental separation, preeclampsia, cesarean section because of hemorrhage, meconium-stained fluid, and shoulder dystocia among the antepartum and intrapartum

TABLE 65.2 Differential Diagnosis of Seizure Etiology**Hypoxic–Ischemic Encephalopathy**

| | |
|--------------------------|---|
| Metabolic derangement | <ul style="list-style-type: none"> • Hypoglycemia • Hypocalcemia • Hypomagnesemia • Hyponatremia • Hypernatremia |
| Cerebrovascular lesions | <ul style="list-style-type: none"> • Perinatal arterial or embolic stroke • Hemorrhage • Cerebral sinus venous thrombosis • Cortical vein thrombosis • Hemorrhagic venous infarction |
| Infection | <ul style="list-style-type: none"> • Bacterial meningitis • Viral encephalitis • TORCH infections |
| Drug exposure/withdrawal | Includes but not limited to: <ul style="list-style-type: none"> • Methadone • Cocaine • SSRIs |

Congenital Brain Malformations

| | |
|-----------------------------------|---|
| Inborn errors of metabolism | Includes but not limited to: <ul style="list-style-type: none"> • Glycine encephalopathy • Aminoacidopathies • Urea cycle defects • Pyridoxine-dependent epilepsy |
| Benign neonatal familial seizures | Caused by a mutation in one of the following genes: <ul style="list-style-type: none"> • <i>KCNQ2</i> • <i>KCNQ3</i> • <i>SCN2A</i> |
| Progressive epilepsy syndromes | Includes but not limited to mutations in the following genes: <ul style="list-style-type: none"> • <i>STXBP1</i> • <i>FOXG1</i> • <i>CDKL5</i> • <i>KCNQ2</i> |

SSRIs, Selective serotonin reuptake inhibitors; TORCH, toxoplasmosis, other agents, rubella, cytomegalovirus, herpes simplex.

factors associated with neonatal seizures (Patterson et al., 1989). Additional factors of diabetes mellitus, advanced maternal age over 40 years, infection, or intrapartum fever were identified in a population-based study (Glass et al., 2009a).

Other Causes of Neonatal Encephalopathy

Hypoxic–ischemic encephalopathy, however, is not the only cause of neonatal encephalopathy consisting of alteration of arousal and muscle tone. Postnatal causes reflected by placental abnormalities, or growth restriction, and factors like intrapartum fever and inflammation and infection can result in a neonatal presentation of encephalopathy with neonatal seizures.

Even in the absence of neonatal meningitis or infection, in one study, intrapartum fever was associated with a fourfold increase in early-onset seizures (Lieberman et al., 2000). Thirty-two neonates without a proximal cause of seizures and 152 controls were assessed;

neonates with seizures were more likely to have been born in the setting of intrapartum fever. It is speculated that circulating maternal cytokines resulted in physiologic events that led to neurologic injury, even in the absence of infection (Lieberman et al., 2000).

Placental findings may represent more chronic disease states, suggesting an antepartum insult in neonates with encephalopathy is not due to birth asphyxia. In one study of 73 neonates, placentas were evaluated in neonates with electrographically confirmed seizures and compared with controls without seizures. Placental lesions consistent with antepartum chronic asphyxial stress such as chronic villitis or maturational changes were increased in those infants with neonatal seizures, as compared with those without (Scher et al., 1998).

Cerebrovascular Lesions

Ischemic or hemorrhagic lesions of either arterial or venous origin are associated with a high risk of seizure in the newborn (Ment et al., 1984; Clancy et al., 1985; Levy et al., 1985; Scher and Beggarly, 1989; Rivkin et al., 1992). In term neonates with perinatal arterial stroke, seizure is the most common presentation, accounting for between 70% and 90%, followed by hypotonia or feeding difficulties (Lee et al., 2005; Grunt et al., 2015). Neonates with cerebral infarction often are otherwise healthy in appearance, with reassuring presentation, not consistent with asphyxia. Use of neuroimaging with magnetic resonance imaging (MRI) is necessary to demonstrate the focal lesion (Osmond et al., 2014; Weeke et al., 2015). Multiple factors are associated with perinatal arterial stroke in aspects affecting the maternal–fetal dyad (Lee et al., 2005).

In preterm infants, intraventricular hemorrhage (IVH) is the most common cause of seizures (Scher et al., 1993; Sheth et al., 1999). IVH is common in preterm neonates and is the cause of seizures in as many as 45% of EEG-confirmed seizures in this population (Scher et al., 1993). IVH typically occurs in the first few days after birth; it can present asymptotically, detected only on screening cranial ultrasounds obtained for this purpose. Some infants, however, will present catastrophically, with bulging fontanelle, hypertonia, apnea, and seizures (Volpe, 2008). Seizures in preterm newborns are thought to be underestimated, as studies prospectively assessing seizure frequency in high-risk preterm neonates find a higher incidence than in those where EEG is obtained in response to a clinical event (Hellstrom-Westas et al., 1991; Scher et al., 1993; Strober et al., 1997).

Seizures caused by IVH may also be present in term neonates; however, further etiologic investigations are imperative, as hemorrhagic transformation of a venous stroke or arteriovenous malformation may be present (Bruno et al., 2014).

Cerebral venous infarction may also result in neonatal seizures (Rivkin et al., 1992). This may occur in the setting of systemic infection, dehydration, or poor feeding with cerebral venous sinus thrombosis. These lesions may result in hemorrhagic transformation and be associated with IVH. In preterm infants, periventricular hemorrhagic infarctions occur within the deep white matter, also referred to as a *grade IV germinal matrix hemorrhage*, and may be complicated by seizures (Strober et al., 1997).

Infants with congenital heart defects, persistent pulmonary hypertension of the newborn, or requiring ECMO have increased risk of seizures caused by recurrent hypoxia hypotensive injury, and emboli. Persistent pulmonary hypertension of the newborn may cause severe and recurrent hypoxia and is associated with cerebrovascular lesions and seizures (Scher et al., 1986). Neurodevelopmental outcomes of neonates with congenital heart defects

are of increasing interest, given advances in cardiothoracic surgery with improved survival of these children. The occurrence of electroclinical and electrographic seizures have been noted in this population, in association with cardiac surgery requiring deep hypothermic circulatory arrest or after prolonged resuscitation (Clancy et al., 2005; Gaynor et al., 2006). In a recently published study, all neonates undergoing cardiac surgery were subsequently placed on continuous EEG monitoring. In this study, 8% of all postoperative patients had seizures, the majority of which were electrographic without clinical correlate (Naim et al., 2015). Infants with seizures had more severe in-hospital course and increased mortality.

ECMO is associated with increased neonatal brain injury and seizures. Hypotension prior to ECMO, or as a complication of ECMO support, places the neonate at risk for watershed distribution injury. The anticoagulation necessary for circuit use may convert an ischemic injury to a hemorrhagic one, with risk of edema or herniation. It is estimated that between 5% and 10% of patients develop seizures during ECMO (Mehta and Ibsen, 2013).

Infection

Central nervous system infections antepartum or postnatally can be associated with neonatal seizures (Kairam and De Vivo, 1981). The TORCH infections (toxoplasmosis, other agents, rubella, cytomegalovirus, and herpes virus) can produce structural brain changes resulting in a predilection for seizures and encephalopathy. These infections are discussed in detail in the Infectious Disease section. Neonatal herpes encephalitis is associated with specific EEG findings, with focal or multifocal epileptiform discharges that may be periodic or quasiperiodic in nature (Mizrahi and Tharp, 1982; Mikati et al., 1990). Enteroviruses may also present with encephalopathy and seizures (Verboon-Macielek et al., 2005, 2006, 2008).

Bacterial meningitis from group B streptococci, *Escherichia coli*, *Listeria monocytogenes*, or *Mycoplasma* may also present with encephalopathy with seizures and may be complicated by abscess formation (Gaschignard et al., 2011; Chu et al., 2014). Some neonates with bacterial meningitis may suffer further complications of arterial stroke or cerebral venous sinus thrombosis, with further neurologic injury (Fitzgerald and Golomb, 2007). A prolonged course of seizures (>72 hours) or markedly abnormal EEG pattern is associated with poor outcome in this setting (Klinger et al., 2000, 2001).

More recently, the Zika virus, a flavivirus transmitted via mosquitoes, has gained attention because of an association with microcephaly in patients born to infected mothers (Schuler-Faccini et al., 2016). Neuroradiologic investigations of these affected neonates demonstrate intracranial calcifications, destructive lesions, or brain malformations including polymicrogyria and pachygyria (de Fatima Vasco Aragao et al., 2016). While reports thus far do not specify neonatal seizures as a presenting feature, one would anticipate a higher risk of this secondary to the associated structural lesions or if a meningoencephalitis remained present.

Metabolic Derangements

Electrolyte disturbances such as hypoglycemia, hypocalcemia, hypomagnesemia, or hyponatremia or hypernatremia may result in seizures; repletion of glucose or electrolytes is imperative for treatment. Further aspects of these etiologies are discussed later.

Hypoglycemia

Hypoglycemia is generally accepted as a glucose level less than 47 mg/dL, although the definition remains controversial (Heck and Erenberg, 1987; Sinclair, 1997; Committee on Fetus and Newborn and Adamkin, 2011). Infants of diabetic mothers and small for gestational age neonates are at risk for transient hypoglycemia, but hyperinsulinism or inborn errors of metabolism should be considered in neonates with persistent hypoglycemia. Hypoglycemia may coexist with hypoxic-ischemic injury or with hypocalcemia, both of which may also result in seizures. Jitteriness, tremors, and abnormal tone may be present in neonates with hypoglycemia, mimicking seizure. Persistent or profound hypoglycemia may result in cerebral injury, classically described as white matter injury or occipital injury (Griffiths and Laurence, 1974; Tam et al., 2008).

Seizures themselves should first be treated by correction of hypoglycemia; seizures may persist despite correction, particularly if cerebral injury occurs. Infants with hypoglycemia and cerebral injury may later develop occipital lobe epilepsy, although the severity of the epilepsy varies (Fong and Harvey, 2014).

Hypocalcemia

Hypocalcemia is defined as a total calcium level of less than 8.0 mg/dL in term neonates and less than 7.0 mg/dL in preterm neonates or an ionized calcium of less than 3.0 mg/dL (0.75 mmol/L) (Beers et al., 1999). Neonates with hypocalcemia may present with seizures secondary to increased excitability of the cell membrane (Nardone et al., 2016), thus resulting in exaggerated startles, jitteriness, myoclonic jerks, or seizures (Keen, 1969; Jain et al., 2010). Hypocalcemia that is not responsive to repletion may be secondary to hypomagnesemia, which should be checked and corrected as appropriate (Jain et al., 2010). Early-onset hypocalcemia may be seen in infants of diabetic mothers, small for gestational age infants, or in hypoxic-ischemic injury, as well as hyperparathyroidism (Jain et al., 2010). Late-onset presentations are often secondary to endocrine etiologies. Other neurologic considerations in late-onset presentation include 22q11 deletion syndromes, which may present with hypocalcemia, cardiac defects, and brain abnormalities; recent studies suggest that neonatal hypocalcemia and resultant seizures may increase the risk of intellectual disability in this population (Cheung et al., 2014).

Hyponatremia and Hypernatremia

Hyponatremia is a cause of seizures across the life span (Nardone et al., 2016); however, it is relatively rare in neonates. When present, this may reflect an inappropriate secretion of antidiuretic hormone in the setting of trauma, asphyxia, or infection (Volpe, 2008). Hypernatremic seizures are also rare in neonates but may be secondary to congenital adrenal disorders or iatrogenic from intravenous solutions with high sodium concentrations.

Drug Withdrawal and Intoxication

Newborns of mothers with polysubstance abuse may be at an increased risk of seizures in the neonatal period. Prenatal exposure to opiates such as methadone or heroin can result in neonatal abstinence syndrome, which, in the most severe cases, can result in seizures (Herzlinger et al., 1977). Neonates born to mothers with acute intoxication of alcohol have a predilection to seizures caused by withdrawal (Pierog et al., 1977). Cocaine, a stimulant with well-studied effects, can produce seizures in neonates either secondary to intoxication caused by maternal ingestion or from

resultant withdrawal (Kramer et al., 1990; Chiriboga et al., 1993). Cocaine may also result in neonatal stroke, which in turn increases the risk of seizures. Exposure to other stimulants, such as methamphetamine, may be associated with a withdrawal syndrome and jitteriness, tremor, and exaggerated startle, but seizures have not been classically reported (Hudak et al., 2012).

Maternal use of SSRIs such as fluoxetine, paroxetine, and sertraline may also result in a withdrawal syndrome in neonates. Clinical symptoms include tremor, jitteriness, vomiting, diarrhea, and sleep disturbance. In some cases, convulsions may be present as a component of the withdrawal syndrome (Sanz et al., 2005). EEG remains imperative in diagnosis, however, as not all abnormal movements noted may have an EEG correlate.

Inadvertent fetal injection with local anesthetic to the pudendal nerve during delivery may result in neonatal intoxication with subsequent seizures (Dodson, 1976; Hillman et al., 1979). Reported newborns presented with apnea, bradycardia, hypotonia, and encephalopathy within the first 6 to 8 hours of life. If the mother received a pudendal nerve block, the newborn's scalp and body should be assessed for potential puncture marks as site of entry. Treatment in this scenario is supportive, with diuresis, acidification of urine, and dialysis as indicated.

Congenital Brain Malformations

Brain malformations occurring from disorders or disruptions in neurodevelopment may result in seizures in the newborn period. These disorders are discussed in greater detail elsewhere but are caused by alterations in stages of induction, segmentation, proliferation, migration, synaptogenesis, and myelination. Seizures may present in the newborn period because of the physiologic stress of birth, which may lower seizure thresholds. Encephalopathy is typically present and may coexist or be mistaken for birth asphyxia. Close attention to physical examination may help in distinguishing these malformations as neonates with lissencephaly or holoprosencephaly have distinct facial features. Neuroimaging with magnetic resonance techniques will verify these abnormalities. Many brain dysgenesis disorders lack specific physical examination findings, and thus neuroimaging is recommended for all neonates with seizures. One study demonstrated that 9% of 356 neonates with seizures were found to have brain malformations (Sheth et al., 1999).

Neonates with brain dysgenesis and seizures in the neonatal period have the greatest likelihood of developing epilepsy among all causes of neonatal seizures (Watanabe et al., 1982).

Inborn Errors of Metabolism

Biochemical abnormalities are rare causes of neonatal seizures, accounting for between 1% and 4% of cases (Volpe, 2008; Vasudevan and Levene, 2013). Although rare, consideration of this etiology is imperative, as specific treatments may be available for some causes, based on the enzymatic defect uncovered. In cases where treatment is not available, prognostic implications remain essential.

Pregnancy and delivery history may be unremarkable; however, the newborn may have encephalopathy, hypotonia, poor feeding, and seizures in the first few days of life to harken the necessity of additional investigation. Other newborns, however, may have difficulty during delivery, thus a high level of clinical suspicion is necessary in neonates with refractory seizures. Signs suggestive of an inborn error of metabolism include seizures that start prenatally,

refractory seizures requiring multiple AEDs, progressive clinical worsening, or deterioration of EEGs (Ficicioglu and Bearden, 2011). Specific neuroimaging may demonstrate characteristic lesions supporting a metabolic etiology. These are nicely reviewed elsewhere (Poretti et al., 2013).

Inborn errors of metabolism causing seizures may be placed in three categories: defects in neurotransmission; disorders of energy production; and disorders associated with a brain malformation, destruction, or dysfunction on a metabolic basis (Van Hove and Lohr, 2011). Examples of each are given below, although extensive review of inborn errors is beyond the scope of this chapter.

Defects in neurotransmission include glycine encephalopathy and pyridoxine-dependent epilepsy. Glycine encephalopathy, also known as nonketotic hyperglycinemia, is due to deficiencies in the ability to cleave glycine. Glycine has both inhibitory and excitatory neurotransmitter activities, and glycine encephalopathy presents with apnea, myoclonic seizures, and burst suppression on EEG. In retrospect, mothers will often note that significant hiccups were present in utero, representing myoclonic seizures. Seizures may initially respond to benzodiazepines, but, long term, patients develop early myoclonic encephalopathy, or Ohtahara syndrome (Van Hove et al., 2002).

Pyridoxine-dependent epilepsy is an uncommon but treatable cause of neonatal seizures, caused by deficiency of antiquitin, an enzyme involved in the lysine catabolic pathway. Newborns may present with seizures, encephalopathy, and hypotonia in the first few days of life (Gospe, 2001 [updated 2014]). In retrospect, mothers may report paroxysmal in utero movements representing seizures. In some patients with pyridoxine-dependent epilepsy, lactic acidosis and other biochemical abnormalities may be present, mimicking features of neonatal encephalopathy secondary to hypoxia or ischemia. Therefore a diagnosis of pyridoxine-dependent epilepsy should be considered in newborns with presumed hypoxic-ischemic encephalopathy and seizures refractory to AEDs. Seizure types and EEG findings may vary between patients, with a predilection toward status epilepticus. It is an autosomal recessive condition, caused by a genetic mutation in *ALDH7A1*, and affected patients have elevated levels of α -amino adipic semialdehyde (AASA) in blood and urine (Stockler et al., 2011). Historically, injection of intravenous pyridoxine in patients with status epilepticus was recommended as it may result in significant improvement of EEG; however, it is now suggested that enteral pyridoxine be administered until biochemical or genetic testing excludes the diagnosis, as immediate intravenous response varies (Bok et al., 2010). Pyridoxine 30 mg/kg divided three times daily is suggested.

Examples of disorders of energy production and utilization include urea cycle defects and glucose transporter type 1 (GLUT1) deficiency. Urea cycle defects may present with encephalopathy and seizures in the first 2 days of life with significant hyperammonemia. Seizures occur as toxic breakdown products accumulate. Testing of serum ammonia and glucose levels, urine organic acids, plasma amino acids, and acylcarnitine levels may reveal the diagnosis. Treatment includes dialysis or exchange transfusion while determining the enzymatic defect, alongside dietary adjustments such as the limitation of protein.

Glucose transport to the brain is mediated by GLUT1. Reduced glucose transport through the blood-brain barrier results in hypoglycorachia (cerebrospinal fluid [CSF] glucose levels <45 mg/dL or a ratio of CSF to serum glucose of <0.4). Patients may present in the first few months of life with significant seizures. Low CSF glucose without alternate explanation should prompt further genetic testing of *SLC2A1*, the major genetic defect found

in this disorder (Wang and de Vivo, 2002). Over the past decade, the phenotype of patients has expanded significantly to include those with movement disorders or early-onset absence epilepsy (Leen et al., 2010). Treatment of GLUT1 deficiency is the ketogenic diet, thereby limiting the necessity of transporting glucose to support brain metabolism.

Metabolic disorders resulting in brain dysgenesis include peroxisomal biogenesis disorders such as Zellweger syndrome. Infants present with characteristic facial features, hypotonia, and encephalopathy alongside seizures in the first days to weeks of life. Neuroimaging reveals neuronal migration defects such as polymicrogyria, while very long chain fatty acids are elevated. Mutations in the *PEX* gene family have been associated with this group of metabolic disorders (Braverman et al., 2016).

Progressive Neonatal Epilepsy Syndromes

Seizures in the neonatal period are rarely the presentation of a chronic epileptic condition (Mizrahi and Clancy, 2000). Affected neonates may endure myoclonic seizures, with early myoclonic encephalopathy, or have frequent or multifocal seizures. Rarely, migratory seizures may be present. When seizures appear progressive, evaluation for underlying etiology should occur. These neonatal syndromes may be termed *early infantile epileptic encephalopathy*, or *Ohtahara syndrome*, and the EEG may demonstrate a suppression-burst pattern or disorganized background. This syndrome typically presents with seizures in the first few weeks to months of life. Some neonates may have demonstrable brain malformations or metabolic disease. Over the past decade, many genes have been associated with early-onset epileptic encephalopathies, including mutations in *ARX*, *CDKL5*, and *STXBPI* among others. These are reviewed elsewhere (Nieh and Sherr, 2014; Gursoy and Ercal, 2016).

Benign Familial Neonatal Seizures

A rare form of neonatal epilepsy that is inherited in an autosomal dominant pattern should be considered in newborns with a positive family history (Pettit and Fenichel, 1980) or after structural, infectious, metabolic, and toxic causes have been ruled out. Often the parents are unaware that they had a seizure in the newborn period until grandparents note this after their grandchild presents with neonatal seizures. Several genes have been implicated, including two potassium channel genes, *KCNQ2* and *KCNQ3*, as well as a sodium channel gene *SCN2A* (Berkovic et al., 2004; Heron et al., 2007; Grinton et al., 2015). *KCNQ2* mutations may also result in a progressive epileptic encephalopathy; thus caution should be exercised in counseling regarding prognosis (Allen et al., 2014). Infants with benign familial seizures typically have a normal interictal pattern on EEG and normal neuroimaging, while those who develop an epileptic encephalopathy tend to have abnormal EEGs with burst suppression, multifocal epileptiform discharges, and basal ganglia hyperintensities (Weckhuysen et al., 2012). Infants with benign neonatal epilepsies tend to have a good response to AEDs, with reports of sodium channel agents being effective in those with *KCNQ2* mutations (Pisano et al., 2015).

Treatment of Seizures

Once the diagnosis of neonatal seizures has been made, management is initiated while the determination of an etiology is under way. Initial efforts should be geared toward detecting correctable causes

of neonatal seizures, such as hypoglycemia, hypocalcemia, or sodium disturbances. Hypoglycemia may be corrected with infusion of 10% dextrose at 2 mL/kg and, depending on the duration of hypoglycemia, may require dextrose infusions to maintain euglycemia. Seizures caused by hypocalcemia should be treated with 10% calcium gluconate in early-onset cases and oral calcitriol or calcium in late-onset cases (Beers et al., 1999). Magnesium levels should be checked and repeated if necessary, as hypomagnesemia may coexist with hypocalcemia. Although rare causes of seizures, hyponatremia or hypernatremia can be managed with determination of the underlying etiology and fluid restriction or replacement dependent on the etiology of sodium dysregulation.

As discussed earlier, pyridoxine-dependent epilepsy is an important etiologic consideration in the newborn presenting with seizures. It is recommended that neonates undergo evaluation for pyridoxine-dependent epilepsy either by testing for the presence of AASA in urine or plasma, measurement of CSF neurotransmitter metabolites (which will show characteristic patterns on chromatography), or testing for mutations in the *ALDH7A1* gene. These laboratory studies should be pursued while the baby is being treated empirically with enteral vitamin B₆ (Bok et al., 2010).

Antiepileptic Drugs

Once metabolic derangements are addressed, treatment of seizures is undertaken with AEDs. As neonates may have both clinical and electrographic seizures, and treatment with AEDs may result in electroclinical dissociation, it is recommended that neonates undergoing treatment with AEDs be monitored through continuous EEG (Shellhaas et al., 2011).

Phenobarbital has remained the first-line choice for treatment of neonatal seizures by most clinicians (Bartha et al., 2007; Blume et al., 2009). Phenobarbital is a barbiturate, acting on the γ -aminobutyric acid (GABA) receptor, enhancing GABA activity. Phenobarbital is typically given as an intravenous bolus of 20 mg/kg, with a maintenance dose of 4 mg/kg per day. The half-life of phenobarbital is quite long in the neonate, approximately 45 to 200 hours (Lockman et al., 1979). Therapeutic levels should be assessed as levels above 40 μ g/mL may not have additional therapeutic benefit (Gilman et al., 1989).

Treatment choices beyond phenobarbital vary greatly between providers (Bartha et al., 2007; Blume et al., 2009). The best evidence for use currently is for phenytoin or fosphenytoin (Painter et al., 1999). Phenytoin should be administered at a dose of 15–20 mg/kg; a water-soluble form that is better tolerated is fosphenytoin, which may be given at the same dose of phenytoin equivalents (Painter et al., 1978). Phenytoin levels are often difficult to maintain, as the medication is rapidly redistributed to the tissues and follows zero-order kinetics (Painter et al., 1981).

Benzodiazepines, such as midazolam, diazepam, or lorazepam, may also be used to control refractory neonatal seizures. Intravenous midazolam has been studied in neonates for safety and efficacy and may be an option for neonates with refractory seizures (Sheth et al., 1996; Castro Conde et al., 2005). Lidocaine, a sodium channel antagonist, has equal efficacy as compared with midazolam for refractory seizures (Shany et al., 2007; Lundqvist et al., 2013; Weeke et al., 2016). This medication is used more commonly in Europe than in the North American continent (Bartha et al., 2007; Vento et al., 2010).

Because of the limited efficacy of AEDs, newer medications are often used off-label by neurologists (Silverstein and Ferriero, 2008). Levetiracetam has been reported in case series to be efficacious, as

has topiramate (Furwentsches et al., 2010; Abend et al., 2011; Glass et al., 2011a). Currently, randomized control trials are under way on a larger scale to assess the safety and efficacy of these agents (Pressler and Mangum, 2013). Bumetanide, a loop diuretic, is a neuronal NKCC (sodium–potassium chloride cotransporter) antagonist and has been shown in preclinical studies to be effective in treating neonatal seizures (Dzhala et al., 2005). The NKCC receptor is expressed in immature neurons, and its activation results in elevated intracellular chloride levels; bumetanide reverses this chloride gradient. A recent open-label feasibility trial to assess its effects in neonates with seizures caused by hypoxic–ischemic encephalopathy did not find an improvement of seizures with bumetanide after treatment with phenobarbital and may have found increased rates of hearing loss (Pressler et al., 2015). Further trials are under way to study bumetanide in this population.

Efficacy of Antiepileptic Drugs

Finding new AEDs for treatment of neonatal seizures is imperative given available data regarding the efficacy of currently used AEDs. Although exact rates vary, studies show a complete response to phenobarbital in less than 50% of neonates (Painter et al., 1978, 1999; Lockman et al., 1979). Clinical studies have varied with regards to endpoint, assessing the cessation of clinical rather than electrographic seizures; however, electrographic seizures may continue despite cessation of clinical seizures (Weiner et al., 1991). As phenobarbital works at the GABA receptor, understanding GABA in the immature brain is imperative in finding appropriate methods in the treatment of seizures. Intracellular chloride concentration determines the strength of GABAergic transmission; in neonates, there are high levels of intracellular chloride, secondary to the developmental expression of the NKCC receptor in these immature neurons (Dzhala et al., 2005; Kahle and Staley, 2008). This developmental expression of NKCC and resultant depolarization of the GABA receptor may be important for calcium-dependent processes such as neuronal migration and synaptogenesis (Brooks-Kayal, 2005). In animal studies, this appears to contribute to the poor efficacy of phenobarbital, such that the addition of bumetanide, an NKCC antagonist, increases the efficacy of phenobarbital in treating seizures (Dzhala et al., 2008).

Discontinuation of Antiepileptic Drugs

How long to treat a neonate with AEDs after resolution of seizures remains an area of uncertainty and controversy (Guillet and Kwon, 2008). It is recognized that the commonly used AEDs complicate assessments of arousal, tone, and feeding abilities because of their sedating qualities. It is now generally recommended that, when possible, AEDs be discontinued in the neonatal intensive care unit (NICU), given the low rate of seizure recurrence after early AED withdrawal. Retrospective studies have demonstrated that there is a low rate of seizure recurrence after early discontinuation of AEDs and that longer length of treatment did not prevent later development of epilepsy (Hellstrom-Westas et al., 1995; Guillet and Kwon, 2007). There is the additional concern of the effects of AEDs on the immature brain. Animal studies show increases in apoptosis after treatment with phenobarbital and phenytoin amongst other AEDs (Mizrahi, 1999; Bittigau et al., 2003).

Additionally, most children with neonatal seizures do not have seizure recurrence in the first year of life (Pisani et al., 2015), and thus AEDs need not be continued. Seizures in infancy that present with specific epilepsy syndromes, such as infantile spasms, require

specific treatments and do not typically respond to the AEDs used in the newborn period. However, neonates with congenital brain malformation or progressive epilepsy syndromes have an ongoing risk for seizures and thus would be expected to continue on AEDs.

Prognosis

A question typically faced by the clinician in the NICU is the prediction of how a newborn will fair in the near and distant future. Immediate concerns in the NICU often include respiratory compromise, either secondary to the underlying etiology or caused by the sedative effects of AEDs. The first neurodevelopmental milestone the newborn often faces is successful feeding, with many neonates requiring extra time or enteral tube support in the NICU. Beyond this newborn period, prognostic considerations remain, particularly with long-term neurodevelopment and epilepsy.

The etiology of a newborn's seizure largely accounts for the overall outcome. Prediction of neurodevelopmental outcomes relies first and foremost on the cause of seizures and is often dependent on advanced neuroimaging that may elucidate congenital malformations or areas of brain injury. Newborns who escape motor impairments in early childhood may still develop more subtle neuropsychological challenges as adolescents or early adults (Temple et al., 1995).

Neurophysiologic data also play a role in prognostication (Monod et al., 1972; Sharp et al., 1981; Sinclair et al., 1999). Those infants with a persistently abnormal EEG pattern such as burst suppression are likely to have a poor outcome, especially when this persists on serial EEGs (Sinclair et al., 1999). Neonates with a normal EEG tend to fair better, while those with moderately abnormal EEGs have a variable outcome (Sharp et al., 1981).

Seizures themselves play an uncertain role in neurodevelopmental outcomes and remain a controversial area in neonatal neurology. Animal models show less vulnerability to seizure-induced brain injury in the neonatal brain as compared with the adult brain (Huang et al., 1999). Despite reduced cell death, it has been shown that seizures in the immature brain result in changes in neural circuitry not seen in the mature brain. In the neonatal rat hippocampus exposed to anoxia, stimulation of electrographic seizures resulted in anoxic depolarization and neuronal death twofold greater than when no seizures were present (Dzhala et al., 2000). Other studies have shown that recurrent seizures in neonatal rats result in spine loss of pyramidal cells in the hippocampus and also affect neurogenesis by altering expression of glutamate and GABA receptors (Holmes, 2009). The effect of recurrent seizures in the neonatal rat results in reduced cell number, despite less cell death (McCabe et al., 2001). These changes in the hippocampus may later result in epilepsy (Holmes, 2009).

There are ethical concerns in studying the effects of neonatal seizures rigorously in the human newborn, and thus limited studies are available. Structural studies have shown that seizure severity is independently associated with brain injury, as measured by magnetic resonance spectroscopy (Miller et al., 2002). Studies assessing the independent role of clinical seizures in the neonate with hypoxic–ischemic encephalopathy have shown mixed results. One study demonstrated lower full-scale intelligence quotient at 4 years of age when correcting for severity of brain injury on MRI (Glass et al., 2009b), while another found no difference on 18-month outcomes on the Bayley Scales of Infant Development (Kwon et al., 2011). The independent role of electrographic seizures remains uncertain. Neonates with status epilepticus confirmed on EEG

have worse neurodevelopmental outcomes and often develop postnatal epilepsy (Pisani et al., 2007). McBride et al. (2000) reported on 40 neonates with electrographic seizures compared with 28 neonates without electrographic seizures, where electrographic seizures were associated with microcephaly, cerebral palsy, and failure to thrive. A recent randomized controlled trial prospectively studied neonates with electroclinical and electrographic seizures, treating one group for electroclinical seizures only, while treating all electrographic seizures (including those without a clinical correlate) in the other group. This study found that newborns in whom all electrographic seizures were treated had a lower seizure burden and received treatment earlier. Additionally, although underpowered, this study suggested that greater seizure burden was associated with greater injury and lower Bayley Scores of Infant Development (Srinivasakumar et al., 2015).

Postnatal Epilepsy

Newborns with seizures acutely in the neonatal period remain at risk for developing epilepsy, defined as recurrent unprovoked seizures, in later life. It is estimated that nearly a quarter of neonates with seizures symptomatic of an underlying cause go on to develop postnatal epilepsy (Clancy and Legido, 1991; Pisani et al., 2004, 2012; Glass et al., 2011). Individual reports vary, but a recent review of published studies found that nearly 20% of newborns with seizures go on to develop postnatal epilepsy, with two-thirds presenting with recurrent seizures in the first year of life (Pisani et al., 2015). Typically, there is a latency period between neonatal seizures and onset of postnatal epilepsy. Neonates with refractory seizures, requiring multiple AEDs, severe brain injury, or those with persistent interictal epileptiform discharges on EEG have a higher risk of developing postnatal epilepsy (Rose and Lombroso, 1970; Watanabe et al., 1982; Clancy and Legido, 1991).

Conclusions

Seizures are a relatively frequent occurrence in the NICU. A high level of suspicion is necessary, alongside ancillary testing to

distinguish seizure from its mimickers. Once diagnosed, management should be geared toward determination of etiology, as well as treatment of seizures. Although it is accepted that status epilepticus should be avoided, the independent impact of neonatal seizures on neurodevelopment remains controversial. There is a growing body of preclinical and clinical data suggesting that electrographic seizures impact later outcomes of neurodevelopment and epilepsy. Our first-line AEDs, however, are inadequate in treating seizures, and further research is under way to find more effective medications.

Suggested Readings

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Risk Assessment and Neurodevelopmental Outcomes

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KEY POINTS

- Preterm and critically ill infants are at high risk for cognitive and motor development problems, neurobehavioral and executive function problems, learning and academic problems, neurosensory problems, and poor functional outcomes.
- Neonatal morbidities, socioeconomic factors, and early interventions can influence long-term outcomes for high-risk infants.
- Many adverse school-age, adolescent, and adult outcomes of high-risk infants cannot be easily predicted in early childhood. Assessments into school age, adolescence, and adulthood are critically important to understand the lifetime trajectory of former high-risk infants.
- Cognitive and neurobehavioral challenges among high-risk children are associated with increased resource utilization and costs.
- All high-risk infants and their families must have access to comprehensive, multidisciplinary developmental follow-up programs and early intervention services in order to optimize both short-term and long-term outcomes.

Outcome Assessment in High-Risk Infants

Who Is the “High-Risk” Infant?

The term *high-risk infant* has been defined in many ways and has been burdened with many negative connotations. However, one general goal for attempting to delineate infants who are high risk is to heighten awareness and focus on those children who may benefit from increased surveillance and early intervention across a number of domains. In a policy statement from the American Academy of Pediatrics (AAP) (AAP, 2008), high-risk infants were defined broadly as including (1) the preterm infant; (2) the infant with special healthcare needs or dependence on technology; (3) the infant at risk because of family issues; and (4) the infant with anticipated early death. Others have underscored the importance of critically assessing risk in any infant admitted to the neonatal intensive care unit (NICU) or special care nursery (Walker et al., 2012). Many clinical conditions and risk factors seen in term-born infants, including but not limited to congenital heart disease (CHD) (Marino et al., 2012), need extracorporeal membrane oxygenation (ECMO) in the neonatal period (Ijsselstijn and Van Heijst, 2014),

and hypoxic-ischemic encephalopathy (HIE) regardless of treatment with therapeutic hypothermia (Shankaran et al., 2005, 2012a) places them at high risk for neurologic, developmental, functional, and health outcome challenges in early childhood and beyond. Unfortunately, although the risks for postdischarge difficulties, in addition to medical morbidities, have been increasingly well-described in the literature for these and other predisposing risk factors, the neurodevelopmental needs of many of these term-born, high-risk infants may be overlooked at hospital discharge, or they may not be appropriately referred to early intervention services even when they are identified as eligible in follow-up (Tang et al., 2012).

Preterm infants are the most recognized and targeted population of high-risk infants. Preterm birth, defined by the World Health Organization (WHO) as delivery before 37 completed weeks of pregnancy, is a growing problem for developing countries across the world (Blencowe et al., 2012). Preterm birth remains a substantial problem for developed nations as well, although the landscape of prematurity appears to be changing. According to the Centers for Disease Control and Prevention National Center for Health Statistics, in the United States, in 2013, the preterm birth rate (proportion of live births <37 weeks' estimated gestational age, [EGA]) was 11.39%, which was very slightly decreased from the 2012 preterm birth rate of 11.55% (Martin et al., 2015). Nevertheless, this represents a considerable decline from the preterm birth rate peak of 12.8% in 2006. Furthermore, rates of preterm birth declined for each of the largest race and Hispanic origin groups and for 49 of the 50 United States over that period. However, the proportion of infants delivered very preterm (VPT, <32 weeks' EGA) stands at 1.92%, which, although decreased from 2.04% in 2006 and 2007, remains relatively unchanged. Similarly, the extremely preterm (EPT, <28 weeks' estimated EGA) birth rate is relatively stable; in 2013 the EPT birth rate was 0.73%, exactly the same as 2012 and 2011, and minimally changed from a peak of 0.77% in 2005. Although a decrease in overall preterm birth rate has been realized, and the VPT and EPT birth rates seem to be miniscule, the impact in terms of total births is important. With nearly 4 million births annually in the United States, approximately 450,000 neonates <37 weeks' EGA, 75,000 very preterm neonates, and 30,000 EPT neonates were born in 2013 alone (March of Dimes Peristats, 2016).

What Is Meant by “Outcomes”?

As survival of even the most EPT and complex infants has improved over the decades, short-term mortality and morbidities have moved from being the only outcomes reported for high-risk infants to being only the first of many outcomes of interest. There is increasing recognition of the critical significance of understanding later outcomes in order to evaluate the true impact of interventions and management approaches in the NICU, to inform counseling, and direct early detection and preventive care. Certainly, for some trials of treatments and management strategies designed to test a hypothesis of improved in-hospital morbidities, the primary outcome may best be short term (Schmidt et al., 2006; SUPPORT Study Group of the Eunice Kennedy Shriver NICHD Neonatal Research Network et al., 2010; BOOST II United Kingdom, Australia and New Zealand Collaborative Groups et al., 2013). However, longer-term follow-up or later primary endpoints may provide valuable additional outcomes data, safety assessments, or information about functional elements (Schmidt et al., 2001, 2012, 2013; Vaucher et al., 2012; BOOST-II Australia and United Kingdom Collaborative Groups et al., 2016).

What are these postdischarge outcomes of potential importance? How are they measured, and what are the barriers or benefits of focusing on various outcomes? The value placed on one outcome or group of outcomes may differ greatly for families, children, and adults who were born high risk, physicians, and other care providers, investigators, educators, and those involved in developing public policy. The importance of various outcomes may also vary substantially among individuals within these groups and across different time points of their lives. Furthermore, later cognitive and behavioral outcomes are complex and influenced by the postdischarge environment, relationships, and biologic factors (Msall and Park, 2008; Treyvaud et al., 2012). Therefore the concept that there is one “best” outcome measure among those born preterm or at high risk is ill conceived.

In the following section, we provide an overview of some general later outcome categories that have been reported and proposed for high-risk infants, particularly for those born preterm or with very low birth weight (VLBW). We begin with special attention to early neurodevelopmental outcomes assessments (18 to 36 months) since these are most frequently reported in trials and prospective observational studies. We discuss challenges and strengths reported at this age, using frequently reported definitions of impairment and disability, and describe the usual battery of tests and assessments. Limitations of standard outcomes definitions and challenges to interpretation of early neurodevelopmental outcome studies are considered. We also highlight abilities and difficulties assessed through school age and adulthood.

Early Neurodevelopmental Outcome Assessments

For the vast majority of interventional trials and prospective observational studies involving high-risk infants, neurodevelopmental outcome at approximately 2 years corrected age, and usually death or “neurodevelopmental impairment” (NDI), is reported as a primary outcome. Although the rationale for the combined outcome is clear in the setting of competing outcomes, there is ongoing and substantial debate regarding the appropriateness of this outcome as a gold standard for all trials (Marlow, 2013, 2014). Furthermore, the NDI outcome is itself a composite outcome, composed of morbidities from neurodevelopmental and sensory domains with

different risk profiles, causal pathways, and predictive validity. There are challenges to interpretation of these data, potential limitations in terms of comparisons across cohorts and across years, and concerns regarding the value of early neurodevelopmental outcomes to predict abilities and challenges in later childhood and beyond.

During a follow-up visit at 2 to 3 years, the traditional battery of tests and assessments includes assessment of motor function, cognitive/developmental capabilities, and neurosensory outcomes including hearing and vision impairments. These general components have been proposed and recommended by expert panels and working groups, including the British Association of Perinatal Medicine (Wang et al., 2006; British Association of Perinatal Medicine, 2008), and within the context of prospective studies and trials (Schmidt et al., 2001; Doyle, 2010a; Moore et al., 2012; Vohr et al., 2012) although the specific evaluations within each area differ among groups and over time. (See later section *Multi-disciplinary Follow-up Care for the High-Risk Infant* for further details.)

Motor Function

Motor impairments including cerebral palsy (CP) are among the most frequently reported neurodevelopmental outcomes for high-risk infants. Motor difficulties may become evident over months or years, yet timely identification of motor difficulties may allow for interventions to improve outcomes, thereby reinforcing the critical importance of vigilant long-term follow-up (see later section *Challenges to and Importance of Follow-Up*). CP is defined as a disorder of movement and posture that involves abnormalities in tone, reflexes, coordination and movement, delays in motor milestone achievement, and aberration in primitive reflexes that is permanent but not unchanging and is caused by a nonprogressive interference, lesion, or abnormality of the developing immature brain (Bax et al., 2005; Fawke, 2007). CP is also categorized by type (spastic, dyskinetic, or dystonic); topography (limbs involvement); and descriptors of extent and pattern of involvement (monoplegia, diplegia, hemiplegia, and quadriplegia). CP sometimes cannot be diagnosed with confidence until after 18 months, although earlier examinations of general movements and other factors may elevate risk profile for CP and can serve to focus and underscore the need for follow-up (Spittle et al., 2011, 2013a).

The Gross Motor Function Classification System (GMFCS) (Palisano et al., 1997, 2007; Rethlefsen et al., 2010) provides a valid and reliable system to classify the extent of activity limitation in CP. It is a five-level system (I–V) used to categorize children up to 18 years of age based on their usual performance, with a focus on functional capabilities, including sitting, mobilizing, walking, and need for assistive devices. Such classification helps clinicians communicate information about severity, choice of interventions, and prognosis in a standardized, easy-to-use, valid, and reliable way.

Higher level on the GMFCS is associated with increasing functional difficulty, but, of note, distinctions between levels I and II are not as significant as differences between other levels, particularly for younger children. Children classified as level I overall are able to walk without restrictions but may have difficulty with the speed, balance, and coordination required for higher-level skills; between 2 and 4 years, they are able to floor sit with both hands free to manipulate objects, move in and out of floor sitting and standing without adult assistance, and they walk as the preferred method of mobility without the need for any assistive mobility device. In contrast, children classified as level V are profoundly

impaired with no means for independent mobility; between 2 and 4 years, this is described as physical impairments that restrict voluntary control of movement and the ability to maintain antigravity head and trunk postures and functional limitations in sitting and standing that are not fully compensated with the use of adaptive equipment.

GMFCS categorization in children less than 2 years old depends predominantly on the amount of support required for the child to sit and also considers more advanced skills such as crawling and walking. Although some children diagnosed before 2 years of age will require reclassification, most are not reclassified by more than one level. The positive predictive value (PPV) of a classification of GMFCS level I, II, or III (child will walk with or without aids) as compared with level IV or V (child will probably need a wheelchair for mobility) is very high (0.96). Thus the GMFCS provides a sound approximation, rather than a definitive final categorization, in this age group (Gorter et al., 2009).

Although CP with severe functional limitations is of great concern, it is relatively rare. An Australian CP registry review from the 1970s to 2004 showed increasing prevalence of CP in the 1970s and 1980s attributed to the increasing survival of EPT infants; however, CP rates stabilized or decreased between the early 1990s and 2004 (Reid et al., 2011; Spittle and Orton, 2014). In children with CP, there may be a spectrum of motor findings, including many motor and coordination deficits that should not be overlooked (Vohr et al., 2005).

There are numerous motor and coordination challenges apart from CP that are reported in high-risk children, including those born preterm. Developmental coordination disorder (DCD) is a diagnosis that is considered best made at or after 5 years, but motor performance assessment tools are available from 3 years. DCD is diagnosed when (1) motor coordination and performance are below that expected for the child's chronologic age and intelligence level, (2) the motor disorder interferes with activities of daily living or academic achievement, (3) it is not due to a general medical or neurologic condition such as CP, and (4) the motor difficulties are in excess of those associated with any intellectual disability that is present. (Sugden et al., 2006; Blank et al., 2012). Current recommendations for diagnosis of DCD include a score less than the 15th percentile on the Movement Assessment Battery for Children (Henderson et al., 2007) or equivalent test such as the Bruininks–Oseretsky Test of Motor Proficiency (Bruininks and Bruininks, 2005), whereas previous guidelines recommended using a score less than the fifth percentile on the Movement Assessment Battery for Children. However, a movement or motor test alone is not sufficient for the diagnosis of DCD, as direct observation or interviews are essential to determine the extent of interference with activities of daily living. It is important to remember that although the motor difficulties associated with DCD are usually considered “minor” motor impairments, particularly in comparison with disabling CP, nonetheless they can have significant impact on the child. These difficulties may include important functional skills such as fine motor skills, speed and accuracy in motor planning, balance, and coordination. Children with DCD or probable DCD have been shown to be at increased risk for reading and attention difficulties, social–emotional and behavior problems, anxiety, speech and language impairment, and other challenges (Kirby and Sugden, 2007; Lingam et al., 2012).

Cognitive Assessment

A central component of a high-risk infant neurodevelopmental follow-up visit has been administration of a standardized

developmental test. These tests are intended to provide a measure of “cognitive” function, although there are widely acknowledged limitations including the evolution of test versions making it difficult to compare across cohorts, preclusion of extrapolation of 2- to 3-year results to intelligent quotient (IQ) at later time points, and challenges to interpretation of results in the preterm population using standardized “cut points” alone and in the absence of a contemporaneous normal birthweight (NBW) term control group (see later section [Limitations and Challenges to Interpreting Early Neurodevelopmental Outcomes Studies](#)).

The Bayley Scales of Infant and Toddler Development (BSID), with a test age range of 1 to 42 months, is now the most widely used developmental test for high-risk infants across the United States and Europe. The original version, released in 1969, was revised in 1993 (Bayley, 1993). The BSID-II had two developmental scores: the Mental Developmental Index (MDI), a composite of cognitive and language tasks, and the Psychomotor Developmental Index (PDI), a composite of fine and gross motor skills. This perceived drawback, as well as the usual drive to revise editions due to the “Flynn effect” (Flynn, 1999), contributed to the development of BSID-III (Bayley, 2006), which contains three main domains, (1) a cognitive composite score, (2) a language composite score (with receptive and expressive subscores), and (3) a motor composite score (with gross and fine motor subscores), in addition to social–emotional and adaptive behavior domains. The goal of the BSID-III was to allow identification of delays, as well as relative strengths and challenges, in specific developmental domains, and to target interventions to areas of need. However, in part because of a change in approach to norming the BSID-III, and also possibly because of separation of the cognitive and language scales, cognitive scores on the BSID-III are substantially higher than anticipated among both preterm high-risk children and term control groups (Anderson et al., 2010; Vohr et al., 2012). These findings have led to concern that the BSID-III underestimates developmental delay if utilizing normative test means alone, which has serious implications for both clinical and research endeavors. Previously, commonly used “cut points” for categorization of “moderate” and “severe” developmental delay or disability were 2 to 3 standard deviations (SD) and greater than 3 SD below the normative mean, respectively; thus for BSID-II, MDI 55 to 70 was considered a moderate delay whereas an MDI less than 55 was considered a severe delay (British Association of Perinatal Medicine, 2008; Vohr et al., 2012). However, in the absence of contemporaneous term control groups, commonly used cut points have shifted in the era of the BSID-III. Some have recommended that BSID-III cognitive and language scores less than 85 or “combined BSID-III” scores less than 80 provide the best definition of “moderate-to-severe” delay for equivalence with BSID-II MDI less than 70 (Johnson et al., 2014), whereas others have modified the threshold for cognitive delay categorization to define moderate delay as 70 to 84, severe delay 55 to 69, and profound delay as less than 54 (Schmidt et al., 2013; Vohr, 2014a).

Another developmental test, the Griffiths Scales, is available in versions for 0–2 years and 2–8 years. The first version was published in 1967, an updated version was available in 1996, and an extended and revised edition (GMDS-ER) was published in 2006. The Griffiths-III was released in 2016 (<http://www.aricd.ac.uk/about-the-griffiths-scales/>).

There are numerous cognitive development tests available to provide full-scale IQ or equivalent that have been used for later childhood assessments, including the Wechsler Preschool and Primary Scale of Intelligence (age range: 2 years 6 months to 7 years 7 months), now in the fourth edition (WPPSI-IV; Wechsler,

2012), the Differential Ability Scales (age range: 2 years 6 months to 17 years 11 months), now in the second edition (DAS-II; [Elliot, 2007](#)), and the Wechsler Intelligence Scale for Children (age range: 6 years 0 months to 16 years 11 months), now in the fifth edition (WISC-V; [Wechsler et al., 2014](#)). The Wechsler Individual Achievement Test (age range: 4 years 0 months to 50 years 11 months), now in the third edition (WIAT-III; [Wechsler, 2009](#)), identifies academic strengths and weaknesses, and has been used in clinical, research, and educational settings.

Hearing and Vision Outcomes

Severe neurosensory impairments, including profound hearing and vision impairment, among preterm infants are now low in incidence but have important long-term consequences. Rates of blindness and significant hearing impairment are inversely related to gestational age ([Hintz et al., 2011](#); [Moore et al., 2012a](#)). Both moderate-to-severe vision and hearing impairment are more common among high-risk infants and with cooccurring characteristics including male gender, multiple birth, CP, hydrocephalus, and seizures ([Davis et al., 2010](#); [Hintz et al., 2006](#); [Synnes et al., 2012](#); [Vohr 2014b](#)).

Early detection of hearing impairment is vital for optimizing speech and language development, and guidelines and recommendations reflect the importance of recognizing potential problems as early as possible. As stated in the AAP Joint Committee on Infant Hearing position statement ([AAP, 2007](#)), infants admitted to the NICU for more than 5 days are to have auditory brainstem response included as part of their predischarge screening so that neural hearing loss will not be missed; for those who fail, referral should be made directly to an audiologist for rescreening and, when indicated, comprehensive evaluation for hearing loss. Reevaluation should occur, regardless of initial evaluation results, based on individual risk factors and readmissions. All infants, including well infants, should have a hearing screening by 1 month of age, with rescreening and referral for audiology evaluation by 3 months of age for those who do not pass the initial screen. In addition, a validated global screening tool is administered to all infants at 9, 18, and 24 to 30 months or sooner when there is concern about hearing or language.

Prematurely born children have an increased risk of various ophthalmic and visual dysfunctions and abnormalities, in particular those children with a history of severe or treatment-requiring retinopathy of prematurity (ROP) and those with severe brain injury. These functional visual challenges include strabismus, problems with acuity, convergence and visual fields, and retinal morphology ([Holmström and Larsson, 2013](#)). It is important to recognize that a short-term outcome of severe ROP in the NICU may not result in the most severe functional vision outcomes in early childhood ([SUPPORT Study Group of the Eunice Kennedy Shriver NICHD Neonatal Research Network et al., 2010](#); [Vaucher et al., 2012](#)). Even children born preterm without a history of ROP or with only mild ROP also have an increased risk of problems. Long-term follow-up studies have demonstrated that up to 50% of VLBW infants have some visual impairments at later school age and that these problems can be associated with learning and academic challenges ([Stephenson et al., 2007](#)). Recommendations from AAP and the American Association of Pediatric Ophthalmology indicate that ophthalmologic follow-up should be undertaken in children with a history of ROP within 4 to 6 months after discharge, regardless of the severity of ROP ([Fierston et al., 2013](#)).

For early neurodevelopmental outcomes studies among preterm infants at 18 to 30 months corrected age, criteria for “profound” or “severe” disability in the hearing domain have generally included

some definition of “no useful hearing” even with aids or “some hearing but loss not corrected by aids,” whether accompanied by specific decibel hearing loss (dBHL) audiologic evaluation cut points (profound >90 dBHL; severe 70–90 dBHL) ([British Association of Perinatal Medicine, 2008](#)) or by examination and observation of bilateral hearing loss not correctable by amplification ([Doyle, 2010b](#); [Vohr et al., 2012](#); [Serenius et al., 2013](#)). Similarly, “severe” disability in the visual domain has generally been defined as functional bilateral blindness, including visual acuity less than 20/200, or inability to perceive light, or only able to perceive light or reflecting objects ([British Association of Perinatal Medicine, 2008](#); [Doyle et al. 2010b](#); [Vohr et al., 2012](#)), or unable to fixate and follow a light with either eye ([Serenius et al., 2013](#)). Definitions of “moderate” hearing and vision impairment differ among studies.

Neurodevelopmental Impairment—Difficulties and Realities of a Composite Outcome

Neurodevelopmental impairment is a composite outcome, combining criteria and cut points from several domains as presented above, including neuromotor, cognitive, hearing, and vision. The relative prevalence of each of these outcomes is not consistent, and rates of each outcome may respond differently to an experimental therapy. This necessarily leads to a number of difficulties in overall interpretation, as well as challenges in generalizability and counseling. Use of composite outcomes during counseling in the prenatal or postnatal setting may not be meaningful to parents and families. The value of each of the components may vary broadly for each individual family, outcomes may be conceived differently, and statistics are probably difficult to grasp ([Janvier et al., 2012](#); [Dupont-Thibodeau et al., 2014](#)).

In addition, significant center variation in 18- to 22-month neurodevelopmental outcomes has been demonstrated even after adjustment for demographic variables, prenatal interventions, and neonatal clinical factors, thus presenting challenges to accurate counseling from multicenter datasets ([Vohr et al., 2004](#)). Unfortunately, robust data from a single center on outcomes of specific high-risk groups are generally unavailable. Furthermore, the largest contributing component to the composite outcome is that of “cognitive” delay or impairment. As explored previously and discussed in more detail later in the section [School Age Outcomes After Prematurity](#), developmental tests at 18 to 30 months are intended to assess cognitive abilities. However, in children born at extremely low birth weight (ELBW) and EPT, scores on these early administered tests predict cognitive scores at school age poorly ([Hack et al., 2005](#); [Roberts et al., 2010](#); [Schmidt et al., 2012](#); [Spencer-Smith et al., 2015](#)).

Death or NDI at 2 to 3 years is a primary or main secondary outcome in a substantial majority of clinical trials and prospective studies in neonatal medicine, particularly for EPT infants ([Marlow, 2014](#)). This combination is understandable because death and NDI are competing outcomes; it is assumed in this context that adverse outcomes (death and NDI) will not be influenced in opposite directions by an intervention and ideally that components of the combined outcome will carry similar value; these tenets may not always be true. Because the incidence of the composite outcome is greater than any individual component, death or NDI may also be a logical statistical choice for powering a trial; however, it may not be the most biologically plausible target. Moreover, although neurodevelopmental outcome at 2 to 3 years is critically important for any prospective observational study or clinical trial of high-risk infants, it may not be the most appropriate primary outcome for every trial.

Limitations and Challenges to Interpreting Early Neurodevelopmental Outcomes Studies

As described previously, early childhood follow-up visits for high-risk infants at 18 months to 3 years typically assess outcomes in multiple domains. Although these evaluations are important and informative, interpretation and comparison of studies are difficult. Many studies report outcomes by categories and frequently include *any* adverse finding to yield an “impaired” or “unimpaired” status. The definitions of “adverse” outcomes may not be consistent across studies; indeed, the definitions of the individual components of “impairment” such as CP, blindness, deafness, and developmental delay often differ across studies. Furthermore, not all prospective studies have enrolled contemporaneous term, NBW controls. Comparing test scores from a VPT or EPT study group with standardized norms instead of scores from a peer, term-born control group has been demonstrated to substantively limit the relevance and veracity of the findings and may have important public policy and resource implications (Marlow et al., 2005; Doyle et al., 2010b; Msall, 2010a; Aylward and Aylward, 2011).

Very early childhood developmental and neurologic outcomes evaluations should only be considered as a first step to comprehensive follow-up; assessments into school age, adolescence, and adulthood are critically important to understand the lifetime trajectory and functional and societal outcomes of former high-risk infants (Hack, 2009; Hack et al., 2012; Saigal et al., 2016). Concerns regarding changes in cognitive abilities over time, and an increasing recognition that physical and environmental effects as well as early intervention approaches may modify recovery, underscore the need for later assessments. Some neurocognitive, executive function and behavioral challenges may only be detected at school age; even recognizing that such learning and attention problems may occur in preterm infants is a critical step to ensuring adequate support and services for families and teachers to help children achieve their best possible outcomes. Evaluation of neuromotor outcomes throughout childhood is also critical. Although most toddler-age and very early childhood outcome studies focus narrowly on the diagnosis of CP, later neuromotor and coordination problems, such as DCD, are prevalent among school-age children born EPT compared with term and can be associated with other functional challenges and academic difficulties. Furthermore, parental perceptions of DCD are not reliable, yet interventions may be able to remediate the functional limitations of DCD, reinforcing the importance of ongoing clinical assessments throughout childhood (Roberts et al., 2011).

Similarly, there are substantial concerns regarding the ability of developmental or cognitive tests at toddler age, particularly the BSID, to detect developmental delay when using standardized test norms. In a group of EPT and ELBW infants at 2 years corrected age, Anderson et al. (2010) found mean BSID-III cognitive scores of 96.9 and motor scores of 100.4 but also substantially higher than expected scores among a term-born control group. If normative BSID-III cut-point criteria alone were applied to the scores of this cohort, it would severely underestimate moderate-to-severe cognitive and motor delay relative to the control group. Vohr et al. (2012) compared mean BSID-II versus BSID-III scores at 18 to 22 months corrected age for prematurity among infants less than 27 weeks' EGA born in National Institute of Child Health and Human Development (NICHD) Neonatal Research Network (NRN) hospitals over two adjacent periods and found mean cognitive composite scores on the BSID-III to be 11 points higher than mean BSID-II MDI scores. Even after adjusting for

differences between groups, BSID-III was a significant factor in a perceived decrease in neurodevelopmental impairment in the more recent time period. Dilemmas also exist with regard to meaningful utilization of BSID-III motor composite and fine motor scaled score normative data and cut points in the context of other gross motor assessments at 18 to 24 months corrected age and the value of these early evaluations to predict later outcomes and intervene appropriately (Duncan et al., 2015). In a prospective, longitudinal EPT cohort from the Victoria Infant Collaborative Study Group (VICS Group), the BSID-III motor scale normative cut points for impairment at 2 years seriously underestimated rates of motor impairment at 4 years (Spittle et al., 2013b). In addition, although the BSID-III cognitive and language scales at 2 years were associated with cognitive functioning at 4 years as assessed by DAS-II, developmental delay at 2 years as determined by BSID-III reference data and normative cut points had low sensitivity in predicting future cognitive, verbal, and nonverbal reasoning impairments at 4 years on the DAS-II (Spencer-Smith et al., 2015). All of these findings have important implications for resource availability and public policy. In countries, states, or regions without specific policies advocating for ongoing, longitudinal assessments and services for children born EPT, the 2-year or 3-year evaluation may be the final opportunity to identify challenges before transitioning to the school system. If ongoing needs are determined only by scoring below a normative cut point on the BSID-III, many children at significant risk for future impairments could be left behind.

Despite these many challenges and provisos, a substantial body of literature exists on early neurologic and cognitive outcomes. An understanding of the range of initial neurodevelopmental outcomes, and the factors associated with adverse outcomes, is crucial for both the family and medical care team. In later sections of this chapter we review early neurodevelopmental outcomes of recent high-risk cohorts, including those born EPT and selected groups of high-risk late preterm and term infants.

Focus on Functional Outcomes

The WHO has defined “disability” as an umbrella term, covering impairments, activity limitations, and participation restrictions. An impairment is a problem in body function or structure; an activity limitation is a difficulty encountered by an individual in executing a task or action; and a participation restriction is a problem experienced by an individual in involvement in life situations. According to The World Report on Disabilities (WHO and World Bank, 2011), approximately 95 million infants, children, and youths 0 to 14 years of age worldwide (5%) live with a disability, of whom 13 million (0.7%) are considered to have severe disabilities. Nearly 800 million (~16%) individuals more than 15 years old live with a disability. The WHO International Classification of Functioning, Disability, and Health (ICF) provides standard language and a framework for the description of health and health-related states that are focused on functioning rather than diagnosis alone (WHO, 2001). It is a classification of health and health-related domains that describes what a person with a health condition can do in a standard environment, as well as how he or she can perform in his or her usual environment. These domains are classified from body, individual, and societal perspectives organized in two parts, each comprising two components. Part 1—Functioning and Disability—includes Body Functions and Structures and Activities and Participation; Part 2—Contextual Factors—incorporates Environmental Factors and Personal Factors. The ICF is meant to

be universally applicable and useful in a range of different sectors, including individual assessments, public policy, and research.

The focus of the ICF is on health and functioning, rather than on disability. In most studies and approaches, particularly in early childhood and even through school age, impairments are identified through tests or examinations, and an individual is classified with regard to that finding alone. In contrast, the ICF approach is to measure functioning in society, regardless of underlying impairments. This allows for a broader view than a traditional classification of health and disability. The ICF shifts the focus from diagnosis or label to impact on function, which may be affected by environment, intervention, and other factors.

In 2011, the WHO approved a “derived” classification, the International Classification of Functioning, Disability and Health for Children and Youth, which conforms to the common classifications of the ICF, with a goal of creating a comprehensive, life-span approach to describing health and functioning. However, attempts to apply these functional outcome classification approaches to very early childhood follow-up studies of high-risk infants have been limited, with some notable exceptions, including Msall and colleagues, who have championed the framework of function, even at early preschool age, as a highly relevant and measurable outcome (Msall, 2005). Among the several instruments available, the Vineland Adaptive Behavior Scale (VABS) is an interview survey for assessing adaptive behavior (Sparrow et al., 2005). In children less than 6 years old, the domains include communication (receptive, expressive language), daily living skills (self-care/personal), socialization (interpersonal relations and play), and motor skills (gross and fine). There is a classroom edition for children 3 years 0 months to 12 years 11 months. The Pediatric Evaluation of Disability Inventory (PEDI) assesses self-care, mobility, and social functional activities in children 6 months to 7 years (Feldman et al., 1990) and has been used extensively in children with severe perceived or known disabilities including those with CP and other physical impairments. The Functional Independence Measure for Children (Wee FIM) is an evaluative measure of basic functional skills that consists of 18 items encompassing three subscales: self-care, mobility, and social cognition. The Wee FIM has been used in children aged 2 to 7 years, including high-risk infants such as those born EPT and those with significant medical risk factors (Ottenbacher et al., 1999; Msall, 2010b). This instrument has been shown to have excellent concurrent validity with the VABS and PEDI and responsiveness to change over time (Msall, 2005). Such instruments are essential for understanding the functional impact of extreme prematurity and critical neonatal illness over the life course.

Outcomes of Preterm Infants Across the Life Spectrum

Early Neurodevelopmental Outcomes of Extremely Preterm Infants

Early neurodevelopmental outcomes of several large cohorts of EPT infants around the world have been reported. These are summarized in Table 66.1 and presented in detail later.

The Victoria Infant Collaborative Study Group

The VICS Group has reported on a series of birth cohorts of EPT neonates born in the state of Victoria (Australia) from 1991 to 1992, in 1997, and in 2005 (Doyle and the VICS Group, 2004; Doyle et al., 2010). Neonates born alive at 22 to 27 completed

gestational weeks and surviving to follow-up received neurodevelopmental assessment at 2 years of corrected age (1991–1992, $n = 219$, 97% of survivors; 1997, $n = 149$, 99% of survivors; 2005, $n = 163$, 95% of survivors). Importantly, contemporaneous NBW controls >36 weeks’ gestation were also enrolled and evaluated. Children were evaluated for blindness, deafness (hearing loss requiring amplification or worse) and developmental delay (using BSID). Cognitive and language composite scores were obtained relative to the mean and SD for the NBW controls, rather than the normative test scores and cut points alone. Neurologic examination for CP was also performed, utilizing the GMFCS, describing “severe” CP as unlikely ever to walk and “moderate” CP as unable to walk at 2 years but likely to walk. Overall disability was considered “severe” for children with severe CP, blindness, or severe developmental delay; “moderate” with moderate CP, deafness, or moderate developmental delay; and “mild” with mild CP or mild developmental delay.

Neurodevelopmental outcomes of the 2005 VICS cohort at 2 years corrected age are shown in Table 66.1. Comparing the 1991 to 1992, 1997, and 2005 cohorts, adverse outcomes were not significantly different across eras, although those in 2005 were less likely to have most adverse outcomes. Of note, for the 1991 to 1992 and 1997 cohorts, rates of any CP were 11% and 12.1%, whereas for the 2005 cohort the CP rate had decreased to 9.8%. Similarly, the 2005 cohort had significantly lower rates of severe developmental delay (3.7%) and severe neurologic disability (3.7%) than the 1997 cohort (14.8% and 15.4%), who in turn had significantly higher rates of these problems than the 1991 to 1992 cohort (7.3% and 7.8%). Moderate or severe developmental delay and moderate or severe disability were also similar overall across cohorts, with a decrease noted between 1997 and 2005 but an increase from 1991 to 1992 to 1997 (moderate or severe developmental delay—1991 to 1992, 18.3%; 1997, 24.2%; 2005, 16%; moderate or severe disability—1991 to 1992, 21%; 1997, 28.2%; 2005, 20.3%). Given these findings, it is not surprising that the mean utility per survivor was higher (better) in 2005 than either of the previous two cohorts at each gestational week from 22 to 26 weeks. Quality-adjusted survival increased with each gestational week, was greater for the 1997 cohort compared with the 1991 to 1992 cohort, and was stable between 2005 and 1997. Focusing on the 2005 cohort, the BSID-III cognitive and language composite mean scores were much higher than would be anticipated for the EPT cohort (97.5 and 93.9) but also higher for the control cohort (108.9 and 108.2); developmental delay for the EPT group would have been greatly underestimated had they not been evaluated relative to a term control group. Nevertheless, more than half of the EPT 2005 cohort had no developmental delay (52.1%) and no disability (50.9%) at 2 years.

Thus these regional, population-based studies reveal that although survival rates and quality adjusted survival for 22- to 27-week EGA infants increased through the 1990s and appear to have stabilized through the 2005 cohort, rates of severe delay and disability at 2 years appear to have improved since the late 1990s. As described later in this chapter, these investigators have also published extensively on school-age, adolescent, and young adult outcomes (Hutchinson et al., 2013; Roberts et al., 2013; Burnett et al., 2014).

EPICure 2

The EPICure 2 Study is a population-based mortality, morbidity, and neurodevelopmental outcomes study (Moore et al., 2012a).

TABLE 66.1
Early Neurodevelopmental Outcomes: Selected Recent Extremely Preterm Cohorts

| Study group description | NICHD NRN (Hintz et al., 2011) | NICHD NRN (Rysavy et al., 2015) | VICS 2005 (Doyle et al., 2010) | Japan NRN (Ishii et al., 2013) | EPICure 2 (Moore et al., 2012a) | Swiss Cohort (Schlapbach et al., 2012) | EXPRESS (Serenius et al., 2013) |
|--|--|---|---|---|---|--|--|
| Birth years | <25 week EGA | 22–26 week EGA | 22–27 $\frac{1}{2}$ week EGA | 22–25 week EGA | 22–26 $\frac{1}{2}$ week EGA | 24–27 $\frac{1}{2}$ week EGA | <27 week EGA |
| Age at follow-up corrected for prematurity | 2002–2004 18–22 months | 2006–2011 18–22 months | 2005 2 years | 2003–2005 36–42 months (chronologic) | 2006 3 years | 2000–2008 2 years | 2004–2007 2.5 years |
| Number (percent follow-up of eligible survivors) | 405 (93%) | 2630 (92%) | 163 (95%) | 562 (72%) | 576 (55%) ^b | 684 (81%) | 415 (90%) |
| Outcomes | | | | | | | |
| Blind | 2.2% | 0.4% | 0 | 4.6% ^c | 1% | Not separately reported | 0.9% |
| Deaf/require aids | 4.3% | 1.4% | 2.5% | Requires aids: 1.7% | Not improved by aids: 0.2% Improved by aids: 5% | Not separately reported | Not improved by aids: 0.2% Improved by aids: 0.7% |
| Developmental/cognitive | BSID-II MDI <70: 51% MDI <50: 19% | BSID-III Cognitive 70–84: 16.5% <70: 9.3% | ^a DQ None/≤1 SD: 52% 1–2 SD: 32% 2–3 SD: 12% >3 SD: 4% | ^a KSPD DQ <70: 35% <50: 11% | ^a Predicted MDI 70–84: 34% <70: 30% | ^e BSID-II MDI <70: 19% PDI <70: 20% | ^a Cognitive/language None: 55% Mild: 25% Moderate: 11% Severe: 9% |
| Cerebral palsy (CP) or motor delay | Moderate: 8.7% Severe: 6.2% | Moderate: 3.4% Severe: 2.5% | Any CP: 9.8% | Any CP: 13.7% Profound CP: 8.2% | Any CP: 14% Moderate motor: 3% Severe motor: 5% | ^e Any: 7.6% | Mild: 2.9% Moderate: 2.9% Severe: 1.3% |
| Disability or impairment | Any NDI: 58.5% Profound NDI: 17.5% None/minimal: 21.9% | Moderate NDI: 23.5% Severe NDI: 13.6% | None: 51% Mild: 29% Moderate: 16% Severe: 4% | ^d Any: 42.4% ^d Profound: 22.6% | None/mild: 75% Moderate: 12% Severe: 13% | Favorable: 64% Moderate: 24% Severe: 11% | None: 42% Mild: 31% Moderate: 16% Severe: 11% |

^aFor VICS, DQ = developmental quotient compared with a contemporaneous NBW control group; for EPICure 2, predicted MDI BSID, 2nd ed, from Bayley-III; for EXPRESS, aggregated Bayley-III cognitive and language score information, with mean and SD relative to a contemporaneous 37–41 week GA control group; for Japan NRN, formal evaluation by the KSPD was available for only 318 participants.

^bFace-to-face study examiner evaluations occurred in 55.3%. Multiple imputation from complete perinatal, neonatal, and sociodemographic information estimated outcomes for the entire cohort.

^cDefined as no functional vision in one or both eyes.

^dIncludes 173 with informal assessments of developmental delay by pediatricians, without formal developmental testing.

^eData regarding CP and BSID-II scores were reported in Schlapbach et al. (2011).

BSID-II, Bayley Scales of Infant and Toddler Development II; BSID-III, Bayley Scales of Infant and Toddler Development III; DQ, developmental quotient; EGA, estimated gestational age; EXPRESS, Extremely Preterm Infants in Sweden Study; GA, gestational age; KSPD, Kyoto Scale of Psychological Development; MDI, Mental Developmental Index; NBW, normal birthweight; NDI, neurodevelopmental impairment; NICHD, National Institute of Child Health and Human Development; NRN, Neonatal Research Network; SD, standard deviation; VICS, Victoria Infant Collaborative Study Group.

All neonates born at 22 to 26 1/7 weeks' gestation in the United Kingdom during 2006 were identified and data about death and morbidities were collected from the delivery room through hospitalization (Costeloe et al., 2012). Comparisons between EPICure 1 (Costeloe et al., 2000) (1995 birth cohort) and EPICure 2, both on short-term and neurodevelopmental outcomes, were limited to those 22 to 25 1/7 weeks' gestation and born in England. For EPICure 2, follow-up was hampered by changes in national research governance procedures in the United Kingdom and increased privacy restrictions by the National Health Service. Therefore tracking success was substantially limited in comparison with the previous study, and multiple imputation was therefore used to estimate outcomes of patients lost to follow-up. Follow-up assessments in EPICure 2 were with the BSID-III at a target age of 36 months, and at a target age of 30 months with the BSID-II in EPICure 1. In order to directly compare results from the two cohorts, combined BSID-III scores were converted to "predicted MDI" (Moore et al., 2012b). "Severe impairment" was defined as nonambulant CP (GMFCS III–V), blindness, profound hearing loss, or developmental quotient less than 3 SD below the mean for age. "Moderate impairment" was defined as ambulant CP (GMFCS 2), functionally impaired vision, hearing loss improved by aids, or developmental score of II–III SD below the mean.

Of the 1031 children in EPICure 2 who survived to follow-up (1041 survivors to discharge, 10 died after discharge), study examiners evaluated 576 (55.3%, median 34 months), and information from an additional 191 were available from local records (18.3%, median 25 months) (Table 66.1). The groups seen in person and not seen in person had similar baseline perinatal and neonatal characteristics, but socioeconomic factors differed; children from families with greater social disadvantage were less likely to have a formal study evaluation.

Among the EPICure 2 group with formal study evaluations, 75% were free from impairment or had mild impairment. Survival without disability for the entire EPICure 2 cohort, including imputed outcomes, among those admitted to the NICU demonstrated increases with each week in gestational age: 22 weeks, 5% (95% confidence interval [CI], 0%–26%); 23 weeks, 15% (95% CI, 10%–21%); 24 weeks, 30% (95% CI, 25%–35%); 25 weeks, 49% (95% CI, 43%–55%); 26 weeks, 62% (95% CI, 57%–67%). In addition, 66% had no hearing, vision, or communication disability, but only 36% of children were reported to have no developmental disability (BSID-II scores >85). However, robust comparisons are difficult, given that patient numbers were quite small, particularly for the less than 23 weeks' EGA group. This finding underscores the grave prognosis for survival among the most premature infants in this cohort; only 18% of less than 23 weeks' EGA neonates admitted alive to an NICU survived to discharge, compared with 48% of 25 weeks' EGA neonates.

Extremely Preterm Infants in Sweden Study

The Extremely Preterm Infants in Sweden Study (EXPRESS) cohort (birth year 2004–2007, <27 weeks' gestation) was followed to a median of 30.5 months, at which point BSID-III, neurologic examinations, and parental questionnaires were administered (Serenius et al., 2013). Of the 707 liveborn infants, 491 survived to follow-up (69%), 415 of 461 who were eligible for inclusion were assessed (90% follow-up rate), and 399 of these completed at least part of the BSID-III. Chart review was available for 41 additional children. The group was skewed to higher gestational age, with only 52 (11%) of the cohort born at 22 or 23 weeks' gestation. Of importance, a control group (37 to 41 weeks' gestation,

matched 2:1 with the preterm group) was recruited by random selection from the Swedish Medical Birth Registry. Severe disability was defined as BSID-III composite cognitive, language, or motor score greater than 3 SD below the mean relative to the control group, severe CP, or bilateral blindness or deafness. Moderate disability was defined as scores between 2 and 3 SD from the mean of any of the BSID-III scales, moderate CP, and moderate visual or hearing impairment. Mild disability was defined as scores between 1 and 2 SD from the mean of any of the BSID-III scales or mild CP.

As summarized in Table 66.1, of those children born EPT, 11.3% had moderate or severe cognitive disability by BSID-III, compared with 0.5% of controls. When considering cognitive, language, or motor scores, 15% of EPT and 3% of control had moderate disability, and 8.9% of EPT and 0.3% of controls had severe disability. The proportion of children with mild or no disabilities increased from 40% at 22 weeks to 83% at 26 weeks. There was a significant decrease in severe disabilities with each increase in gestational week, with an odds ratio (OR) 0.58 (99% CI, 0.39–0.86), $P < .001$. Overall, 42% of the EPT children in this group had no disability at 30 months, compared with 78% of control children. The majority of the 58% disabled EPT children had mild disability (31%). In further analyses, Serenius et al. (2015) explored whether intensity of perinatal care in regions across Sweden was associated with an increased risk of death or NDI at 2.5 years. The investigators found significant variation in obstetric and neonatal intervention practices. In regions with more aggressive perinatal intervention practices, the risk of death or NDI at 2.5 years was reduced, but only for the 22 to 24 weeks' group. Furthermore, there was no increase in NDI among survivors associated with more aggressive perinatal practices.

Japan Neonatal Research Network

The NRN of Japan reported outcomes of neonates born alive at 22 to 25 weeks' gestation during 2003 to 2005 (Ishii et al., 2013). Neurodevelopmental assessments were performed at 36 to 42 months chronologic age and consisted of a neurologic examination, functional assessment for vision and hearing, and cognitive evaluation by the Kyoto Scale of Psychological Development (KSPD). A developmental quotient (DQ) was derived, with a mean SD score of 100.6 ± 13.4 . NDI for this study was defined as any CP with GMFCS II–V, hearing impairment, visual impairment, or KSPD DQ score of less than 70. Profound impairment was defined as DQ less than 50 or CP with GMFCS IV or V.

Highlights of outcomes are shown in Table 66.1. Among 782 survivors at 3 years, 562 (71.9%) were evaluated in a follow-up visit. Follow-up rate among survivors declined over gestational age weeks, from 85.2% (23/27) for those born at 22 weeks' gestation, to 69.6% (240/345) for those born at 25 weeks' gestation. Among those examined, CP of any severity was diagnosed in 13.7%, and profound CP was seen in 8.2%, with higher rates noted at lower gestational ages (22 weeks, 17.4%; 23 weeks, 10.2%). Only 318 children were formally assessed by the KSPD (56.6% of those followed), whereas 173 were evaluated as delayed or not delayed by judgment of a pediatrician. If judged as "delayed" by a pediatrician, the DQ was assumed to be less than 50 for the purposes of this study; therefore rates of profound NDI or profound developmental delay may be overestimated when including these informal evaluations. Among those formally assessed, 34.6% had developmental delay of any severity (KSPD <70), and 11% were profoundly delayed (KSPD <50). For the children evaluated by a pediatrician only, 37% were assessed as delayed. Overall, when including any

assessment, NDI of any severity was reported for 208/491 (42.4%) of survivors. The composite outcome of death or any NDI was reported in 45.7% (483/1057) and death or profound NDI in 36.5% (386/1057).

Eunice Kennedy Shriver NICHD Neonatal Research Network Follow-Up Study Group

The NICHD NRN was initiated in 1986 as a multicenter effort in the United States with the main objective of providing a registry of uniformly collected baseline and morbidity and mortality data information to provide the basis for planning and implementing clinical trials. The NICHD NRN Follow-Up Study Group was later added to provide neurodevelopmental follow-up for trials and for those meeting NRN Follow-Up Study criteria, which now includes infants less than 27 weeks' gestation.

The NICHD NRN reported 18 to 22 months' corrected age outcomes of neonates inborn at an NRN site at less than 25 weeks' gestation during two epochs, 1999 to 2001 (epoch 1) and 2002 to 2004 (epoch 2) (Hintz et al., 2011). The follow-up visit included the BSID-II, as well as neurosensory and motor examinations. NDI was defined as MDI or PDI less than 70, moderate-to-severe CP (nonambulatory or requiring assistive devices), bilateral blindness (absence of functional vision in both eyes), or bilateral severe hearing loss requiring amplification. Profound NDI was defined as MDI less than 50, or GMFCS level 4 or 5. Unimpaired or minimally impaired was defined as having none of the following: moderate-to-severe CP, bilateral severe hearing loss or blindness, MDI or PDI less than 85. Follow-up rates for both epochs were approximately 90%. Only approximately 25% of both follow-up groups were composed of children born at less than 23 weeks' gestation.

Although results showed an apparent absolute increase in NDI from 50.1% to 58.7% between epoch 1 and 2, epoch was not found to be associated with NDI on multivariable analyses. Profound NDI was not significantly increased between epoch 1 (16.8%) and epoch 2 (17.5%). Rates of adverse outcomes were higher for children of less than 23 weeks' gestation than 24 weeks' gestation in both epochs, although patient numbers were small for rarer outcomes. Despite more aggressive perinatal management with increased cesarean section delivery (40.9%–48.8%) and dramatic reduction in postnatal steroid use (63.5%–32.8%) from epoch 1 to 2, no significant improvement in neurodevelopmental outcomes was observed. However, this study differed from others in that it narrowly focused on those less than 25 ½ weeks, and they were evaluated at an earlier age. These cohorts were also not population-based but rather representative of the academic centers in the NRN. As with previous analyses from this group (Vohr et al., 2004), center differences in outcomes were observed, suggesting that variability beyond what can be explained by perinatal and neonatal risk factors plays an important role in outcomes.

Between-hospital variation in survival and NDI at 18 to 22 months was further explored by Rysavy et al. (2015) among infants inborn at an NRN site before 27 weeks' gestation between 2006 and 2011. Impairment was defined similarly as above, with the exception that BSID-III cognitive scores of less than 70 were categorized as severe NDI, and scores of 70–84 were categorized as moderate NDI. Outcomes were evaluated in relation to hospital rates of "active treatment," defined as potentially lifesaving treatments initiated after delivery. For all infants, overall mean rates of survival without severe impairment were 3.4%, 17.9%, 44.7%, 61.1%, and 75.6% for 22, 23, 24, 25, and 26 weeks' gestation. However, there were significant differences among hospital rates

of active treatment for those born at 22 to 24 weeks, which accounted for a substantial proportion of variation in outcomes for children born in that gestational age range. The BSID-III was utilized during this study period, and there was no term control group.

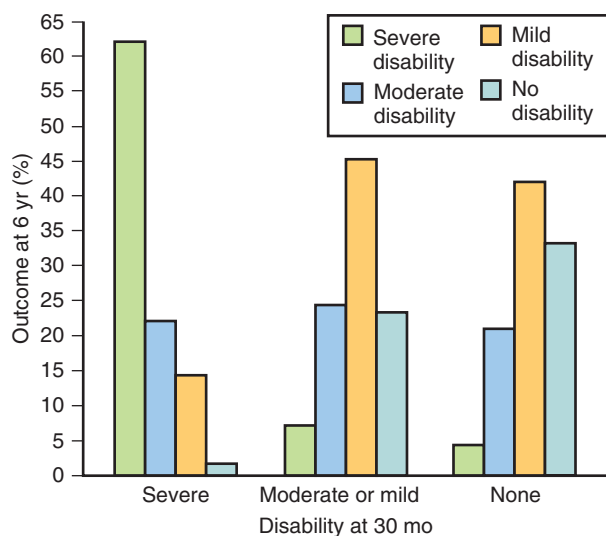
Swiss National Extremely Preterm Cohort

The Swiss national EPT cohort study was a prospective, longitudinal study of infants live-born between 24 ½ and 27 ½ weeks' gestation in Switzerland between 2000 and 2008 (Schlapbach et al., 2012). Outcomes at 18 to 24 months corrected age were reported only for infants evaluated at sites utilizing the BSID-II. Severe disability was defined as CP with GMFCS level III–V, MDI or PDI less than 55, absence of useful hearing even with aids, or bilateral blindness. Moderate disability was defined as CP with GMFCS level II, MDI or PDI 55–69, hearing loss corrected with aids, moderately reduced vision but better than severe, or unilateral blindness with good vision in the contralateral eye. Favorable outcome was defined as survival without moderate or severe disability. Of 1266 liveborn infants, 844 survived to 18 to 24 months, of whom 684 had follow-up, including BSID-II testing (81%). For the cohort overall, 166 (24.3% of those with follow-up) were reported to have moderate disability, and 78 (11.4%) had severe disability. In a previous report from this group that included neonates born through December 31, 2007, CP and BSID-II scores were reported (Schlapbach et al., 2011). Of 541 children assessed at 18 to 24 months corrected age, 41 (7.6%) had CP of any severity. Of those with BSID-II scores, PDI less than 70 and MDI less than 70 were present in 99/485 (20.4%) and 99/527 (18.8%), respectively.

School-Age Outcomes After Prematurity

School-age survivors of prematurity have high rates of NDI, with CP rates of 9%–10% and cognitive disability rates of 4%–36%, depending on the population evaluated, outcome definitions, and age at assessment (Jarjour, 2015). Infants who have severe cognitive, motor, or neurosensory impairments in early years (2 to 3 years of life) are nearly always found to have moderate or severe impairments at school age (Marlow et al., 2005; Kodric et al., 2014). In the EPICure cohort of neonates born at less than 25 weeks' gestational age, 86% of children with severe disability at 30 months continued to have moderate or severe disability at 6 years (Fig. 66.1) (Marlow et al., 2005). More commonly though, school-age ELBW or very preterm survivors have more mild impairments that are difficult to classify correctly at earlier ages. These impairments include mild cognitive impairment (IQ 1–2 SD below the mean or 70–84); learning, emotional, behavior, motor coordination, and executive function disorders; and poor academic achievement.

Cognitive delay is the most common impairment in children who were born VPT. In a 2002 systematic review that included 1556 preterm infants and 1720 controls at 5 to 14 years of age, controls had significantly higher cognitive scores (mean difference 10.9 points) (Bhutta et al., 2002). The most immature infants have the highest risk for poor cognitive outcome; at 6 years 49% of boys and 32% of girls in the EPICure cohort (≤25 weeks' gestation at birth) had cognitive impairment (Marlow et al., 2005). Moderate and late preterm infants may even have an increased risk for poor cognitive functioning compared with full-term peers at school age (de Jong et al., 2012). Importantly, biologic factors such as sex, birth weight, and race are predictive of early



• **Fig. 66.1** Severity of disability at 6 years, based on classification at 30 months corrected age. Data are based on 236 children who were born at 25 or fewer completed weeks of gestation in the United Kingdom and Ireland in 1995. (Reproduced from Marlow N, Wolke D, Bracewell MA, Samara M; EPIcure Study Group. Neurologic and developmental disability at six years of age after extremely preterm birth. *N Engl J Med*. 2005;352:9–19. With permission from the Massachusetts Medical Society.)

cognitive outcomes (Linsell et al., 2015). However, as children enter school age, parental education is more predictive of cognitive outcome than biologic or perinatal factors. Furthermore, children with social advantages (two-parent household, educated parents, employed parents) demonstrate more cognitive gains through early childhood than those without social advantages (Manley et al., 2015).

Similarly, in 14 studies of school-age academic achievement, VLBW or VPT infants have scores on math, reading, and spelling tests that are 0.5–0.75 SD below term peers (Aarnoudse-Moens et al., 2009). Performance on academic assessments is also closely correlated with both birthweight and gestational age (Bhutta et al., 2002; Aarnoudse-Moens et al., 2009). Thus the most immature neonates born at less than 25 weeks' gestation have eight times higher odds of serious impairment in academic achievement than matched term controls (Marlow et al., 2005).

Measures of executive function (verbal fluency, working memory, and cognitive flexibility) also demonstrate decreases of about 0.5 SD between VPT or VLBW infants and term controls (Anderson and Doyle, 2004; Aarnoudse-Moens et al., 2009). These, however, do not seem to be correlated with birthweight or gestational age and may not be influenced by socioeconomic status (Anderson and Doyle, 2004).

At school age, children who were born preterm are more frequently reported by both their parents and their teachers to have behavior problems. VPT and even late preterm infants are more likely to have internalizing or externalizing symptoms on the Child Behavior Checklist than term counterparts (Bhutta et al., 2002; Delobel-Ayoub et al., 2009; Talge et al., 2010). Internalizing symptoms manifest as shyness, social maladaptation, anxiety, and withdrawn behavior (Anderson and Doyle, 2003; Aylward, 2005). It is critical to note that behavioral problems commonly occur in children with cognitive problems. In the French regional EPIPAGE cohort of children born at less than 33 weeks' gestation, there was a direct correlation between cognitive performance and

behavioral problems (Delobel-Ayoub et al., 2009). Infants with an IQ greater than 2 SD below the mean had 2.6 times higher odds of behavioral problems than those with an IQ less than 1 SD below the mean. However, cognitive disabilities did not explain the higher rates of behavioral problems in preterm-born children, because preterms remained at higher risk even after adjustment for cognitive performance. Behavioral problems in this cohort were more common in boys and in children with more complex medical histories, socioeconomic risk factors, and poor maternal well-being.

Neurobehavioral or psychiatric problems, including autism spectrum disorders (ASDs) and attention deficit-hyperactivity disorder (ADHD), are reported with higher frequency in school-aged former preterm children than in the general population (Bhutta et al., 2002). In the Extremely Low Gestational Age Newborn (ELGAN) study of neonates born before 28 weeks' gestation, 7% were diagnosed with ASD at school age (Kuban et al., 2016). Boys were twice as likely as girls to have ASD in this cohort. Moderate and late preterm infants may also be more likely to have ASD: one study reported that these children have a 1.55 higher odds of being diagnosed with autism by 10 years than full-term born children (Buchmayer, 2009). In preterm children, risk of ASD seems to be particularly increased among those who were small for gestational age, those with congenital malformations, low 5-minute Apgar scores, intracranial bleeding, cerebral edema, or seizures in the neonatal period and those born to mothers with preeclampsia (Buchmayer et al., 2009).

In a systematic review, preterm-born children had a relative risk (RR) of being diagnosed with ADHD of 2.64 (95% CI, 1.85–3.78) when compared with term-born children (Bhutta, 2002). In a large Swedish cohort, odds of ADHD at school age decreased with increasing gestational age until term corrected age (Lindström et al., 2011). Odds of ADHD were 2:1 among the most immature (23–28 weeks' gestation) infants and 1:1 among early term (37–38 weeks' gestation) infants. In the most preterm children, risk of ADHD does not seem to be significantly impacted by socioeconomic factors. However, sociodemographic risk—particularly low maternal education—may increase the risk for ADHD in moderately preterm children born to women with lower education (Lindström et al., 2011). Across the entire gestational age spectrum, school-age boys are about four times more likely to be diagnosed with ADHD than girls.

Because adverse developmental and neurobehavioral outcomes are considerably more common among preterm infants, it is essential for the pediatrician caring for former preterm school-age children to have a high index of suspicion for these problems. Early access to services is essential for optimizing outcomes. Many developmental and behavioral problems are more common among preterm-born male children than female children. Furthermore, while risk is highest in the most EPT infants, moderately preterm and late preterm infants are also at higher risk for adverse outcome at school age than term-born counterparts. School-age problems in the older preterm infant include fine and gross motor coordination problems, academic difficulties, attention problems, and need for special support in school (Huddy et al., 2001). As moderate and late preterm infants far outnumber VPT infants, it is critical to be aware of the likelihood of such outcomes and address any concerns or potential problems expeditiously. Cognitive and neurobehavioral problems in all high-risk children significantly increase special education costs and resource utilization at school age and strongly influence outcomes later in life.

Adolescent and Adult Outcomes After Prematurity

The advent of assisted ventilation and then surfactant and antenatal steroids in the late 20th century led to the first substantive generations of ELBW survivors. These surviving preterms are now adolescents and adults, whose outcomes are of importance not only to pediatricians but also to the adult physicians who will care for them for the remainder of the life course. The association between increased risk for all categories of adverse outcomes and decreasing gestational age continues into adolescence and adulthood. Medical sequelae of prematurity include impact on respiratory, cardiovascular, and renal function. Surviving ELBW infants are far more likely to have CP, blindness, and deafness compared with term-born matched controls (Doyle et al., 2010). As these are typically fixed impairments, rates in adulthood closely mirror rates at school age. Furthermore, over time, ELBW survivors have higher rates of other visual impairments, including refractive errors and late retinal detachment (Saigal et al., 2007). In adulthood, preterm survivors are more likely to receive disability pensions than term-born controls (Doyle et al., 2010).

VPT, VLBW, and moderately preterm-born adolescents are less likely to complete high school than adolescents born at full term (Hack et al., 2002; Lindström et al., 2007; Mathiasen et al., 2009). Furthermore, VLBW-born or VPT-born adults are less likely to seek education beyond high school (Hack et al., 2002; Cooke, 2004; Mathiasen et al., 2009). In adulthood, preterm-born cohorts have worse performance on tests of academic achievement than term-born controls (Hack et al., 2002). Few studies have closely evaluated cognitive function in preterm survivors. IQ is generally reported to be about 0.5 SD below term controls, but differences depend on the population studied. Predictors of adolescent and young adult IQ include maternal educational level, birthweight, and gestational age. For example, two studies have reported IQ of 87 versus 92 in adolescents with a birthweight of less than 1500 g and 94 versus 108 in adolescents with a birthweight of less than 1000 g, when compared with full-term controls (Hack et al., 2002; Lefebvre et al., 2005).

Even in adults without neurosensory or cognitive impairment, impairments in learning or executive function may interfere with educational and vocational achievement. Executive function deficits primarily involve impairments in response inhibition and mental flexibility (Nosarti et al., 2007). As discussed above, ASD and ADHD are more common among both VPT or VLBW children and even moderate/late preterm children; these disorders likely persist into adulthood (Mostert et al., 2008; Lindström et al., 2009). Reports of increased rates of schizophrenia, anxiety, and depression among preterm-born adults are inconsistent and conflicting (Hack et al., 2004; Mostert et al., 2008). In a large Norwegian study, RR of ASD was 9.7 times higher among adults born before 28 weeks' gestation as compared with those born at term (Mostert et al., 2008). Furthermore, RR of having a disorder of psychological development, behavior, and emotion resulting in a need to claim disability benefits was 10.5 times higher among those born before 28 weeks' gestation.

Overall, 23 to 27 weeks' gestation-born adults are 7.5 (95% CI, 5.5–10.0) times more likely to have a medical disability affecting the ability to work, as compared with term-born adults (Mostert et al., 2008). Among those who do not have a medical disability, VPT-born adults have significantly lower educational level, have lower income, and are less likely to get married and become parents (Mostert et al., 2008; Saigal et al., 2016). RR of having at least

one child among men born at 22 to 27 weeks' gestation is 0.24, RR among women is 0.33 (Swamy, 2008). Furthermore, preterm-born women are more likely to have preterm babies. Preterm-born adults are less likely to move outside their parents' home or cohabitate with a partner (Swamy et al., 2008; Mathiasen et al., 2009). Net income is significantly lower among VPT-born adults (Mathiasen et al., 2009; Saigal et al., 2016). On the positive side, preterm and VLBW-born adults are consistently reported to have lower rates of risk-taking, including smoking, alcohol, and drug use, and delinquent behavior (Hack et al., 2004; Saigal et al., 2016). However, this may be due to difficulty with socialization related to higher rates of internalizing behaviors.

Health-related quality of life is an important consideration as our most fragile and vulnerable patients age into adulthood (Dinesen and Greisen, 2001; Saigal et al., 2006). Only a few small studies have evaluated the long-term impact of prematurity on self-reported quality of life. Saigal and colleagues assessed health-related quality of life in a population of ELBW neonates born in 1977 to 1982 when the participants were 24 years old. Despite more functional limitations (cognition, sensation, mobility, and self-care), the ELBW participants did not report significantly different health-related quality of life from term-born controls. Later, in their thirties, the same cohort indicated lower self-esteem along with lower measures of multiple functional and health-related outcomes (Saigal et al., 2016). Similarly, in a small Danish cohort of young adults, objective quality of life (based on societal standards) was lower in those born VPT or with a chronic health problem (Dinesen and Greisen, 2001). However, subjective quality of life (based on individual life preferences and experiences) was not significantly different.

Thus the impact of prematurity on medical, developmental, psychological, and functional domains persists throughout the life course. It is critical to note that the most EPT (22–25 weeks' gestation) infants are only now surviving into adulthood. Adolescent and adult impact of survival at the limits of viability remain to be fully described in the current era of neonatal medicine.

Risk Factors for Adverse Outcomes in Preterm Infants

As described above, preterm infants as a group are at risk for multiple adverse outcomes throughout childhood and into adulthood. These risks are modified by patient characteristics, morbidities, and complications that occur during the neonatal period. Important neonatal morbidities that directly impact outcomes of preterm infants include brain injury, bronchopulmonary dysplasia (BPD), ROP, infection, necrotizing enterocolitis (NEC), and poor growth and nutrition. We end this section by addressing the impact of socioeconomic factors on outcomes.

Brain Injury

Cranial Ultrasound

Cranial ultrasound (CUS) has been used to image preterm infant brain injury since the late 1970s (Pape et al., 1979; Slovis et al., 1981). With the development of a standardized grading system for intracranial hemorrhage (ICH) (Papile et al., 1978), CUS quickly became and remains the neuroimaging standard of care for preterm infants (Ment et al., 2002). Although many still seem to rely heavily on simply the presence of grade 3 ICH, intraparenchymal hemorrhage (IPH), or cystic periventricular leukomalacia (PVL) to counsel families about the neurodevelopmental outcomes

of their preterm infants, the complexity of interpretation of CUS findings, and limitations of prediction of outcomes with any single neuroimaging or other finding, should give clinicians cause for prudence and careful consideration.

Virtually every major study of early neurodevelopmental outcomes among preterm and ELBW infants has confirmed a strong association between major CUS abnormalities and adverse neurologic and developmental outcomes. Definitions of CUS abnormalities as well as specific outcomes differ among studies; however, most consider IPH, ventriculomegaly (VM), or cystic changes, regardless of laterality or extent of the findings, to be severe abnormalities. In some, persistence of periventricular echodensity or “flaring” is included (de Vries et al., 2004, 2011; Ancel et al., 2006). The diagnosis reported in studies is frequently based on the results from a single CUS, either the “worst” or the “final” imaging study, but some prospective cohorts include serial imaging.

The focus of many studies has been on exploring the association of major CUS findings with CP. The ELGAN study followed infants of less than 28 weeks’ gestation from 14 institutions across five states in the United States from 2002 to 2004 (Kuban et al., 2009; O’Shea et al., 2009). Three study CUSs were performed during hospitalization and by multiple radiologists. BSID-II and standardized neurologic examinations for CP were performed at 2 years. The investigators found strong independent associations between CUS findings and CP. About half of the children with CUS echolucency or VM developed CP, and late occurrence of VM, bilateral echolucency, and IPH or PVL were strongly predictive of quadriplegia. However, almost half of the children with CP at 2 years had completely normal CUS, and the PPV of VM or echolucency for moderate or severe CP was poor. Isolated intraventricular hemorrhage (IVH) was not strongly predictive of CP.

Furthermore, CUS findings alone are poorly predictive of early developmental outcomes or later childhood cognitive and learning outcomes (Hack et al., 2000; Wood et al., 2005), and 30%–40% of those with “normal” CUS have neurodevelopmental challenges at 18 to 30 months (Laptook et al., 2005). The ELGAN study group showed that IVH was associated with increased risk for motor or developmental impairment at 2 years *only* when accompanied or followed by white matter lesions, highlighting the limited predictive value of both early CUS findings and IVH alone. In longer-term follow-up studies of the EPIPAGE cohort born at 24 to 28 1/7 weeks’ gestation, major CUS abnormalities remained strongly associated with CP (O’Shea et al., 2012). However, approximately 40% of those with major neonatal CUS abnormalities had no significant cognitive or learning challenges identified at 8 years, whereas 30%–40% of those with no neonatal CUS abnormalities had moderate-to-severe challenges (Beaino et al., 2010). This underscores the need for long-term surveillance through childhood for all born EPT. Nevertheless, in skilled hands, with meticulous technical attention and serial CUS imaging, much can be seen beyond ICH by CUS. In a single center, deVries et al. (2004) reported 76% sensitivity and 95% specificity of CUS abnormalities for CP at 2 years for patients of less than 32 weeks’ EGA. Of importance, among those with major CUS abnormalities who developed CP, approximately 30% were noted only after 28 days. Major CUS abnormalities were also not strongly associated with cognitive delay at 2 years. More recent findings from this group, using magnetic resonance imaging (MRI) at term equivalent age to refine specific CUS findings, have resulted in PPV and negative predictive value of 96% and 69%, respectively, for CP at 2 years among preterm infants (de Vries et al., 2011).

CUS is an operator-dependent modality, imaging procedures and views differ among institutions and studies, and there is no uniform approach to serial imaging protocols. Although interrater reliability and accuracy are very good to excellent for severe ICH, agreement is only fair or poor for subtler findings and PVL alone (Hintz et al., 2007). Cerebellar hemorrhage, a finding that may be missed without appropriate CUS views, is increasingly recognized to be associated with neurodevelopmental disabilities in children born preterm (Limperopoulos et al., 2007, 2014). Transient lesions may be missed, including echodense periventricular lesions or collapsing small cystic lesions (Pierrat et al., 2001). Isolated IPHs and of course large IVHs can be seen by CUS; however, not all “severe” hemorrhages can be considered equivalent in terms of association with early neurodevelopmental outcomes. Characteristics of the hemorrhage including laterality, midline shift, and extent of hemorrhage (Bassan et al., 2007; Davis et al., 2014), as well as the presence or absence of other adverse clinical factors (Merhar et al., 2012), impact prediction of neurodevelopmental outcomes.

Magnetic Resonance Imaging

Brain MRI has been used more extensively in recent years among preterm infants, both for research and for clinical indications. MRI provides a more comprehensive and detailed picture of the brain, with better delineation of deep structure and cortical injury. Potentially most importantly, MRI provides improved detection of white matter injury (WMI), which is common among preterm infants at term corrected age. Identification of WMI is critically important to understanding the structure–function relationship of the developing preterm brain, influences on later neuromotor and cognitive outcomes, and developing future neuroprotective strategies (Volpe et al., 2011). Subtle WMI on MRI is associated with reduced total brain and gray matter volumes, reduced cerebellar volume, and reduced basal ganglia and thalamic volume, which in turn are associated with childhood developmental impairments among preterm infants. These findings and others provide evidence that WMI in the preterm is associated with brain maturational disturbances, suggesting an overall link to impaired neural connectivity (Dean et al., 2014). Thus clinical investigations have focused on whether MRI may provide enhanced prognostic information.

Early studies attempting to compare term equivalent MRI with CUS predictive capabilities were limited by study size and different approaches to timing of imaging (Roelants-van Rijn et al., 2001; Mirmiran et al., 2004; Sie et al., 2005). Subsequently, WMI scoring approaches have been developed, and larger cohort studies have been published, among the first of which was a multicenter effort in Australia and New Zealand comparing serial CUS with near-term MRI findings and their association with 2-year outcomes in 167 infants of less than 30 weeks’ EGA (Woodward et al., 2006). This study demonstrated that moderate-to-severe WMI on near-term MRI was significantly associated with neuromotor delay and CP, independent of CUS findings and other risk factors. Increasing WMI severity was also related to lower BSID-II MDI scores, but an independent association of moderate-to-severe WMI with severe cognitive delay was not detected. However, CUS was assessed only with regard to early findings including grade of ICH and periventricular cystic changes, and a substantial proportion of infants with moderate-to-severe WMI by MRI did not have adverse 2-year outcomes. The NICHD Neuroimaging and Neurodevelopmental Outcomes study was a prospective study of early and late CUS and near-term MRI, including 480 infants of less than 28 weeks’

gestation, with outcomes including BSID-III assessed at 18 to 22 months (Hintz et al., 2015a). In multivariable models, both late CUS findings reflective of WMI and MRI findings of significant cerebellar injury remained independently associated with adverse neurodevelopmental outcomes. In models that did not include late CUS, MRI findings of both moderate-to-severe WMI and significant cerebellar lesions were independently associated with adverse outcomes. Early CUS findings were not associated with adverse outcomes when any late neuroimaging was taken into account. These results demonstrate the need to understand the evolution of brain injury over time rather than to rely on early findings. Similarly, in a prospective study of serial CUS and near-term MRI among neonates of less than 27 weeks' gestation in Sweden, associations between MRI findings and 30-month outcomes were shown, but the investigators determined that any substantial abnormalities on MRI were detected by the late CUS done on the same day (Skiöld et al., 2013). Diffuse excessive high signal intensity on near-term MRI has been shown not to be associated with adverse early childhood outcomes in several studies (Jeon et al., 2012; Skiöld et al., 2012).

In summary, despite what appears to be substantial experience with CUS and conventional brain MRI in preterm infants, controversies and questions remain as to which studies to perform, when to perform them and under what circumstances, and relative values in prognosis. These are not simple questions, as the "value" of additional information may vary by clinical circumstances and for individual parents and physicians (Janvier and Barrington, 2012). Cerebellar injury seen by MRI but not by CUS may be associated with higher risk for neurodevelopmental abnormalities (Tam et al., 2011; Hintz et al., 2015a), although the importance of punctate lesions remains unclear (Steggerda et al., 2013). Other studies have shown that MRI may provide additive information to predict neuromotor outcomes (de Vries et al., 2011), complementary to specific findings such as periventricular echodensities by CUS (Sie et al., 2005) or neurologic examination (Spittle et al., 2009; Skiöld et al., 2013). Neonatal sepsis and NEC have been linked with progressive or higher rates of WMI on MRI, and adverse 2-year outcomes associated with these morbidities (see later) may be mediated by WMI (Glass et al., 2008; Shah et al., 2008). Nevertheless, guidelines published in 2002 do not recommend near-term MRI for routine preterm infant neuroimaging (Ment et al., 2002), and a recent publication has suggested avoidance of routine term equivalent conventional brain MRI for screening purposes because there is "insufficient evidence that the practice improves long term outcomes" (Ho et al., 2015).

Future studies should further focus on identifying specific high-risk groups of preterm infants for which MRI would definitively improve prediction of neurodevelopmental outcomes and allow for risk stratification for neuroprotective or interventional studies. Investigations with advanced magnetic resonance techniques, including diffusion tensor imaging, functional connectivity MRI, surface morphometry, and volumetric methods, hold enormous promise to help to explore these questions (Anderson et al., 2015).

Bronchopulmonary Dysplasia

Bronchopulmonary dysplasia (BPD), typically defined as oxygen dependence at 36 weeks' postmenstrual age, is the most common morbidity of prematurity. BPD occurs in nearly half of neonates born at less than 28 weeks' gestation, and rates may be increasing over time (Stoll et al., 2015). BPD is associated with adverse

respiratory, developmental, educational, and health economic outcomes. EPT infants with BPD have increased coughing, respiratory medication use, hospitalizations, and impact of respiratory disease on the family at 18 to 22 months corrected age, compared with those without BPD (Stevens et al., 2014). A metaanalysis of pulmonary function testing in children born preterm with BPD, those born preterm without BPD, and children born at term demonstrated significantly decreased percentage forced expiratory volume in one second, with a further decrease in those with BPD (Kotecha et al., 2013). Several investigators have reported poor neurodevelopmental outcomes among infants with BPD, compared with preterm infants without BPD (Lifschitz et al., 1987; Portnoy et al., 1988; Grégoire et al., 1998; Majnemer et al., 2000; Roberts et al., 2009; Lodha et al., 2014; Schmidt et al., 2015). BPD is a consistent independent predictor of adverse developmental outcomes at 2 and 5 years (Schmidt et al., 2012). The adjusted odds ratio for death or disability at 5 years is 2.3 (95% CI, 1.8–3.0) times higher among very preterm born children with BPD than among those without BPD (Schmidt et al., 2015). Reports of cognitive outcomes at 8 years suggest that preterm-born children with BPD have lower IQs than those without (Robertson et al., 1992; Hughes et al., 1999).

Multiple large randomized trials have attempted to decrease rates of BPD in EPT neonates by applying interventions or drugs either in the delivery room or during the neonatal hospitalization. While some interventions or medications successfully decrease rates of BPD few have demonstrated improved developmental outcomes. Vitamin A reduces BPD or death in ELBW infants (RR 0.89) but does not improve developmental outcomes at 18 to 22 months (Ambalavanan et al., 2005). Postnatal corticosteroids reduce BPD but significantly increase risk for CP if given in the first 4 days of life. Use of corticosteroids after 7 days of life reduces BPD without increasing risk for CP (Halliday et al., 2003a, 2003b). Caffeine reduces BPD at 36 weeks, CP at 2 years, and severity of motor impairment at 5 years (Schmidt et al., 2006, 2007, 2012). In multiple randomized trials, surfactant therapy for respiratory distress syndrome reduces both air leak syndromes and mortality (Bahadue and Soll, 2012). It may reduce mild disability at 1 year but does not reduce moderate or severe disability at 1 year or any adverse outcomes at 2 years (Sinn et al., 2002).

Lastly, although antenatal corticosteroids reduce many complications of prematurity, including mortality and respiratory distress syndrome, it is unclear whether they improve rates of chronic lung disease (Roberts and Dalziel, 2006; Carlo et al., 2011). In the most immature (≤ 25 weeks' gestation) infants, antenatal steroids may increase rates of BPD, perhaps by leading to increased survival of high-risk infants (Carlo et al., 2011). Yet, prenatal steroid treatment is associated with a reduction in developmental disability or developmental impairment in childhood survivors (Roberts and Dalziel, 2006; Carlo et al., 2011). This includes decreased rates of cognitive impairment and CP among infants of less than 25 weeks' gestation who are exposed to prenatal steroids (Carlo et al., 2011).

Numerous neonatal trials have evaluated various methods of respiratory support in the delivery room and during the hospitalization. Early extubation to or complete reliance on noninvasive support such as continuous positive airway pressure (CPAP) has become standard of care (Pfister and Soll, 2012). However, none of these respiratory interventions has led to significant improvements in developmental outcomes (Vaucher et al., 2012).

Retinopathy of Prematurity

ROP occurs in about 60% of neonates born at less than 28 weeks' gestation in the United States, and stage 3 or higher ROP disease occurs in about 15% of those neonates (Stoll et al., 2015). Incidence of both ROP and high-grade ROP possibly requiring treatment increase significantly with decreasing gestational age (Stoll et al., 2015). With the evolution of cryotherapy, laser therapy, and antivascular endothelial growth factor (VEGF) pharmacologic treatment, blindness caused by ROP in the developed world is now rare. Nevertheless, on a global level, as many as 20,000 children annually are blind from ROP (Blencowe et al., 2013). Prematurely born children who are treated for ROP are at clear risk for long-term visual morbidities. However, infants with ROP that do not require therapy or lead to blindness are also at risk for long-term visual problems. At a 6.5-year follow-up of the Swedish EXPRESS cohort of neonates born at less than 27 weeks' gestation, 38% had at least some ophthalmologic abnormality, including blindness, strabismus, and refractive errors (Hellgren et al., 2016). Visual problems were strongly associated with ROP treatment and lower gestational age.

In addition to visual disabilities, severe ROP is associated with important nonvisual disabilities. At 5 years, stage 4 or 5 ROP or treated ROP is an independent predictor of poor neurodevelopmental outcome (Schmidt et al., 2015). This effect is similar in magnitude to and additive to the risks associated with severe brain injury or BPD (see earlier). These infants have more than four times higher odds of motor and cognitive disability than infants without severe ROP (Schmidt et al., 2014). In addition, they are more likely to have impairment in multiple domains.

The primary strategy utilized by neonatologists to reduce ROP has been restriction of supplemental oxygen. In a metaanalysis of five oxygen saturation targeting trials in EPT infants, targeting lower oxygen saturations (85%–89%) as compared with higher oxygen saturations (91%–95%) was associated with a somewhat lower risk of ROP (risk ratio 0.72, 95% CI 0.50–1.04) (Manja et al., 2015). Offsetting this potential benefit, the low saturation targeting strategy was also associated with an increase in risk of death before discharge (risk ratio 1.18, 95% CI 1.03–1.36) though not a significant difference in death by 18 to 24 months. Thus careful targeting of oxygen saturations alone is insufficient to eliminate risk of ROP in EPT infants, and low oxygen saturation targeting is unlikely to prevent enough ROP to justify potential risks.

The effect of ROP treatment itself may also have important implications for both visual and developmental outcomes. Laser therapy is the current standard treatment, but injection of anti-VEGF agents is increasingly used to treat acute ROP. In one small randomized trial, an anti-VEGF agent appeared to be superior to laser therapy for treatment of Zone 1 ROP (Mintz-Hittner et al., 2011). However, because of concerns about systemic absorption of the drug, late proliferative vascular changes that lead to retinal detachment, and only minimal differences in refractive outcomes at 2.5 years in the Mintz-Hittner et al. (2011) trial, neonatologists and ophthalmologists remain apprehensive about use of these drugs (Darlow, 2015). Such concerns are augmented by a small 2016 case series of 2-year outcomes in infants treated with laser, anti-VEGF drug, or both (Lien et al., 2016). Infants treated with both therapies had significantly higher rates of mental and psychomotor impairment on the BSID-II at 2 years than infants treated with laser. There was no difference between the laser and anti-VEGF-treated infants. Further research is needed to identify both novel

strategies for prevention of ROP as well as treatments that are safe and lead to improved visual outcomes without increasing adverse developmental outcomes.

Infection

Preterm infants are at high risk for both perinatally acquired and postnatally acquired infections. Due in large part to intense efforts to reduce iatrogenic late-onset sepsis, rates of neonatal infection have fallen in recent years. Nevertheless, about a quarter of EPT infants (born ≤ 28 weeks' gestation) have culture-positive sepsis while in the NICU (Schlapbach et al., 2011; Stoll et al., 2015). Similar to other neonatal morbidities, risk for late-onset sepsis increases significantly as gestational age decreases. Infection is associated with poor growth, and poor head growth in particular, which is an independent predictor of adverse outcome in preterm infants (see later) (Ehrenkranz et al., 2006). Furthermore, infection is associated with increased risk for low cognitive performance, CP, and vision impairment at 18 to 22 months (Stoll et al., 2004; Schlapbach et al., 2011). When adjusted for multiple factors predictive of CP, 2-year-old children with a history of extreme prematurity and confirmed sepsis have more than three times higher odds of CP (Schlapbach et al., 2011). By 5 years of age, both early-onset and late-onset sepsis in VPT infants are associated with significantly increased odds of CP but may not be associated with cognitive impairment (Mitha et al., 2013).

Infants with candida sepsis and/or meningitis and infants with bacterial meningitis experience the most significant increase in risk for poor developmental outcomes in early childhood (Bassler et al., 2009; Adams-Chapman et al., 2013). Ultimately, however, the overall risk for neurodevelopmental impairment (any one of cognitive impairment, CP, or vision or hearing impairment) associated with neonatal infection is slightly less significant than risks associated with severe IVH, ROP, or BPD (Bassler et al., 2009; Schlapbach et al., 2011).

Necrotizing Enterocolitis

NEC is diagnosed in about 7%–13% of neonates born at less than 28 weeks (Stoll et al., 2015). Infants with NEC are at increased risk for death, cognitive delay, CP, severe vision or hearing impairment, and the composite outcome of developmental impairment at 18 months (Schulzke et al., 2007; Bassler et al., 2009). In one large observational study, nearly all of this increase in risk for adverse outcome is associated with NEC requiring surgical intervention (Hintz et al., 2005). Surgical NEC and spontaneous intestinal perforation are associated with more than doubled odds of neurodevelopmental impairment among survivors (Wadhawan et al., 2014). However, similar to infection, the overall risk for adverse outcomes associated with NEC is less significant than risks associated with severe IVH, ROP, or BPD (Bassler et al., 2009).

Prenatal corticosteroid treatment leads to a significant reduction in NEC (Roberts and Dalziel, 2006; Carlo et al., 2011). Despite this reduction in NEC, as noted earlier, the impact of prenatal steroids on developmental outcomes remains uncertain. Exclusive maternal milk feeding reduces risk for NEC and improves developmental outcomes, although it is unclear that these two benefits are directly associated with one another. Several additional interventions show promise for further decreasing rates of NEC, including lactoferrin, probiotics, and use of donor milk when maternal milk is unavailable. However, there are currently insufficient data about either short-term efficacy or long-term impact on developmental

outcomes for any of these therapies to be adopted as standard of care (Sari et al., 2012; Akar, 2016).

Growth and Nutrition

A focus on growth and nutrition both in the NICU and after discharge is increasingly recognized as essential for optimization of longer-term developmental outcomes. In a large cohort study, neonates born between 501 and 1000 g were divided into quartiles of in-hospital growth velocity (Ehrenkranz et al., 2006). Increasing quartile of in-hospital growth velocity was associated with decreasing risk for CP and developmental outcomes more than 2 SD below the mean at 18 to 22 months corrected age, even after adjustment for other factors predictive of poor growth and development. Preterm infants who fail to thrive in the first 8 months after discharge have poor developmental outcomes compared with those who demonstrate catch-up growth or maintain an appropriate growth trajectory (Hack et al., 1982). Poor head growth in particular is highly predictive of adverse developmental outcomes until at least 8 to 9 years old (Hack et al., 1991).

Because of the strong associations between nutrition and growth during the first year of life and developmental outcomes, multiple strategies to improve growth have been evaluated. Early aggressive nutrition in the NICU currently includes early administration of parenteral nutrition, early enteral nutrition with maternal milk, and fortification of enteral milk feedings. While many of these strategies successfully improve growth, there is little evidence to date that these interventions improve developmental outcomes (Poindexter et al., 2006; Brown et al., 2016).

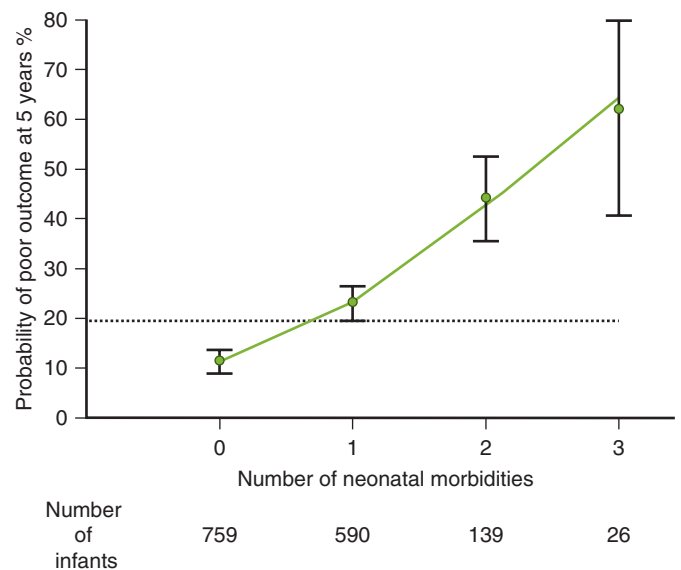
Socioeconomic Status

The critical importance of socioeconomic factors in the long-term development of high-risk infants cannot be overemphasized (Hack et al., 1992). Socioeconomic factors that influence cognitive outcomes include parent education, having two parents in the household, neighborhood, and social and racial backgrounds (Aylward, 2005; Manley et al., 2015). Furthermore, these factors influence the chance that a child's cognitive status will improve over the first years of life (Manley et al., 2015). Similarly, adverse socioeconomic factors such as younger maternal age and poor maternal well-being are strongly associated with increased behavioral problems at 5 years (Delobel-Ayoub et al., 2009).

As high-risk children get older, perinatal factors have progressively less influence on outcome, while parental education remains highly important (Linsell et al., 2015). Older children with functional limitations are more likely to live in families with limited resources, have less access to health care, and are exposed to less healthy home environments (Hogan et al., 2000). Decreased access to necessary health care is usually due to cost. Therefore just as for biologic risk factors, social risk factors including poor parental education, single parent household, low social class, and maternal well-being must be taken into consideration when assessing an individual child's risk for adverse developmental and behavioral outcome.

Summary

In summary, multiple adverse events during the neonatal course can impact longer-term developmental risk. Each of these events is associated with a unique level of risk and may impact development in somewhat different ways. Furthermore, in some situations these morbidities may interact with one another to additionally increase risk. The most significant risk factors for poor developmental



• **Fig. 66.2** The probability of neurodevelopmental impairment (NDI) in the Caffeine for Apnea of Prematurity Trial participants ($n = 1514$) at 5 years corrected age, based on the number of neonatal morbidities. Morbidities included were severe brain injury, bronchopulmonary dysplasia, and severe retinopathy of prematurity. The error bars represent 95% confidence intervals, the sloping green solid line indicates predictions based on a fitted morbidity count model, and the horizontal black dotted line indicates overall probability of NDI in the entire cohort. (From Schmidt B, Roberts RS, Davis PG, et al. Prediction of late death or disability at age 5 years using a count of 3 neonatal morbidities in very low birth weight infants. *J Pediatr*. 2015;167:982–986. With permission from Elsevier.)

outcomes at least until 5 years of age are severe IVH, ROP, and BPD (Fig. 66.2) (Schmidt et al., 2015). Each of these morbidities is associated with a linear, additive increase in risk for adverse developmental outcome. Neonatal infection—particularly fungal infection and meningitis—and NEC—particularly surgical NEC—add to the prediction of poor outcome. Poor growth is independently predictive of adverse outcome. Additional neonatal factors that increase risk for poor outcome include surgical ligation of the patent ductus arteriosus, general anesthesia, seizures, serious pulmonary hemorrhage, small for gestational age, and congenital abnormalities. Lastly, the family's socioeconomic status is of increasing importance as children grow. Assessment of the number and severity of neonatal risk factors will aid in counseling of families about likely developmental outcomes and determination of possible need for services during early childhood.

Other Infants at High Risk for Adverse Outcomes

Though the primary focus of the current chapter is risk assessment and outcomes of preterm and low birth weight infants, select groups of late preterm and full-term infants have significantly increased risk for poor outcomes and are addressed below.

Hypoxic–Ischemic Encephalopathy

Infants with perinatal depression or HIE are at risk for poor outcomes because of a neurologic insult suffered around the time of birth. Diagnosis of HIE is made by a standardized neurologic

examination consistent with encephalopathy, history of an acute perinatal event, low Apgar scores, and evidence of acidosis from either the umbilical cord or an infant blood sample obtained soon after birth.

More than 1000 infants with moderate or severe HIE have been enrolled in randomized trials of therapeutic hypothermia or “cooling” for HIE. These trials have demonstrated that cooling significantly reduces mortality (RR 0.75, 95% CI 0.63–0.88) and decreases rates of survival with major disability at 18 months of age (RR 0.68, 95% CI 0.56–0.83) (Tagin et al., 2012). Benefits of cooling persist at least until school age. Follow-up of two randomized trials of therapeutic hypothermia for HIE reveals lower rates of death or severe disability, CP, and moderate or severe disability, in addition to improved motor function scores, at 6 to 7 years (Shankaran, 2012a; Azzopardi et al., 2014). Despite the success of this intervention, about half of children with moderate or severe HIE still die or suffer long-term neurologic impairment. CP is diagnosed in nearly 20% and developmental delay in more than 22% of surviving cooled infants (Tagin et al., 2012).

The relationships between several clinical factors and outcomes of infants with HIE have been studied. Certainly, the degree of encephalopathy based on the original clinical assessment is closely correlated with both risk for mortality and developmental outcome. Other early clinical signs predictive of poor longer-term outcome are administration of chest compressions for greater than 1 minute at birth, onset of breathing greater than 30 minutes after birth, and base deficit of greater than 16 at any time (Shah et al., 2006). Serial clinical examinations are more predictive of outcome than any single examination. Improvement in the clinical examination during cooling or by 72 hours of life and normal examination at discharge are associated with decreased rates of death or disability by 18 months (Gunn et al., 2008; Shankaran et al., 2012b).

Children undergoing cooling are often monitored with either electroencephalography (EEG) or amplitude-integrated electroencephalography (aEEG). Prolonged discontinuity on EEG is associated with brain injury on MRI and poor developmental outcomes (Dunne et al., 2017). Burst suppression, low voltage, and flat trace predict developmental outcomes at greater than 12 months (Awal et al., 2016). However, it is uncertain whether aEEG or EEG data add significantly to the initial clinical assessment based on severity of encephalopathy in the era of therapeutic hypothermia (Shalak et al., 2003; Shankaran et al., 2011). Particularly in cooled infants, serial evaluation of aEEG background may improve prediction of poor outcome just as serial clinical examinations improve prediction (Thoresen et al., 2010). About half of newborns with HIE have seizures on EEG (Boylan et al., 2015). When managed with cooling, infants with HIE have lower overall seizure burden and shorter duration of seizures. The majority of these seizures are subclinical. The AAP recommends that centers offering therapeutic hypothermia have monitoring with aEEG or EEG available (Committee on Fetus and Newborn et al., 2014).

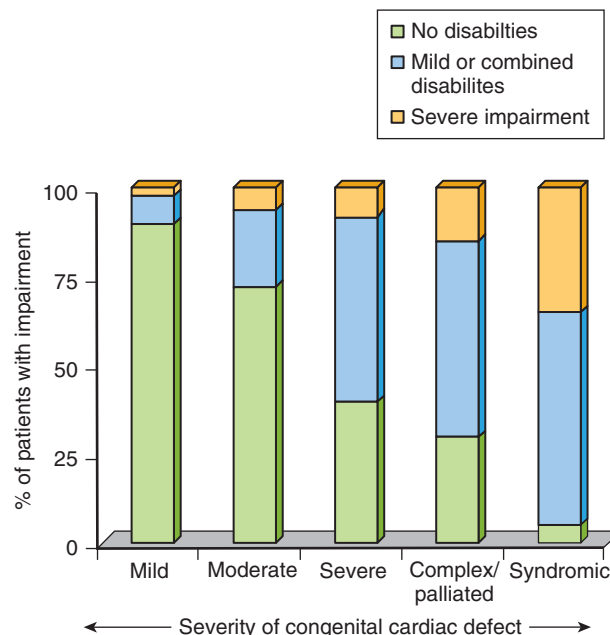
MRI-detected patterns and extent of brain injury are predictive of developmental outcome both at 2 years and at school age in HIE (Shankaran et al., 2015). In particular, injury to the basal ganglia and/or thalami is highly predictive of poor outcome (Bonifacio et al., 2015). Interpretation of MRI findings depends on timing of the examination after birth, because sequelae of the initial insult may evolve over the first days to weeks of life. Infants treated with cooling are more likely to have normal MRI than those who are not cooled, but an MRI without abnormal findings does not guarantee “normal” developmental outcome after HIE

(Rollins et al., 2014). Therefore all hospitals that provide cooling should also be capable of providing longitudinal neurodevelopmental monitoring and care (Papile et al., 2014).

Congenital Heart Disease

Over the past several decades, improved surgical techniques and medical management have resulted in significantly improved survival rates for the 0.6%–0.9% of infants who are born with CHD (Marino et al., 2012; Latal, 2016). This has led to increasing interest in the longer-term developmental and quality of life outcomes of children and young adult survivors of CHD. Noncardiac complications of CHD include cognitive, academic, financial, and psychological problems. Prevalence of poor outcome increases with severity of the heart lesion (Wernovsky, 2006). Furthermore, children who have genetic abnormalities or prematurity in addition to CHD have increased risk for developmental delays compared with those without these confounding conditions (Fig. 66.3).

In children with CHD, gross motor problems are often evident in early infancy and during the toddler period but appear to improve over time. Rates of CP are low, especially in comparison with other high-risk groups such as EPT infants or full-term infants with HIE. Intelligence in children with a history of CHD is decreased from expected values by 5 to 10 points and is less than 70 in about 10%–20%. This is associated with increased rates of language and learning problems at school age, emotional and behavioral problems in up to 40%, visuomotor and fine motor coordination problems, and significant impact on executive function. Survivors of CHD often require habilitative services, including therapies and special education. As in adults who were born preterm, persistence of these problems over time is likely to impact academic achievement, employment, and quality of life.



• **Fig. 66.3** Relationship between severity of congenital heart lesion in children with and without syndromes and severity of neurodevelopmental impairment. (Modified from Wernovsky G. Current insights regarding neurological and developmental abnormalities in children and young adults with complex congenital cardiac disease. *Cardiol Young*. 2006;16:92–104. With permission from Cambridge University Press.)

Adverse neurologic or developmental outcome in CHD is associated with several factors that are known in the neonatal and perioperative period. Just as clinical examination in HIE is predictive of longer-term outcome, so too is a careful neurologic examination of the infant with CHD (Khalil et al., 2014). Neurobehavioral problems are often evident even before surgical palliation of the CHD. Seizures occur in more than 10% of infants who undergo bypass surgery and are associated with poor outcomes, including mortality (Clancy et al., 2005; Naim et al., 2015). Duration of intensive care stay is a strong risk factor for adverse cognitive outcome up to 8 years, likely because it is a surrogate measure of overall severity of illness in this population (Newburger et al., 2003).

Infants with CHD are often born with brains that are both small and immature for their gestational age, which is thought to lead to increased vulnerability during the perioperative period. On MRI imaging, brain injury is detected both preoperatively and postoperatively and predominantly consists of WMI. However, the degree of WMI has not yet been correlated with developmental outcomes in the CHD population (Latal, 2016). A 2012 position statement from the American Heart Association and the AAP stratifies children based on risk for poor outcome and suggests age-based approaches to surveillance, screening, and evaluation of children with CHD (Marino et al., 2012). Because the first generations of survivors of CHD are now entering adulthood, more work to describe the developmental, functional, and quality of life outcomes of CHD during adulthood is urgently needed.

Extracorporeal Membrane Oxygenation

ECMO is a lifesaving therapy for patients with severe cardiac or respiratory failure that cannot be managed with conventional medical therapies. Almost half of all patients treated with ECMO are neonates, and about three-quarters survive to discharge (Mok et al., 2016). Both survival and longer-term developmental outcome after ECMO depend on the original indication for ECMO, pre-ECMO course, and complications while on ECMO. Highest ECMO survival rates are in infants with meconium aspiration syndrome, and lowest survival rates are in infants with congenital diaphragmatic hernia. Lower gestational age and birthweight are associated with higher risk of both complications and mortality. The primary complication of concern while on ECMO is intracranial bleeding.

Children who were treated with ECMO are at higher risk for later respiratory morbidity; hearing loss; poor motor, cognitive, and visuomotor performance; and behavioral problems. A randomized trial of therapeutic hypothermia during ECMO failed to demonstrate any neuroprotective effects at 2 years (Field et al., 2013). However, without ECMO these children are likely to still be at high risk for many of these outcomes and are likely to have higher rates of mortality. For example, infants with congenital diaphragmatic hernias who do not undergo ECMO have lower developmental outcomes at 1 year than expected norms, and 13% have severe cognitive, motor, or language delays (Danzer et al., 2015).

Summary

In summary, infants in each of these high-risk categories should be referred for early intervention at hospital discharge. In addition, most should be followed throughout the early childhood years by a multidisciplinary neurodevelopmental follow-up clinic that is capable of comprehensive surveillance, screening, diagnosis, and management. Monitoring should include traditional cognitive and motor assessments, behavioral evaluations, and surveillance for

neurosensory deficits. This proactive approach will ensure that these high-risk infants achieve their greatest potential as they mature through childhood and adolescence.

Postdischarge Management of the High-Risk Infant

Discharge Planning for the High-Risk Infant

Proper discharge planning will ensure a smooth transition home from the hospital both for the high-risk infant and for the family. A parent conference to review the infant's hospital course and plans for discharge will provide an opportunity to discuss the infant's progress and goals for discharge, identify risk factors for developmental challenges, assess the parents' understanding, and make clear plans for discharge. In review of the infant's various risk factors, an honest but sensitive discussion about the range of possible outcomes will help put risk for neurodevelopmental disabilities into perspective. Parents should be reassured wherever possible and given opportunity to hope.

Discharge teaching includes routine well-baby care, cardiopulmonary resuscitation, use of any special equipment or medication, and anticipatory guidance about car seats, safe sleep, and other common topics. When infants are discharged on medication or equipment, parents must demonstrate safe administration of the medication and use of the equipment. A pediatrician must be identified before discharge, and the inpatient team must personally communicate all relevant data about the child's hospital course and postdischarge plans with the pediatrician. The AAP has established a policy for the screening and surveillance of developmental concerns in the primary care setting (AAP, 2006). Please see Box 66.1 for general recommendations for primary caregivers who care for high-risk infants after discharge. In addition, infants who have had complications during the hospital course may require ongoing subspecialty care from various specialists. Lastly, all high-risk infants should be evaluated on an intermittent basis by a comprehensive developmental follow-up clinic and early intervention program. These services are an essential part of the continuum of care provided to high-risk infants and their families.

Multidisciplinary Follow-Up Care for the High-Risk Infant

Criteria for referral of high-risk infants for comprehensive developmental follow-up vary widely, based on available resources, funding, and geography. The comprehensive developmental follow-up program serves several important functions. High rates of follow-up and consistent collection of outcomes data allow a follow-up program to provide the NICU with accurate local data for specific conditions. Close relationships with community health, educational, and social services promote coordination of intervention services, based on individual child and family needs. Most importantly, the follow-up program provides coordinated and comprehensive care that will help the family optimize the child's growth and development; help the child integrate into the family, school and community; and allow timely intervention when necessary to reduce future medical, social, and emotional costs. These goals are promoted by the recognition that each child is an individual with unique qualities, strengths, and challenges and that each family differs in background, social supports, finances, coping mechanisms, and expectations for their child's future.

• BOX 66.1 Considerations for the Primary Care Physician Caring for the High-Risk Infant After Discharge

1. **Growth** – Monitor weight, length, and head circumference in all children and especially in children with BPD. Follow feeding skills closely.
2. **Neurosensory** – Diagnostic audiology assessment by 24–30 months (Harlor and Bower, 2009). Continued ophthalmology screenings and evaluation postdischarge for children with or at risk for ROP (Fierson, 2013).
3. **Immunizations** – Routine immunizations according to birth date, rather than corrected age. Influenza immunization yearly for child and caregivers. Palivizumab for all high-risk infants and young children per AAP recommendations (AAP, 2014).
4. **Neurodevelopmental** – Screen for neurodevelopmental problems, including ADHD and ASD, behavioral problems, and developmental delays.
5. **Motor** – Perform careful neuromotor examination and refer to physical or occupational therapy if not meeting milestones or abnormal examination.
6. **Learning** – Refer for evaluation for learning and executive function disorders if children struggle in school.
7. **Sociodemographic** – Assess for sociodemographic factors that may adversely impact outcomes, including parental anxiety and depression. Provide access to social supports as indicated.

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AAP, American Academy of Pediatrics; ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorders; BPD, bronchopulmonary dysplasia; ROP, retinopathy of prematurity.

The appropriate evaluations for a follow-up clinic visit will change as the child grows, develops, and increasingly interacts with the environment. Recognition of impairments, disabilities, and handicaps relies in part on parental reports, an appreciation of normal variability in developmental patterns, and the examiner's assessment skills and ability to determine the significance of abnormalities or deviations from normal development. The foundation of each follow-up evaluation is one of the basic tools of medicine—the history and physical examination. The focus of the history is on behavior, including sleep, feeding, temperament and any behavioral concerns, and developmental milestones. The physical examination is expanded to include a neurodevelopmental evaluation with assessment of posture; muscle tone; primitive, protective, and deep tendon reflexes; and functional abilities (Amiel-Tison and Gosselin, 2001). A detailed, structured neurologic examination as early as full-term corrected age in high-risk infants is highly correlated with neuromotor outcomes at least until 1 year of age. Many multidisciplinary clinics include an early childhood psychologist who evaluates cognition, learning, and other functions either at all visits or at targeted visits—for instance, at 12 and 24 months corrected age.

Gross and fine motor abilities are evaluated to assess motor development and to screen for CP (see section on [Early Neurodevelopmental Outcome Assessments](#) earlier) and minor neuromotor dysfunction. Children with minor neuromotor dysfunction have mild or no delay and mild neuromotor abnormalities on examination. However, minor neuromotor dysfunction may still have significant functional impact, such as impact on speech, feeding, and motor coordination. As children get older, disorders of motor coordination (developmental coordination disorder) can influence ability to participate in activities in multiple domains, including home and school.

Motor problems are often the first to be recognized and are associated not only with later motor problems but also later cognitive disability (Allen and Capute, 1989). Cognitive and language problems are recognized later in the first or second year. Delay in language abilities can be related to cognitive delay, hearing impairment, language disorder, or ASD. The first step in evaluation of a child with apparent language delay is a diagnostic audiology evaluation (Harlor and Bower, 2009). Neurobehavioral problems may become apparent in toddler-age children; motor coordination, learning, and executive function disorders are generally recognized at school age. Once identified, problems should be addressed with parents, including a nonmedical description of any diagnoses and description of specific intervention strategies.

Assessment of development and timely acquisition of milestones in preterm infants raise the controversial question of whether to correct for the degree of prematurity. This issue is most important early in life and in the most premature infants. Therefore early in life, expectations and results of developmental assessments are “adjusted” for prematurity. As children grow, the difference between the adjusted and chronologic age becomes less significant, so correction is no longer as critical. Researchers and clinicians have different perspectives on when—if ever—to stop correcting for prematurity. Within an individual clinic, however, a standard should be set and followed by all clinicians for all patients.

Referral for Early Intervention Services

All preterm children and high-risk children with abilities in any domain that are consistently below expectations based on their age should be referred for early intervention. Eligibility for surveillance or services for high-risk infants or children differs by geography. In the United States, the Individuals with Disabilities Education Act requires states to provide early intervention services for infants and toddlers up to 3 years of age with developmental delays and with conditions that lead to developmental delays. However, states differ in definitions of delay, standards with regard to correction for prematurity, and services offered (Shackelford, 2006).

Infants may be referred to early intervention by the NICU, follow-up clinic, pediatrician, or family. Each child who is referred is entitled to a multidisciplinary assessment and a service coordinator to facilitate the assessment and services. Early intervention programs recognize the importance of (1) viewing each child as a unique individual, (2) evaluating not only needs but also strengths, (3) including the family in the planning process, and (4) coordinating all intervention services. The choice of interventions is determined by the individual child's developmental profile and health, the needs of the family, and available resources.

Early intervention helps ensure that children reach their maximum potential but does not necessarily prevent disability (Spittle et al., 2015). Research about the efficacy of early intervention

includes many different interventions, applied with different intensity, in different populations, and for different lengths of time. Hearing and visual impairments are responsive to early intervention, and early therapies can significantly improve long-term functioning and quality of life. In general, early intervention programs have been shown to have positive effects on developmental and neurologic outcomes for preterm infants during infancy and early childhood (Spittle et al., 2015). Specifically, early intervention is associated with improved behavioral outcomes, reduced anxiety and depression for primary caregivers, and cognitive benefits through preschool age. Importantly, early intervention programs focused on the parent–child relationship are more effective than programs focused on the child or the parent alone.

Early intervention has less of an impact on motor outcomes and no impact on rates of CP. However, a few recent small studies of focused, intensive, goal-oriented therapy for infants at high risk for CP have demonstrated improved motor outcomes (Prosser et al., 2012; Eliasson and Holmefur, 2015; Morgan et al., 2016). No long-term studies of such interventions for infants at risk for CP have been completed to date. Thus it remains uncertain whether any specific forms of early intervention or disability-focused therapies have significant long-term impact on functional motor outcomes at school or at home. However, once a child has a diagnosis of CP or DCD, targeted interventions are then aimed at prevention of further delay and compensating for deficits, to optimize the child's function and independence (Majnemer, 1998). A recent review of therapies for children with DCD identified that the task-oriented approach, which includes cognitive approaches with a focus on specific aspects of a motor skill, was an effective way of teaching motor skills in DCD (Smits-Engelsman et al., 2013). Continued research is essential to identify the best therapies, including timing, intensity, and duration, to optimize outcomes for preterm and high-risk infants and children.

Challenges to and Importance of Follow-Up

There is a growing reliance on complete evaluation and reporting of neurodevelopmental outcomes in prospective studies and trials and an increasing and appropriate recognition that it is crucial to fully understand outcomes beyond the initial hospitalization and even beyond early childhood. Yet, the challenges of following a cohort for years or decades and the potential barriers to achieving reliable results are numerous. Long-term follow-up requires time, dedication, and persistence from both follow-up staff and from families. Achieving a high follow-up rate is essential to limit bias. Some studies report that infants who fail to keep follow-up appointments, or are followed only with great difficulty, are more likely to have developmental impairment; however, a systematic review suggests just the opposite (Tin et al., 1998; Callanan et al., 2001; Guillén et al., 2012). Social and demographic disparities and population differences have been shown to be associated with increased attrition (Aylward et al., 1985; Guillén et al., 2012; Kuppala et al., 2012; Hintz 2015b), which is of concern not only because of the potential bias introduced but also because those children and families lost to follow-up could potentially benefit most from supports and services.

Nevertheless, depending on the group from which a cohort is drawn and the reason for its creation, questions related to generalizability of findings may be inherent, regardless of outstanding follow-up rates. Thus population-based or large regional-based cohorts such as EPICure (Marlow et al., 2005), VICS (Doyle et al., 2010), and the EXPRESS group (Serenius et al., 2013) may be

considered the ideal model of prospective observational studies of high-risk infants. Caution is advised when comparing outcomes of different cohorts over time, because of differences in the enrolled populations and attrition rates, changes in versions of or use of different instruments, and varying approaches to use of term control groups.

Outside the scientific framework of follow-up for research purposes, there is a substantial *clinical* need for high-risk infant follow-up. The purpose and potential value of this difficult undertaking are manifold. As survival of even the most extremely preterm and complex infants has improved, there is increasing recognition of the importance of neurodevelopmental outcomes, rather than short-term endpoints alone, as a measure of the impact of interventions and management strategies in the NICU. Much has been invested in assuring the survival of these high-risk infants and must be invested similarly to assure that all of these children reach their best potential. In addition to traditional quality improvement measures to decrease in-hospital morbidities that are associated with adverse neurodevelopmental outcomes, early detection, preventive care, and intervention programs inclusive of both infants and families hold the best promise for changing the trajectory of outcomes.

Within an ecologic framework, the home environment and relationships are the most immediate and proximal influences on child development (Bronfenbrenner, 1986). More distal factors, such as family income and broader community factors, influence children's development both directly and indirectly through interaction with other proximal environmental factors. In understanding the developmental systems approach and recognizing the profound potential to influence brain development, positive outcomes can be understood in terms of improvements in developmental pathways associated with parental sensitive–responsiveness and child participation in intensive intervention-oriented child care (Milgrom et al., 2010; Kolb and Gibb, 2011; Guralnick, 2012).

Without a doubt, implementing a comprehensive system of NICU-to-community early and preventive interventions for high-risk infants is a time-intensive and resource-intensive undertaking. But we are unlikely to truly transform long-term care and improve the lifetime outcomes of high-risk infants without such an investment in the future.

Suggested Readings

- Anderson PJ, De Luca CR, Hutchinson E, Roberts G, Doyle LW, Victorian Infant Collaborative group. Underestimation of developmental delay by the new Bayley-III Scales. *Arch Pediatr Adolesc Med.* 2010;164(4):352-356.
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67

Breastfeeding

LYDIA FURMAN AND RICHARD J. SCHANLER

KEY POINTS

- Hospital policies must support mothers who intend to breastfeed and should reflect and incorporate the Baby-Friendly Hospital Initiative's Ten Steps to Successful Breastfeeding.
- Breastfeeding advocacy should reach into the community: breastfeeding is a public health issue with evidence-based health and economic benefits.
- All pediatricians must be knowledgeable about lactation management and be prepared to lead healthcare teams that support the breastfeeding dyad.
- Every home-going infant should receive medical care at a pediatric office that has seamless access to appropriately trained lactation specialists.
- Breastfeeding education and support should be offered by trained peers and medical health providers, both prepartum and postpartum, both in the community and in medical settings, and to the mother, her partner, and all in her support system.
- Changes in national health policy, such as availability of free lactation services and paid maternity leave, hold promise for reducing health disparities associated with breastfeeding rates.
- Each neonatal intensive care unit should have protocols and guidelines to support lactation for mothers of hospitalized neonates.

Exclusive breastfeeding through 6 months of age, with continued breastfeeding to 12 months and beyond, is recommended for all infants by the World Health Organization (WHO; <http://www.who.int/topics/breastfeeding/en/>), the American Academy of Pediatrics (AAP; <http://www.aap.org/breastfeeding/>), and other professional organizations (World Health Organization, 2002; AAP, 2015; ACOG, *Optimizing Support for Breastfeeding*, 2016). Successful lactation depends on supportive attitudes of pediatric and obstetric providers, evidence-based hospital practices, and the awareness that many mothers will need assistance to establish and maintain breastfeeding. Much information must be shared with new parents in the short postpartum hospital stay, so both prenatal and postdischarge breastfeeding education and support are essential. Caregivers should be trained to support and document breastfeeding, and newborns should have early follow-up at 3 to 5 days of age with a knowledgeable healthcare provider and continuing lactation help (AAP, 2015).

Rates of Breastfeeding in the United States

While breastfeeding was the norm in the early part of the 20th century, rates declined after World War II, likely because of women returning to the workforce as well as to the availability of commercial infant formulas. Recognition of breastfeeding benefits grew during the 1970s, and rates more than doubled in the United States from 24.7% in 1971 to 59.7% in 1984 (Wright, 2001). The US Centers for Disease Control and Prevention began monitoring annual breastfeeding rates through the National Immunization Survey (NIS) in 2001 and administered the first national survey of maternity practices related to breastfeeding, the Maternity Practices in Infant Nutrition and Care Survey, in 2007 (Breastfeeding Data and Statistics, 2017). These figures can be compared with the Department of Health and Human Services' Healthy People 2020 breastfeeding goals (Table 67.1); goals added for 2020 relate to reducing formula supplementation for breastfed infants, increasing the proportion of workplaces with onsite lactation support, and increasing the proportion of live births at facilities with best practices supporting lactation (to 8.1%) (Breastfeeding Data and Statistics, 2015; US Department of Health and Human Services, 2015). In 2011 the US Department of Health and Human Services issued *The Surgeon General's Call to Action to Support Breastfeeding*, which reviewed research on the multiple benefits of breastfeeding and described steps that family members, healthcare providers, researchers, employers, and communities can take to support breastfeeding (DHHS, 2011). While some states and population subgroups have met initiation and continuation goals, disparities related to race, ethnicity, age, and socioeconomic status have not been eliminated (CDC, 2015). Non-Hispanic African Americans and economically disadvantaged populations have the lowest breastfeeding rates for all measures at all infant ages, and women who are younger, unmarried, receive or are eligible for support from the Special Supplemental Nutrition Program for Women, Infants, and Children (WIC), and who have a lower educational level are less likely to initiate or continue breastfeeding (Table 67.2; CDC, 2015). Culturally specific feeding beliefs also influence breastfeeding choice (Cassidy and El Tom, 2015). An example from the Navajo culture is the belief that either negative or positive maternal emotions can be transmitted through breast milk (Wright et al., 1993). Disparities in breastfeeding rates are an important arena for public health initiatives (US Department of Health and Human Services, 2013).

TABLE 67.1**Healthy People 2020 Infant Feeding Goals and Rates of Breastfeeding in the United States 2004 to 2012**

| Healthy People 2020 Objectives | 2004 | 2006 | 2008 | 2010 | 2012 |
|--|---|------------|------------|------------|------------|
| Breastfeeding Goals | | | | | |
| 81.9% initiation postpartum | 73.1 ± 0.8 | 74.0 ± 0.9 | 74.6 ± 0.9 | 76.7 ± 1.2 | 80.0 ± 1.2 |
| 46.2% exclusive breastfeeding at 3 months | 31.5 ± 0.9 | 33.6 ± 1.0 | 34.3 ± 1.0 | 37.1 ± 1.4 | 43.3 ± 1.6 |
| 25.5% exclusive breastfeeding at 6 months | 12.1 ± 0.7 | 14.1 ± 0.8 | 14.6 ± 0.8 | 17.2 ± 1.2 | 21.9 ± 1.4 |
| 60.6% any breastfeeding at 6 months | 42.1 ± 0.9 | 43.5 ± 1.1 | 43.5 ± 1.1 | 47.5 ± 1.4 | 51.4 ± 1.5 |
| 34.1% any breastfeeding at 12 months | 21.4 ± 0.8 | 21.4 ± 0.8 | 21.4 ± 0.8 | 25.3 ± 1.3 | 29.2 ± 1.4 |
| Additional Goals | | | | | |
| 14.2% of breastfed infants within first 48 h | 23.5 ± 1.0 | 24.2 ± 1.1 | 25.1 ± 1.1 | 22.8 ± 1.4 | 19.1 ± 1.3 |
| 38.0% of workplaces with lactation support | (25% reporting in 2009 serving as the baseline; other data not available) | | | | |

Numbers represent the percentage of US children who were breastfed, by birth year, National Immunization Survey, United States (percent age ± half 95% confidence interval); see site for additional methodological details of the survey, and see <http://www.cdc.gov/breastfeeding/data/reportcard.htm> for annually updated breastfeeding rates.

From HealthyPeople.gov. Maternal, Infant and Child Health. <http://www.healthypeople.gov/2020/topics-objectives/topic/maternal-infant-and-child-health/objectives>; and Centers for Disease Control and Prevention. Breastfeeding among U.S. children born 1999-2005, CDC National Immunization Survey. http://www.cdc.gov/breastfeeding/data/NIS_data/index.htm.

• BOX 67.1 Ten Steps to Successful Breastfeeding

- Step 1: Have a written breastfeeding policy that is routinely communicated to all healthcare staff.
- Step 2: Train all healthcare staff in the skills necessary to implement this policy.
- Step 3: Inform all pregnant women about the benefits and management of breastfeeding.
- Step 4: Help mothers initiate breastfeeding within 1 hour of birth.
- Step 5: Show mothers how to breastfeed and how to maintain lactation even if they are separated from their infants.
- Step 6: Give newborns no food or drink other than breast milk, unless medically indicated.
- Step 7: Practice rooming-in—allow mothers and infants to remain together 24 hours a day.
- Step 8: Encourage breastfeeding on demand.
- Step 9: Give no pacifiers or artificial nipples to breastfeeding infants.
- Step 10: Foster the establishment of breastfeeding support groups, and refer mothers to them on their discharge from the hospital or birth center.

From the Baby Friendly Hospital Initiative “Ten Steps to Successful Breastfeeding for Hospitals” as outlined by UNICEF/World Health Organization. Available at <https://www.babyfriendlyusa.org/about-us/baby-friendly-hospital-initiative/the-ten-steps>.

Interventions to Support Breastfeeding Initiation, Exclusivity, and Continuation

In response to extensive formula marketing in the developing world with resulting high rates of infant morbidity and mortality, the World Health Assembly created the International Code of Marketing of Breastmilk Substitutes (1981) to promote ethical marketing of formula products (World Health Organization, 1981). In 1992 United Nations International Children's Emergency Fund and the World Health Organization (WHO) developed the Baby-Friendly Hospital Initiative (BFHI), an international program to promote breastfeeding-supportive policies for birthing hospitals (Box 67.1; Willumsen, 2013). The BFHI “10 steps” in combination with an “11th step,” the WHO International Code of Marketing of Breastmilk Substitutes, which protects against free provision and

advertising of breast milk substitutes (i.e., formula), effectively increased breastfeeding rates worldwide (World Health Organization, UNICEF and Wellstart International, 2009). Adherence to these evidence-based maternity practices significantly increases the likelihood of mothers initiating breastfeeding, exclusively breastfeeding, and breastfeeding through 6 months (DiGirolamo et al., 2008; Sinha et al., 2015; Yotebieng et al., 2015). While implementation of all ten steps is optimal, breastfeeding rates increase with the number of steps practiced, including with a single step, such as initiating breastfeeding within 1 hour of birth with skin-to-skin contact (Kramer et al., 2001; Philipp et al., 2001; Rosenberg et al., 2008; Moore et al., 2012; Olaiya et al., 2016). Possible expansion of a modified Ten Steps to Successful Breastfeeding program to neonatal intensive care units (NICUs) has been proposed (Nyqvist et al., 2013; World Health Organization, 2015). State health departments have begun to create programs to support Ten Steps to Successful Breastfeeding practices (e.g., <http://texastenstep.org/>), and the number of birthing hospitals designated as baby friendly (by Baby-Friendly USA Inc.) is increasing (Baby-Friendly, 2015).

Systematic reviews suggest that breastfeeding support is optimal when offered prepartum and postpartum and in multiple settings: essential interventions include counseling by peers and health providers, BFHI practices, and actions that mobilize community awareness (Sinha et al., 2015). Counseling and education in the home and family environment promote breastfeeding initiation, exclusivity, and continuation; family and social support alone, surprisingly, had no significant impact (Sinha et al., 2015).

WIC initiatives, including enhanced maternal food packages for breastfeeding mothers and peer counseling (Loving Support), hold promise, as do the Affordable Care Act mandates covering breastfeeding education and supplies and reasonable break time for “nonexempt” hourly employees to express milk (Sections 2713 and 4207) (Murtagh and Moulton, 2011; Hawkins et al., 2015). Workday strategies that include feeding the infant directly from the breast appear more effective than pumping only (Fein et al., 2008). However, maternal employment outside the home, in general, is associated with decreased breastfeeding initiation and continuation (Ogbuanu et al., 2011; CDC, 2016). Public policy changes can increase national breastfeeding rates: provision of paid maternity

TABLE
67.2

Rates of Any and Exclusive Breastfeeding by Sociodemographic Factors Among Children Born in 2012

| Sociodemographic Factors | n | ANY BREASTFEEDING | | | n | EXCLUSIVE BREASTFEEDING | |
|---|--------|-----------------------------------|--------------------------------|---------------------------------|--------|-------------------------------------|-------------------------------------|
| | | Ever Breastfed % ± Half 95% CI | At 6 Months % ± Half 95% CI | At 12 Months % ± Half 95% CI | | Through 3 Months % ± Half 95% CI | Through 6 Months % ± Half 95% CI |
| US National | 15,141 | 80.0 ± 1.2 | 51.4 ± 1.5 | 29.2 ± 1.4 | 14,768 | 43.3 ± 1.6 | 21.9 ± 1.4 |
| Race/Ethnicity | | | | | | | |
| Hispanic | 2788 | 82.4 ± 2.8 | 51.4 ± 3.7 | 27.9 ± 3.6 | 2749 | 40.3 ± 3.8 | 20.8 ± 3.3 |
| Non-Hispanic white | 8811 | 83.0 ± 1.3 | 55.8 ± 1.8 | 32.8 ± 1.8 | 8546 | 48.0 ± 1.9 | 24.4 ± 1.7 |
| Non-Hispanic black | 1476 | 66.4 ± 3.8 | 35.3 ± 4.0 | 16.9 ± 3.1 | 1460 | 33.4 ± 4.1 | 13.9 ± 2.9 |
| Non-Hispanic Asian | 683 | 83.2 ± 7.6 | 65.6 ± 7.7 | 42.3 ± 7.0 | 662 | 46.5 ± 7.5 | 26.9 ± 7.1 |
| Non-Hispanic Hawaiian/ Pacific Islander | 96 | 83.9 ± 14.1 | 32.6 ± 19.0 | 14.4 ± 9.0 | 95 | 43.3 ± 24.1 | 11.8 ± 9.5 |
| Non-Hispanic American Indian/Alaska Native | 217 | 71.5 ± 12.6 | 28.8 ± 11.7 | 17.9 ± 8.9 | 212 | 27.4 ± 11.8 | 12.5 ± 6.5 |
| Two or more races | 1070 | 75.4 ± 6.5 | 46.2 ± 6.2 | 25.3 ± 5.4 | 1044 | 41.4 ± 6.3 | 23.0 ± 5.6 |
| Maternal Education | | | | | | | |
| Less than high school | 1575 | 69.1 ± 3.8 | 40.3 ± 4.5 | 21.2 ± 3.9 | 1559 | 32.8 ± 4.5 | 16.1 ± 3.9 |
| High school graduate | 2755 | 71.1 ± 3.1 | 38.2 ± 3.3 | 20.0 ± 2.8 | 2696 | 33.5 ± 3.1 | 16.3 ± 2.5 |
| Some college/technical school | 3924 | 81.2 ± 2.3 | 45.9 ± 3.2 | 24.2 ± 2.8 | 3814 | 41.2 ± 3.2 | 19.5 ± 2.7 |
| College graduate | 6887 | 91.2 ± 1.1 | 70.3 ± 1.9 | 43.2 ± 2.3 | 6699 | 57.2 ± 2.2 | 30.6 ± 2.2 |
| Maternal Age | | | | | | | |
| <20 years | 103 | 58.6 ± 16.1 | 17.4 ± 10.0 | 4.3 ± 2.8 | 103 | 28.3 ± 13.1 | 8.0 ± 7.7 |
| 20–29 years | 5443 | 75.2 ± 2.0 | 40.6 ± 2.4 | 21.0 ± 2.1 | 5315 | 37.8 ± 2.4 | 18.8 ± 2.1 |
| ≥30 years | 9595 | 84.1 ± 1.6 | 60.2 ± 2.0 | 35.8 ± 2.0 | 9350 | 47.8 ± 2.1 | 24.5 ± 1.8 |
| Poverty Income Ratio^a | | | | | | | |
| <100 | 3840 | 71.4 ± 2.6 | 37.7 ± 2.9 | 19.8 ± 2.5 | 3768 | 31.7 ± 2.9 | 15.6 ± 2.3 |
| 100–199 | 2952 | 79.0 ± 2.6 | 49.1 ± 3.3 | 27.9 ± 3.1 | 2880 | 40.5 ± 3.3 | 19.9 ± 2.8 |
| 200–399 | 4028 | 86.0 ± 2.0 | 59.5 ± 2.9 | 36.4 ± 3.0 | 3920 | 54.5 ± 3.0 | 27.1 ± 3.0 |
| 400–599 | 2379 | 88.2 ± 2.1 | 66.3 ± 3.5 | 38.1 ± 3.8 | 2317 | 53.7 ± 3.8 | 30.2 ± 3.9 |
| ≥600 | 1942 | 90.9 ± 2.1 | 70.4 ± 3.5 | 39.1 ± 4.2 | 1883 | 54.4 ± 4.3 | 27.9 ± 4.1 |
| Marital Status^b | | | | | | | |
| Married | 10,842 | 87.0 ± 1.2 | 62.3 ± 1.8 | 37.8 ± 1.8 | 10,534 | 51.5 ± 1.9 | 27.2 ± 1.7 |
| Unmarried | 4299 | 68.3 ± 2.6 | 33.1 ± 2.7 | 14.7 ± 2.2 | 4234 | 29.7 ± 2.6 | 13.2 ± 2.1 |
| Receiving WIC Support | | | | | | | |
| Yes | 6676 | 73.1 ± 1.9 | 39.1 ± 2.2 | 19.3 ± 1.9 | 6549 | 33.4 ± 2.2 | 15.6 ± 1.8 |
| No, but eligible | 1096 | 80.3 ± 6.7 | 58.2 ± 6.5 | 40.9 ± 6.0 | 1065 | 51.4 ± 6.5 | 29.7 ± 5.9 |
| Ineligible | 7304 | 90.5 ± 1.1 | 68.4 ± 1.9 | 41.6 ± 2.2 | 7096 | 56.8 ± 2.2 | 30.0 ± 2.2 |

The breastfeeding rates are based on dual-frame (landline and cellular telephone) samples from the 2013 National Immunization Surveys and the 2014 National Immunization Survey. See the survey methods for details on study design. Exclusive breastfeeding is defined as only breast milk—no solids, no water, and no other liquids.

^aRatio of self-reported family income to federal poverty threshold for number in household.

^bUnmarried includes never married, widowed, separated, and divorced.

CI, Confidence interval; WIC, Special Supplemental Nutrition Program for Women, Infants, and Children.

From National Immunization Survey, Centers for Disease Control and Prevention, Department of Health and Human Services (http://www.cdc.gov/breastfeeding/data/nis_data/rates-any-exclusive-bf-socio-dem-2012.htm). Sample sizes appearing in the National Immunization Survey breastfeeding tables are slightly smaller than the numbers published in other National Immunization Survey publications because in the Division of Nutrition, Physical Activity, and Obesity breastfeeding analyses, the sample was limited to records with valid responses to the breastfeeding questions.

leave for employed women increases breastfeeding exclusivity and duration, and longer maternity leaves are associated with increased breastfeeding initiation (Fein et al., 2008; DHHS, 2011; Ogbuanu et al., 2011; Breastfeeding Promotion and Employment, 2016; Huang and Yang, 2015; Nandi et al., 2016). The United Nations International Labor Organization recommends 18 weeks of paid maternity leave, a “reasonable goal” that could push the United States closer to Healthy People breastfeeding targets (DHHS, 2011; US Department of Health and Human Services, 2017).

The Evidence to Support Breastfeeding

More than 1 decade ago, exclusive breastfeeding through 6 months of age was identified as the most effective international preventive health intervention for children younger than 5 years, with the potential to prevent 13% of deaths of children younger than 5 years worldwide (Jones et al., 2003). Human milk has widely acknowledged benefits in high-, middle-, and low-income countries with respect to infant nutrition, infant survival, gastrointestinal function, host defense, neurodevelopment, and psychological well-being. The benefits of exclusive breastfeeding, as well as a “dose-dependent” effect of breastfeeding duration and of partial breastfeeding, are well documented (Sankar et al., 2015). Maternal health benefits increase with the number of months of lactation and are also well described (Chowdhury et al., 2015). There is insufficient evidence to endorse formula supplementation as a change in practice from exclusive breastfeeding (Becker and Remington, 2014). However, health providers can elicit parents’ perspectives about the challenges of exclusive breastfeeding and provide scientifically based guidance and personalized support, recognizing that any breast milk (including colostrum) gives a meaningful health benefit (Flaherman and Fuentes-Afflick, 2014). The success of the United Nations Millennium Development Goals, with a reduction in the number of deaths of children younger than 5 years from 12.7 million per year in 1990 to 5.9 million per year in 2015, is multifactorial, yet breastfeeding plays a large current and larger potential role (World Health Organization Media Centre, 2015). Exclusive breastfeeding (0 to 5 months) is increasing by 1% annually in the Millennium Development Goals–tracked countries, with median coverage of 39% (Requejo et al., 2015). Each of the 2030 United Nations Sustainable Development Goals, for example, “Zero Hunger,” can be linked to and supported by breastfeeding. The global societal and economic benefits of full breastfeeding are remarkable and are yet to be fully realized.

Studies Supporting Benefits of Breastfeeding

Methodological issues impact research on health outcomes related to breastfeeding. These include (1) duration of breastfeeding recall, which is inversely related to accuracy (Burnham et al., 2014), (2) use of standardized definitions for breastfeeding duration, exclusivity, and method of feeding (expressed or at breast), which facilitates comparisons (Noel-Weiss et al., 2012), (3) the need to identify and adjust for confounders such as socioeconomic status, (4) study design (e.g., retrospective or cross-sectional studies are weaker than prospective longitudinal ones), and (5) concerns for reverse causality. Since it is nearly impossible and undoubtedly unethical to randomize mothers to breastfeed or not, unmeasured confounding occurs; randomization by health system has been one alternative approach (Kramer et al., 2001; Yotebieng et al., 2015). The preponderance of evidence supports the benefits detailed in the following sections.

Nutritional Aspects

The human milk model is used to design the composition of breast milk substitutes, because the goal for infant nutrition through the first year is to mimic the body composition of the breastfed infant. Human milk has a dynamic nutrient composition that changes throughout lactation, over the course of a day, and within a feeding and differs between women. Components of human milk have multiple nutritional and immunologic functions. A reference tabulation of the composition of human milk comparing early and more mature milk is given in Table 67.3 (Picciano, 2001). In the first few weeks after birth, the total protein content of milk from mothers who deliver prematurely (preterm milk) is greater than that of milk obtained from women delivering at term (term milk), and the total protein content in both declines similarly to approach that in what is called *mature milk* (Ballard and Morrow, 2013). Milk protein content is not related to maternal diet but increases with maternal body mass index (BMI) (Nommsen et al., 1991).

The protein quality of human milk (whey 70%, casein 30%) differs from that of bovine milk (82% casein, 18% whey). Caseins are proteins with low solubility in gastric acid, while whey proteins remain in solution after acid precipitation. The whey protein fraction is more easily digested and promotes rapid gastric emptying. It also provides lower concentrations of phenylalanine, tyrosine, and methionine and higher concentrations of taurine than the casein fraction and serves as a model for enteral and parenteral amino acid mixtures. The major whey protein in human milk is α -lactalbumin. Lactoferrin, lysozyme, and secretory immunoglobulin A (sIgA) are specific immunoactive human whey proteins that resist proteolytic digestion and thus serve as a first line of defense by lining the gastrointestinal tract (Brandtzaeg, 2010).

The lipid system in human milk, responsible for providing approximately 50% of the calories in the milk, is structured to facilitate fat digestion and absorption. The lipid system is composed of an organized milk fat globule, bile salt–stimulated lipase, and a pattern of fatty acids (high in palmitic [C16:0], oleic [C16:1 ω 9], linoleic [C18:2 ω 6], and linolenic [C18:3 ω 3] acids) characteristically distributed on the triglyceride molecule (C16:0 at the 2-position of the molecule). That distribution is unique to human milk. When triglycerides are hydrolyzed in the intestine, free fatty acids are released. Palmitic acid, in particular, is a prevalent fatty acid that has a predilection to bind minerals such as calcium to form insoluble “soaps.” The soap formation therefore reduces fat absorption and mineral (calcium) absorption. The palmitic acid in human milk triglyceride, however, is mainly found at the 2-position. Human milk lipases hydrolyze the triglyceride molecule but leave the palmitic acid bound to the glycerol backbone. The resulting monoglyceride is well absorbed. The fat blends in formula must be modified to compensate for poor fat absorption, so they contain greater quantities of medium-chain-length fatty acids, which are absorbed passively, in an attempt to mimic the superior fat absorption from human milk.

The pattern of fatty acids in human milk is also unique in its composition of very long chain polyunsaturated fatty acids. Arachidonic acid (C20:4 ω 6) and docosahexaenoic acid (C22:6 ω 3), found in human milk but not bovine milk and added to formula, are constituents of retinal and brain phospholipid membranes and have been associated with improved visual function and, potentially, neurodevelopmental outcome (Koletzko et al., 2008). The fat content of milk varies with maternal diet and throughout the feed, with hindmilk (the last part of the feed) containing

**TABLE
67.3****Representative Values for Constituents of Human Milk**

| Constituent | Early Milk | Mature Milk | Constituent | Early Milk | Mature Milk |
|---|------------|-------------|--------------------------------|------------|-------------|
| Energy (kcal/L) | | 650–700 | Linoleic acid (C18:2 ω6) | 8.9 | 11.3 |
| Carbohydrate | | | Arachidonic acid (C20:4 ω6) | 0.7 | 0.5 |
| Lactose (g/L) | 20–30 | 67 | Water-Soluble Vitamins | | |
| Glucose (g/L) | 0.2–1.0 | 0.2–0.3 | Ascorbic Acid (mg/L) | | 100 |
| Oligosaccharides (g/L) | 22–24 | 12–14 | Thiamin (μg/L) | 20 | 200 |
| Total nitrogen (g/L) | 3.0 | 1.9 | Riboflavin (μg/L) | | 400–600 |
| Nonprotein nitrogen (g/L) | 0.5 | 0.45 | Niacin (mg/L) | 0.5 | 1.8–6.0 |
| Protein nitrogen (g/L) | 2.5 | 1.45 | Vitamin B ₆ (mg/L) | | 0.09–0.31 |
| Total protein (g/L) | 16 | 9 | Folate (μg/L) | | 80–140 |
| Casein (g/L) | 3.8 | 5.7 | Vitamin B ₁₂ (μg/L) | | 0.5–1.0 |
| β-Casein (g/L) | 2.6 | 4.4 | Pantothenic acid (mg/L) | | 2–2.5 |
| κ-Casein (g/L) | 1.2 | 1.3 | Biotin (μg/L) | | 5–9 |
| α-Lactalbumin (g/L) | 3.62 | 3.26 | Fat-Soluble Vitamins | | |
| Lactoferrin (g/L) | 3.53 | 1.94 | Retinol (mg/L) | 2 | 0.3–0.6 |
| Albumin (g) | 0.39 | 0.41 | Carotenoids (mg/L) | 2 | 0.2–0.6 |
| sIgA (g/L) | 2.0 | 1.0 | Vitamin K (μg/L) | 2–5 | 2–3 |
| IgM (g/L) | 0.12 | 0.2 | Vitamin D (μg/L) | | 0.33 |
| IgG (g/L) | 0.34 | 0.05 | Vitamin E (mg/L) | 8–12 | 3–8 |
| Total lipids (%) | 2 | 3.5 | Minerals | | |
| Triglyceride (% total lipids) | 97–98 | 97–98 | Calcium (mg/L) | 250 | 200–250 |
| Cholesterol ^a (% total lipids) | 0.7–1.3 | 0.4–0.5 | Magnesium (mg/L) | 30–35 | 30–35 |
| Phospholipids (% total lipids) | 1.1 | 0.6–0.8 | Phosphorus (mg/L) | 120–160 | 120–140 |
| Fatty Acids (wt%) | 88 | 88 | Sodium (mg/L) | 300–400 | 120–250 |
| Total saturated fatty acids (%) | 43–44 | 44–45 | Potassium (mg/L) | 600–700 | 400–550 |
| Palmitic acid (C16:0) | | 20 | Chloride (mg/L) | 600–800 | 400–450 |
| Monounsaturated fatty acids (%) | | 40 | Iron (mg/L) | 0.5–1.0 | 0.3–0.9 |
| Oleic acid (C18:1 ω9) | 32 | 31 | Zinc (mg/L) | 8–12 | 1–3 |
| Polyunsaturated fatty acids (%) | 13 | 14–15 | Copper (mg/L) | 0.5–0.8 | 0.2–0.4 |
| Total ω3 fatty acids (%) | 1.5 | 1.5 | Manganese (μg/L) | 5–6 | 3 |
| Linolenic acid (C18:3 ω3) | 0.7 | 0.9 | Selenium (μg/L) | 40 | 7–33 |
| Eicosapentaenoic acid (C22:5 ω3) | 0.2 | 0.1 | Iodine (μg/L) | | 150 |
| Docosahexaenoic acid (C22:6 ω3) | 0.5 | 0.2 | Fluoride (μg/L) | | 4–15 |
| Total ω6 fatty acids (%) | 11.6 | 13.06 | | | |

^aThe cholesterol content of human milk ranges from 100 to 200 mg/L in most samples of human milk after day 21 of lactation.

IgG, Immunoglobulin G; IgM, immunoglobulin M; Kcal, kilocalorie; sIgA, secretory immunoglobulin A.

Modified from Picciano MF. Appendix: representative values for constituents of human milk. *Pediatr Clin North Am.* 2001;48:263–272 and Schanler RS, Krebs N, Mass S, eds. *AAP/ACOG Breastfeeding Handbook for Physicians*. Elk Grove, IL: American Academy of Pediatrics; 2013:77.

two to three times more than the initial milk (foremilk) (Saarela et al., 2005).

The carbohydrate composition of human milk is important as a nutritional source of lactose and for the presence of oligosaccharides. A softer stool consistency, more nonpathogenic fecal flora, and improved mineral absorption have each been attributed to small quantities of unabsorbed lactose. Human milk oligosaccharides are bioactive carbohydrate polymers (also including glycoproteins) that help protect the infant because their structure mimics specific bacterial antigen receptors and prevent bacterial attachment to the host mucosa. Some examples of human milk oligosaccharides (HMOs) include fucosylated glycans that specifically inhibit binding by *Haemophilus influenzae*, *Campylobacter jejuni*, and some viral agents (Morrow et al., 2005). HMOs are also prebiotics that stimulate the growth of nonpathogenic bifidus bacteria.

The concentrations of calcium and phosphorus in human milk are significantly lower than in other milks; calcium and phosphorus are present in more bioavailable forms bound to digestible proteins and in complexed and ionized states (Neville and Watters, 1983). Thus the bone mineral content of breastfed infants is similar to that of infants fed formula (Venkataraman et al., 1992). The concentrations of copper and zinc, despite their decline throughout lactation, appear adequate to meet the infant's nutritional needs (Lonnerdal and Hernell, 1994). The concentration of iron, however, does not meet the term infant's needs beyond 4 to 6 months, so beginning at about 6 months, either an oral iron supplement or iron-containing complementary feedings are indicated to prevent subsequent iron-deficiency anemia; preterm and low birth weight infants should receive iron supplementation from birth (Greer and Suttie, 1988; Baker et al., 2010; Qasem et al., 2015).

The content of vitamin K in human milk in comparison with fortified formulas is very low, and a single injection of vitamin K should be given at birth to all infants to prevent vitamin K–deficiency bleeding, a rare condition for which breastfed infants are at greatest risk (Greer and Suttie, 1988; Shearer, 2009). The breastfed baby is particularly at risk because of the low vitamin K content of human milk and its association with the development of an intestinal microflora that makes less vitamin K. The content of vitamin D in human milk is dependent on maternal vitamin D status. However, many women have insufficient vitamin D stores, and vitamin D deficiency leading to rickets has been reported in breastfed infants, especially those with dark skin pigmentation and/or minimal exposure to sunlight (Weisberg et al., 2004). Oral vitamin D supplementation is indicated and can be accomplished either by giving the infant 400 international units (IU) daily or by advising the breastfeeding mother to take 6400 IU daily herself (Wagner and Greer, 2008; Hollis et al., 2015).

Nutritional Implications for the Premature Infant

Maternal breast milk is the preferred enteral feeding for premature infants, although its nutritional adequacy may be limited for several reasons (Henderson et al., 2007). The protein content of milk from mothers delivering prematurely is greater than that of milk from mothers delivering at term but never meets the needs of smaller and more immature preterm infants (Polberger and Raiha, 1995). Fat is the most variable nutrient in human milk, and its content differs throughout the day, from mother to mother, within a single milk expression, and during lactation (Neville et al., 1984). Further, since human milk is not homogenized, the fat content separates from the body of milk during standing and may be lost

with multiple milk transfers and tube feedings (Greer et al., 1984; Schanler, 1988). Gastrointestinal advantages of human milk as compared with preterm formula include faster gastric emptying, less feeding intolerance, and fewer days to full enteral feeds (Schanler et al., 1999; Tudehope, 2013; Assad et al., 2016). However, the concentrations of protein, sodium, and zinc decline throughout lactation, and the nutrient needs of the premature infant remain higher than those of term infants until after term postmenstrual age. Therefore in contrast to the term infant, this physiologic decline in milk protein and micronutrient content precedes any reduction in the premature infant's needs. For the very low birth weight (VLBW; <1500 g) infant, the feeding of unfortified human milk results both in protein insufficiency, manifest by declines in growth rates and lowered biochemical indicators (blood urea nitrogen [BUN] and serum prealbumin concentrations), and inadequate bone health, marked by declines in serum phosphorus concentration and increases in alkaline phosphatase activity, as compared with feeding of preterm formula (Rowe et al., 1979; Cooper et al., 1984; Kuschel and Harding, 2004).

Thus VLBW infants should receive human milk fortifier, a multinutrient supplement designed to meet their nutritional needs and prevent clinical deficiency diseases and growth failure. Meta-analysis comparing feeding of fortified (versus unfortified) human milk shows an increase in short-term weight, length, and head circumference growth without adverse effects (Kuschel and Harding, 2004). In addition, bone density, nitrogen balance, and BUN values are all increased with fortified human milk versus unfortified milk (Quigley and McGuire, 2014). Fortifier adds not only protein, calcium, phosphorus, and calories but also other micronutrients (Table 67.4). A new class of fortifiers derived from pasteurized donor human milk has been developed (Sullivan et al., 2010). The use of an exclusive human milk diet, made with human milk–based fortifier instead of bovine milk–based human milk fortifier, has been shown to result in lower rates of necrotizing enterocolitis (NEC) (Sullivan et al., 2010).

Human milk fortifier is usually added once the premature infant is tolerating tube feeding, and its use is continued until the infant has achieved all oral feedings, a weight of 1800 g, or is near to discharge from the hospital (Tudehope, 2013; Tudehope et al., 2013; Underwood, 2013). Evidence regarding postdischarge multinutrient fortification of breast milk is limited, and hence metaanalysis does not provide convincing guidance (Young et al., 2013). However, the infant will likely benefit from an individualized plan that includes supplementation or fortification, maternal lactation support, and monitoring to achieve a weight gain of more than 20 g/day until “catch up” to growth for corrected age has been achieved, linear growth of 0.5 cm/week, and an alkaline phosphatase level less than 450 IU/L (Groh-Wargo and Thompson, 2014). Usually the enriched diet is maintained for 3 to 6 months, but excessive growth (i.e., crossing more than two channels upward) should be avoided.

Host Defense: Prevention of Infections

Bioactive factors such as sIgA, lactoferrin, lysozyme, oligosaccharides, growth factors, and cellular components augment the infant's active host defenses (Table 67.5; Ballard and Morrow, 2013). Infants also receive specific passive immunity via the enteromammary immune system in which the mother produces sIgA antibody in response to foreign antigens, and specific antibodies are then elaborated at mucosal surfaces and in her breast milk (Kleinman and Walker, 1979).

TABLE 67.4 Nutrient Composition of Preterm Human Milk With and Without Fortifier

| | Preterm Human Milk (1 Week) | Mature Preterm Human Milk (1 Month) | Mature Preterm Human Milk Plus Human Milk Fortifier ^a |
|--------------------------------|-----------------------------|-------------------------------------|--|
| Volume (mL) | 100 | 100 | 100 |
| Energy (kcal) | 67 | 69 | 83 |
| Protein (g) | 2.4 | 1.5 | 2.5–2.6 |
| Whey/casein (%) | 70/30 | 70/30 | 70/30 |
| Fat (g) | 3.8 | 3.6 | 4.0–4.6 |
| Medium-chain triglycerides (%) | 2 | 2 | 11–17 |
| Carbohydrate (g) | 6.1 | 6.7 | 7.1–8.5 |
| Lactose (%) | 100 | 100 | 80–85 |
| Calcium (mg) | 25 | 29 | 119–146 |
| Phosphorus (mg) | 14 | 9.3 | 59–76 |
| Magnesium (mg) | 3.1 | 2.4 | 3.4–9.4 |
| Sodium (mEq, mmol) | 2.2 | 0.9 | 1.6 |
| Potassium (mEq, mmol) | 1.8 | 1.3 | 2–2.9 |
| Chloride (mEq, mmol) | 2.6 | 1.5 | 1.9–2.6 |
| Zinc (μg) | 500 | 215 | 935–1215 |
| Copper (μg) | 80 | 51 | 95–221 |
| Vitamin A (IU) | 560 | 227 | 847–1177 |
| Vitamin D (IU) | 4 | 1.2 | 122–151 |
| Vitamin E (mg) | 1.0 | 0.3 | 3.5–4.9 |

^aEnfamil human milk fortifier (Mead Johnson Nutritionals, Evansville, Indiana) and Similac human milk fortifier (Ross Laboratories, Columbus, Ohio) are powdered formulations that use bovine protein (based on addition of four packets to 100 mL mature preterm human milk). Liquid bovine milk protein–based fortifiers (Enfamil human milk fortifier acidified liquid and Similac human milk fortifier concentrated liquid) and liquid human milk–based fortifiers are also available (Prolacta+ H²MF[®] all-liquid milk fortifier [<http://www.prolacta.com/>]). Liquid fortifiers displace and hence reduce part of the volume of human milk that the infant receives. IU, International unit; Kcal, kilocalorie; mEq, milliequivalent.

From Gross SJ, David RJ, Bauman L, Tomarelli RM. Nutritional composition of milk produced by mothers delivering preterm. *J Pediatr*. 1980;96:641–644; Butte NF, Garza C, Johnson CA, Smith EO, Nichols BL. Longitudinal changes in milk composition of mothers delivering preterm and term infants. *Early Hum Dev*. 1984a;9:153–162; Specker BL, Greer F, Tsang RC. Vitamin D. In: Tsang RC, Nichols BL, eds. *Nutrition During Infancy*. Philadelphia, PA: Hanley & Belfus; 1988: 264–276; Schanler RJ. Water-soluble vitamins for premature infants. In: Tsang RC, Uauy R, Koletzko B, Zlotkin S, eds. *Nutrition of the Preterm Infant. Scientific Basis and Practical Guidelines*. Cincinnati, OH: Digital Educational Publishing; 2005:173–199; and Schanler RJ, Atkinson SA. Human milk. In: Tsang RC, Uauy R, Koletzko B, Zlotkin S, eds. *Nutrition of the Preterm Infant. Scientific Basis and Practical Guidelines*. Cincinnati, OH: Digital Educational Publishing; 2005:333–356.

In both developing and developed countries, there is a reduction in all-cause infection-related mortality attributable to breastfeeding, with a clear “dose response.” The dose response has been categorized as exclusively, predominantly, partially, or never breastfed. The relative risk of death due to all infectious causes is significantly greater in infants aged 0 to 5 months who were never breastfed

(relative risk [RR] 8.66) or who were partially breastfed (RR 4.56) and even who were predominantly but not exclusively breastfed (RR 1.7), each in comparison with exclusively breastfed infants aged 0 to 5 months. Children aged 6 to 23 months who were never breastfed, as compared with ever breastfed, had a 2.1 times higher risk of death (Sankar et al., 2015). In developing countries the incidence, prevalence, and mortality because of diarrheal illnesses are significantly decreased by breastfeeding. When compared with exclusive breastfeeding, mortality caused by diarrhea was higher among infants who were never breastfed (RR 10.52), partially breastfed (RR 4.62), or predominantly breastfed (RR 2.28) (Lamberti et al., 2011). These data demonstrate the “dose dependent” benefit of breast milk for prevention of death caused by diarrheal illness. Never breastfed infants aged 0 to 11 months, as compared with those who were predominantly breastfed, were also at greater risk of death from diarrhea illness (RR 11.73) (Lamberti et al., 2011). Even when only infants in the developed world are considered, the risk of nonspecific gastrointestinal disease is lower among infants aged 0 to 11 months who were ever breastfed as compared with those who were never breastfed (Ip et al., 2007). Exclusive breastfeeding for the first 6 months of life reduces the risk of otitis media in children younger than 2 years, and both longer duration and any versus no breastfeeding are protective (Bowatte et al., 2015); this benefit may extend through age 6 years (Li et al., 2014). Worldwide, the risk of acute lower respiratory tract infection (LRTI) caused by respiratory syncytial virus was increased by not breastfeeding (Shi et al., 2015); the risk of death because of pneumonia was higher for both never versus exclusively breastfed infants aged 0 to 5 months and for never versus ever breastfed children aged 6 to 23 months (Lamberti et al., 2013). In developed countries, after adjustment for day care, smoke exposure, and socioeconomic status, the risk of hospitalization because of LRTI was significantly lower for infants exclusively breastfed for 4 months versus those receiving no breast milk (Ip et al., 2007; Table 67.6).

Clinical studies throughout the world and over several decades have also suggested a decrease in the rates of morbidities in premature infants fed human milk (Narayanan et al., 1980; Hylander et al., 1998; El-Mohandes et al., 1998; Ronnestad et al., 2005). Pasteurized donor milk, rather than preterm formula, is now recommended for infants whose mothers are unable to provide full volumes of breast milk for their premature infant (Arslanoglu et al., 2013; Quigley and McGuire, 2014; AAP, 2015). Methodological issues, including differing definitions of “human milk” (mother’s own vs donor, frozen vs fresh, mixed with formula) and of human milk intake volume (mL/kg per day vs daily percentage of enteral or all intake) create difficulties in the interpretation of results, yet data from studies that quantified human milk intakes suggest that the protective effect is (1) optimized by an exclusive human milk diet and (2) likely dose dependent.

Premature infants who receive their own mother’s milk have a significantly lower risk of NEC after adjustment for gestational age, and metaanalysis confirms the magnitude of these results (Ip et al., 2007; Sisk et al., 2007; Meinen-Derr et al., 2009). Donor human milk is also protective against NEC in premature infants when compared with formula (see later) (Arslanoglu et al., 2013; Quigley and McGuire, 2014; Kantorowska et al., 2016). The risk of late-onset sepsis is likely reduced by human milk feeding (Schanler et al., 1999; Furman et al., 2003; Ronnestad et al., 2005; Cossey et al., 2013; Pammi and Weisman, 2015). NICU length of stay is shorter for human milk–fed infants: a direct relationship between lower risk of morbidity and dose of human milk (19% lower risk

TABLE 67.5 Selected Bioactive Factors in Human Milk**Immunoglobulins**

| | |
|-------------------------------------|---|
| Secretory IgA | Specific antigen-targeted antiinfectives |
| IgG | Antimicrobial |
| IgM | Complement activation |
| Lactoferrin | Immunomodulation, iron chelation, antimicrobial action, antiadhesive, trophic for intestinal growth |
| Lactadherin | Antiviral and antiinflammatory |
| Lysozyme | Bacterial lysis, immunomodulation |
| κ -Casein | Antiadhesive, bacterial flora |
| Oligosaccharides | Stimulate colonization by beneficial organisms |
| Gangliosides and glycosaminoglycans | Prevent infections |
| Cytokines ^a | Antiinflammatory, proinflammatory, epithelial barrier function, recruit neutrophils |
| Macrophages | Antiinfectious and activate T cells |

Growth Factors

| | |
|--|--|
| Epidermal growth factor | Luminal surveillance, repair of intestine |
| Transforming growth factor | Promotes epithelial cell growth (transforming growth factor- α) Suppresses lymphocyte function (transforming growth factor- β) |
| Nerve growth factor | Promotes growth of neurons |
| Vascular endothelial growth factor | Promotes angiogenesis and tissue repair |
| Insulin-like growth factor | Promotes growth |
| Macrophage migratory inhibitory factor | Prevents macrophage movement |

Hormones

| | |
|--------------|---|
| Adiponectin | Reduction of infant BMI, antiinflammatory |
| Leptin | Regulation of infant BMI and appetite |
| Calcitonin | Promotes development of enteric neurons |
| Somatostatin | Regulates growth of gastric epithelium |

Enzymes

| | |
|--|---|
| Platelet-activating factor acetylhydrolase | Blocks action of platelet-activating factor |
| Glutathione peroxidase | Prevents lipid oxidation |
| Nucleotides | Enhance antibody responses, bacterial flora |
| Vitamin A, E, C | Antioxidants |
| Glutamine (amino acid) | Intestinal cell fuel, immune responses |
| Lipids | Antiinfective properties |
| Mucins 1 and 4 | Block infectious agents |

^aIncludes interleukins 6, 7, 8, and 10, tumor necrosis factor- α , transforming growth factor- β , and interferon gamma.

BMI, Body mass index.

Modified from Schanler RS, Krebs N, and Mass S, eds. *AAP/ACOG Breastfeeding Handbook for Physicians*. Elk Grove, IL: American Academy of Pediatrics; 2013:77 and Ballard O, Morrow AL. Human milk composition: nutrients and bioactive factors. *Pediatr Clin North Am*. 2013;60:49–74.

for each additional 10 mL of human milk/kg/day) and significantly lower NICU costs has been documented, and exclusive human milk feeding, as compared with all other diets, was associated with reduced risk of NEC ($P < .011$), fewer days to full feeds ($P < .001$), and lower hospitalization cost (savings up to \$106,968 per

infant) (Patel et al., 2013; Assad et al., 2016). The rates of both any-stage and severe retinopathy of prematurity are decreased by human milk feeding, with a dose response noted (Zhou et al., 2015). Exclusive human milk feeding is also associated with reduced risk of bronchopulmonary dysplasia (Spiegler et al., 2016).

TABLE 67.6 Benefits of Breastfeeding for Infants and Mothers

| Condition | Comparison | Odds Ratio | 95% Confidence Interval |
|--|--|------------|-------------------------|
| Maternal Benefits | | | |
| Breast cancer | All for each additional 12 months of lactation | 0.74 | 0.69–0.79 |
| Ovarian cancer | | 0.63 | 0.56–0.71 |
| Hypertension | | 0.89 | 0.84–0.93 |
| Hyperlipidemia | | 0.81 | 0.76–0.87 |
| Cardiovascular disease | | 0.80 | 0.65–0.97 |
| Type 2 diabetes | | RR 0.91 | 0.86–0.96 |
| Infant/Child Benefits | | | |
| Infectious Diseases | | | |
| All cause 0–5 months | Never vs exclusive BF | RR 8.66 | 3.19–23.5 |
| All cause 6–23 months | Never vs ever BF | RR 2.09 | 1.68–2.60 |
| Otitis media <2 years | Exclusive BF ≥6 months | 0.57 | 0.44–0.75 |
| | Ever vs never BF | 0.67 | 0.56–0.80 |
| Gastrointestinal disease 0–11 months | Ever vs never BF | 0.36 | 0.32–0.41 |
| RSV LRTI | Never vs ever BF | 2.24 | 1.56–3.20 |
| Pneumonia deaths 0–5 months worldwide | Never vs exclusive BF | RR 14.97 | 0.67–332.74 |
| Pneumonia deaths 6–23 months worldwide | Never vs ever BF | RR 1.92 | 0.79–4.68 |
| Respiratory disease hospitalization | Exclusive BF for 4 months vs never BF | RR 0.28 | 0.14–0.54 |
| Noninfectious Conditions | | | |
| <Childhood leukemia | BF for at least 6 months | 0.81 | 0.73–0.89 |
| Child dental caries | Up to 12 months of BF | 0.50 | 0.25–0.99 |
| Asthma age 5–18 years | Ever vs never BF | 0.88 | 0.82–0.95 |
| | More vs less BF | 0.90 | 0.84–0.97 |
| Eczema <2 years | Exclusive BF for 4 months | 0.74 | 0.57–0.97 |
| Allergic disease <5 years | | 0.79 | 0.63–0.98 |
| SIDS | Exclusive BF | SOR 0.27 | 0.24–0.31 |
| | Ever vs never BF | SOR 0.55 | 0.44–0.69 |
| ROP | | | |
| Severe ROP | Exclusive HM feeding vs formula feeding | 0.10 | 0.04–0.29 |
| Any ROP | Any HM feeding vs formula feeding | 0.42 | 0.08–2.18 |
| Necrotizing enterocolitis | ≥50% HM feeding in first 14 days vs less than 50% HM feeding | 0.17 | 0.04–0.68 |

Univariable summary odds ratio for exclusive breastfeeding and multivariable summary odds ratio for any breastfeeding.

BF, Breastfeeding; HM, human milk; LRTI, lower respiratory tract infection; ROP, retinopathy of prematurity; RR, relative risk; RSV, respiratory syncytial virus; SIDS, sudden infant death syndrome; SOR, summary odds ratio.

Chronic Conditions of Childhood

Data from epidemiologic studies suggest that certain chronic disorders have a lower incidence in children who were breastfed as infants; however, causal relationships between breastfeeding and the health outcomes of interest are difficult to infer because of the nature of the studies that can be performed (Table 67.6). Among

these are inflammatory bowel disorders and type 1 diabetes, each with limited or conflicting evidence for a protective effect (Klement et al., 2004; Patelarou et al., 2012; Silano et al., 2016). A 19% reduced risk of childhood leukemia has been associated with breastfeeding for 6 months or more (Amitay and Keinan-Boker, 2015). Dental caries are reduced by longer duration of breastfeeding, although breastfeeding beyond 12 months may actually increase

risk (Tham et al., 2015). Data are conflicting regarding allergic disease, but metaanalysis shows reduced risk of asthma at age 5 years to age 18 years for ever versus never breastfeeding and for more versus less breastfeeding, with no impact of stratification by family atopy (Lodge et al., 2015). Reduced risk of eczema before age 2 years, but not after, was associated with exclusive breastfeeding for 3 to 4 months (Lodge et al., 2015). Breastfeeding does not specifically reduce the risk of food allergies, although it appears to reduce the risk of generalized allergic disease before age 5 years (Lodge et al., 2015).

There appears to be an inverse relationship between exclusivity and duration of breastfeeding and the risk of obesity and overweight in childhood and adulthood (metaanalyses showing pooled odds ratio (OR) of 0.74; 95% confidence interval (CI) 0.70–0.78) (Ip et al., 2007; Horta et al., 2015b). This association is stronger for studies assessing exclusive breastfeeding and for those reporting children as compared with adults and is weaker for studies with large numbers of participants (>1500) and for those that adjusted the data for relevant confounders, including maternal BMI, birth weight for gestational age, and socioeconomic factors. Although it is difficult to eliminate the possibility of residual confounding by unmeasured lifestyle factors, high-quality studies from high-, medium-, and low-income settings show a 13% reduction in the risk of obesity with breastfeeding (summary OR 0.87; 95% CI 0.76–0.99) (Ip et al., 2007; Horta et al., 2015b). Biologically plausible mechanisms for this effect include more optimal self-regulation of energy intake when feeding at the breast (Li et al., 2012), higher protein/energy intake among formula-fed than breastfed infants leading to neonatal obesity and hence childhood obesity (Heinig et al., 1993; Rolland-Cachera et al., 1995; Stettler et al., 2002), differences in release of insulin and other gut and pancreatic hormones in formula-fed versus breastfed infants that may alter fat deposition patterns (Lucas et al., 1980), and reduced risk of type 2 diabetes mellitus with breastfeeding (Horta et al., 2015b). Additionally, hormones in breast milk, including leptin, adiponectin, and ghrelin, appear to regulate appetite, growth, fat deposition, and energy balance, which may explain differences in early body composition and later BMI between breastfed and formula-fed infants (Savino et al., 2009; Kon et al., 2014). Neither blood pressure nor cholesterol levels in later life appear to be impacted by breastfeeding (Owen et al., 2008; Horta et al., 2015b).

Neurobehavioral Aspects

Whether an improvement in cognitive ability is attributable to breastfeeding and/or human milk has been difficult to determine. Disagreement has remained even when (1) results are adjusted for the key confounders of maternal intelligence, education, and socioeconomic class, and (2) only studies meeting rigorous standards are included (Ip et al., 2007). However, metaanalysis using strict study criteria found that breastfeeding was positively associated with an average gain of 3.44 points (95% CI 2.30–4.58 points) on intelligence testing in childhood and adolescence for breastfed individuals. When only the highest-quality studies ($n = 4$ with sample size >500, breastfeeding recall time <3 years, controlled for maternal intelligence) were examined, the gain on intelligence testing was still significant (1.76 points; 95% CI 0.25–3.26 points) (Horta et al., 2015a).

Significantly improved neurodevelopment among extremely low birth weight infants as measured by the Bayley Scales of Infant Development II at age 18 to 22 months and age 30 months has

been correlated with the receipt of fortified human milk during hospitalization. The magnitude of the effect at 18 to 22 months was greatest in the highest quintile (>80%), who averaged 110 mL/kg per day of human milk, and at 30 months the effect persisted such that for every 10 mL/kg per day increase in human milk, the Mental Developmental Index increased by 0.59 points, the Psychomotor Developmental Index increased by 0.56 points, and the total behavior percentile score increased by 0.99 points (Vohr et al., 2006, 2007).

Mother–infant bonding is also enhanced during breastfeeding (Feldman et al., 2007; Galbally et al., 2011). The likely biologic basis for this universal observation is oxytocin, which causes the milk ejection reflex during nursing, and serves as a central neurotransmitter that directly affects maternal nurturing behaviors, mother–infant social interaction, gaze, vocalizations, and affectionate touch (Britton et al., 2006).

Infant and Childhood Mortality

The risk of all-cause death worldwide was higher among children aged 0 to 5 months for those who were not breastfed (RR 14.4), partially breastfed (RR 4.8), and predominantly breastfed (RR 1.5) as compared with those who were exclusively breastfed (Sankar et al., 2015). Children aged 6 to 23 months who were not breastfed had higher all-cause mortality than those who continued breastfeeding; mortality decreased with exclusivity and duration of breastfeeding, but any breastfeeding was protective (Sankar et al., 2015). Breastfeeding also appears protective against sudden infant death syndrome (SIDS) (Hauck et al., 2011). On metaanalysis the risk of SIDS was lowered for exclusively breastfed infants and for infants with any breastfeeding (Hauck et al., 2011). Although a direct mechanism is not known, the effect is strong and dose dependent and does not appear only as a marker for confounders such as passive smoking or demographic factors.

Maternal Benefits

There are numerous benefits of breastfeeding for the mother (Table 67.6). Lactation has a beneficial effect on lipid and glucose metabolism and is associated with significant reduction in the risk of type 2 diabetes and cardiovascular morbidities (Schwarz et al., 2009; Aune et al., 2014; Chowdhury et al., 2015). Multiple studies have documented a significantly decreased incidence of premenopausal breast and ovarian cancer in women who have breastfed (Collaborative Group on Hormonal Factors in Breast Cancer, 2002; Chowdhury et al., 2015). Neither rapid postpartum weight loss nor changes in bone mineral density are attributable to breastfeeding; however, the risk of postpartum hemorrhage may be decreased by breastfeeding combined with skin-to-skin care, although additional study is needed (Chowdhury et al., 2015; Abedi et al., 2016). Postpartum depression both predicts and is predicted by cessation of breastfeeding; it is not known whether breastfeeding can directly reduce postpartum depression (Dias and Figueiredo, 2015). Exclusive breastfeeding delays resumption of normal ovarian cycles and the return of fertility, probably because of an elevated prolactin level (McNeilly, 1993). The lactational amenorrhea method, defined by full breastfeeding (round-the-clock), no resumption of menses, and infant age less than 6 months, is a highly effective global contraceptive program with efficacy rates of 98.5%–100% (Peterson et al., 2000; WHPO, 2017). Breastfeeding can therefore contribute to child spacing, which additionally improves maternal and child health.

Societal Impact of Breastfeeding

From a national economic perspective, it has been estimated that if all infants enrolled in the WIC program were breastfed exclusively for the first 6 months of life, food package costs would be reduced by 18%, realizing approximately \$1.2 million of savings (Institute of Medicine, 2010; Hartmann et al., 2012; US Department of Agriculture, Food and Nutrition Service, 2015). Additional savings can accrue from a reduction in household expenditure on formula and healthcare costs, with less parental work absence because of the decreased rates of illness experienced by breastfed infants; costs significantly drop with every additional month of breastfeeding and each month delay in return to work after 3 months (Cattaneo et al., 2006). Cost analyses estimating the burden of suboptimal breastfeeding for maternal health alone predicted a \$733.7 million direct loss to society; similar analyses showed that if 80% of US families exclusively breastfed for 6 months, \$10.5 billion would be saved and 741 infant deaths would be prevented (Bartick and Reinhold, 2010; Bartick et al., 2013). Thus extraordinary societal and economic incentives support attainment of full breastfeeding for each infant–mother dyad.

Contraindications to Breastfeeding

Few true contraindications to breastfeeding exist (Box 67.2; AAP/ACOG, 2012; CDC, 2016). Mothers with fever or other minor illness should be permitted to breastfeed, since the infant has already been exposed to the infectious agent and will be able to benefit from the mother's developing immunity if breastfeeding can continue. Few maternal medications contraindicate breastfeeding, and alternative choices are available in most cases; the National Institutes of Health US National Library of Medicine Drugs and Lactation Database (<https://toxnet.nlm.nih.gov/newtoxnet/lactmed.htm>) is the optimal resource and should be consulted (US National Library of Medicine, 2015). Mothers who have undergone breast reduction or breast implant surgery can breastfeed but should work closely with a lactation specialist because milk supply may be negatively impacted if ducts or nerves were severed during surgery.

Anatomy and Physiology of Lactation

Breast milk is produced by the mammary alveolar cells of the breast after childbirth. The mammary gland is a highly evolved skin gland, and its rudiments are first seen during the sixth week in utero. Mammogenesis, or breast development, begins during puberty with increased breast size due mainly to estrogen and lobuloalveolar development facilitated predominantly by progesterone. During pregnancy, with the support of these hormones and others, including prolactin and placental lactogen, breast glandular tissue further differentiates, and the alveolar epithelium proliferates and then becomes secretory. Research using ultrasound technology (Fig. 67.1) shows that the milk ducts branch close to the nipple, that their number is lower and more variable than previously believed, that most glandular tissue is close to the nipple, and that the “lactiferous sinuses,” which were thought to store milk, do not exist (Ramsay et al., 2005). These findings have implications for hand expression of breast milk, since inadvertent pressure on the breast close to the nipple may actually inhibit milk flow, as well as for breastfeeding after breast augmentation or reduction surgery, since major nerves and milk ducts may have been unintentionally severed or damaged during surgery, with risk of reduced milk supply.

• BOX 67.2 Contraindications to Breastfeeding

Maternal

- Untreated active maternal military tuberculosis: Refrain from breastfeeding or infant contact until treated and no longer contagious, approximately 2 weeks.
- Active herpetic lesions on the breast: Refrain from breastfeeding until active lesions on breast and nipple have resolved. Vaginal herpes is not a contraindication.
- Active varicella (chickenpox) lesions on the breast: Express milk until lesions are crusted over; administer varicella immunoglobulin to infant.
- Active human immunodeficiency virus (HIV) infection: Active HIV infection is not an absolute contraindication in developing countries.^a
- Active human T-lymphotrophic virus (types 1 and 2) infection: Use of illicit drugs is an absolute contraindication.^b
- Drug-free methadone-maintained women can breastfeed.
- Cancer chemotherapy or radiation treatment: For the duration of treatment, seek consultation for diagnostic studies using radiation to determine duration for which expressed milk must be discarded.
- Acute maternal illness with swine flu (H1N1 flu):^c
 - If the mother is ill, her infant should ideally be fed the mother's expressed breast milk by someone who is not ill.

Infant

- Galactosemia: Lactose cannot be ingested and is the carbohydrate of breast milk.
- Other inborn errors of metabolism require consultation regarding the specific metabolic defect(s).

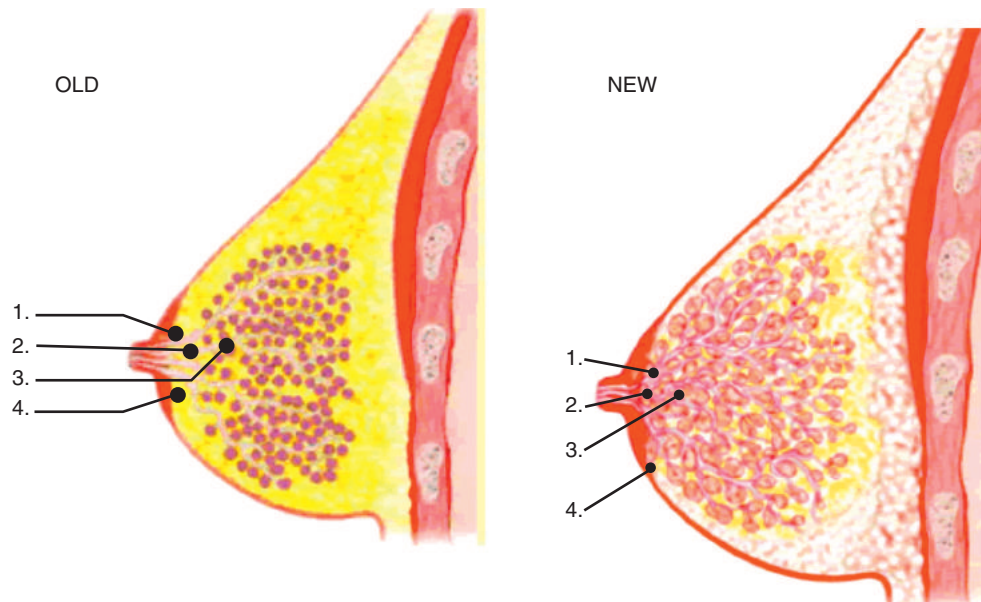
^a“Since 2010, the World Health Organization has recommended that mothers who are HIV-infected take ARVs [anti-retroviral medications] and exclusively breastfeed their babies for 6 months, then introduce appropriate complementary foods and continue breastfeeding up to the child's first birthday.... Even when ARVs are not available, mothers should be counseled to exclusively breastfeed for 6 months and continue breastfeeding thereafter unless environmental and social circumstances are safe for, and supportive of, feeding with infant formula.” World Health Organization recommendation available at <http://www.who.int/mediacentre/factsheets/fs342/en/>.

^bThe American Academy of Obstetricians and Gynecologists (Committee opinion no. 637: Marijuana use during pregnancy and lactation. American College of Obstetricians and Gynecologists. Obstet Gynecol 2015;126:234–8) and the American Academy of Pediatrics (<http://pediatrics.aapublications.org/content/135/3/584>) strongly discourage cannabis use during lactation; the chapter authors acknowledge evidence is limited and individualized care is important.

^cSee the Centers for Disease Control and Prevention recommendations: “2009 H1N1 Flu (Swine Flu) and Feeding Your Baby: What Parents Should Know” at <http://www.cdc.gov/h1n1flu/infantfeeding.htm>.

Data from Kimberlin DW, Brady MT, Jackson MA, Long SS. Red book: 2015 report of the Committee on Infectious Diseases. Elk Grove, IL: American Academy of Pediatrics, 2015; Division of Nutrition, Physical Activity, and Obesity, National Center for Chronic Disease Prevention and Health Promotion, Centers for Disease Control and Prevention. Diseases and Conditions. When should a mother avoid breastfeeding? <http://www.cdc.gov/breastfeeding/disease>; 2016; and Hill M, Reed K. Pregnancy, breast-feeding, and marijuana: a review article. Obstet Gynecol Surv. 2013;68:710–718.

Lactogenesis begins by midpregnancy, although actual milk secretion does not occur at this time because of high circulating levels of progesterone (and probably estrogen). During pregnancy, secretory differentiation of the mammary epithelial cells into lactocytes that have the ability to produce milk components occurs: this is known as *lactogenesis I*. At the end of gestation the alveoli are filled with proteins, including sIgA, and leukocytes and desquamated cells: this glandular fluid is colostrum. Lactogenesis II, or secretory activation of the lactocytes (also known as *the milk coming in*), usually occurs between day 2 and day 8. This phase, defined by the copious onset of milk secretion, is triggered by birth and the drop in progesterone level associated with removal of the placenta. Adequate circulating prolactin and cortisol levels are required, and other hormones, including insulin and thyroid hormone, likely play a supporting role. During this time daily milk volume increases from about 50 to



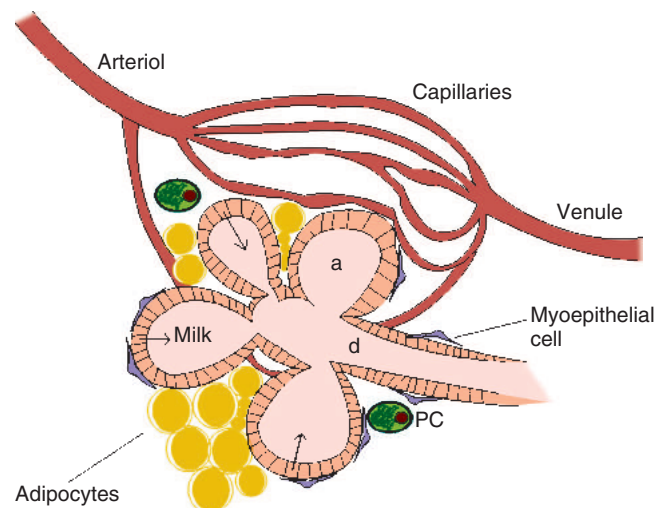
• **Fig. 67.1** Anatomy of the breast. (From Medela AG. Old vs new anatomy. (Available at <http://www.medelabreastfeedingus.com/for-professionals/cbe-information/106/breast-anatomy-research>.) 1-Ducts branch closer to nipple, 2-The conventionally described lactiferous sinuses do not exist, 3-Glandular tissue is found closer to the nipple, 4-Subcutaneous fat is minimal at the base of the nipple.

500 mL, and transitional milk is produced, a descriptor for milk that is literally transitioning in composition from colostrum to mature milk (Neville, 1999; Neville et al., 2001; Pang and Hartman, 2007).

The control of lactogenesis is completely hormonal. Evidence suggests that milk removal or infant suckling is not needed for the programmed changes of lactogenesis, although milk removal may improve the efficiency of early milk secretion (Neville et al., 2001). Delayed lactogenesis II has been associated with placental retention (failure of progesterone level to decline postpartum), hypothyroidism, cesarean delivery, and premature delivery; there are also associations with factors related to glucose metabolism, including maternal insulin-dependent diabetes, elevated maternal BMI, and higher infant birth weight (Nommsen-Rivers et al., 2012).

If the mother begins to feed her infant at the breast (or to express milk from the breast), then the next phase of lactation, called *galactopoiesis* or *maintenance of milk secretion*, starts at approximately day 9 postpartum and continues until weaning and involution of the breast. The control of galactopoiesis is both endocrine and autocrine. Prolactin and cortisol are necessary for milk production: prolactin binds to epithelial cell receptors and is responsible for activating the milk protein genes for α -lactalbumin and casein. Milk synthesis remains near 700 to 800 mL/day throughout lactation, but the actual volume of milk is dependent on milk removal. With incomplete emptying of the breast because of infrequent or inefficient infant suckling, milk synthesis is adjusted downward because of a locally produced whey protein called *feedback inhibitor of lactation* (FIL). FIL appears to act on alveolar cell receptors to decrease their sensitivity to prolactin and therefore to decrease milk production. The cellular mechanisms for milk synthesis and secretion are described elsewhere (Neville, 1999; Neville et al., 2001).

Milk that was accumulated in the alveoli cannot flow passively into the ducts. Actual milk ejection, or galactokinesis, is dependent on the hormone oxytocin, which is produced in the posterior pituitary. Oxytocin secretion is stimulated by infant suckling and other sensory inputs that mimic the infant such as cry, sight, smell, and touch. Stretch receptors in the canalicular ductal system are



• **Fig. 67.2** The milk secreting unit: the terminal duct lobular unit. a, Alveoli, d, ductule, PC, plasma cells. (From Neville MC. Milk secretion: an overview. Available at http://www.health-e-learning.com/articles/Neville_MILK_SECRETION_2008.pdf.)

activated, and afferent nerve endings send a signal to the central nervous system. The alveolar lactocytes are wrapped with myoepithelial cells that lack innervation but have oxytocin receptors (Fig. 67.2). In the presence of oxytocin, they squeeze milk into the ducts, leading to milk ejection. Prolactin originates in the anterior pituitary, and although secretion is also stimulated by suckling, prolactin production is not inhibited by pain and stress like that of oxytocin, and the milk ejection reflex, or “let down,” does not directly depend on it.

During weaning or extended periods of infrequent suckling at the breast, levels of prolactin drift downward. The tight junctions between alveolar lactocytes appear to open and allow passage of

electrolytes, including sodium and chloride. Elevated breast milk sodium level is associated with several clinical situations, including involution and remodeling of the mammary gland, which occurs with cessation of milk secretion (Lawrence et al., 2016).

Management of Breastfeeding

The management of lactation begins before pregnancy (AAP/ACOG, 2012; ACOG, *Optimizing Support for Breastfeeding*, 2016). Formal prenatal breastfeeding education (BFHI step 3), in addition to personalized information offered during prenatal visits, positively impacts exclusive breastfeeding and breastfeeding at 6 months (Academy of Breastfeeding Medicine [ABM] Clinical Protocol 19, Breastfeeding Promotion in the Prenatal Setting, <http://www.bfmed.org/Resources/Protocols.aspx>; Lawrence et al., 2016). Ideally breastfeeding education, and the function of the mammary glands, should be taught in secondary school and continued throughout the reproductive life cycle. In support of this approach, maternal prenatal intention to breastfeed appears to be the best predictor of breastfeeding initiation and duration (Donath et al., 2003; Stuebe and Bonuck, 2011). Obstetric providers should initiate the discussion of breastfeeding as an evidence-based choice and should encourage and support mothers in setting their own breastfeeding goals.

Hospital Care

Hospital management of delivery room and postpartum mother–infant routines (Box 67.1) has also been shown to impact rates of breastfeeding (Kramer et al., 2001; Philipp et al., 2001; DiGirolamo et al., 2008; Rosenberg et al., 2008; AAP, *Breastfeeding and the use of Human Milk*, 2012; Moore et al., 2012; Sinha et al., 2015; Yotebieng et al., 2015; Olaiya et al., 2016). As summarized by the ABM Clinical Protocol 5, Peripartum Care, and Clinical Protocol 7, Model Hospital Policy (<http://www.bfmed.org/Resources/Protocols.aspx>), written hospital guidelines should promote and support breastfeeding for each mother–infant dyad per recommendations of the American Academy of Pediatrics and WHO, maximize each mother’s ability to meet her own breastfeeding goals, and maximize each infant’s opportunity to receive full feeds of breast milk (BFHI step 1) (AAP, *Breastfeeding and the use of Human Milk*, 2012; Feldman-Winter et al., 2012; Sample Hospital Breastfeeding Policy for Newborns, 2016). The BFHI 10 steps serve as a useful and evidence-based guide to hospital policy (Box 67.1). Each evidence-based step may require changes in entrenched hospital routines that are not conducive to breastfeeding. For example, mothers should be encouraged to put the infant to breastfeed within the first hour after birth (BFHI step 4). Newborns that are placed skin-to-skin on the mother’s abdomen a few minutes after birth are able to initiate a “crawl” toward the breast and nipple, latch on, and begin sucking (<http://www.breastcrawl.org/video.shtml>). The necessary medical routines of infant identification, drying, warmth, assessment, and administration of prophylactic eye drops and vitamin K can be safely delayed until after the first breastfeeding for healthy infants (AAP, *Breastfeeding and the use of Human Milk*, 2012).

Initial Management

Normal healthy infants are able to latch on to the breast in the first hour after birth. The goals for the infant during the postpartum stay include (1) achievement of latch on to the breast within the

• BOX 67.3 Risk Factors for Lactation Failure or Difficulty

Maternal Factors

Maternal chronic illness (e.g., cystic fibrosis, diabetes type 1 or 2)

Anatomic

Prior breast surgery

Inverted nipples, nipple size mismatch with infant mouth

Tubular or variant breast shape (size per se is not a factor)

Obesity

History of breastfeeding difficulty

Perinatal complications (e.g., hemorrhage, hypertension/preeclampsia, shock, infection)

Cesarean delivery

Multiples (twins, triplets, etc.)

Mother–infant separation

Secondary—nipple trauma, nipple pain, engorgement

Infant Factors

Premature delivery <34 weeks

Late preterm delivery (34 to 36 weeks’ gestation)

Early term delivery at 37 weeks

Birth weight large or small for gestational age

No effective latch in the first 24 hours

Anatomic or congenital anomaly

Trisomy

Major congenital malformation

Micrognathia, Pierre Robin sequence

Cleft palate or lip

Macroglossia or ankyloglossia

Neurologic abnormality with hypotonia or hypertonia

Neonatal intensive care unit admission/perinatal complications (e.g., hypoxic–ischemic encephalopathy, pneumonia)

Sleepy infant for any cause

Mother–infant separation

Secondary—supplementation more than once in 24 hours, weight >7% below birth weight, excessive pacifier use

first few hours after birth, (2) ability to transfer milk (or colostrum) adequately at each feeding, (3) feeding at the breast 8 to 12 times per 24 hours as cued by infant hunger (rooting, fist sucking, alerting), (4) nursing at both breasts each feeding for up to 15 to 20 minutes per side or until there are signs of infant satiety (fewer sucking bursts, longer pauses, sleepiness), and (5) the mother feeling comfortable with the infant feeding at the breast. Maternal and infant risk factors for lactation difficulty (Box 67.3) should be identified within the first few hours after birth so that interventions to promote maternal and infant health and the breastfeeding process can be promptly initiated, and this assessment process should be ongoing throughout the hospital stay.

Latch On

“Good latch” is the cornerstone of successful breastfeeding because it permits adequate milk transfer to the infant and prevents nipple pain for the mother. Brief videos are available that demonstrate how to assist a mother achieve latch (*Basics of Breastfeeding*). Nursing at the breast is fundamentally different from bottlefeeding in that the infant extracts the milk from behind the nipple while nursing rather than sucking on the nipple to elicit flow. There are several ways to hold an infant to breastfeed, but to achieve good latch, the mother and infant should be tummy-to-tummy, regardless

of the holding position, with the infant facing the mother's body (AAP/ACOG, 2012). The mother can hold her breast well behind the areola using a "C hold" with four fingers under the nipple and the thumb above. She then strokes the infant's mid upper lip with her nipple, and once rooting is elicited and the infant opens its mouth, she can promptly bring the infant in so it takes in the breast, including the areola, not just the nipple. The mother's nipple is protected since it is well back in the infant's mouth, and the infant's jaw massages milk from behind the areola. The infant's nose and chin will contact the mother's skin, and the mother should experience a tugging sensation without pain as the infant suckles with this deep latch. Many women acknowledge that breastfeeding is initially painful because of challenges with establishing good latch and because of the uterine contractions elicited by oxytocin during "let down," but if breast or nipple pain persists, prompt breastfeeding assistance is needed.

The First 2 Weeks to 2 Months

Each hospital should establish breastfeeding support groups or work with organized community support groups so that families have a resource on leaving the hospital (BFHI step 10). All breastfed infants should be seen by a knowledgeable healthcare professional at 3 to 5 days of age to recognize and to avoid potential problems of dehydration and severe jaundice (AAP, 2015).

The nursing history differs from infant to infant and over any 24-hour period, so although nursing 8 to 12 times per day is the average, some infants will "cluster feed," nursing more frequently for short periods. Without anticipatory guidance, new mothers may compare their infants with bottlefed infants and misinterpret the normal frequency of breastfeeding to mean that they have insufficient milk. As infants get older, they nurse more efficiently, and the frequency and duration of feedings decrease. New parents may expect their baby to cry when it is hungry and need guidance that crying is a late sign of hunger and can result in an infant who is difficult to calm and latch. Earlier hunger signs include rooting, finger and fist sucking, and lip smacking. Pacifier use is associated with a reduction in the risk of SIDS. However, pacifiers should not be introduced to breastfeeding infants until breastfeeding is well established, at about 3 to 4 weeks of age, because frequent use of a pacifier can conceal hunger cues, and the offering of artificial nipples may interfere with latch, both of which can adversely impact maternal milk supply (Hauck et al., 2011; Task Force on Sudden Infant Death Syndrome, 2011).

Once lactogenesis stage II is completed (the "milk has come in"), an infant who did not lose excessive weight and who is nursing effectively should obtain enough milk to begin gaining weight, 15–30 g per day, by day 4 or 5. At this rate, most breastfed infants will exceed their birth weight by 10 to 14 days and gain 20–30 g per day for the first 2 months. A breastfed infant who weighs less than birth weight at 2 weeks requires evaluation and intervention. Anticipatory guidance should include information about growth spurts, in which infants are restless and breastfeed more often than usual for 2 to 3 days. These typically occur at approximately ages 10 days, 3 weeks, 6 weeks, 3 months, and every few months and may be exhausting for the mother. Since milk supply depends on infant demand, permitting the infant to breastfeed frequently is optimal. In addition, screening for maternal depression, which is associated with a decreased duration of breastfeeding, and for other maternal concerns, such as sore nipples, is critical (AAP/ACOG, 2012; Dias and Figueiredo, 2015).

Growth of the Breastfed Infant

The rate and pattern of weight gain of breastfed infants differs from that of infants fed formula, and several studies have concluded that the breastfed infant's growth should be considered normative (Butte et al., 1984b, 1990; Dewey et al., 1991, 1992). The WHO Multicentre Growth Reference Study was undertaken between 1997 and 2003 and gathered primary longitudinal growth data from about 8500 healthy and optimally breastfed children of differing ethnic, racial, and cultural backgrounds (Brazil, Ghana, India, Norway, Oman, and the United States). WHO subsequently published an international growth reference (<http://www.who.int/childgrowth/>) based on the growth of these healthy and fully breastfed infants that benchmarks growth and development from birth to age 5 years, replacing older references (de Onis et al., 2006). See the Centers for Disease Control and Prevention growth charts, which use the WHO standards (http://www.CDC.gov/growthcharts/who_charts.htm).

Clinicians are occasionally in doubt as to when and how to intervene if a breastfed infant is not gaining adequate weight (Powers, 2001). A newborn younger than 2 weeks must be evaluated if its weight is more than 10% below birth weight. A newborn who fails to regain birth weight by 2 weeks of age or is not gaining a minimum of 20 g per day should be evaluated. The infant with growth faltering, when the weight for age (or weight for length) is less than two standard deviations below the mean or weight for age crosses more than two percentile channels downward on the growth chart, should also be evaluated. Infant physical examination and assessments of milk supply, intake, appropriateness of complementary foods, maternal history and behaviors, and the home environment are all part of the evaluation. Test weighing with an electronic scale before and immediately after a feeding is one method of measuring milk intake (Meier et al., 1990). Pumping after a feeding is a method to assess residual milk. Causes of growth problems not specifically related to breastfeeding must be considered, for example, cystic fibrosis or cardiac disease in the infant or severe postpartum depression in the mother.

Tongue Tie

Tongue tie, or ankyloglossia, is a congenital disorder with a male predominance (3:1), found in 4%–16% of infants, in which there is a short lingual frenulum that may restrict tongue movement and impact function, including breastfeeding. Poor latch with maternal nipple pain and insufficient milk transfer may result, although less than half of infants with even moderate to severe tongue tie appear to be affected (Ngerncham et al., 2013). As described in ABM Clinical Protocol 11, Neonatal Ankyloglossia (<http://www.bfmed.org/Resources/Protocols.aspx>), standardized clinical tools to assess the degree of tongue tie and its impact on breastfeeding include the Hazelbaker assessment tool for lingual frenulum function (Amir et al., 2006). One-week prefrenotomy and postfrenotomy changes in sucking pattern and milk transfer were demonstrated on submental ultrasonography (Geddes et al., 2008); however, whether or not frenulotomy (division of the frenulum), frenulectomy (removal of the frenulum), or frenuloplasty (frenulotomy with stitches) clearly improves breastfeeding has been controversial. Systematic reviews including five randomized controlled studies, of which four used validated measures of latch as a primary outcome, concluded that frenotomy in selected infants appears to provide objective improvements in breastfeeding

measures, with subjective improvement in maternal pain (Brooks et al., 2014; Francis and Krishnaswami, 2015).

Breastfeeding the Late Preterm Infant

Late preterm infants (34–36 weeks' gestation) are at high risk of complications of insufficient breast milk intake, including dehydration, hypernatremia, severe jaundice, and poor weight gain, and their mothers are at high risk of lactation failure due to immature and ineffective infant sucking, as reviewed in ABM Clinical Protocol 10, Breastfeeding the Late Preterm Infant (<http://www.bfmed.org/Resources/Protocols.aspx>) (Raju, 2006; Meier et al., 2013). Within hours of birth the mother can be taught to hand express colostrum to feed the baby, and within 24 hours of birth she can express breast milk every 2 to 3 hours after breastfeeding using a hospital-grade electric pump. Infant interventions include waking the infant to feed and providing supplementation with or after each feeding at least every 2 to 3 hours with expressed colostrum or breast milk (mixed with formula if there is not yet enough breast milk). This “trio” of nursing at the breast, followed by supplementation (or simultaneous if a supplemental nursing system is used) and then maternal milk expression, forms “triple feeds,” a bridge to successful lactation for the at-risk mother–infant dyad. In this manner the mother's milk supply is not at risk or diminished because of ineffective or infrequent sucking, and the physiologically immature infant is guaranteed sufficient intake. A breastfeeding management plan should be established before discharge.

Breastfeeding the Very Low Birth Weight Premature Infant

Mothers of VLBW and premature infants face multiple barriers to breastfeeding, including potentially incomplete mammary gland growth and inadequate priming of the mammary epithelium, mother–infant separation, infant fragility, the need to maintain milk supply by milk expression for weeks to months, and fatigue and stress, which may inhibit lactation (Jones and Spencer, 2007). Mothers can be encouraged to begin milk expression shortly after delivery and maintain a pumping frequency of eight times per day to optimize and maintain milk production (Furman and Minich, 2002; Jones and Spencer, 2007). Milk volume on day 4 may be predictive of supply at 6 weeks (Hill et al., 1999); those who combine massage or hand expression (“hands-on pumping”) with use of a double electric breast pump can exceed term milk volumes and may increase the caloric density of their milk (Jones et al., 2001; Morton et al., 2009, 2012). Lactation counseling does not increase maternal stress and anxiety and can increase the number of mothers who initiate lactation and the volume of breast milk their infants receive (Sisk et al., 2006). Multifaceted approaches to lactation support, including education of staff and parents, skin-to-skin (kangaroo) care, supportive hospital policies, peer support groups with transportation assistance, access to breast pumps, and lactation consultant availability, have all been demonstrated to increase rates of breast milk provision for VLBW infants (Meier et al., 2004; Dereddy et al., 2015).

Maternal Breastfeeding Issues

Nipple Pain

Sore nipples and pain are the most common complaints of breastfeeding mothers in the immediate postpartum period. Early,

mild discomfort is common, but severe nipple pain, the presence of cracks or bruises, discomfort that continues throughout a feeding, or pain that is not reducing by the end of the first 2 weeks is not normal. The most common cause of nipple pain is difficulty with breastfeeding technique, specifically improper latch, and prompt skilled help is the primary intervention. Limited milk transfer occurs when the infant is attached incorrectly, resulting in poor infant weight gain and impaired milk production. Other potential causes of nipple pain include overzealous breast cleansing, use of preparations that irritate the skin, skin trauma leading to impetigo, *Candida* infection, and rarely dermatologic conditions. Treatment for nipple pain depends on the underlying cause. If severe trauma exists, it may be necessary either to express milk or use a nipple shield with guidance from a lactation specialist until the nipple has healed; other therapies are not evidence based (Dennis et al., 2014). Ultrathin nipple shields may also help the infant latch on to inverted, flat, or engorged nipples, may reduce rapid milk flow, may facilitate milk transfer for premature infants, and may support the transition back to the breast for bottlefed infants.

Engorgement and Blocked Ducts

Physiologic breast fullness occurs because of vascular congestion during lactogenesis II. Pathologic engorgement, in which the infant cannot initially remove milk, is the firm, diffuse, and painful overfilling and edema of breasts usually caused by rapid increase in milk volume or a skipped feeding. As described in ABM Clinical Protocol 20, Engorgement (<http://www.bfmed.org/Resources/Protocols.aspx>), the best treatments for engorgement are a warm shower and gentle hand massage to soften the areola and permit the infant to attach and frequent effective breastfeeding (or hand milk expression if the infant is not able to breastfeed) followed by cool packs for 5 minutes after each feeding. “Reverse pressure softening,” a simple technique in which gentle pressure pushes fluid backward and upward in the breast to reduce areolar edema and permit latch, may also be effective (Cotterman, 2011). Engorgement should not be confused with a plugged milk duct, which can result in a localized lump in one area of the breast, usually because of infrequent or ineffective feedings or local pressure because of constrictive clothing. Treatment includes warm packs, gentle massage from the painful area toward the nipple, frequent effective feedings using different infant positions to facilitate emptying, and discontinuation of tight or underwire bras.

Mastitis

As a single area of localized warmth, tenderness, edema, and erythema in one breast more than 10 days after delivery, mastitis may present with a sudden onset of breast pain, myalgia, and fever or with flulike symptoms. The infection commonly enters through a break in the skin, usually a cracked nipple; however, milk stasis from engorgement or obstruction from plugged ducts can also lead to mastitis. As reviewed in ABM Protocol 4, Mastitis (<http://www.bfmed.org/Resources/Protocols.aspx>), treatment includes antibiotics and continuation of breastfeeding with frequent feeding (or pumping) to allow drainage. Additional therapy includes good fluid intake, bed rest, and pain control.

Low Milk Supply

Actual low milk supply most often results from infrequent and/or ineffective feedings at the breast, introduction of formula

supplementation, which decreases breastfeeding frequency, or early (before 3 weeks) introduction of bottlefeeding, each of which can decrease milk supply because of inadequate milk removal. Primary treatment includes increased effectiveness and frequency of milk removal; galactagogues such as fenugreek (although not evidence based) may be helpful if use is temporary and supervised (Mortel and Mehta, 2013); ABM Clinical Protocol 9, Galactagogues (<http://www.bfmed.org/Resources/Protocols.aspx>), provides an additional summary. Maternal factors that reduce milk supply include primary or secondary hypoprolactinemia, absence of an intact adenohypophyseal axis, severe maternal illness, including sepsis or hemorrhage, prior breast surgery, use of estrogen-containing contraceptives, and severe fatigue, stress, or pain. It is not clear if maternal smoking actually reduces milk supply or is a behavioral risk factor for decreased duration of breastfeeding. Perceived low milk supply, in which the infant has a normal growth pattern but the mother believes she does not have sufficient milk, can result from growth spurts leading to increased infant demand, changes in the efficiency of the breast around 4 weeks leading to decreased apparent fullness before nursing, or increased efficiency of infant nursing beginning at 2 to 3 months leading to less frequent or shorter breastfeeding sessions. Infant weight check is diagnostic. Maternal depression may also contribute to maternal perception of low milk supply and is associated with early cessation of breastfeeding, so screening and treatment are essential (Dias and Figueiredo, 2015).

Contraception

The lactational amenorrhea method (LAM) is considered a sufficient criterion for “how to be reasonably sure a woman is not pregnant,” a formal endorsement of its contraceptive efficacy (Centers for Disease Control and Prevention, 2013) (Box 67.4). LAM and nonhormonal or barrier methods of contraception do not impact breast milk supply and are recommended if acceptable to the mother and her partner. For breastfeeding mothers, according to the US Medical Eligibility Criteria (MEC 1-4) grading system, intrauterine devices (IUDs), progestin-only contraceptive pills, implants (etonogestrel), and injectables (depot medroxyprogesterone acetate [DMPA]) are classified as MEC2 for use from 10 minutes after placental delivery to 1 month postpartum and MEC1 for use 1 month or more postpartum. The use of combined oral contraceptives (COCs) is contraindicated less than 21 days postpartum (MEC 3) (Centers for Disease Control and Prevention, 2013). Although the US Selected Practice Recommendations for Contraceptive Use, 2013, provides guidance to health providers, evidence is incomplete regarding the impact of hormonal methods on milk supply. A recent systematic review on this topic concluded that evidence was limited and conflicting and that the trials were of moderate to low quality: two of eight trials found a negative effect on breastfeeding duration (of COCs vs placebo and hormonal vs nonhormonal IUDs), and two of six trials found lower milk volume (with COCs vs placebo) (Lopez et al., 2015). A review focused on progestin-only contraception generally did not show an adverse impact on breastfeeding outcomes; however, evidence regarding the effect of early postpartum DMPA is methodologically weak (Brownell et al., 2012; Phillips et al., 2016.) Centers for Disease Control and Prevention guidelines (<http://www.cdc.gov/reproductivehealth/unintendedpregnancy/usmec.htm>) and ABM Clinical Protocol 13, Contraception and Breastfeeding (www.bfmed.org/Resources/Protocols.aspx), offer additional detail.

• BOX 67.4 US Medical Eligibility Criteria for Contraceptive Use

| | |
|-------|---|
| MEC 1 | No restriction (method can be used) |
| MEC 2 | Advantages generally outweigh theoretical or proven risks |
| MEC 3 | Theoretical or proven risks usually outweigh the advantages |
| MEC 4 | Unacceptable health risk (method not be used) |

From Centers for Disease Control and Prevention. U.S. medical eligibility criteria for contraceptive use, 2010: adapted from the World Health Organization Medical Eligibility Criteria for Contraceptive Use, 4th edition. MMWR Morb Mortal Wkly Rep. 2010;59(RR04):1–85.

Maternal Employment Outside the Home

Mothers who seek to combine breastfeeding and bottlefeeding or who face returning to work can offer one bottle of expressed milk daily from the time breastfeeding is established at 2 to 3 weeks of age: clinicians and mothers report that newborns who are unaccustomed to a bottle may refuse one if it is not introduced before 1 month of age. Although exclusive breastfeeding through 6 months with addition of complementary feeds and continued breastfeeding until 1 to 2 years of age is an evidence-based medical recommendation, mothers may face personal and work constraints. Health insurance plans now must cover the cost of a breast pump (Healthcare.gov, 2016). Although a double electric pump is considered the “gold standard” for optimal milk expression, a recent review found that low-cost interventions, including hand expression, breast warming, and relaxation tapes, may be as or more effective in specific situations (Becker et al., 2015). Supportive nonjudgmental information about flexible ways to combine working outside the home with breastfeeding should be offered. In addition to expressing and storing breast milk, mothers may want to identify reduced or flexible hours, take the infant to work, or breastfeed during breaks at a nearby childcare facility; working 19 hours or less per week was associated with a higher likelihood of continued breastfeeding regardless of the duration of maternity leave (Li et al., 2008; Galtry, 2015; Xiang et al., 2016).

Jaundice and Breastfeeding

“Breast-nonfeeding jaundice” and “breast milk jaundice” are distinct clinical entities, with differing approaches (Academy of Breastfeeding medicine protocol Committee, ABM Clinical Protocol 22, Jaundice, <http://www.bfmed.org/Resources/Protocols.aspx>; Preer and Philipp, 2011).

Breast-Nonfeeding Jaundice

Severe jaundice is the most frequent reason for readmission of late preterm and term infants, most of whom are breastfeeding. If there is any delay in lactogenesis II or if the infant has ineffective milk removal or infrequent sucking, less milk will be ingested and consequently less milk will be produced, leading to a cycle of decreased demand and decreased supply, with consequent “starvation jaundice” or breast-nonfeeding jaundice. The most common risk factor for inadequate milk removal is late preterm birth. The neonatal intestine has highly active glucuronidases, which cleave conjugated bilirubin to unconjugated bilirubin. The unconjugated bilirubin is readily reabsorbed and recirculated to the liver for conjugation. Milk alleviates bilirubin recirculation by providing calories and

gastrocolic stimulation. Thus treatment focuses on improving maternal lactation and providing milk (preferably expressed or donor human milk or formula) to the infant. Phototherapy should be initiated as per guidelines (Academy of Breastfeeding Medicine Protocol Committee, 2010).

Reports from the US Pilot Kernicterus Registry note that more than 90% of cases of kernicterus have been in breastfed infants, with late preterm infants and those with glucose 6-phosphate dehydrogenase (G6PD) deficiency overrepresented (Johnson, 2009). Primary prevention includes establishment of optimal lactation and measurement of total serum bilirubin concentrations at discharge and at the 3- to 5-day follow-up visit (AAP, 2015). Guidelines for families and providers are now also available (<http://www.cdc.gov/ncbddd/jaundice/index.html>).

Breast Milk Jaundice

In breastfed infants, total serum bilirubin concentrations remain elevated, and in a few infants this may last as long as 12 weeks. In formula-fed infants, serum bilirubin concentration declines, reaching values of less than 1.5 mg/dL by the 11th or 12th day after birth. It has been suggested that elevation in serum bilirubin concentration may be protective against oxidative injury since bilirubin has been shown to be an effective antioxidant in vitro. Infants with breastfeeding jaundice appear healthy and, other than jaundice, have completely normal physical examination findings and are growing normally. Mature human milk contains an unidentified factor that enhances the intestinal absorption of bilirubin in a susceptible host infant to produce jaundice. As the production of the factor diminishes over time and the liver matures, the serum bilirubin concentration eventually returns to normal. A direct bilirubin level should be measured to exclude biliary atresia, liver disease, sepsis, and other conditions, and an evaluation should exclude other causes of prolonged unconjugated hyperbilirubinemia such as galactosemia, hypothyroidism, urinary tract infection, pyloric stenosis, or low-grade hemolysis due to G6PD or other causes. Parents should be reassured, since there is no harm to the infant, and breastfeeding should not be interrupted unless the bilirubin level rises to reach phototherapy level (>20 mg/dL) (Preer and Philipp, 2011).

Collection and Storage of Human Milk

Electric breast pumps enable optimal milk production for mothers separated, either by employment or hospitalization, from their infant. General techniques for ensuring cleanliness during milk expression begin with good hand washing with soap and water; collection kits should be rinsed, cleaned with hot soapy water, and air dried. Bacteriologic testing is generally not necessary for milk collected for feeding to a mother's own infant. Milk can remain unrefrigerated for 6 to 8 hours, in an insulated cooler bag for 24 hours, and in a refrigerator for 5 days, each without significant bacterial proliferation (Centers for Disease Control and Prevention, 2016; Academy of Breastfeeding Medicine Protocol Committee, 2010). As described in ABM Clinical Protocol 8, Human Milk Storage (www.bfmed.org/Resources/Protocols.aspx), freezing is the preferred method of storing milk that will not be fed: single milk expressions should be packaged separately and labeled with the date (and the name of the infant if for center or hospital use). Unlike heat treatment, freezing preserves many of the nutritional and immunologic benefits of human milk, and pasteurization of the mother's own milk offers no additional benefit (Cossey et al.,

2013). When frozen appropriately, milk can be stored for as long as 6 months. Milk should never be thawed in a microwave oven, should be used within 24 hours, and should not be refrozen (Centers for Disease Control and Prevention, 2016).

Donor Human Milk

Donor milk generally refers to milk donated to a milk bank by a mother with excess supply, usually a woman who delivers a term infant and generally later in lactation. After rigorous screening processes, the milk is heat treated at 62.5°C for 30 minutes, the Holder pasteurization method. Donor milk is used primarily in NICUs to support the feeding of preterm neonates whose mothers have inadequate milk production to meet their neonates' needs (Arslanoglu et al., 2013). The Human Milk Banking Association of North America is a nonprofit entity founded in 1985 that has set guidelines for collection and pasteurization of milk and for use in NICUs. In 2016 there were 18 Human Milk Banking Association of North America milk banks in the United States and Canada (<https://www.hmbana.org/>).

The Holder pasteurization process results in a milk that is free of microbial contamination, but biologically and immunologically active factors may be affected adversely, including immunoglobulins, lactoferrin, lysozyme, erythropoietin, lipase, and insulin. The heat treatment does not affect oligosaccharides (which may be active in preventing NEC) (Ewaschuk et al., 2011; Arslanoglu et al., 2013). The donor milk carbohydrate content is generally preserved, but protein content may be low because milk is donated later in lactation from term mothers. The fat content of donor milk is also low because milk is not homogenized, and there are multiple transfers of the milk with loss of fat at each step (García-Lara et al., 2013).

Clinical studies on the use of donor human milk as compared with formula in the NICU suggest that it supports lower rates of NEC and better feeding tolerance but poorer growth and more biochemical abnormalities in nutritional status (Boyd et al., 2007; Quigley and McGuire, 2014). A randomized trial of donor milk versus preterm formula as supplements for inadequate volumes of the mother's own milk was conducted in preterm infants (Schanler et al., 2005). That appropriate matched study found no difference in the major outcome of NEC and/or late-onset sepsis between study groups. A parallel group that continued to receive only their mother's milk had significantly lower rates of these outcomes (Sullivan et al., 2010). The human milk groups in that study, however, also received bovine-based milk products containing intact bovine protein. An exclusive human milk diet, appropriately fortified with a human milk-derived fortifier, resulted in markedly lower rates of NEC and surgery for NEC, fewer parenteral nutrition days, and lower mortality compared with a diet containing intact bovine milk fortifier and/or formula (Sullivan et al., 2010; Cristofalo et al., 2013; Abrams et al., 2014). Thus donor milk is an alternative to the mother's own milk but not a replacement for it. Furthermore, a recent report showing that use of donor milk was associated with increasing use of mother's own milk suggests that use of donor milk may serve as a bridge to use of mother's own milk (Kantorowska et al., 2016).

The business of donor milk purchase by hospitals (and individuals) has created an industry of for-profit human milk banking (Prolacta Bioscience Monrovia, CA and Medolac Laboratories Lake Oswego, OR) that relies on paid donors: political and social issues include concern for diversion of milk from nonprofit banks. Milk selling and sharing over the Internet for personal use has become a relatively common practice outside medical venues; however, evidence regarding

spoilage, impurity (mixing with bovine milk), and contamination (infectious agents, caffeine, smoke exposure) is very discouraging, and the Food and Drug Administration advises against this practice (<http://www.fda.gov/ScienceResearch/SpecialTopics/PediatricTherapeuticsResearch/ucm235203.htm>; Geraghty et al., 2011; Keim et al., 2015).

Conclusion

In summary, evidence is compelling that breastfeeding significantly reduces all-cause mortality and the number and severity of infections in term and preterm infants in developed and developing countries. Strong evidence demonstrates that breastfeeding also promotes maternal health. Breastfeeding is also likely associated with long-term beneficial effects on infant health, growth, and development. Substantial increases are needed in the rates of breastfeeding, particularly among racial, ethnic, and socioeconomic subpopulations that have the highest infant morbidity and mortality rates. Healthcare practitioners are uniquely positioned to influence women in their decision to breastfeed and should not only collaborate with lactation specialists but should also be prepared to assist in the management of breastfeeding problems themselves. Discussion regarding the benefits of breastfeeding and the risks of formula permit the mother to make an informed infant feeding choice, and for many women, physician support is critical.

Suggested Readings

- American College of Obstetricians and Gynecologists' Committee on Obstetric Practice; Breastfeeding Expert Work Group. Optimizing support for breastfeeding as part of obstetric practice. ACOG Committee opinion no. 658. *Obstet Gynecol.* 2016;127:e86-e92.
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Suggested Educational Resources on the Web

- This list is neither exhaustive nor presented in a specific order but is intended as an introduction to Web-based educational materials that promote or inform about breastfeeding. All listed resources were cost-free at the time of this publication.
- Academy of Breastfeeding Medicine has clinical protocols related to breastfeeding, which are not intended as standards of care but offer informative guidelines. These are available at <http://www.guideline.gov> and at <http://www.bfmed.org/Resources/Protocols.aspx>.
- American Academy of Pediatrics has created The Breastfeeding Residency Curriculum Online Resource for program directors and other faculty. This resource offers "tools and resources about breastfeeding including clinical and cultural cases, prepared presentations about breastfeeding management, and evaluation and tracking tools" and is available at <http://www2.aap.org/breastfeeding/curriculum/>.
- Baby-Friendly USA Inc. is the official body for the Baby-Friendly Hospital Initiative in the United States. The website <https://www.babyfriendlyusa.org/> is an entry point for beginning work toward designation.
- Expanding Clinicians' Roles in Breastfeeding Support is a two-part online tutorial that provides 3 hours of content material that is compatible with the Baby-Friendly Hospital Initiative and serves to meet physician education requirements for Baby-Friendly Hospital Initiative certification: <http://www.hriainstitute.org/breastfeedingcme/>. The Stanford School of Medicine, Newborn Nursery at Lucile Packard Children's Hospital website "is designed to support the educational goals of our pediatric trainees" and is a resource for all healthcare providers promoting breastfeeding. Multiple video clips are included: <http://newborns.stanford.edu/Breastfeeding/>.
- LactMed is a database with information on the safe use of medications during lactation and is located on the National Library of Medicine's ToxNet at <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?LACT>.
- LaLeche League is an international organization dedicated to providing information and support to breastfeeding mothers: <http://www.llli.org/>.
- Wellstart International's *Lactation Management Self-Study Modules Level 1* (4th ed) is an educational tool with clinical cases in a question-answer format and is intended for health professionals interested in breastfeeding. The link <http://www.wellstart.org> provides access to a free download for the first three modules, with a webpage of links to promote breastfeeding at <http://www.wellstart.org/links.html>.

Complete references used in this text can be found online at www.expertconsult.com

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Enteral Nutrition for the High-Risk Neonate

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KEY POINTS

- Early protein intake is associated with improved growth and neurodevelopmental outcomes in premature infants; achieving adequate intake requires a combined parenteral and enteral approach.
- Maternal human milk is the preferred diet for preterm infants; further research is needed to develop evidence-based clinical guidelines for the use of donor human milk when maternal milk is unavailable.
- For very low birth weight infants, unfortified human milk contains insufficient protein, energy, and many essential micronutrients, requiring supplementation with milk fortifier and/or multivitamin products.
- Postnatal growth failure remains a common complication of preterm birth and is associated with adverse outcomes; optimization of growth outcomes requires attention not only to weight gain but also to appropriate linear growth and body composition both in the neonatal intensive care unit and following hospital discharge.

Providing optimal enteral nutrition to high-risk premature neonates is a difficult clinical challenge. To achieve optimal growth, nutritional needs in the early neonatal period are greater than at any other time of life. Premature delivery results in decreased nutrient deposition in the infant. Critical illness and immature gut motility and function often preclude intended delivery of nutrition. Despite the lack of evidence, fear of necrotizing enterocolitis (NEC) if feedings are advanced too quickly may also limit provision of optimal enteral nutrition. Consequently, the premature infant requires specialized nutritional support to meet these great demands for growth.

Current recommendations for provision of enteral nutrition to premature infants are based on the illusive goal of duplicating rates of intrauterine accretion of the fetus at the same postmenstrual age. However, this goal is rarely accomplished, and the incidence of postnatal growth failure in very low birth weight (VLBW) infants remains unacceptably high. Data from the Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network revealed growth failure at 36 weeks' postmenstrual age in 79% of VLBW infants (Stoll et al., 2010). Infants who experience one or more major morbidities such as bronchopulmonary dysplasia (BPD), severe intraventricular

hemorrhage, NEC, or late-onset sepsis are at increased risk of growth failure. The association between suboptimal postnatal growth and poor neurodevelopmental outcomes is especially worrisome and emphasizes the importance of optimizing nutritional support for premature infants in the neonatal intensive care unit (NICU).

Special considerations regarding nutrient needs of premature infants arise at birth. Because of limited body stores, increased energy expenditure, severity of illness, and/or immaturity and inability to tolerate enteral feedings, premature infants are given parenteral nutrition immediately after birth. However, to meet nutrient requirements, a combined approach with both parenteral and enteral nutrition is imperative to achieve optimal growth outcomes and minimize morbidities such as NEC. This chapter describes the basis of recommendations for enteral nutrient support for high-risk neonates. In addition, evidence regarding the initiation and advancement of enteral feedings in premature infants is discussed. Finally, special considerations for enteral nutrition after discharge from the NICU are reviewed.

Macronutrient Requirements

Protein

When fetal life is interrupted by premature birth, significant protein deficits can occur and may be difficult if not impossible to recoup (Embleton et al., 2001). Several observational studies have found an association between early protein intake and postnatal growth in extremely premature infants (Berry et al., 1997; Olsen et al., 2002). In addition, there is increasing evidence that the amount of protein intake early in life correlates with improved neurodevelopmental outcomes. To optimize growth outcomes, particular focus must therefore be given to enteral protein intake.

The most commonly accepted goal of provision of enteral nutrition to premature infants is to achieve growth comparable with that of the fetus. At 26 weeks' gestation the fetus accretes approximately 2.2 g/kg per day; of protein by term, this amount declines to approximately 0.9 g/kg per day. Protein losses are inversely related to gestational age, providing an explanation for the higher protein requirements in extremely premature neonates. Accretion of protein is dependent on protein quantity and quality, energy intake, and underlying disease states (such as sepsis or

TABLE 68.1 Estimated Protein and Energy Requirements to Achieve Fetal Growth

| Weight (g) | Protein (g/kg per day) | Energy (kcal/kg per day) | Protein/Energy (g/100 kcal) |
|------------|------------------------|--------------------------|-----------------------------|
| 500–700 | 4.0 | 105 | 3.8 |
| 700–900 | 4.0 | 108 | 3.7 |
| 900–1200 | 4.0 | 119 | 3.4 |
| 1200–1500 | 3.9 | 125 | 3.1 |
| 1500–1800 | 3.6 | 128 | 2.8 |
| 1800–2200 | 3.4 | 131 | 2.6 |

Based on the factorial method (Ziegler et al., 2002).

surgical stress), as well as concomitant medications (such as systemically acting steroids, fentanyl, and insulin).

Protein requirements in premature infants have been estimated with use of a variety of methods, including fetal animal models, the factorial approach, and estimates based on the composition of human milk. Protein requirements estimated with the factorial approach for premature infants weighing 500–1200 g are shown in Table 68.1; with increasing body weight, protein requirements determined by the factorial approach decrease (Ziegler et al., 2002). Protein and energy needs should be considered hand in hand because protein synthesis requires energy. Extremely premature infants require a higher protein-to-energy ratio for optimal growth. Protein retention, or balance, is generally a function of protein intake if energy intake is adequate. Enteral protein requirements are calculated to be higher than parenteral ones because only approximately 85% of enteral protein is absorbed. In contrast to empiric methods, this approach does not take into consideration nutrient requirements for catch-up growth.

On the basis of currently available evidence and consensus of an expert panel, 3.5–4.5 g of protein per kilogram per day is recommended for fully enterally fed VLBW infants (Koletzko et al., 2014). Further studies are required to more clearly define the upper limit of enteral protein intake in premature neonates to optimize growth and neurodevelopmental outcomes.

Energy

In utero the fetus utilizes both glucose and amino acids as a source of energy. If energy intake is not adequate, protein utilization is not efficient, resulting in lower retention of nitrogen. As shown in Table 68.1, Ziegler et al. (2002) have estimated that energy requirements are lower in infants with lower body weight. Coupled with the inverse relationship between protein requirements and weight, the protein-to-energy ratio required to achieve fetal growth is highest in the most premature infants. The recommended energy intake for enterally fed premature infants ranges between 110 and 130 kcal/kg per day (Koletzko et al., 2014).

The optimal ratio of enteral protein to energy intake must be defined not only in terms of optimizing weight gain but also by that which achieves optimal body composition. Consequently, attempting to duplicate the intrauterine environment may not be appropriate for extrauterine life, given differences in nutrient supply

and metabolism. Changes in body composition in response to energy intake are an important consideration, because excessive energy intake can contribute to excessive fat deposition, and recent studies have suggested that rapid weight gain may be associated with adverse outcomes.

The energy needs of the neonate are derived from a computation of the energy expenditure, energy storage, and energy losses. Energy expenditure consists of the energy needed to cover the resting metabolic rate, activity, thermoregulation, and the energy cost of growth. Energy storage consists of the energy (fat and lean mass) deposited for growth. Energy losses are usually due to incomplete absorption of nutrients and are greater in premature infants than in term infants or adults. The largest component of the total estimated energy requirement is that needed for the resting metabolic rate. When nourished parenterally, the premature infant has less fecal energy loss, generally fewer episodes of cold stress, and somewhat lesser activity, so the actual energy needs for growth are lowered to approximately 85–95 kcal/kg per day. In the case of chronic disease, such as BPD, the resting energy expenditure rises significantly. Total energy needs in premature infants with BPD are increased because of greater energy expenditure, activity, and fecal energy losses. It is not surprising to find that these infants may require 150 kcal/kg per day to achieve weight gain.

Carbohydrates

The main carbohydrate in human milk is lactose, supplying nearly half of the total calories. Lactase (β -galactosidase) is an intestinal enzyme that hydrolyzes lactose to glucose and galactose in the small intestine. Despite lower levels of intestinal lactase activities in premature infants, they are able to efficiently digest lactose. Nonetheless, many infant formulas designed for premature infants supply glucose polymers. Glucose polymers are digested by α -glucosidases; the activity level of these enzymes approximates adult levels much sooner than does that of β -galactosidase, which theoretically makes glucose polymers easier for the premature infant to digest than lactose. Glucose polymers also have an advantage in that they increase caloric density without a rise in osmolality. The recommended carbohydrate intake for premature infants is 11.6–13.2 g/kg per day (Koletzko et al., 2014). This amount of intake will provide sufficient glucose to meet the needs for total energy expenditure.

Fat

Fat provides a substantial source of energy for growing premature infants. Premature infants have low levels of pancreatic lipase, bile acids, and lingual lipase. Human milk, however, supplies a variety of lipases, including lipoprotein lipase, bile salt esterase, and nonactivated lipase. The composition of dietary fat affects absorption and digestion. The absorption of fatty acids increases with decreasing chain length and with the degree of unsaturation. Consequently, medium-chain triglycerides (carbon chain length of 6–12) are hydrolyzed more readily than long-chain triglycerides. In contrast to formulas designed for term infants, premature infant formulas supply medium-chain triglycerides. Human milk supplies 8%–12% of fat as medium-chain triglycerides. The recommended intake for lipid in enterally fed premature infants ranges between 4.8 and 6.6 g/kg per day (Koletzko et al., 2014). Of this amount, medium-chain triglycerides should account for less than 40% of total intake.

Micronutrients, Vitamins, Minerals, and Trace Element Requirements

Calcium and Phosphorus

Calcium and phosphorus are primary components of the skeleton, accounting for 99% and 85%, respectively, of bone mass. The goal for premature infant nutrition is to achieve a bone mineralization pattern similar to that in the fetus and to avoid osteopenia and fractures. The peak fetal calcium accretion rate occurs in late gestation through active calcium influx (Bhatia et al., 2013). Infants will have greater calcium and phosphorus needs when exposed to diuretics, which increase renal excretion of these minerals. Phosphorus requirements may be greater in the setting of a history of placental insufficiency, and calcium requirements may be greater for infants exposed to theophylline or steroids.

Preterm human milk contains approximately 250 mg of calcium and 140 mg of phosphorus per liter. Metaanalysis of breast milk content studies reveals calcium and phosphorus levels that are similar in preterm and term milk (Gidrewicz and Fenton, 2014). The calcium and phosphorus contents of enteral formula products designed for premature infants in the United States are significantly greater. In human milk, calcium and phosphorus exist in ionized and complexed forms that are easily absorbed. Thus in the design of commercial formulas, greater quantities of these minerals are added to compensate for their poorer bioavailability, so additional supplementation is not necessary (Bhatia et al., 2013). However, distinct from the term infant, the premature infant requires significantly greater quantities of calcium and phosphorus than can be provided in human milk.

For the human milk–fed premature infant, calcium and phosphorus are deficient throughout lactation, and the levels are far below those necessary to achieve respective intrauterine accretion rates. Deficient intakes of calcium and phosphorus are associated with biochemical markers such as low serum and urine phosphorus concentrations, elevated serum alkaline phosphatase activity, and elevated serum and urine calcium concentrations. Usually, serum phosphorus concentrations are the best indicators of calcium and phosphorus status in human milk–fed premature infants, and serum phosphorus concentration below 4 mg/dL should be followed up carefully. Monitoring of these laboratory markers should be undertaken during hospitalization of VLBW infants (Abrams and Committee on Nutrition, 2013). The American Academy of Pediatrics (AAP) Committee on Nutrition issued guidelines that laboratory monitoring of VLBW infants should begin at 4 to 5 weeks after birth. Prolonged deficiency of these minerals tends to stimulate bone resorption to normalize serum calcium concentrations. This bone activity is often correlated with elevated serum alkaline phosphatase activity. It has been reported that most premature infants who had an elevated serum alkaline phosphatase activity were those fed human milk. Moreover, follow-up evaluations of the same infants at 9 and 18 months noted that linear growth was significantly lower in the group that had the higher serum activity of alkaline phosphatase in the neonatal period. A high alkaline phosphatase value in the neonatal period is a negative predictor of height in 9- to 12-year-old adolescents (Fewtrell et al., 2000). Serum alkaline phosphatase levels greater than 800 international units (IU)/L or clinical concern for fractures should lead to a radiographic work-up for rickets. Dual-energy X-ray absorptiometry and quantitative ultrasonography are increasingly used as tools for studying metabolic bone disease in preterm infants but have variable clinical availability (Rack et al., 2012; Rehman

and Narchi, 2015). Diagnosis of rickets by US neonatologists is most commonly performed by X-ray (Kelly et al., 2014).

The supplementation of human milk with both calcium and phosphorus not only improves the net retention of both minerals but also increases bone mineral content. Current management of human milk–fed premature infants emphasizes the need for supplements of both calcium and phosphorus. A linear relationship exists between calcium (or phosphorus) intake and net retention in enterally fed premature infants. Premature infants receiving unfortified human milk never achieve intrauterine accretion rates for calcium and phosphorus. Daily intakes of calcium at approximately 200 mg/kg per day and phosphorus at 100 mg/kg per day can be achieved with the use of specialized human milk fortifiers (HMFs) and preterm formulas, thus making it possible to meet intrauterine estimates. HMFs contain highly soluble calcium glycerophosphate, promoting calcium retention (Bhatia et al., 2013). However, term infant formulas and specialized (not “preterm”) formulas provide inadequate quantities of calcium and phosphorus to meet the needs of growing premature infants. Several factors affect the absorption of calcium and phosphorus, including postnatal age and intake of calcium, phosphorus, lactose, fat, and vitamin D. Calcium absorption increases with a low-pH environment and high-casein, high-lactose formula. Loss of calcium content is seen with continuous gavage feeding of breast milk (Rogers et al., 2010). Vitamin D, however, is responsible for only a small component of calcium absorption in premature infants.

The time to supply sufficient calcium and phosphorus stores for premature infants is during the initial hospitalization, before their discharge and the beginning of exclusive breastfeeding. However, because of the need for prolonged parenteral nutrition and the inability to provide “catch-up” quantities of calcium and phosphorus in milk, some infants may benefit from additional calcium and phosphorus after hospital discharge through the use of transitional formulas. Preterm infants discharged on an exclusive breast milk diet may require continued monitoring of biochemical markers.

Magnesium

Approximately 60% of body magnesium is in bone. Magnesium has the highest fetal accretion rate in the third trimester. Preterm human milk contains approximately 30 mg of magnesium per liter. The absorption of magnesium is significantly greater from unfortified human milk than from formula. The needs of preterm infants are 8–15 mg/kg per day (Bhatia et al., 2013), and net magnesium retention in human milk–fed premature infants meets intrauterine estimates. Hypomagnesemia may be present in infants of diabetic mothers and is frequently transient. Sometimes hypomagnesemia may reduce parathyroid hormone secretion and responsiveness and will require correction to achieve normocalcemia.

Trace Elements

Premature infants are particularly susceptible to trace element deficiencies given that most accrual occurs during the last third of pregnancy. The literature on trace mineral requirements for premature infants is sparse, as are studies to support evidence-based guidelines for provision of these nutrients (Finch, 2015).

Zinc

Several factors affect the zinc needs of the enterally fed premature infant. Fetal accretion of zinc is approximately 0.85 mg/kg per

day. Growth is a major determinant of zinc needs. Premature infants receiving pooled pasteurized human milk (zinc intake of approximately 0.7 mg/kg per day) are in negative zinc balance for 60 days postnatally and never meet the intrauterine accretion rate. In contrast, intakes of 1.8–2 mg/kg per day are associated with a net retention of zinc that surpasses intrauterine accretion rates. A zinc-to-copper ratio of less than 20:1 is recommended for preterm infants.

The major excretory route is via the gastrointestinal (GI) tract. Infants with large GI fluid losses may become zinc deficient, and patients with short bowel syndrome may require 400–800 µg/kg per day. The classic signs of zinc deficiency include an erythematous dermatitis over mucous membranes, facial areas, and the extremities. Symptomatic zinc deficiency presents with susceptibility to infection, impaired wound healing, and failure to thrive.

Plasma zinc values lower than 50 µg/dL are highly suggestive of deficiency, but this is not a reliable biomarker for marginal deficiency, and levels may be falsely elevated in times of bone remodeling. Blood zinc levels are lower in extremely critically ill preterm infants (Wang et al., 2015). Levels should be measured in the context of large stool or ostomy losses. A very low activity of serum alkaline phosphatase, a zinc-dependent enzyme, is also suggestive of deficiency. Reports of symptomatic zinc deficiency in unsupplemented human milk–fed premature infants serve as a reminder of the decline in milk zinc concentration as lactation advances.

Copper

No universally accepted methods exist to assess copper status clinically. Balance study data provide only an estimate of copper retention at one point in time. Premature infants receiving pooled pasteurized human milk (copper intake of approximately 85 µg/kg per day) are in negative copper balance for 30 days postnatally and never meet the intrauterine accretion rate. Copper concentration is high in early breast milk and decreases throughout lactation. Human milk fortifier supplies additional copper. Symptoms of copper deficiency include osteopenia, neutropenia, and hypochromic anemia. Copper retention is negatively correlated to zinc intake and postnatal age (Bhatia et al., 2013). Copper deficiency may also present as metabolic bone disease with osteoporosis, metaphyseal changes, and physeal disruption (Marquardt et al., 2012). Because copper is excreted in bile, cases of severe cholestasis warrant limitation of copper intakes.

Selenium

Selenium is an essential trace element and is actively transported from the mother to the fetus (Kantola et al., 2004). It may have an important role in oxidative damage from organic hydroperoxides and may be protective of the toxicity of maternal exposure to polycyclic aromatic hydrocarbons, which may be one contributor to preterm delivery (Huel et al., 2000). Low maternal selenium status in early gestation may increase the risk of preterm premature rupture of membranes (Rayman et al., 2011). In randomized controlled trials the incidence of premature rupture of membranes was lower in a group of pregnant women who received selenium supplementation (Tara et al., 2010). Further research is needed to clarify the role of selenium in adverse pregnancy outcomes as most study designs are limited in the ability to determine causal relationships (Mariath et al., 2011). A Cochrane review of three eligible trials (two of which were done in geographic regions with low population selenium levels) reported an association between selenium supplementation to premature infants and a reduced risk of sepsis (Darlow and Austin, 2003).

Iron

The iron needs of the premature infant are determined by birth weight, initial hemoglobin concentration, rate of growth, and magnitude of iron loss and/or volume of transfused blood. Iron endowment may be diminished by growth restriction and placental insufficiency in preterm infants (McCarthy et al., 2016). Optimization of umbilical cord clamping practices results in increased iron stores (Oh et al., 2011; Rabe et al., 2012). Postnatal iron metabolism occurs in three phases. In the first phase, there is decreased erythropoiesis. The hemoglobin concentration declines to a nadir, physiologic anemia of prematurity, which is at approximately 2–3 months of postnatal age. In the second phase, the hemoglobin concentration rises as active red cell production is occurring. In this phase, iron is needed. The third phase is an exhaustion of iron stores, or late anemia of prematurity, observed if iron supplementation is inadequate.

The concentration of iron in human milk declines throughout lactation. Premature infants fed human milk are in negative iron balance, which, in the absence of transfusion, can be corrected with iron supplements. Iron absorption also appears to be facilitated by a modest degree of anemia. The most recent recommendation from the AAP Committee on Nutrition is to begin enteral iron supplementation of 2 mg/kg per day by 1 month of age in preterm infants fed human milk (Baker et al., 2010). This may be delayed in preterm infants who have received multiple erythrocyte transfusions, as each milliliter of transfused blood delivers 1 mg of elemental iron, leading to risk of excessive iron stores (Park and Kim, 2015; Rehman and Narchi, 2015). Enteral iron supplementation is safer than parenteral iron dosing. Formula-fed premature infants should receive iron-fortified formula from the onset of milk feeding. At 6 months of age, term infants should receive 11 mg/day (Baker et al., 2010). Iron supplementation significantly decreases the prevalence of iron-deficiency anemia (Long et al., 2012).

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Although it is clear that preterm infants will require iron supplementation, the optimal timing of initiation of supplementation and the duration of use are less clear (Mills and Davies, 2012; Taylor, Kennedy, 2013). There is mixed evidence on the effects of iron supplementation and improved neurodevelopmental outcomes (Long et al., 2012). Dysregulation of the iron ion homeostasis pathway mediating oxidative damage may contribute to the mechanism for retinopathy of prematurity pathophysiology (Luo et al., 2015). Latent iron deficiency is associated with abnormal auditory neural maturation in late preterm infants (Choudhury et al., 2015). Early iron supplementation results in a higher nadir of hemoglobin and serum ferritin concentrations but may also result in iron overload (Jin et al., 2015).

Sodium and Potassium

Premature infants generally need more sodium per unit of body weight than is needed by term infants. Historical studies proposed that this increased need was due to immature renal sodium conservation mechanisms, but more recent studies of very preterm infants have revealed defective aldosterone secretion with conserved renal aldosterone sensitivity (Martinerie et al., 2015). Sodium wasting is inversely related to gestational age. A study comparing daily sodium intakes of 2.9 and 1.6 milliequivalent (mmol)/kg in premature infants suggested that the former intake provided more appropriate serum sodium concentrations. Hyponatremia also may occur in premature infants primarily fed human milk because the sodium content of preterm milk continues to decline throughout lactation (Dutta et al., 2014), although studies have revealed that there is a significant increase in the sodium concentration of donor

breast milk after 1 year postpartum (Perrin et al., 2017). Early sodium supplementation prevents hyponatremia in very preterm infants and may enhance weight gain (Isemann et al., 2016). The need for these electrolytes may increase during or after diuretic use. Infants with short gut or ostomies may have increased sodium needs, and urinary sodium measurements are a good correlate for growth (Mansour et al., 2014).

Vitamins

The fat-soluble vitamins A, D, E, and K are stored in the body, and large doses may result in toxicity. Water-soluble vitamins—thiamine, riboflavin, niacin, vitamin B₆, folate, vitamin B₁₂, pantothenic acid, biotin, and vitamin C—are not stored in the body, and excess intakes are excreted in the urine or bile (vitamin B₁₂). The intake of water-soluble vitamins should therefore be monitored at frequent intervals to avoid deficiency states. Vitamin A and riboflavin concentrations decline in human milk under conditions of light exposure and after passage through feeding tubes. As a consequence of exposure to air, ascorbic acid concentrations are lower in pooled human milk. Pasteurization of donor breast milk leads to reduction in vitamin D content (Gomes et al., 2016). Supplementary vitamins are provided in HMFs and in preterm formulas. A multivitamin supplement should be given to preterm infants once feedings change to unfortified human milk or standard formula.

Vitamin A

The reported vitamin A content of preterm breast milk is variable but is similar to that of term breast milk. The content is highest in colostrum (400–600 IU/dL) and in breast milk with a higher fat content and declines in mature lactation (60–200 IU/dL). The system of handling of breast milk, including bottlefeeding, may decrease the concentration of retinol (Francis et al., 2012).

The optimal dosing of vitamin A supplementation needs further study (Moya, 2014). Metaanalyses reveal that vitamin A supplementation may result in a modest reduction in the risk of BPD or death, as low tissue vitamin A and retinol-binding protein levels are associated with decreased clearance of lung secretions and decreased ability to repair lung tissue (Darlow and Graham, 2011). However, these studies are confounded by the use of dexamethasone in preterm infants, which results in a transient rise then fall in serum retinol and retinol-binding protein levels. Furthermore, there is evidence that there is a higher risk of sepsis with vitamin A administration (Mactier, 2013; Ubers et al., 2014).

CONTROVERSY BOX

There is emerging evidence that retinoic acid, a vitamin A metabolite, may have a role in prevention of retinopathy of prematurity (Mactier et al., 2012; Wang et al., 2014).

Vitamin D

Conflicting guidelines are proposed by different professional organizations regarding the optimal dosing of vitamin D. The AAP Committee on Nutrition issued guidelines establishing the amount of recommended vitamin D as 400 IU/day for infants. The recommendation applies to infants receiving human milk and those who are consuming less than 1 quart of infant formula per day and is based in part on the risk of rickets in exclusively breastfed infants who do not receive supplementation with 400 IU of vitamin D per day. This level of supplementation is sufficient to meet a target

plasma 25-hydroxyvitamin D concentration of 50 mmol/L in most infants (Abrams and Committee on Nutrition, 2013; McCarthy et al., 2013). However, the Endocrine Society recommends that infants may require up to 1000 IU/day to meet a target plasma 25-hydroxyvitamin D concentration of 75 mmol/L for nonskeletal health benefits (Nehra et al., 2013).

Antiepileptic drugs such as phenytoin and phenobarbital may affect vitamin metabolism. Ethnicity has a role in serum 25-hydroxyvitamin D levels, with Hispanic infants having a lower umbilical cord blood level (Abrams et al., 2012). Preterm infants are at higher risk of being born with lower 25-hydroxyvitamin D umbilical cord serum levels (Burris et al., 2014).

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Certain vitamin D receptor polymorphisms are associated with increased frequency of BPD (Koroglu et al., 2014). Lower maternal and neonatal serum 25-hydroxyvitamin D levels are associated with BPD in preterm infants (Cetinkaya et al., 2015; Fettah et al., 2015). Recent studies have revealed that vitamin D supplementation in black preterm infants is associated with more recurrent wheezing (Hibbs et al., 2015). A randomized clinical trial of different vitamin D dosing strategies for black preterm infants is under way.

Vitamin E

A single enteral dose of vitamin E supplementation raises the serum levels of α -tocopherol, the biologically active form, in preterm infants (Bell et al., 2013). Colostrum has high concentrations of α -tocopherol in both term and preterm milk (Grilo et al., 2013). The system of handling breast milk, including bottlefeeding, may decrease the concentration of α -tocopherol (Francis et al., 2012). There is a high prevalence of vitamin E deficiency in VLBW infants until term-corrected gestational age (Kositamongkol et al., 2011). Vitamin E supplementation reduces the risk of retinopathy of prematurity and intracranial hemorrhage and increases the risk of sepsis in VLBW infants (Brion et al., 2003). Long-term (>6 months) α -tocopherol supplementation in extremely low birth weight (ELBW) infants may increase performance intelligence quotient (Kitajima et al., 2015).

Vitamin K

In the United States, phytonadione (vitamin K) is routinely administered at birth by intramuscular injection to prevent hemorrhagic disease of the newborn. There are oral dosing regimens reported in the literature, but there is a lack of evidence to support routine alternative use (Ipema, 2012). Genetic polymorphisms in the vitamin K–dependent coagulation system may make some preterm infants be at higher risk of developing intraventricular hemorrhage (Schreiner et al., 2014). Proteins induced by vitamin K absence are the most sensitive indicators of vitamin K status, but prothrombin time and coagulation studies are commonly used.

Options for Enteral Nutrition

When clinicians are considering enteral feeding in preterm infants, there are several basic choices that they must make. First and foremost is the choice of base diet for the infant, with three options commonly used: maternal milk, donor human milk, or preterm formula. Once this decision has been made, clinicians must decide (1) when to initiate enteral feeding, (2) how to advance the feeding volumes, and (3) how to feed the infant (by mouth, by gravity bolus via a nasogastric (NO)/orogastric (OG) tube, by timed or continuous infusion via NG/OG tube). Because of the specific

nutritional needs of preterm infants (primarily the requirement for higher protein and mineral intake than that provided by human milk alone), there is an additional decision to be made, and that is determining when human milk fortification will be initiated and how to manage ongoing milk fortification.

Human Milk

Maternal Milk

Maternal human milk is the preferred diet for preterm infants, in almost all circumstances. Observational studies conducted in the past 30 years have demonstrated that preterm infants fed maternal milk experience health benefits compared with those fed preterm formula, including reduced risk of common complications of prematurity such as late-onset sepsis, NEC, and BPD (Lucas and Cole, 1990; Schanler et al., 1999, 2005; Vohr et al., 2006; Sisk et al., 2007; Maayan-Metzger et al., 2012). Additionally, VLBW infants fed maternal milk have been shown to require fewer rehospitalizations (Vohr et al., 2006) and to have superior developmental outcomes at 2 to 8 years of age (Lucas et al., 1992), with demonstration of a significant dose–response relationship (Vohr, 2007). Maternal milk diets are recommended for preterm infants by the AAP, the World Health Organization (WHO), and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (Agostoni et al., 2010; Section on Breastfeeding, 2012). Although rates of initiation of breast milk expression are increasing among mothers of VLBW infants (Colaizy and Morris, 2008), mothers are sometimes unable to produce the required volume of milk (Jegier et al., 2013) or have medical contraindications to breastfeeding (Neifert, 2001). In these circumstances, donor human milk or preterm formula must be used to replace or supplement maternal milk. This section will address human milk, both maternal and donor.

Human Milk Nutrient Content

Protein

In the first few weeks after birth the protein content of milk from mothers who deliver premature infants (preterm milk) is greater

than that of milk obtained from mothers who deliver term infants (term milk). The protein content of both preterm milk and term milk declines over time, such that beyond 2 weeks it levels off to that which is termed *mature milk*. The quality of protein—the proportion of whey and casein—in human milk is particularly suitable for the premature infant. Human milk contains 70% whey and 30% casein, whereas bovine milk contains 18% whey and 82% casein. The whey fraction of milk consists of soluble proteins that remain in solution after acidification and thus are digested more easily. Human milk promotes more rapid gastric emptying than both casein- and whey-dominated bovine milk.

The major human whey protein is α -lactalbumin, a nutritional protein for the infant and a component of mammary gland lactose synthesis. Lactoferrin, lysozyme, and secretory immunoglobulin A (sIgA) are specific human whey proteins that are particularly resistant to hydrolysis and, as such, line the GI tract to play a primary role in host defense. These proteins can therefore provide protection for the premature infant who is exposed to multiple pathogens in the nursery environment.

Although the protein content of human milk from mothers who deliver prematurely is initially higher than the protein content of human milk from mothers who deliver at term, the protein content of preterm human milk declines over time, much more quickly than has been generally thought. Pooled data from multiple small trials in the 1980s have demonstrated an initial protein content of preterm human milk of approximately 1.9 g/dL at 1 week after birth, declining to 1.2 g/dL by 30 days, and these values are typically used for calculations of estimated protein content of human milk fed to preterm infants (Schanler and Oh, 1980; Gross et al., 1981; Lemons et al., 1982; Picciano, 2001). Similarly, the protein content of milk produced by mothers delivering at term is typically estimated to be 1 g/dL. Gidrewicz and Fenton (2014) published a metaanalysis of 41 studies of human milk composition performed over 30 years, representing more than 3000 mothers. They report protein content of preterm milk in the first week of lactation as 2.2 g/dL, declining to 1.4 g/dL by week 3, and 1 g/dL by week 10 (Table 68.2). These values should be kept in mind when one is estimating protein intake of preterm infants fed human milk.

TABLE 68.2 Composition of Preterm and Term Human Milk

| | Energy (kcal/dL) | Protein (g/dL) | Fat (g/dL) | Lactose (kcal/dL) | Oligosaccharides (g/dL) |
|----------------|------------------|----------------|---------------|-------------------|-------------------------|
| Preterm | | | | | |
| Week 1 | 60 (45–75) | 2.2 (0.3–4.1) | 2.6 (0.5–4.7) | 5.7 (3.9–7.5) | 2.1 (1.3–2.9) |
| Week 2 | 71 (49–94) | 1.5 (0.8–2.3) | 3.5 (1.2–5.7) | 5.7 (4.1–7.3) | 2.1 (1.1–3.1) |
| Week 3–4 | 77 (61–92) | 1.4 (0.6–2.2) | 3.5 (1.6–5.5) | 6.0 (5–7) | 1.7 (1.1–2.3) |
| Week 10–12 | 66 (39–94) | 1.0 (0.6–1.4) | 3.7 (0.8–6.5) | 6.8 (6.2–7.2) | NA |
| Term | | | | | |
| Week 1 | 60 (44–77) | 1.8 (0.4–3.2) | 2.2 (0.7–3.7) | 5.8 (4.2–7.4) | 1.9 (1.1–2.7) |
| Week 2 | 67 (47–86) | 1.3 (0.8–1.8) | 3.0 (1.2–4.8) | 6.2 (5–7.3) | 1.9 (1.1–2.7) |
| Week 3–4 | 66 (48–85) | 1.2 (0.8–1.6) | 3.3 (1.6–5.1) | 6.7 (5.3–8.1) | 1.6 (1–2.2) |
| Week 10–12 | 68 (50–86) | 0.9 (0.6–1.2) | 3.4 (1.6–5.2) | 6.7 (5.3–8.1) | NA |

Values are given as the mean ± two standard deviations.
NA, Not available.
Modified from Gidrewicz DA, Fenton TR. A systematic review and meta-analysis of the nutrient content of preterm and term breast milk. *BMC Pediatr*. 2014;14:216.

Fat

Fat provides 50% of the energy in human milk. The lipid system in human milk is structured in a way that facilitates fat digestion and absorption. In human milk, fat exists as organized fat globules containing an outer protein coat and an inner lipid core. The type of fatty acids (high palmitic 16:0, oleic 18:1, linoleic 18:2 ω -6, and linoleic 18:3 ω -3), their distribution on the triglyceride molecule (16:0 at the 2-position of the molecule), and the presence of bile salt-stimulated lipase are important components of the lipid system in human milk. Fat content of preterm milk is higher than that of term milk in the first 2 weeks (2.2–3.5 g/dL in preterm milk vs 1.8–3.0 g/dL in term milk) (Gidrewicz and Fenton, 2014). However, fat is the most variable macronutrient in human milk (Table 68.2). Fat content of human milk differs among women, changes during the day, rises slightly during lactation, and increases dramatically within a single milk expression. The variability in total fat content is unrelated to maternal dietary fat intake. Because it is not homogenized, the fat separates out of human milk on standing. The separated fat may adhere to collection containers, feeding tubes, and syringes and thus may not be delivered to the infant, compromising energy intake.

The variability in the fat content of human milk may be used to advantage in the premature infant. Most milk transfer during a feeding occurs in 10 to 15 minutes, but continued milk expression yields a milk with a progressively higher fat content—the hindmilk—than the earlier foremilk. The fat content of hindmilk may be 1.5- to 3-fold greater than that of foremilk. The use of hindmilk in selected cases may provide the premature infant with additional energy. Hindmilk and foremilk contain similar concentrations of nitrogen, calcium, phosphorus, sodium, and potassium. Copper and zinc concentrations decline by approximately 5% from foremilk to hindmilk.

The differences between foremilk and hindmilk should also be considered in terms of the distribution of calories. Fat and protein account for 42% and 12%, respectively, of the calories in foremilk and 55% and 9% of the calories in hindmilk. The long-term feeding of hindmilk thus could have a negative effect on protein status. A greater proportion of protein calories (10%–12%) is recommended for premature infants. The variability in the fat content of human milk may be used to advantage in the premature infant.

Carbohydrate

The major carbohydrate in human milk is lactose, a disaccharide composed of the monosaccharides galactose and glucose. Lactose content is similar between preterm human milk and term human milk and does not vary with the duration of lactation as protein and fat contents do. Small amounts of free glucose and galactose are also present in human milk. Human milk also contains a large amount of oligosaccharides (human milk oligosaccharides [HMO]), complex sugars composed of disaccharide chains built of lactose, galactose, *N*-acetylglucosamine, and *N*-acetylneuraminic acid, modified by fucose and sialic acid. HMOs represent the largest carbohydrate fraction in human milk other than lactose (5%–10% of total carbohydrate) and are often present in similar amounts as protein (Table 68.2). These carbohydrates are not metabolized by infants as fuel but reach the distal part of the gut intact, where they serve as host defense and prebiotic agents, influencing gut microbial colonization patterns (Bode, 2015).

Essential Fatty Acids

The essential fatty acids, linoleic and linolenic acids, are present in ample quantities in human milk and commercial formula.

Without an adequate intake of these fatty acids, essential fatty acid deficiency (thrombocytopenia, dermatitis, increased infections, and delayed growth) can develop in as little as 1 week. Only 0.5 g/kg per day of essential fatty acids (~4% of total energy intake) will prevent the deficiency. α -Linolenic acid is an important precursor for synthesis of both eicosapentaenoic acid and docosahexaenoic acid (DHA). The very long chain polyunsaturated fatty acids arachidonic acid (AA) (20:4 ω -6) and DHA (22:6 ω -3) are found in human milk but not bovine milk and are components of phospholipids found in brain, retina, and red blood cell membranes. AA and DHA functionally have been associated with body growth, vision, and cognition. In addition, the fatty acids are integral parts of prostaglandin metabolism. When their diet was supplemented with polyunsaturated fatty acids, formula-fed premature infants had red blood cell concentrations of DHA paralleling those of similar infants fed human milk. Follow-up studies of such supplemented infants suggest improvements in visual acuity compared with infants that received no supplementation but of similar magnitude to that in infants fed human milk (Carlson et al., 1996). Improvement in cognitive measures during the first year of life has also been shown. Both AA and DHA are now added to premature formula. The recommended intakes for DHA and AA are 11–27 mg/100 kcal and 16–39 mg/100 kcal, respectively (Agostoni et al., 2010; Lapillonne et al., 2013; Uauy and Mena, 2015).

Carnitine

Carnitine is synthesized from lysine and methionine and serves as an important effector of fatty acid oxidation in the mitochondria. The provision of carnitine in the diet results in improved fatty acid oxidation. Human milk contains abundant carnitine, and all infant formulas are supplemented with carnitine.

Human Milk Enzymes

Human milk contains enzymes that aid the infant in nutrient digestion. α -Amylase, the enzyme responsible for most of polysaccharide digestion, is not fully developed at birth even in term infants, who have only 0.2%–0.5% of adult activity. Mammary amylase is active at the pH of both the stomach (3.5) and the duodenum (5.3) and can aid the deficient infant in the digestion of glucose polymers and starches. Although human milk does not contain substrate for α -amylase, this enzyme may aid in digestion of feedings, including infant formula or HMFs that contain complex carbohydrates. Lipases (similar to pancreatic lipase) are present in human milk and aid in digestion of triglycerides such that a significant fraction are broken down into free fatty acids and glycerol before digestion in the small intestine. Bile salt-stimulated lipase, a lipase present in human milk, is highly active because of its wide substrate specificity: it hydrolyzes monoacylglycerols, diacylglycerols, and triacylglycerols, as well as cholesterol esters. This enzyme is also stable in the duodenum and resistant to the low pH of the stomach (Lonnerdal, 2003).

Vitamins and Minerals

The vitamin and mineral content of preterm human milk, as well as currently available multicomponent human milk fortifiers, are shown in Table 68.3.

Donor Human Milk

Recent recommendations have been published by the European Society for Paediatric Gastroenterology, Hepatology and Nutrition, the AAP Section on Breastfeeding, and the WHO endorsing the

**TABLE
68.3****Vitamin and Mineral Content of Human Milk, Raw and After Addition of Human Milk Fortifier Products**

| | PTHM | Similac HMF Liquid, 4 Packets ^a | Similac HMF Liquid, 4 Packets, + 100 mL PTHM ^a | Enfamil HMF Acidified Liquid, 4 Packets ^b | Enfamil HMF Acidified Liquid, 4 Packets, + 100 mL PTHM ^b | Prolact+4 H ² MF ^c | PTHM + Prolact+4 H ² MF (4:1 Ratio) ^c |
|---|------|--|---|--|---|--|---|
| Nutrients | | | | | | | |
| Energy (kcal) | 67 | 27.4 | 94 | 30 | 97 | 28 | 82 |
| Volume (mL) | 100 | 20 | 120 | 20 | 120 | 20 | 80 + 20 |
| Protein (g) | 1.4 | 1.4 | 2.8 | 2.2 | 3.6 | 1.2 | 2.3 |
| Fat (g) | 3.9 | 1.1 | 5.0 | 2.3 | 6.2 | 1.8 | 4.9 |
| Carbohydrate (g) | 6.6 | 3.2 | 9.8 | <1.2 | <7.8 | 1.8 | 7.1 |
| Vitamins | | | | | | | |
| Vitamin A (IU) | 390 | 788 | 1178 | 1160 | 1550 | 61 | 373 |
| Vitamin D (IU) | 2 | 140 | 142 | 188 | 190 | 26 | 27.6 |
| Vitamin E (IU) | 1 | 3.9 | 4.9 | 5.6 | 6.6 | 0.4 | 1.2 |
| Vitamin K (μg) | 0.2 | 9.7 | 9.9 | 5.7 | 5.9 | <0.2 | <0.36 |
| Thiamin (vitamin B ₁) (μg) | 21 | 192 | 213 | 184 | 205 | 4.1 | 20.9 |
| Riboflavin (vitamin B ₂) (μg) | 48 | 492 | 540 | 260 | 308 | 15 | 53.4 |
| Vitamin B ₆ (μg) | 15 | 198 | 213 | 140 | 155 | 4.1 | 16.1 |
| Vitamin B ₁₂ (μg) | 0.05 | 0.33 | 0.38 | 0.64 | 0.69 | 0.05 | 0.09 |
| Niacin (μg) | 150 | 4176 | 4326 | 3700 | 3850 | 52.4 | 172 |
| Folic acid (μg) | 3.4 | 27.3 | 30.7 | 31 | 34.4 | 5.4 | 8.1 |
| Pantothenic acid (μg) | 181 | 1236 | 1417 | 920 | 1101 | 75 | 220 |
| Biotin (μg) | 0.4 | 30.3 | 30.7 | 3.4 | 3.8 | 0.2 | 0.52 |
| Vitamin C (μg) | 11 | 30.9 | 41.9 | 15.2 | 26.2 | <0.2 | <9 |
| Choline (mg) | 9.4 | 3.7 | 13.1 | NA | NA | NA | NA |
| Inositol (mg) | 14.7 | 6.9 | 21.6 | NA | NA | NA | NA |
| Minerals | | | | | | | |
| Calcium (mg) | 25 | 140.4 | 165.4 | 116 | 141 | 103 | 123 |
| Phosphorus (mg) | 13 | 80 | 93 | 63 | 76 | 53.8 | 64.2 |
| Magnesium (mg) | 3.1 | 8.6 | 11.7 | 1.84 | 4.9 | 4.7 | 7.2 |
| Iron (mg) | 0.12 | 0.43 | 0.55 | 1.76 | 1.9 | 0.1 | 1.1 |
| Zinc (mg) | 0.34 | 1.2 | 1.5 | 0.96 | 1.3 | 0.7 | 0.97 |
| Manganese (μg) | 0.67 | 8.5 | 9.2 | 10 | 10.7 | <12 | <12.5 |
| Copper (μg) | 64.4 | 58.9 | 123.3 | 60 | 124.4 | 64 | 116 |
| Iodine (μg) | 11 | 1.9 | 12.9 | NA | NA | NA | NA |
| Selenium (μg) | 1.5 | 0.82 | 2.3 | NA | NA | NA | NA |
| Sodium (mg) | 25 | 21.6 | 46.6 | 27 | 52 | 37 | 57 |
| Potassium (mg) | 57 | 82.8 | 139.8 | 45 | 102 | 34.4 | 80 |
| Chloride (mg) | 55 | 53.6 | 108.6 | 28 | 83 | 38.6 | 82.6 |
| Osmolality (mOsm/kg H ₂ O) | 290 | 160 | 450 | 144 | 434 | NA | NA |

^a<http://abbottnutrition.com/brands/products/similac-human-milk-fortifier-hydrolyzed-protein-concentrated-liquid>.

^b<https://www.meadjohnson.com/pediatrics/us-en/product-information/products/premature/enfamil-human-milk-fortifier-acidified-liquid#nutrients-sup-sup>.

^c<http://www.prolacta.com/Data/Sites/14/media/PDF/mkt-180-prolact-hmf-nutrition-labels.pdf>.

HMF, Human milk fortifier; IU, international unit; NA, not available; PTHM, preterm human milk.

use of donor human milk in preterm infants when maternal milk is unavailable, either as a sole diet or as a supplement to maternal milk (World Health Organization, 2011; Section on Breastfeeding, 2012; ESPGHAN Committee on Nutrition et al., 2013). Despite these recommendations and widespread use, large-scale studies of the use of donor human milk in VBLW infants during the era of routine human milk fortification are lacking. In 2011 the US Surgeon General called for further research to identify areas where the evidence regarding donor milk is inconclusive and to develop evidence-based clinical guidelines for its use (US Department of Human & Health Services, 2014).

Most donor human milk currently used in NICUs in North America is dispensed from the 26 member banks of the nonprofit Human Milk Banking Association of North America (HMBANA), which collectively dispensed 4.2 million ounces of milk in 2015. The rapid rise in donor milk use is apparent when one considers that in 2007 HMBANA dispensed 1 million ounces of milk, demonstrating that demand increased fourfold in 8 years. Milk processed and dispensed by HMBANA is obtained from healthy donors, most of whom delivered term infants and who undergo extensive behavioral and serologic screening (Human Milk Banking Association of North America, 2015). Donors are screened for human immunodeficiency virus, human T-lymphotropic virus1, human T-lymphotropic virus2, hepatitis B, hepatitis C, and syphilis. Testing may be performed via antibody screening or nucleic acid polymerase chain reaction as desired by each bank. Milk is processed by the pooling of donations from 3–10 donors and pasteurization of the milk by heating it to 62°C for 30 min (Holder pasteurization). After pasteurization a sample of milk from each pool is sent for bacteriologic screening. HMBANA member banks maintain records allowing donors and recipients to be matched. Smaller contributions to the volume of donor milk used in US NICUs are made by two commercial producers, Prolacta Bioscience (City of Industry, California) and Medolac Laboratories (Lake Oswego, Oregon). These companies use proprietary processes for pasteurization and donor screening.

Differences Between Maternal and Donor Human Milk

Donor human milk and maternal milk differ in some important aspects, and these differences must be kept in mind when one is choosing donor milk as a diet for preterm infants. Since donor human milk is obtained primarily from mothers who delivered term infants, this milk is lower in protein than preterm maternal milk. Calculations of protein and energy intakes in preterm infants are often conducted with an estimate of 1.4 g/dL for protein content. Donor milk, however, typically contains less protein than this, with studies reporting values of 0.9–1.1 g/dL, with more than 30% of samples in one study of 273 donors having a protein content of less than 1 g/dL (Wojcik et al., 2009). In addition to most donor milk being term human milk, the handling needed to process and feed donor milk causes additional nutrient loss, with 9% protein loss and 13% fat loss between raw milk and milk that has been pasteurized and subjected to a sham nasogastric bolus tube feeding (Vieira et al., 2011). Fat content of donor human milk is similar to that of maternal milk, with less variability per batch of HMBANA donor milk than individual expression of maternal milk because of the pooling process. Some HMBANA milk banks collect and pool milk from mothers who deliver preterm and dispense it after pasteurization as preterm milk to provide higher protein content for preterm infants. Additionally, some HMBANA milk banks analyze the nutrient content of their pooled milk, labeling containers with energy (kcal/dL) and/or protein (g/dL) content, although this practice is neither universal nor mandated

by HMBANA. Prolacta Bioscience and Medolac Laboratories both label their commercial products with nutrient content.

In addition to the nutritional differences noted earlier, some of the unique host defense mechanisms present in human milk are inactivated by Holder pasteurization and freezing. Forty percent to 60% of the sIgA and 60% of the lactoferrin in human milk are inactivated by Holder pasteurization, as are all live white blood cells. Lysozyme and oligosaccharides, other antiinfective protective factors in human milk, are unaffected. There are no published data regarding the effects of the proprietary pasteurization processes used by Prolacta Bioscience and Medolac Laboratories on host defense compounds in donor human milk. Bile salt–stimulated lipase and amylase are also inactivated by Holder pasteurization.

Initiation, Mode, and Advancement of Enteral Feedings

The initiation, mode, and rate of advancement of enteral feeding all remain a topic of controversy in neonatology. The literature does not definitively inform practice, resulting in substantial variation among NICUs. The main concern regarding enteral feeding is the presumed association of enteral feeding and NEC, with early initiation and/or rapidly advancing feeding perceived to increase the risk of this complication of prematurity.

Current practice favors early minimal enteral feeding, also referred to as *trophic feeding* or *GI priming*, as lack of enteral feeding has been shown to delay gut maturation in preterm infants and results in negative effects. Fasting results in intestinal atrophy, diminished intestinal weight and size (in animal models), delayed maturation of intestinal enzyme function, increase in gut permeability and bacterial translocation, and delay in maturation of the intestinal motor function. Additionally, delay in enteral feeding can extend the duration of parenteral nutrition, with its well-described risks, including metabolic disturbance, direct hyperbilirubinemia and cholestasis, and late-onset sepsis.

Several Cochrane reviews, published since 2013, have investigated various aspects of feeding initiation and advancement in preterm infants (Table 68.4), with underwhelming results. These issues have been difficult to study in a rigorous manner but have been addressed in a series of Cochrane systematic reviews (Kennedy et al., 2000; Tyson, Kennedy, 2000). The primary goal is to determine the optimal feeding regimen that does not increase the incidence of NEC. Comparisons of (1) early (<4 days of age) trophic versus delayed initiation of feeding, (2) early (<4 days) versus delayed progressive feeding advancement, and (3) slow (<24 mL/kg per day) versus faster feeding volume advancement yielded no association between any of these interventions and the incidence of NEC. Early progressive feeding was associated with a 2-day shorter hospital stay, and slow feeding volume advancement was associated with increased risk of invasive infection, not including NEC. The authors of all these metaanalyses concluded that early trophic feeding, early progressive feeding, and faster feeding volume advancement are not associated with increased risk of NEC, but that evidence for benefit or harm from any of these feeding techniques is not available. They urge caution in interpretation of studies and recommend further randomized trials (Morgan et al., 2013, 2014, 2015).

Tube Feeding

Preterm infants are often unable to feed by mouth at the breast or via a bottle at birth because of developmental immaturity. A

TABLE 68.4 Evidence for Early Trophic Feeding, Delayed Progressive Feeding, and Slow Advancement of Feeding Volumes in Very Low Birth Weight Infants: Metaanalyses

| Metaanalysis | Primary Comparison | Population | Results |
|----------------------|--|--|---|
| Morgan et al. (2013) | Trophic feeding at <4 days of age for at least 7 days vs enteral fasting | Nine trials 754 VLBW or very preterm infants | No difference in days to full feeds (MD -1.05, 95% CI -2.61 to 0.51), risk of NEC (RR 1.07, 95% CI 0.67 to 1.70) |
| Morgan et al. (2014) | Delayed (>4 days) progressive feeding vs early (<4 days) feeding | Nine trials 1106 infants, few <28 weeks or <1000 g | Decreased length of stay for the early feeding group (MD 2.11 days, 95% CI 0.31 to 3.9, $P = .02$) No difference in risk of NEC (RR 0.93, 95% CI 0.64 to 1.34), mortality (RR 1.18, 95% CI 0.75 to 1.88) |
| Morgan et al. (2015) | Slow (<24 mL/kg per day) vs faster advancement of feeding | Nine trials 949 infants, most 1000 to 1500 g at birth | Higher incidence of invasive infection in the slow advancement group (RR 1.46, 95% CI 1.03 to 2.06, $P = .03$) No difference in risk of NEC (RR 1.02, 95% CI 0.64 to 1.62), mortality (RR 1.18, 95% CI 0.9 to 1.53) |

CI, Confidence interval; MD, mean difference; NEC, necrotizing enterocolitis; RR, risk ratio; VLBW, very low birth weight.

metaanalysis of small studies suggested that infants with postmenstrual age as low as 28 weeks could safely be exposed to breastfeeding (Lucas and Smith, 2015) and that introduction of oral feeding can be accomplished at 30 to 31 weeks' postmenstrual age (Simpson et al., 2002). However, extremely preterm infants may be unable to even begin oral feeding attempts for many weeks after birth and thus are dependent on NG or OG tube feeding. NG/OG feedings may be administered via a timed infusion, with use of an electronic pump, or via gravity bolus. Timed infusions can be periodic, occurring at a specified interval and lasting a specific duration, with a period of rest between feedings, or continuous, in which milk is infused slowly 24 hours a day with no rest period. There are theoretical benefits and risks from both methods of feeding, although research is sparse, with small trials. Continuous feedings may improve energy absorption while decreasing energy expenditure when compared with bolus feedings (Grant, Denne, 1991), improve growth, and reduce feeding intolerance (Toce et al., 1987). Conversely, continuous feeding may disrupt normal cyclical patterns of GI hormone release stimulated by intermittent feeding (Aynsley-Green et al., 1982). Bolus feeding promotes the normal physiologic surge of GI hormones, which may enhance maturation of the preterm GI tract (Aynsley-Green et al., 1990), but immature GI function, including delayed gastric emptying and abnormal intestinal motility, may result in feeding intolerance. With both tube feeding methods, nutrient delivery can be impaired by losses of nutrients that remain in feeding tubes and infusion systems rather than reaching the infant. Fat, particularly, is known to adhere to feeding equipment, decreasing energy delivery (Rayyan et al., 2015). When gravity bolus, 30-min pump infusion, and continuous infusion were compared with simulated feeding techniques using fortified human milk, the least amount of fat was lost with gravity bolus (6%), and the greatest amount was lost with continuous infusion (25%) (Rogers et al., 2010).

Human Milk Fortification

Although human milk is the appropriate and preferred diet for preterm infants, these infants are at risk of growth failure if unadulterated milk is used. VLBW (<1500 g) infants, particularly those born early in the third trimester of pregnancy, have much higher calorie, protein, and mineral needs than term infants because of the rapid growth and development that occurs at the end of

pregnancy. Therefore it is standard practice to add multicomponent HMF to human milk fed to VLBW infants to provide adequate nutrients (primarily protein and minerals) to support growth. Fortification is initiated when infants are tolerating a significant volume of human milk intake, typically between 50 and 120 mL/kg per day, and the timing is highly variable by NICU, with no evidence to support any specific target volume for initiation. HMFs are typically produced from bovine milk and contain standard doses of hydrolyzed or intact protein, as well as carbohydrate, fat, electrolytes, minerals, and vitamins. There is also a HMF available that is made from donor human milk. The nutrient, vitamin, and mineral contents of the three most commonly used HMFs in the United States in 2016 are reported in Table 68.3 as an example. Bovine fortifiers are available in both liquid and powder form, although liquid form is preferred for NICU use because of safety concerns regarding the sterility of powder products. Human milk-derived HMF is a liquid product. Although when used according to manufacturer directions all products result in fortified milk containing approximately 24 kcal/oz, protein content differs substantially, which clinicians must be aware of when managing growth in VLBW infants who have high protein requirements.

The recommended target enteral protein intake to achieve growth in this population is 3.5–4.5 g/kg per day with energy of 120–130 kcal/kg per day. When HMF is added to preterm human milk (with protein content assumed to be at least 1.4 g/dL), all three current products achieve appropriate protein intake (3.9–5.4 g/kg per day). However, if the protein content of the base milk is lower, such as in donor human milk from term donors or maternal milk after the first 3 weeks of life, protein intake may be insufficient. Thus the step of adding standard amounts of HMF according to manufacturer directions may not be sufficient; growth must be monitored with additional fortification provided as necessary.

Standard Fortification

The process described earlier is referred to as *standard fortification*, wherein a standard composition of human milk is assumed, and protein and energy intakes are estimated with these values. Growth is monitored, and if it is insufficient, additional products are added to increase the estimated protein and/or energy content of the milk, with use of additional HMFs (bovine or human derived) or single-component protein, carbohydrate, or fat supplements. These interventions are driven solely by infant growth response to feeding.

Because of unknown and certain variability in human milk nutrient content, standard fortification is imprecise but has been shown to be successful in achieving growth in some studies using both bovine (Colaizy et al., 2012) and human milk–derived HMF (Hair et al., 2013).

Adjustable Fortification

Adjustable fortification is a modification of standard fortification in which infant blood urea nitrogen (BUN) level is monitored as a biochemical marker for adequate protein intake. When the weekly obtained BUN level is lower than the desired range (9–14 mg/dL) (Alan et al., 2013), additional protein is added until BUN reaches the target level. In one small study, use of this fortification technique resulted in improved weight and head circumference growth, as well as higher Bayley Scales of Infant and Toddler Development scores at 18 months compared with the use of standard fortification (Ergenekon et al., 2013).

Target (or Individualized) Fortification

Both standard and adjustable fortification are imprecise, in that nutrient content of the human milk used is estimated with the use of standard assumptions, which are certainly not accurate in every case. The technique of target fortification accounts for the variability in protein, energy, fat, and carbohydrate content in human milk by measuring these nutrients directly through regular milk analysis using near-infrared or mid-infrared analysis instruments. Specific doses of fortifier, either single component or multicomponent, are added to bring the nutrient content of the milk to the desired targets. This approach when used to determine energy density of human milk, with supplementation of the milk with a human milk cream product when the energy density was less than 20 kcal/oz, resulted in superior weight and length gain compared with weight and length gain in a control cohort of infants treated with standard fortification and no milk analysis (Hair et al., 2014). Another group of investigators used this approach to determine fat, protein, and carbohydrate content of 12-hour pools of maternal milk and fortified each macronutrient to target levels. They found this approach resulted in appropriate weight gain and to be clinically feasible (Rochow et al., 2013).

Infant Nutrition and Growth

A growing body of literature suggests that early nutrition has long-term implications for health and development of all infants and particularly preterm infants. In most scenarios the primary goal of postnatal nutrition for the preterm infant is to match as closely as possible expected in utero growth and development. Ideally this would also optimize long-term developmental outcomes. However, feeding the preterm infant is associated with several challenges. Physiologic weight loss in the first few days after birth sets preterm infants up for “catch-up growth,” and it is extremely difficult to provide nutrition (and minerals, in particular) at a rate that matches in utero accretion rates. On the other hand, potential risks of overnutrition may include short-term risk such as increased rates of NEC and longer-term risks such as metabolic syndrome. Most data supporting current nutritional practice in this population are derived from observational studies. The inherent limitations of this type of study design influence the conclusions that can be drawn about the links between nutrition, morbidities, growth, and outcomes.

After birth, preterm infants may lose up to 15%–20% of their birth weight and then regain their birth weight by about 2 weeks

of life. After this physiologic weight loss and regain of birth weight, VLBW infants often gain weight at approximated intrauterine rates. However, the impact of poor growth during the first few weeks persists. By term-corrected age, most preterm infants remain far below the reference weight of an infant born at the same postmenstrual age (Ehrenkranz et al., 1999).

Postnatal growth failure in the preterm infant, defined as body weight or length below the 10th percentile of expected intrauterine growth at the time of hospital discharge, is extremely common. The rates of reported growth failure depend on the population studied and generally increase with decreasing gestational age and birth weight and increase with the severity of illness. Among more than 24,000 infants in the Pediatrix Medical Group, Inc. database who were born before 34 weeks gestation, 28% had weight below the 10th percentile at discharge (Clark et al., 2003). Early postnatal growth failure may increase the risk of long-term adverse effects, such as cardiovascular disease and adult-onset diabetes (De Curtis and Rigo, 2004). Significant variation between hospitals in the degree of postnatal growth failure suggests that this outcome is modifiable and may at least in part reflect differences in nutritional practice (Bloom et al., 2003; Cooke et al., 2004).

Extremely preterm infants with a lower growth trajectory while in the hospital are more likely to have weight, length, and head circumference below the 10th percentile at 18 months’ corrected age (Ehrenkranz et al., 2006). Both in the United States and in other countries, infants born preterm have growth failure at least into adolescence and likely beyond (Ford et al., 2000). The most extremely preterm infants remain smallest and in particular may have the smallest head circumferences throughout childhood (Ericson and Kallen, 1998).

Longitudinal growth is slower in infants with morbidities of prematurity (Ehrenkranz et al., 1999; Clark et al., 2003). On the other hand, infants with morbidities such as lung disease or NEC may be fed less aggressively because of their underlying illness (Ehrenkranz et al., 2011). Infants with morbidities of prematurity continue to experience poor growth through at least 2 years, which is only in part explained by the lower growth velocity during the first several months of life (Bertino et al., 2007; Ramel et al., 2012).

Extrauterine growth failure among preterm infants has been a target of multiple randomized trials and nutritional interventions in the past several decades. These have led to the current “aggressive” approach to nutrition for very preterm infants, including institution of intravenous protein supplementation of 3.5g/kg per day at birth or as soon as possible within the first day of life, trophic enteral feeding—ideally with expressed maternal milk—as soon as possible, and early fortification of both human milk and preterm formula feedings (Ehrenkranz, 2007). This approach improves energy and nitrogen balance, promotes earlier regain of birth weight, and does not increase the risk of acidosis, NEC, sepsis, or other adverse clinical outcomes. Several studies suggest that lean growth and early catch up are optimized with a high-protein regimen. In ELBW infants, early enteral provision of protein significantly increases weight, length, and head circumference at least until 36 weeks’ postmenstrual age (Poindexter et al., 2006). Shorter duration of dependence on parenteral nutrition is associated with improved longitudinal growth. Nevertheless, “normal” immediate postnatal weight loss is augmented by a suboptimal nutritional state during the first several weeks of life, and growth failure remains common. An observational study demonstrated that postnatal weight loss can be limited to the first few days (and limited to ~8% of birth weight) with optimized early parenteral and enteral

nutrition, including a goal of achieving 120 kcal/kg per day and 3.8 g/kg per day of protein by the end of the first week (Senterre, 2011).

Infants with intrauterine growth restriction (IUGR) likely have different metabolic programming and therefore different nutritional requirements from appropriately grown infants. Term infants born with IUGR have higher rates of adult-onset disease, including hypertension, heart disease, and diabetes, and early death (Barker, 1990; Phenekos, 2001). Unfortunately, similar data on IUGR preterm infants are not available. Little is known about how to identify or select these infants, and no studies have specifically targeted the nutritional needs of this population. Therefore the nutrition and growth targets of the IUGR preterm infants are currently similar to those of the appropriate for gestational age preterm infant. The risks of slow growth and persistent small size for age, which are common in the IUGR population, likely outweigh the risks associated with rapid early catch-up growth. Ultimately, adult weight and risk of long-term metabolic consequences are more likely to be related to parental weight, adult weight, and lifestyle choices than to aggressive early nutrition (Greer, 2007) in the IUGR infant.

While growth failure in the preterm or IUGR infant remains a significant concern, growth that is too rapid in infancy is also associated with adult-onset diseases, including obesity. This phenomenon, in which early “programming” leads to future adverse effects, is commonly termed the *Barker hypothesis* (Barker et al., 1993). Large term infants are more likely to be overweight as adults (Stettler, Iotova, 2010). In a multicenter study of more than 19,000 term-born American children, growth in the first 4 months was associated with risk of overweight at 7 years (Stettler et al., 2002). However, it is unlikely that these studies of term infants apply to preterm infants, in whom the benefits of brisk early growth may outweigh any potential risks. There is little evidence that current attempts to maximize growth in the NICU “programs” very preterm or critically ill infants for later adverse metabolic consequences. In fact, accelerated or catch-up growth in preterm infants may be associated with improved outcomes.

Assessing Growth and Body Composition in Infants

Infant growth is generally assessed by anthropometry, in which body weight, length, and head circumference are measured. These are compared with established reference data or plotted on a set of growth curves. In general, growth curves for preterm infants are based on expected intrauterine growth. Others are based on postnatal growth data. Several sets of curves have been established for preterm infants; differences are related to population characteristics and sample size, as well as neonatal nutrition practices. In general, curves for both male and female infants allow the clinician to assess growth parameters relative to gestational age and sex-matched norms. Importantly, preterm infants do not always have proportional growth—or proportional growth failure—in the NICU. Linear growth often falters more than weight and may take longer to recover (Ramel et al., 2012).

Olsen et al. (2015) published body mass index (BMI) curves for preterm infants. BMI curves allow the clinician to assess the proportion of weight to length and to distinguish proportionate from disproportionate growth, most commonly when weight increases faster than length. Similarly to other growth and body composition parameters, BMI varies with sex and gestational age.

While a preterm infant is hospitalized, growth should be monitored serially and plotted on a set of standardized growth curves. These curves should include weight, length, head circumferences, and possibly BMI. Nutrition should be adjusted to target expected intrauterine growth, which is about 20 g/kg per day for the smallest infants (Ziegler et al., 2002).

Changes in body composition over the first months and years of life are associated with nutritional programming of adult morbidities. Assessment of anthropomorphic measures, including BMI, does not inform the clinician about the relative contributions of bone mass, fat mass, and lean mass to body size. Data on the contributions of bone, fat, and lean mass may better reflect the nutritional state of an infant and could potentially inform decisions about appropriate provision of nutrients and goals for growth.

Many preterm infants have poor growth into adolescence, with lower BMI and lower body weight. They have equal fat mass and waist circumference, with an increase in relative abdominal adiposity compared with term-born children. Adiposity may also differ in different groups of preterm infants. For instance, small for gestational age preterm infants have lower fat mass between term-corrected age and 3 months of life but catch up after that point to match appropriate for gestational age born preterm infants (Roggero et al., 2007). Critically ill preterm infants may have increased central or abdominal adiposity, which is a marker of insulin resistance, versus subcutaneous adiposity. This has unclear implications for short-term and long-term health. Increased abdominal adiposity, rather rapid increases in body weight or the absolute body weight itself, may be the factor that puts some preterm infants at increased risk of adult-onset diseases (Fewtrell et al., 2004; Uthaya, 2005).

Little else is known about the changes in body composition in preterm infants and how to distinguish “normal” from “abnormal.” Yet, an understanding of body composition is essential for comparing quality versus quantity of weight gain. An appropriate technique for assessment of body composition must be standardized, valid, reliable, portable, inexpensive, and noninvasive. Ideally, such technology would be used serially to assess response to interventions (Roggero et al., 2007). Several techniques for assessment of body composition, including dual-energy X-ray absorptiometry and magnetic resonance imaging, are available for research use but cannot practicably be applied in clinical settings, particularly in critically ill neonates. Until additional data on “normal” trajectories of body composition in preterm infants and appropriate technologies for assessment of body composition exist, body composition cannot be used to guide nutrition interventions in the NICU.

Growth and Developmental Outcomes in Preterm Infants

Optimal nutrition is not only essential to match appropriate growth trajectories and minimize risk for adult-onset diseases. Early growth and nutrition impact developmental outcomes throughout childhood. The most likely explanation for this long-term impact of infant nutrition is that the first year of life represents a “critical window” of brain growth. In a multicenter cohort study, Ehrenkranz et al. (2006) divided ELBW infants into quartiles of in-hospital growth velocity rates. They identified correlations between the quartile of in-hospital growth velocity and the risk of cerebral palsy and developmental outcomes more than two standard deviations below the mean at 18–22 months’ corrected age. These relationships persisted when they were adjusted for multiple potential confounders, such as center and severity of illness.

Postdischarge growth is likely at least as important for developmental outcomes as in-hospital growth. Preterm infants who experience catch-up growth by 8 months' corrected age have better developmental outcomes than those who do not catch up or fail to thrive by 8 months (Hack et al., 1982). By 8–9 years' corrected age, head growth less than 2 standard deviations below the mean for age at 8 months' corrected age is independently associated with lower intelligence quotient and lower reading, mathematics, spelling, and language scores (Hack et al., 1991). In a cohort of 62 VLBW infants followed up prospectively until 24 months' corrected age, poor linear growth velocity in this period was associated with lower cognitive performance at 24 months (Ramel et al., 2012).

It remains unknown which specific nutritional interventions in hospital and after discharge would optimize both growth and developmental outcomes in the preterm infant. For example, although parenteral provision of amino acids before as compared with after 5 days of life improves in-hospital growth in ELBW infants, it does not seem to improve developmental outcomes at 18 months' corrected age (Poindexter et al., 2006). In two randomized trials of fortified versus term formula at discharge for preterm infants, infants fed fortified preterm formula had improved growth at 18 months (Lucas et al., 2001). However, there was no difference in developmental scores at 18 months in either trial.

Continued research will be essential to establish which growth targets are associated with the best long-term developmental and health outcomes in preterm infants and which nutritional strategies best achieve these targets.

Postdischarge Nutrition for the Premature Infant

When preterm infants are discharged, they have accumulated energy, protein, and mineral deficits. In addition, they are generally discharged before their original anticipated birth date. Together, these factors lead the preterm infant to have higher nutritional needs after discharge than healthy appropriate for gestational age term infants. Targeting of an appropriate trajectory is essential because postnatal growth through age 1 year in infants born before 32 weeks has a positive relationship with height, weight, and BMI until at least 19 years (Euser et al., 2005).

At least one small, randomized trial (Koo and Hockman, 2006) has demonstrated improved growth among preterm infants fed standard, rather than fortified, formula after discharge. On the other hand, several randomized trials have demonstrated improved growth and mineral accretion among both well former preterm infants and infants with BPD when fed fortified formula feedings after discharge (Brunton et al., 1998; Lucas et al., 2001). Carver et al. (2001) performed a small trial in which infants with birth weights less than 1800 g were randomized to receive term formula or a nutrient-enriched (22 kcal/oz) formula until 12 months' corrected age. High rates of loss to follow-up limit the conclusions that can be drawn from this trial. However, infants who received the nutrient-enriched formula seemed to have improved proportional growth, because they weighed more at 6 and 12 months' corrected age, were longer at 6 months' corrected age, and had better head circumference growth at term and at 1, 2, 6, and 12 months' corrected age. The nutrient-enriched formula may have been particularly beneficial among infants with birth weight less than 1250 g. Bishop et al. (1993) found increased bone mineral content up to 9 months' corrected age in infants who received nutrient-enriched formula compared with term formula.

Fewer data are available to support decisions about fortification of maternal milk after discharge of the preterm infant, particularly when families express a goal of nursing rather than bottlefeeding their infants. Infants fed unfortified maternal milk have slower growth and lower bone mass than those fed formula after discharge. Fortification of maternal milk is most commonly accomplished by addition of a postdischarge formula to maternal milk to increase energy, protein, calcium, and phosphorus intake. Such fortification improves mineral accretion but may not impact growth in the long term. Nevertheless, on the basis of the existing literature, "fortified" or high-calorie/high-protein milk feeding has become the standard for the preterm infant at discharge, whether feeding is with human milk or formula. Fortification should be continued until at least 6 months' corrected age to optimize catch-up growth. After that time, overly aggressive nutrition may carry an increased risk of adult-onset disease (De Curtis and Rigo, 2004).

Postdischarge formulas supply more energy (22 kcal/oz), protein (2.8 g/100 kcal), calcium, phosphorus, and zinc than term formulas. Compared with term formula, these formulas provide 49% more protein, 10% more calories, 48% more calcium, 62% more phosphorus, and 75% more zinc. Even at intake volumes of up to 200 mL/kg per day, neither maternal milk nor preterm formulas contain the recommended allowance of vitamin D (Greer, 2007). Thus vitamin D should be provided as a supplement to all infants both during the hospitalization and after discharge. In infants younger than 1 year, 1 L of vitamin D–fortified infant formula or cow's milk per day will provide sufficient vitamin D to discontinue supplementation.

Postdischarge nutrition should be discussed during the discharge planning process with the families of premature infants. In addition, these plans should be discussed with the primary medical caregiver to ensure a smooth transition to the outpatient setting. Premature infants who are formula fed should be fed nutrient-enriched postdischarge formula for the first year of life. The duration of use will differ depending on the severity of postnatal growth failure, bone health, and proportional growth after NICU discharge. Likewise, growth of premature infants who on discharge are receiving human milk should be closely monitored to ensure optimal proportional growth.

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Parenteral Nutrition for the High-Risk Neonate

SCOTT C. DENNE

KEY POINTS

- A high proportion of extremely low birth weight (ELBW) infants experience poor growth in the neonatal intensive care unit. Nutritional deficits that rapidly accumulate shortly after birth are a major factor in these growth outcomes.
- Nutritional deficits can be minimized and growth outcomes improved by beginning parenteral nutrition soon after birth. Initial support should include glucose at 4–7 mg/kg per min, amino acids at 2–3 g/kg per day, and lipids at 2 g/kg per day.
- Goals for full parenteral nutrition support in ELBW infants are 90–100 kcal/kg per day (13–15 g/kg per day glucose, 3–4 g/kg per day lipid, 3.5–4.0 g/kg per day amino acids).
- There is increasing attention to fish oil–based lipid solutions, which appear to ameliorate parenteral nutrition–associated liver disease in infants that require long-term intravenous nutrition. These products are currently not commercially available in the United States. Randomized trials evaluating fish oil–based and other new parenteral nutrition solutions in preterm infants are greatly needed.

Effective nutritional support of premature and critically ill infants is largely dependent on parenteral nutrition, especially in early postnatal life. In practice, the supply of nutrients to preterm neonates—especially extremely low birth weight (ELBW) infants—is often inadequate, and these infants accumulate major deficits in early postnatal life (Berry et al., 1997; Embleton et al., 2001; Olsen et al., 2002). A high proportion of ELBW infants exhibit poor growth in the neonatal intensive care unit (NICU), with those at the lowest gestational age and birth-weight at greatest risk (Clark et al., 2003; Fanaroff et al., 2007). However, growing evidence indicates that early use of parenteral nutrition may minimize protein losses and improve growth outcomes (Wilson et al., 1997; Thureen et al., 2003; Poindexter, 2005; Stephens et al., 2009). For example, Wilson et al. (1997), in a randomized clinical trial in 125 sick very low birth weight (VLBW) infants, demonstrated that early aggressive parenteral nutrition combined with early enteral feeding reduced growth failure without an increased incidence of adverse clinical consequences or metabolic derangements. Multiple observational studies have produced similar results (Poindexter, 2005; Valentine et al., 2009; Cormack et al., 2013; Rigo and Senterre, 2013; Bolisetty et al., 2014; Morgan et al., 2014). In addition to

improved growth outcomes, Stephens et al. (2009) found an association between increased protein and energy intake in the first week of life and higher Bayley Mental Development Index scores at 18 months' corrected age. Parenteral nutrition solutions, although still imperfect, have improved markedly from the early days of use, and complications are less common. At present, however, improved growth outcomes in preterm infants continue to require a consistent effort at providing parenteral nutritional support, especially in early postnatal life. This means initiating parenteral nutrition within the first 24 hours, continuing until enteral nutrition supplies at least 75% of the total protein and energy requirements, and reinstituting parenteral nutrition quickly whenever enteral feeding is suspended.

Components of Parenteral Nutrition

Protein

The initial goal of parenteral nutrition is to minimize losses and preserve existing body stores; this is particularly important for protein. Protein losses are significant in all neonates in the absence of amino acid intake, and these losses are the highest in the most immature neonates. For example, 26-week-gestation infants lose 1.5 g/kg per day of body protein; protein losses in term infants are approximately half that rate (0.7 g/kg per day) (Denne et al., 1996). These high rates of loss in extremely premature infants result in substantial protein deficits. If extremely premature infants are provided with no amino acid supply, they lose over 1.5% of their body protein per day when they should be accumulating protein at a rate of 2% per day. After only 3 days of no protein intake, a 10% protein deficit results.

Fortunately, there is good evidence that early amino acid intake can compensate for high rates of protein loss and thus preserve body protein, even at low caloric intakes (Saini et al., 1989; Van Lingen et al., 1992; Rivera et al., 1993; Kashyap and Heird, 1994). Amino acid intakes of 1.1–2.3 g/kg per day at caloric intakes of 30–50 kcal/kg per day change the protein balance from significantly negative to neutral or positive in sick VLBW infants (Saini et al., 1989; Van Lingen et al., 1992; Rivera et al., 1993). In addition, Thureen et al. (1998) conducted a randomized trial of 1 g/kg per day versus 3 g/kg per day amino acid intake immediately after birth in extremely premature infants. Despite a modest caloric intake in both groups (approximately 50 kcal/kg per day), protein

accretion was significantly greater in the higher amino acid intake group. In all these studies evaluating the effect of early amino acid intake in premature infants, no differences in ammonia concentrations or acid–base status were observed between infants who received amino acids and those who did not (Saini et al., 1989; Van Lingen et al., 1992; Rivera et al., 1993; Paisley et al., 2000). In addition, these studies demonstrated no relationship between amino acid intake and blood urea nitrogen (BUN), although other studies reported modestly elevated BUN levels in neonates receiving higher amino acid intakes at 7 days of age (Clark et al., 2007; Blanco et al., 2008). The fact that BUN concentrations do not usually correlate with amino acid intake in early postnatal life suggests these levels are related primarily to fluid status and that increased BUN levels should not be used as an indication of protein excess. These data indicate that providing parenteral amino acids at a rate of 2–3 g/kg per day as soon as possible after birth (within hours) can preserve limited body protein stores in sick premature and ELBW infants, even at low caloric intakes.

It is important to point out that even though parenteral amino acid administration is beneficial at low caloric intakes, increasing caloric intake is likely to improve protein accretion. Older studies in premature infants have suggested that increasing caloric intake from 50–80 kcal/kg per day can significantly improve protein balance (Pineault et al., 1988). Based on currently available data, 70–80 kcal/kg per day may be sufficient to maximize protein accretion. However, additional energy beyond this amount probably is necessary to produce appropriate fat accretion (see “Energy” section later).

The ultimate goal of parenteral amino acid administration is to achieve the rate of fetal protein accretion. Based on a variety of studies measuring protein losses and balance, 3.5–4.0 g/kg per day of amino acids is a reasonable estimate of parenteral protein requirements in ELBW infants (Ziegler, 2007) (Table 69.1). Some evidence suggests that up to 4.0 g/kg per day of

amino acids is well tolerated by ELBW infants (Porcelli and Sisk, 2002). For premature infants with birthweights over 1000 g, estimated parenteral protein requirements are 3.0–3.5 g/kg per day. Estimates for term infants are 2.5–3 g/kg per day. Parenteral protein intake recommendations for premature infants are shown in Table 69.1.

The composition of currently available amino acid solutions is shown in Table 69.2. These amino acid solutions were designed to mimic plasma amino acid concentrations in healthy 30-day-old breastfed term infants (TrophAmine, B. Braun Medical Inc.) or fetal or neonatal cord blood amino acid concentrations (Primene, Baxter Corporation). No convincing information exists to support the superiority of one neonatal amino acid solution over another.

Although the current neonatal amino acid solutions represent a substantial advance over previous casein hydrolysates and early crystalline amino acid mixtures, these solutions do not contain all the amino acids. Glutamine, an amino acid abundantly supplied by breast milk and potentially conditionally essential in premature infants, is not included in any available amino acid solution because of issues of stability. However, the National Institute of Child Health and Human Development Neonatal Research Network conducted a multicenter randomized clinical trial of parenteral glutamine supplementation and found that parenteral glutamine supplementation did not decrease mortality or the incidence of late-onset sepsis in ELBW infants (Poindexter et al., 2004). In addition, glutamine had no effect on enteral feeding tolerance, incidence of necrotizing enterocolitis, or growth. Tyrosine has very limited solubility, so little is included in current amino acid solutions. TrophAmine contains a soluble derivative of tyrosine (*N*-acetyltyrosine), but this derivative appears to have poor bioavailability. A variety of studies in premature infants suggest that the tyrosine supply may not be optimal in current amino acid solutions (Brunton et al., 2000). Cysteine is not included in most amino acid solutions because it is not stable for long periods. However,

TABLE
69.1

Suggested Daily Parenteral Intakes for Extremely Low and Very Low Birth Weight Infants

| Component (units/kg per day) | ELBW | | | VLBW | | |
|---------------------------------|--------------------|-------------------------|---------|--------------------|-------------------------|---------|
| | Day 0 ^a | Transition ^b | Growing | Day 0 ^a | Transition ^b | Growing |
| Energy (kcal) | 40–50 | 70–80 | 90–100 | 40–50 | 60–70 | 90–100 |
| Protein (g) | 2.0–3.0 | 3.5 | 3.5–4.0 | 2.0–3.0 | 3.0–3.5 | 3.0–3.5 |
| Glucose (g) | 7–10 | 8–15 | 13–17 | 7–10 | 8–15 | 13–17 |
| Fat (g) | 2 | 2–3 | 3–4 | 2 | 2–3 | 3 |
| Na (mEq) | 0–1 | 2–4 | 3–7 | 0–1 | 2–4 | 3–5 |
| Potassium (K) (mEq) | 0 | 0–2 | 2–3 | 0 | 0–2 | 2–3 |
| Chloride (mEq) | 0–1 | 2–4 | 3–7 | 0–1 | 2–4 | 3–7 |
| Calcium (mg) | 20–60 | 60 | 60–80 | 20–60 | 60 | 60–80 |
| Phosphorus (mg) | 0 | 45–60 | 45–60 | 0 | 45–60 | 45–60 |
| Magnesium (mg) | 0 | 3.0–7.2 | 3.0–7.2 | 0 | 3.0–7.2 | 3.0–7.2 |

ELBW, Extremely low birth weight; VLBW, very low birth weight.

^aRecommended parenteral intakes on the first day of life.

^bPeriod of transition to physiologic and metabolic stability. For most premature neonates, this occurs between 2 and 7 days.

TABLE 69.2 Composition of Commercial Parenteral Amino Acid Solutions

| Amino Acid ^a | CONCENTRATION (MG/DL) | | | |
|-------------------------|-----------------------|-----------------------|-------------------------------|--------------------------------|
| | Aminosyn-PF (Hospira) | TrophAmine (B. Braun) | Primene (Baxter) ^b | Premasol (Baxter) ^b |
| Histidine | 312 | 480 | 380 | 480 |
| Isoleucine | 760 | 820 | 670 | 820 |
| Leucine | 1200 | 1400 | 1000 | 1400 |
| Lysine | 677 | 820 | 1100 | 820 |
| Methionine | 180 | 340 | 240 | 340 |
| Phenylalanine | 427 | 480 | 420 | 480 |
| Threonine | 512 | 420 | 370 | 420 |
| Tryptophan | 180 | 200 | 200 | 200 |
| Valine | 673 | 780 | 760 | 780 |
| Alanine | 698 | 540 | 800 | 540 |
| Arginine | 1227 | 1200 | 840 | 1200 |
| Proline | 812 | 680 | 300 | 680 |
| Serine | 495 | 380 | 400 | 380 |
| Taurine | 70 | 25 | 60 | 25 |
| Tyrosine | 44 | 240 ^c | 45 | 240 ^c |
| Glycine | 385 | 360 | 400 | 360 |
| Cysteine | — | <16 | 189 | <16 |
| Glutamic acid | 820 | 500 | 1000 | 500 |
| Aspartic acid | 527 | 320 | 600 | 320 |

^aAll amino acid mixtures shown are 10% solutions.^bPrimene available in Canada; Premasol available in the United States.^cMixture of L-tyrosine and N-acetyltyrosine.

a cysteine hydrochloride supplement that can be added to the parenteral nutrition solution just before delivery is commercially available. There is supporting evidence that when cysteine hydrochloride supplements are added to parenteral nutrition, nitrogen retention is improved in premature infants (Soghier and Brion, 2006). The addition of cysteine hydrochloride also improves the solubility of calcium and phosphorus in parenteral nutrition solutions and also may improve the status of the important antioxidant glutathione. For these reasons, the addition of cysteine hydrochloride (40 mg/g of amino acid, up to a maximum of 120 mg/kg) is recommended. Cysteine hydrochloride can result in metabolic acidosis, but this possibility can be appropriately countered by the use of acetate in the parenteral nutrition solution as a buffer (Peters et al., 1997).

Energy

The initial goal of parenteral nutrition in early postnatal life is to provide sufficient energy intake to at least match rates of energy expenditure, to preserve body energy stores. Measures of energy expenditure in premature infants have ranged between 30 and

70 kcal/kg per day; energy expenditure increases with energy intake and with advancing postnatal age (Bauer et al., 2003a, 2003b; Torine et al., 2007; Weintraub et al., 2009). Energy expenditure also appears to be greater at lower birthweights (Weintraub et al., 2009). An intake of approximately 70 kcal/kg per day is a reasonable clinical goal to achieve neutral or slightly positive energy balance, although because of glucose and lipid intolerance, this intake may not be achievable for a number of days after birth. Nevertheless, maximizing energy intake within the limits of glucose and lipid tolerance can minimize accumulating energy deficits. It is also important to note that common clinical conditions such as sepsis and chronic lung disease can significantly increase energy expenditure, which can further exaggerate energy deficits (Bauer et al., 2003c; Torine et al., 2007).

To support normal rates of growth, a positive energy balance of 20–25 kcal/kg per day must be achieved (Denne, 2001). This requires 90–100 kcal/kg per day for preterm infants with birthweights of less than 1000 g and 100–110 kcal/kg per day for ELBW infants (Table 69.1). A parenteral intake of 80–90 kcal/kg per day is most often sufficient for term infants. Most of the parenteral calories are best supplied by a balanced caloric intake of lipid and glucose. Parenteral energy requirements are less than those required for enteral nutrition because no energy is lost in the stools. Recommendations for parenteral energy intake are shown in Table 69.2.

Glucose

Glucose is typically the first parenteral nutrient provided to the preterm infant, and glucose administration is initiated minutes after birth to maintain glucose homeostasis and preserve endogenous carbohydrate stores. Although the precise definitions of hypoglycemia and hyperglycemia remain a topic of debate, maintaining glucose concentrations of above 40 mg/dL and below 150–200 mg/dL is a reasonable clinical goal (Cornblath et al., 2000). Hypoglycemia is easily avoided in preterm infants by maintaining a constant intravenous (IV) glucose delivery, but hyperglycemia is more often problematic, especially in ELBW infants shortly after birth. Hyperglycemia is very common in this population in early postnatal life, with up to three-quarters of ELBW infants having glucose concentrations exceeding 150 mg/dL and a third of infants frequently having glucose concentrations over 180 mg/dL (Blanco et al., 2006; Beardsall et al., 2008).

Glucose infusion rates of 4–7 mg/kg per minute (70–110 mL/kg per day of 10% dextrose in water [D₁₀W]) are appropriate starting points for most infants. These rates of glucose infusion approximate or slightly exceed the rate of endogenous glucose release from the liver in term and premature infants with birthweights above 1000 g; therefore these rates of glucose infusion serve to preserve the limited carbohydrate stores in these infants. For ELBW infants, a rate of 8–10 mg/kg per minute is required to match endogenous glucose production (Hertz et al., 1993). Unfortunately, many infants will not tolerate this rate of glucose infusion for several days without developing hyperglycemia. Because ELBW infants can have fluid requirements in excess of 100 mL/kg per day, beginning with 5% dextrose may be necessary to maintain glucose infusion rates in the range of 4–7 mg/kg to achieve glucose homeostasis.

A gradual increase in glucose intake over 2–7 days, up to 13–17 g/kg per day, is usually tolerated when the glucose is combined with amino acid intake. An infusion rate of 18 g/kg per day is a reasonable maximum for IV glucose delivery, because

higher rates probably exceed the glucose oxidative capacity (Jones et al., 1993; Chessex et al., 1995). Exceeding glucose oxidative capacity will drive extensive lipogenesis, an energy-expensive process. Supplying appropriate amounts of glucose rarely requires glucose solution concentrations in excess of 12.5%, unless infants are fluid restricted. Recommendations for glucose intake during parenteral nutrition are provided in Table 69.1.

Some ELBW infants have difficulty tolerating even moderate rates of glucose delivery. This problem can usually be overcome by a temporary reduction in the glucose infusion rate. The use of insulin in this situation remains a controversial practice. Collins et al. (1991), in a small randomized controlled trial, demonstrated increased weight gain in infants who received insulin infusions. No differences in head circumference or length were observed between these infants and controls, suggesting that insulin may have produced increases in fat mass but not in lean tissue. Poindexter et al. (1998) evaluated the effect of insulin on protein metabolism using a euglycemic hyperinsulinemic clamp. Insulin infusion resulted in no improvement in protein balance and unexpectedly produced significant lactic acidosis. An international randomized clinical trial was conducted to determine whether early insulin therapy would reduce hyperglycemia and improve outcomes in VLBW infants (Beardsall et al., 2008). The study demonstrated no improvements in mortality, sepsis, growth, intracranial disease, necrotizing enterocolitis, or chronic lung disease and was terminated early because of futility concerns. Although insulin reduced hyperglycemia, it also resulted in increased episodes of hypoglycemia. Further, the mortality at 28 days was higher in the insulin group. Another randomized controlled trial examined the effect of insulin in hyperglycemic VLBW infants who were randomized to receive insulin either when glucose levels exceeded 150 mg/dL or when glucose levels exceeded 180 mg/dL (Alsweliler et al., 2012). The primary outcome of linear growth was significantly reduced in the more aggressive insulin use group, along with a two times greater rate of hypoglycemia. At present, there is no evidence supporting a clinical benefit from routine insulin administration in VLBW infants. Nevertheless, there are rare VLBW infants who remain hyperglycemic despite very low glucose infusion rates; these infants may require exogenous insulin, beginning at 0.05 unit/kg per hour for a short period of time, to produce normoglycemia.

Meeting the goal of 13–17 g/kg per day of IV glucose will result in a caloric intake of 45–60 kcal/kg per day, which is insufficient by itself to meet total energy needs. IV lipids are necessary to supply the rest of the nonprotein calories. A balanced glucose and lipid approach to supplying nonprotein calories has a number of advantages: it better approximates the carbohydrate-to-fat ratio in enteral feedings, it may improve overall protein accretion, and it minimizes overall energy expenditure (Nose et al., 1987; Van Aerde et al., 1989).

Lipids

IV lipids are made up of triglycerides, phospholipids from egg yolk to emulsify, and glycerol, which is added to achieve isotonicity. IV lipid solutions commercially available in the United States are derived from soybean oil (Intralipid, Hospira, Inc.) or a combination of soybean oil and safflower oil (Liposyn II, Baxter Corporation); these solutions contain long-chain triglycerides. Differences in lipid source result in a slightly different fatty acid profile; the compositions of IV lipid solutions are shown in Table 69.3. All available IV lipid products have a fatty acid profile substantially different from that of human milk.

TABLE 69.3

Composition of Parenteral Lipid Emulsions

| | Intralipid 20% | Liposyn II 20% | Omegaven ^a |
|---------------------|----------------|----------------|-----------------------|
| Oil | | | |
| Soybean | 20 | 10 | – |
| Safflower | | 10 | – |
| Fish | – | – | 10 |
| Fats (%) | | | |
| Linoleic | 50 | 65 | 0.1–0.7 |
| α -Linolenic | 9 | 4 | <0.2 |
| EPA | – | – | 1.3–2.8 |
| DHA | – | – | 1.4–3.1 |
| Arachidonic acid | – | – | 0.1–0.4 |
| Glycerol | 2.3 | 2.5 | 2.5 |
| Egg phospholipid | 1.2 | 1.2 | 1.2 |
| Phytosterols, mg/L | 348 + 33 | 383 | 0 |

DHA, Docosahexaenoic acid; EPA, eicosapentaenoic acid.

^aOmegaven is not approved for use in the United States and is only available under experimental or compassionate use protocol. Omegaven is only available as a 10% solution (10 g lipid per 100 mL).

There is increasing attention on fish oil–based lipid solutions, although none of these products are currently available in the United States. A recent systematic review of fish oil–based emulsions concluded that these products are effective in reducing existing parenteral nutrition–associated liver disease (PNALD) but do not seem to prevent PNALD in neonates (Park et al., 2015). Evaluating fish oil–based and other new parenteral lipid solutions is an area of active interest and will require large randomized trials of premature infants.

IV lipid solutions contain lipid particles similar in size to endogenously produced chylomicrons. These particles are hydrolyzed by lipoprotein lipase into free fatty acids. Lipoprotein lipase activity and triglyceride clearance are reduced in preterm infants of less than 28 weeks' gestation (Brans et al., 1990). Although heparin can release lipoprotein lipase from the endothelium into the circulation, at present no evidence exists that this increases lipid utilization in preterm infants (Spear et al., 1988). In the absence of any information demonstrating clinical benefit of heparin administration, the routine addition of heparin in lipid infusions is not recommended.

Linoleic and linolenic acids cannot be endogenously synthesized and therefore are essential fatty acids for humans. Biochemical evidence of essential fatty acid deficiency may be noted in preterm neonates within 72 hours of birth (Foote et al., 1991). Essential fatty acid deficiency can be avoided if 0.5–1.0 g/kg per day of IV lipid is provided. Additional IV lipid beyond these amounts is necessary if the energy requirements of preterm infants are to be met in early postnatal life.

There is good evidence that early administration of IV lipid to preterm infants is safe and well tolerated. Drenckpohl and colleagues demonstrated that beginning 2 g/kg of lipid on day 1 significantly increased the energy intake of VLBW infants, and hypertriglyceridemia occurred in only 15% of infants

(Drenckpohl et al., 2008). A more recent study showed improved nitrogen balance when lipids were begun at 2g/kg on day 1 in VLBW infants (Vlaardingerbroek et al., 2013). A systematic review and metaanalysis of early versus late IV lipid use in premature infants showed no detrimental effects of early lipids on either death, bronchopulmonary dysplasia, or sepsis (Vlaardingerbroek et al., 2012). However, a beneficial effect from early lipids on long-term growth could not be demonstrated.

In view of the available data, beginning 2g/kg of IV lipid on day 1 in ELBW and VLBW infants is a recommended clinical practice. This approach will support essential fatty acid needs and minimize energy and protein deficits.

The rate of IV lipid infusion is important, and plasma lipid clearance is improved when IV lipid is given as a continuous infusion over 24 hours (Putet, 2000). Triglyceride concentrations are most often used as an indication of lipid tolerance, and maintaining triglyceride concentrations below 150–200 mg/dL seems reasonable; however, there are no available outcome data supporting this practice. Recommendations for parenteral lipid intake are provided in Table 69.1.

Numerous studies have documented the superiority of 20% over 10% lipid emulsions (Putet, 2000). Lipid clearance is improved with the 20% solutions because they have half the amount of phospholipid emulsifier relative to the same amount of triglycerides. Phospholipids can combine with cholesterol to form lipoprotein X, which ultimately interferes with the clearance of infused triglycerides. Consequently, the use of 10% lipid emulsions should be avoided. A 30% lipid solution has recently become available and may confer even more advantages, although currently there are no comparative data.

IV lipid emulsions may undergo lipid peroxidation, with the formation of organic free radicals, potentially initiating tissue injury. Light, especially phototherapy, may play some role in increasing lipid peroxidation in IV lipid emulsions (Neuzil et al., 1995). However, multivitamin preparations included in the IV solutions are major contributors to generation of peroxides, and lipid emulsions may have only a minor additive effect (Lavoie et al., 1997). Some small studies have suggested that light protection may reduce chronic lung disease, but these results have not been consistent (Chessex et al., 2007; Bassiouny et al., 2009; Sherlock and Chessex, 2009). On the basis of these findings, some clinicians protect IV lipid solutions from light, although the importance or clinical efficacy of this practice remains in doubt. Only a large randomized clinical trial is likely to resolve this issue (Sherlock and Chessex, 2009).

Carnitine facilitates transport of long chain fatty acids through the myocardial membrane and thereby plays an important role in their oxidation. Premature infants receiving parenteral nutrition have low carnitine levels, but the clinical significance of this finding remains uncertain. Metaanalysis of the studies evaluating carnitine supplementation in parenteral nutrition showed no evidence of effect on ketogenesis, lipid utilization, or weight gain (Cairns and Stalker, 2000). At present, insufficient information is available to support a recommendation for the routine supplementation of carnitine for parenterally fed neonates.

Electrolytes, Minerals, Trace Elements, and Vitamins

Sodium needs are low in the first few days of life because of the expected free water diuresis. For ELBW infants, addition of sodium to the parenteral nutrition solution may not be necessary until about day 3 of life. It is, however, necessary to frequently measure

sodium concentrations and water balance. After the initial diuresis, 2–4 milliequivalent (mEq)/kg per day is usually sufficient to maintain serum sodium in the normal range, but ELBW infants sometimes require higher sodium intakes to compensate for larger renal sodium losses. Chloride requirements follow the same time course as for sodium requirements and are also usually 2–4 mEq/kg per day. Once electrolytes are added to the parenteral nutrition solution, chloride intake should not be less than 1 mEq/kg per day, and all chloride should not be omitted when sodium bicarbonate or acetate is given to correct metabolic acidosis. Potassium requirements are also low in the first few days of life, and potassium should probably be omitted from parenteral solutions in ELBW infants until renal function is clearly established. Potassium intake of 2–3 mEq/kg per day is usually adequate to maintain normal serum potassium concentrations.

Parenteral nutrition solutions usually require the addition of anions, as either acetate or chloride. In general, excess anions should be provided as acetate to prevent hyperchloremic metabolic acidosis. A randomized controlled clinical trial demonstrated that acetate in parenteral nutrition solutions effectively ameliorates this metabolic acidosis (Phelps and Cochran, 1989).

Supplying calcium and phosphorus in parenteral nutrition remains a significant clinical challenge because of limited solubility. It is currently not possible to supply enough calcium and phosphorus to support adequate bone mineralization in preterm infants using the solutions available in the United States. In other countries, organophosphate preparations are available (e.g., glycerophosphate), and calcium and phosphorus can be supplied in parenteral nutrition solutions in amounts that approximate enteral intakes. Precipitation of calcium and phosphorus remains an issue in the United States, however, and the solubility of calcium and phosphorus in parenteral nutrition solutions depends on temperature, type and concentration of amino acid, glucose concentration, pH, type of calcium salt, sequence of addition of calcium and phosphorus to the solution, the calcium-to-phosphorus ratio, and the presence of lipid. Adding cysteine to parenteral nutrition solutions lowers the pH, which improves calcium and phosphorus solubility. Intakes of 60–80 mg/kg per day of elemental calcium (1.5–2.0 mmol/kg per day) and 48–60 mg/kg per day of phosphorus (1.5–2.0 mmol/kg per day) have been recommended for premature infants receiving parenteral nutrition (Atkinson and Tsang, 2005). A calcium-to-phosphorus ratio of 1.7:1 by weight (1.3:1 by molar ratio) may be optimal for bone mineralization, but it appears that neonates can tolerate and adjust to molar ratios over the range of 0.8 to 1.5 (Atkinson and Tsang, 2005). In general, calcium and phosphorus should be added to parenteral nutrition solutions in early postnatal life. Magnesium is also a necessary nutrient and should be supplied at 3.0–7.2 mg/kg per day. Calcium, phosphorus, and magnesium serum concentrations should be frequently monitored.

Recommendations for trace elements in term and preterm infants are primarily derived from the American Society for Clinical Nutrition guidelines from 1988 (Greene et al., 1988), with some recent updates by the American Society of Parenteral and Enteral Nutrition (Vanek et al., 2012; Finch, 2015). There is reasonable consensus that zinc and selenium should be included early in parenteral nutrition solutions (Finch, 2015). Other trace elements are probably not needed until after the first 2 weeks of life. The recommended intakes of trace elements for term and preterm infants are shown in Table 69.4.

Zinc and copper are available in the sulfate form and can be added separately to parenteral solutions. Several pediatric trace metal solutions are available that contain zinc, copper, magnesium,

TABLE 69.4 Recommended Parenteral Intake of Trace Elements for Term and Preterm Infants

| Trace Element | Term (μg/kg per day) | Preterm (μg/kg per day) |
|------------------------|----------------------|-------------------------|
| Chromium ^a | 0.20 | 0.05–0.30 |
| Copper ^b | 20 | 29 |
| Iron ^c | – | – |
| Fluoride ^d | – | – |
| Iodide | 1 | 1 |
| Manganese ^b | 1 | 1 |
| Molybdenum | 0.25 | 0.25 |
| Selenium ^a | 2 | 1.5–4.5 |
| Zinc ^e | 250 | 400 |

^aRenal dysfunction can cause toxicity.

^bImpaired biliary excretion can cause toxicity.

^cIron not recommended unless on parenteral nutrition for longer than 2 months (estimated daily intravenous requirement is 250–670 μg/kg for term infants and 100–200 μg/kg for preterm infants).

^dBecause of a lack of information on the compatibility of fluoride with TPN and on the contamination level of fluoride in TPN solutions, firm recommendations cannot be made; with long-term TPN (longer than 3 months), a dosage of 500 μg/day may be important in preterm infants, who already have a higher incidence of subsequent dental caries.

^eThe only trace element recommended on day 1 of parenteral nutrition. If the infant requires TPN for longer than 3 months, the dosage must be reduced to 100 μg/kg/day.

Data from Vanek VW, Boren P, Buchman A, et al. A.S.P.E.N. position paper: recommendations for changes in commercially available parenteral multivitamin and multi-trace element products. *Nutr Clin Pract.* 2012;27:440–491.

and chromium in various proportions; these solutions are usually provided at 0.2 mL/kg per day. When trace metal solutions are used, additional zinc is usually needed to provide the recommended intake for preterm infants. In infants with cholestasis, copper and manganese should be discontinued, and chromium and selenium should be used with caution and in smaller amounts in infants with renal dysfunction. At present, parenteral iron is recommended only when preterm infants are nourished exclusively by parenteral solutions for the first 2 months of life.

The recommended intakes of vitamins for term and preterm infants on parenteral nutrition are shown in Table 69.5. Currently only two pediatric multivitamin preparations are available. These preparations provide somewhat higher amounts of most of the B vitamins relative to the recommendations.

Complications of Parenteral Nutrition

Although a wide variety of complications associated with parenteral nutrition were reported in the early days of use, most of these are now rare with current parenteral solutions. Many of the complications (electrolyte and glucose imbalance) can be prevented or corrected by manipulating the constituents of the infusate. The primary complications of parenteral nutrition as currently used are cholestasis and those related to the infusion catheter.

Cholestatic jaundice as a result of hepatic dysfunction is a well-recognized complication of parenteral nutrition. The initial histologic lesion is cholestasis, both intracellular and intracanalicular, followed by portal inflammation and progression to bile duct proliferation after several weeks of parenteral nutrition. Cholestasis most often

TABLE 69.5 Recommended Parenteral Intake of Vitamins for Term and Preterm Infants

| Vitamin | Term (daily dose) | Preterm (dose/kg per day) ^a |
|------------------------------|-------------------|--|
| Fat Soluble | | |
| Vitamin A (IU) | 2300 | 700–1500 |
| Vitamin D (IU) | 400 | 40–160 |
| Vitamin E (IU) | 7 | 2.8–3.5 |
| Vitamin K (μg) | 200 ^b | 10 ^b |
| Water Soluble | | |
| Vitamin B ₆ (μg) | 1000 | 150–200 |
| Vitamin B ₁₂ (μg) | 1 | 0.3 |
| Vitamin C (mg) | 80 | 15–25 |
| Biotin (μg) | 20 | 5–8 |
| Folic acid (μg) | 140 | 56 |
| Niacin (mg) | 17 | 4.0–6.8 |
| Pantothenate (mg) | 5 | 1–2 |
| Riboflavin (μg) | 1400 | 150–200 |
| Thiamin (μg) | 1200 | 200–350 |

^aMaximum not to exceed dosage for term infant.

^bThis does not include the 0.5 to 1 mg of vitamin K to be given at birth.

Data from Vanek VW, Boren P, Buchman A, et al. A.S.P.E.N. position paper: recommendations for changes in commercially available parenteral multivitamin and multi-trace element products. *Nutr Clin Pract.* 2012;27:440–491.

resolves after discontinuation of parenteral nutrition and initiation of enteral feedings. Some rare instances of irreversible liver failure have been documented, but this seems to occur only after several months of use.

The etiology of PNALD cholestasis is unknown and most likely multifactorial. The patients at greatest risk are critically ill premature infants who are susceptible to multiple insults, such as hypoxia, hemodynamic instability, and sepsis. The most frequently identified risk factors in parenteral nutrition–associated cholestasis are duration of parenteral nutrition, degree of immaturity, and delayed enteral feeding (Steinbach et al., 2008). There is also evidence that being small for gestational age is also an independent risk factor for PNALD (Robinson and Ehrenkranz, 2008). A recent systematic review estimated parenteral nutrition–associated cholestasis in premature infants at 25%, but there is substantial variation across studies (Lauriti et al., 2014).

There is growing evidence implicating soybean-derived emulsions in the pathogenesis of PNALD. Soybean-derived lipid solutions contain phytosterols, high concentrations of the proinflammatory omega-6 polyunsaturated fatty acids (PUFAs), and low concentrations of antioxidants (i.e., alpha tocopherol). A variety of both animal and human studies have demonstrated that these components, both alone and in combination, contribute to PNALD (Nandivada et al., 2013).

There are now multiple studies, mostly observational, that demonstrate improvement in PNALD in infants using the fish oil–based product Omegaven (Gura et al., 2008; Diamond et al., 2009; Puder et al., 2009; Park et al., 2015). Puder et al. (2009)

performed an open-label trial of Omegaven in 42 infants with short bowel syndrome with PNALD and compared them with a similar cohort of short bowel syndrome infants who received only soy-based IV lipids. The group receiving Omegaven had lower rates of mortality and liver transplantation and a higher rate of cholestasis reversal. However, this therapy is currently only available in the United States through research or compassionate use protocols. Data from randomized clinical trials are needed before recommending changes in clinical practice to include Omegaven in the treatment of PNALD.

Some infants with cholestasis will require continued parenteral nutrition. In these infants, the use of small-volume enteral feedings in combination with parenteral nutrition may stabilize or improve hepatic function. The use of phenobarbital and ursodeoxycholic acid has been shown to be beneficial in some studies of older children and adults. However, a study in preterm infants demonstrated that tauroursodeoxycholic acid did not prevent the development of parenteral nutrition–associated cholestasis and was ineffective in reducing cholestasis once it occurred (Heubi et al., 2002). At present, the routine use of ursodeoxycholic acid or phenobarbital in parenteral nutrition–associated cholestasis cannot be recommended.

There is emerging information suggesting that lipid restriction may prevent or ameliorate PNALD; however, not all studies have shown this effect (Calkins et al., 2014; Jakobsen et al., 2015). Given the fundamental role of lipid in supporting caloric needs and brain development, lipid restriction should not be routinely used for ELBW and VLBW infants. This strategy might be considered for infants with long-term dependency on parenteral nutrition, but additional studies are needed.

Catheter-related complications remain an important problem associated with parenteral nutrition; the major complication is infection. Two of the most common bacterial pathogens are *Staphylococcus epidermidis* and *Staphylococcus aureus*. Fungal infections also occur, with *Candida albicans* and *Malassezia furfur* being the most common agents. The incidence of sepsis during parenteral nutrition is higher at the lower gestational ages and also increases with the duration of parenteral nutrition. Parenteral nutrition–associated sepsis is likely to be a product of many factors, not the least of which is that the most immature and critically ill patients are most likely to receive parenteral nutrition for prolonged periods. In infants who have developed cholestasis while receiving parenteral nutrition, the rate of sepsis may be increased. At present, avoiding parenteral nutrition–associated infections is best accomplished by meticulous attention to sterile technique in catheter care, early initiation and advancement of enteral nutrition, and prompt removal of the catheter when adequate enteral nutrition is achieved. Prophylactic low-dose vancomycin may diminish the incidence of parenteral nutrition–associated sepsis, but in view of concerns about toxicities and the potential for antibiotic resistance, this approach cannot be recommended (Craft et al., 2004).

Complications specifically related to the catheter have also been reported. Broviac catheters are difficult to place and are associated with thrombosis in neonates (Sadiq et al., 1987). In most NICUs, Broviac catheters have largely been replaced by small-bore Silastic catheters placed percutaneously. However, all central catheters, including the small-bore variety, have produced life-threatening complications. Pericardial tamponade and significant pleural effusions are known complications of the use of central catheters in neonates (Hermansen and Hermansen, 2005). Although these are uncommon events, clinical awareness and early recognition of these complications can prevent mortality.

Use of Parenteral Nutrition in the Neonatal Intensive Care Unit: A Practical Approach

The preceding portion of this chapter has presented the scientific basis for recommendations regarding provision of parenteral nutrition to neonates. The following paragraphs present a practical approach to the administration of parenteral nutrition, with a particular emphasis on ELBW infants.

Every clinician caring for ELBW infants must recognize the urgent need to initiate IV amino acids shortly after birth. As mentioned previously, the ELBW infant loses 1.5% of total body protein each day that amino acids are withheld. Consequently, the goal of early parenteral nutrition should be to limit catabolism and preserve endogenous protein stores. Numerous studies have clearly demonstrated both the safety and efficacy of early amino acids in accomplishing this goal, even at low caloric intakes.

We recommend starting 3.0 g/kg per day of amino acids on the first day after birth. This can be accomplished simply by adding one of the crystalline amino acid solutions designed for use in neonates (Aminosyn-PF, Hospira, Inc.; Primene, Baxter Corporation; Premasol, Baxter Corporation; or TrophAmine, B. Braun Medical, Inc.) to glucose to use as the initial maintenance fluid in ELBW infants. We recommend developing a neonatal amino acid stock solution, made in advance in the pharmacy. The solution contains amino acids in a 7.5% dextrose that, when delivered at 60 mL/kg per day, provides 3 g/kg per day of amino acids. Additional fluids with or without electrolytes and/or a higher concentration of dextrose are “Y’d in,” with adjustments as needed for the individual infant’s fluid, dextrose, and electrolyte requirements, eliminating the need to discard the bag of parenteral nutrition fluid for such changes in status. It is important to note that this stock solution should not be increased beyond 60 mL/kg per day; any alterations to total fluids must be made with ancillary fluids. This mixture of glucose and amino acids can be given via a peripheral IV line, umbilical venous line, or percutaneous central venous catheter. Increased use of percutaneously placed central venous catheters has certainly facilitated early and widespread use of parenteral nutrition in premature infants. In our nursery, strong consideration is given to percutaneous central venous line placement in ELBW infants early in their postnatal course.

To meet growth requirements, 3.5–4.0 g/kg per day of amino acids is required. It is important to point out that such amounts are merely estimates, and protein requirements to sustain optimal growth in ELBW infants might be even higher. Once administration of amino acids is initiated, intake can be advanced to meet requirements for growth over a relatively short period. We typically advance amino acid intake 3.5 g/kg per day by the second day of life. Given the available data, we also recommend the addition of cysteine to the amino acid solution (40 mg/g of amino acids, to a maximum of 120 mg). However, we delay adding cysteine until other electrolytes are included in the parenteral nutrition solution so that acetate can be added to buffer the cysteine acid load.

Glucose should be supplied in a quantity sufficient to maintain normal plasma glucose concentrations. As discussed previously, glucose production and utilization rates are highest in the most premature infants; their glucose needs are in the range of 6–8 mg/kg per minute, whereas the term infant’s needs are approximately 3–4 mg/kg per minute. Giving 10% dextrose at 100 mL/kg per day provides a glucose infusion rate of 7 mg/kg per minute. Starting infants with birthweights less than 1000 g on 5% dextrose is likely to be prudent if their total fluid requirements are greater than 120–150 mL/kg per day.

TABLE 69.6 Caloric Value of Parenteral Nutrition Solutions

| Composition ^a | Kcal/kg per day | % of Nonprotein Calories |
|--|-----------------|--------------------------|
| Example 1: Total Fluids at 110 mL/kg per day | | |
| 10% dextrose | 37 | 55 |
| 3 g/kg per day lipid | 30 | 45 |
| 3.5 g/kg per day amino acids | 14 | — |
| Total | 81 | — |
| Example 2: Total Fluids at 80 mL/kg per day | | |
| 12.5% dextrose | 34 | 53 |
| 3 g/kg per day lipid | 30 | 47 |
| 3.5 g/kg per day amino acids | 14 | — |
| Total | 78 | — |
| Example 3: Total Fluids at 140 mL/kg per day | | |
| 12.5% dextrose | 60 | 67 |
| 3 g/kg per day lipid | 30 | 33 |
| 3.5 g/kg per day amino acids | 14 | — |
| Total | 104 | — |

^aDextrose: 3.4 kcal/g; protein: 4 kcal/g; lipid (20% emulsion): 10 kcal/g.

Lipids should be started within the first 24 hours. We typically start lipids at 2.0 g/kg per day and advance by 0.5–1.0 g/kg per day to a usual maximum of 3 g/kg per day while monitoring and maintaining serum triglycerides at less than 200 mg/dL. Given the numerous advantages over 10% solutions, 20% lipid emulsions should always be used. To facilitate clearance and to avoid impairment of oxygenation, lipids should be infused over a 24-hour period. There is currently no evidence to support the use of cyclic infusion in the acute setting of the NICU.

Caloric goals during parenteral nutrition are lower than with enteral feeds. To achieve optimal protein retention, approximately 70–80 kcal/kg per day is a reasonable goal. To optimize growth, somewhat higher caloric intakes may be necessary. The nonprotein balance between carbohydrate and lipid should be approximately 60:40. These goals can usually be achieved using glucose solutions with concentrations no greater than 12.5% (Table 69.6).

There is a paucity of data related to monitoring laboratory tests during provision of parenteral nutrition. Suggested monitoring for infants receiving parenteral nutrition is shown in Table 69.7. Not all of these laboratory tests may be appropriate in ELBW infants because of constraints related to blood sampling.

The use of parenteral nutrition should be accompanied by the early initiation of enteral feeds (ideally on the first day). Parenteral nutrition should be continued until enteral feedings are well established and providing approximately 100–110 kcal/kg per day, although availability of IV access may necessitate earlier termination of parenteral nutrition in some circumstances. As enteral feeds are advanced, the protein and lipid contents of the parenteral nutrition can be gradually decreased. In addition, careful and prompt attention to reinstitution of parenteral nutrition during episodes of intolerance

TABLE 69.7 Suggested Monitoring During Parenteral Nutrition

| Parameter | Frequency |
|---|--------------------------------------|
| Weight | Daily |
| Length and head circumference | Weekly |
| Serum glucose | 1×/shift during week 1, then daily |
| Serum Na, K, Cl, BUN, Ca, P, Mg, hematocrit | 2–3×/week during week 1, then weekly |
| Alkaline phosphatase, ALT (SGPT), GGT, fractionated bilirubin | Weekly |

ALT, Alanine transaminase; BUN, blood urea nitrogen; GGT, gamma-glutamyl transferase; SGPT, serum glutamate-pyruvate transaminase.

of enteral feeds cannot be overemphasized. Infants with intolerance of enteral feeds in whom nothing-by-mouth (NPO) status is frequently necessary present an additional challenge. In such infants, it may be prudent to determine full-volume parenteral nutrition needs as for NPO status and to then run the parenteral nutrition solution at a lower rate if enteral feeds are administered. With this approach, if a change to NPO status becomes necessary after administration of the parenteral nutrition fluid has begun, the volume can be safely increased without compromising caloric and protein intake.

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Gastrointestinal Tract Development

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KEY POINTS

- A series of folding, lengthening, and luminal dilation events result in the formation of the foregut (esophagus, stomach, duodenum, liver, and pancreas); the midgut (jejunum, ileum, ascending colon, and transverse colon); and hindgut (descending colon, sigmoid colon, and rectum).
- The molecular mechanisms regulating gastrointestinal (GI) tract tissue development and cellular differentiation involve genetically controlled interactions between trophic compounds and cellular receptors and between nuclear transcription factors and target gene DNA.
- GI microbiota play important roles in gut development.

Structural and Functional Development

Understanding the development of the human gastrointestinal (GI) tract, from both an anatomic and cellular basis, has a long history, dating back more than a century. This knowledge encompasses prenatal in utero and postnatal processes. Changes in both morphogenesis and cellular differentiation drive structural formation of the GI tract in the developing embryo. Digestive function continues to develop following birth. Advances in molecular biology have revealed that specific aspects of GI tract development involve genetically controlled interactions between trophic compounds and cellular receptors, as described later for the different organs of the GI tract.

From a global perspective, the GI tract results from embryonic invagination and folding during week 4 of gestation. Eventually, the buccopharyngeal and cloacal membranes rupture, permitting a direct communication between the fetal GI tract and the in utero environment. A series of folding, lengthening, and luminal dilation events result in the formation of the foregut (esophagus, stomach, duodenum, liver, and pancreas); the midgut (jejunum, ileum, ascending colon, and transverse colon); and hindgut (descending colon, sigmoid colon, and rectum). With additional elongation and growth during the first trimester, the developing gut migrates into the umbilical cord. It returns to the fetal abdominal cavity and rotates counterclockwise around the axis of the superior mesenteric artery at approximately 20 weeks' gestation (Fig. 70.1). Thus by the second trimester, the basic morphogenesis of the fetal GI tract is complete. Additional in utero and postnatal functional maturation is still necessary. For example, GI motility is markedly

disorganized at 24 weeks' gestation. Although dysmotility is a normal developmental "milestone" in the fetal environment, uncoordinated peristalsis in the extremely low birth weight infant is associated with significant postnatal problems.

Knowledge of the cellular and molecular processes of early morphogenesis is rapidly expanding, and these processes involve crosstalk between maternal and fetal factors during development. We first discuss the embryology of the GI tract, in advance of discussing functional development including digestion.

Foregut

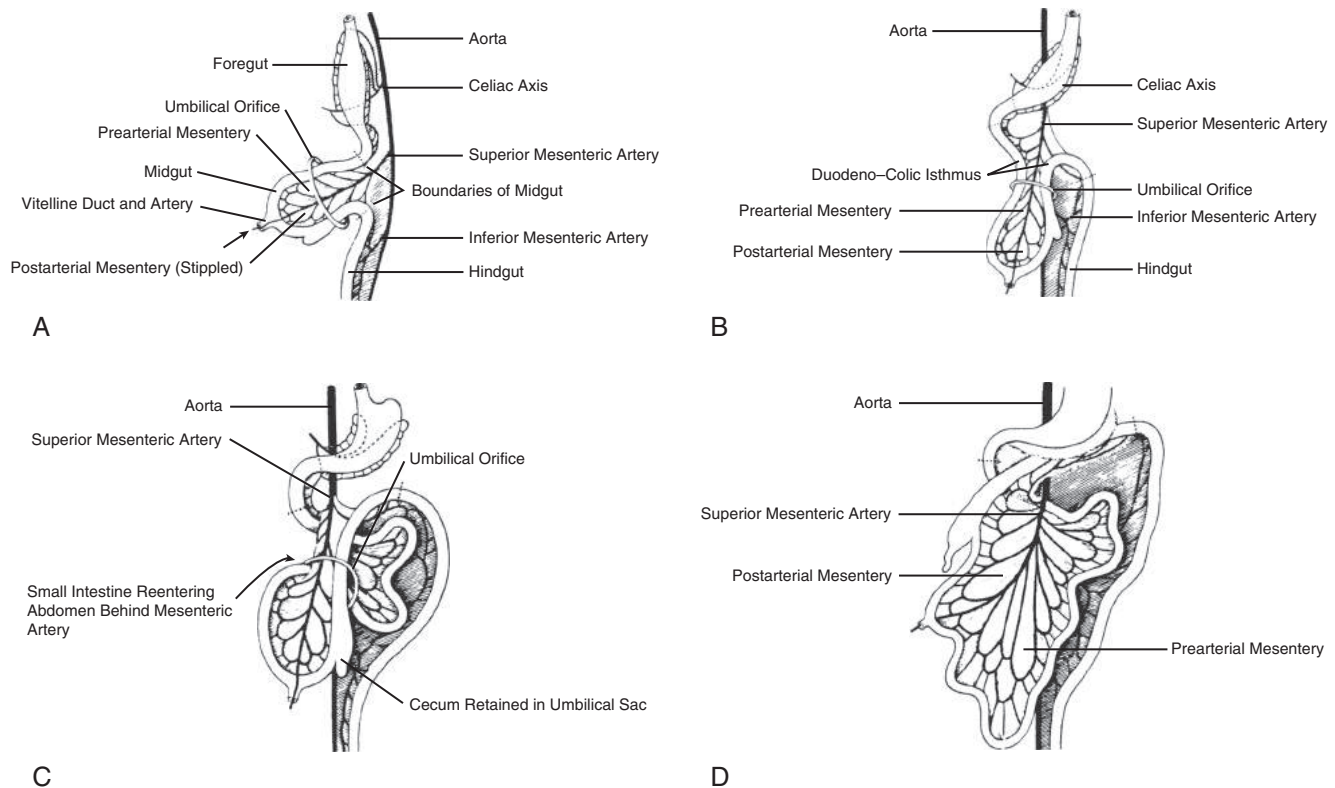
Esophagus

The normal morphogenesis of the foregut can be subdivided into five successive developmental stages (Kluth et al., 2003). In the first stage, occurring at days 22 to 23 weeks' gestation, the primitive foregut differentiates into ventral and dorsal structures, termed the *lung field* and *esophageal area*, respectively. The esophageal area has one cell layer consisting of 10 somites. In the second stage, the lung bud develops from the caudal lung field proximal to the liver. Next, in the third stage, longitudinal ridges appear inside the lumen of the developing foregut, which results in a distinct and separate dorsal esophageal area. The fourth stage involves proliferation of the longitudinal ridges, resulting in a tracheo-esophageal septum. Subsequently, apoptosis in the central section of the septum begins the initial separation of the dorsal and ventral compartments. In the fifth stage, there is the formation of definitive respiratory and esophageal structures between weeks 6 and 7 of gestation.

Researchers utilizing *Drosophila* and murine models have identified a myriad of key signaling pathways and transcription factors in foregut development (Jacobs et al., 2012). For example, the hedgehog signaling pathway appears to be essential for maintaining stem cell niches as well as directing developmental axes (Fig. 70.2) (Lees et al., 2005). Hedgehog binds to a cell membrane receptor Patched (Ptc) resulting in downstream signaling and transcription factor-mediated cell differentiation. Defects in this pathway are associated with congenital anomalies of the foregut.

Stomach

By week 6 of gestation, the fetal stomach is well defined. The muscular layers (the inner circular and outer longitudinal) become visible by 9 weeks. By week 12 of gestation, the gastric mucosa



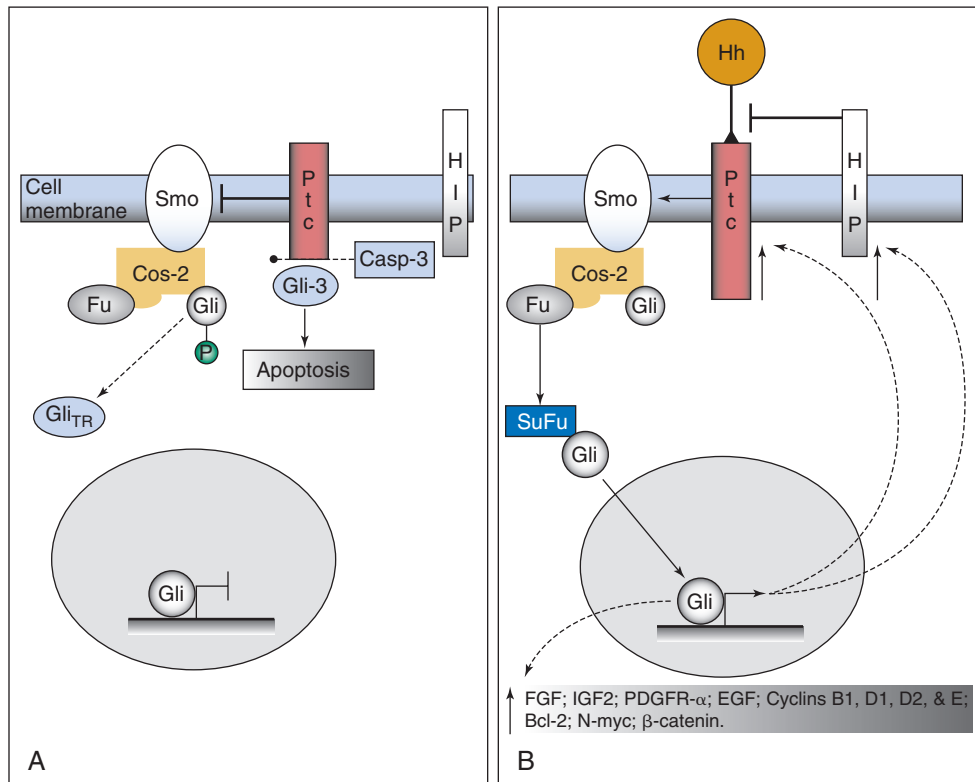
• **Fig. 70.1** Diagrams Showing Normal Rotation Of Alimentary Tract. (A) Fifth week of intrauterine life (*lateral view*). The foregut, midgut, and hindgut are shown with their individual blood supply supported by the common dorsal mesentery in the sagittal plane. The midgut loop has been extruded into the umbilical cord. (B) Eighth week of intrauterine life (*anteroposterior view*). The first stage of rotation is being completed. Note the narrow duodenocolic isthmus from which the midgut loop depends and the right-sided position of the small intestine and left-sided position of the colon. Maintenance of this position within the abdomen after birth is termed *nonrotation*. (C) About the 10th week of intrauterine life, during the second stage of rotation (*anteroposterior view*). The bowel in the temporary umbilical hernia is in the process of reduction; the most proximal part of the prearterial segment entering the abdomen to the right of the superior mesenteric artery is held forward close to the cecum and ascending colon, permitting the bowel to pass under it. As the coils of small intestine collect within the abdomen, the hindgut is displaced to the left and upward. (D) Eleventh week of intrauterine life at the end of the second stage of rotation. From its original sagittal position, the midgut has rotated 270 degrees in a counterclockwise direction about the origin of the superior mesenteric artery. The essentials of the permanent disposition of the viscera have been attained. (A to D from Gardner CE Jr, Hart D. Anomalies of intestinal rotation as a cause of intestinal obstruction. *Arch Surg.* 1934;29:942–946. Copyright 1934, American Medical Association.)

has differentiated into the various types of epithelium: the zymogen, endocrine, mucous, and parietal cells. By 16 weeks, all of these cells are secreting their respective cellular products.

Several trophic factors have been shown to be involved in gastric epithelial differentiation. Crosstalk between transforming growth factor- β 1 and basement membrane protein laminins are important mediators of gastric epithelial polarity (Basque et al., 2002). Fibroblast growth factors 10 and 2, proteins in the hedgehog pathway, are strong determinants of gastric epithelial differentiation, in particular with parietal cell differentiation (Spencer-Dene et al., 2006). Investigators have identified and characterized several other key signaling pathways and transcription factors involved in gastric morphogenesis and cellular differentiation (Kim and Shivdisani, 2016).

Liver and Pancreas

The liver and pancreas develop from two different anatomic domains of the definitive endodermal epithelium of the embryonic foregut. Fate-mapping experiments demonstrate that the liver arises from precursor cells in the developing ventral foregut as well as from a small group of endodermal cells tracking down the ventral midline. During foregut closure, the medial and lateral domains come together. After the initial differentiation, several transcription factors shape the developing liver precursor cells into hepatocytes and bile duct cells (Fig. 70.3) (Zaret and Grompe, 2008; Gordillo et al., 2015). The Wnt/ β -catenin pathway is an integral regulator of liver zonation and proliferation, undergoing tight spatiotemporal regulation. This occurs in part through expression of R-spondin (RSPO) ligands binding to LGR4/5 receptors, leading to increased Wnt



• **Fig. 70.2** Hedgehog Signaling in Mammalian Cells. In the absence of hedgehog Hh (A), the receptor Patched (*Ptc*) exerts an inhibitory effect on Smoothened (*Smo*), a transmembrane protein with homology to G protein-coupled receptors. In the presence of Hh ligand-binding (B), the inhibitory action of *Ptc* on *Smo* is released. The full Gli product is now stabilized and transferred to the nucleus. Once in the nucleus, the full Gli product binds to and upregulates transcriptional targets, including *Ptc* and another Hh-binding protein, *HIP*. In this manner, excess Hh is sequestered and the pathway regulated.

signaling through E3 ubiquitin ligases, zinc and ring fingers (ZNRF3, RNF43). Experimentally, recombinant RSPO1 accelerates liver regeneration following partial hepatectomy, sparking promise for new therapies in liver injury (Planas-Paz et al., 2016).

The pancreas is also induced in lateral endoderm domains, adjacent and caudal to the lateral liver domains, and in cells near the dorsal midline of the foregut. In an even more complex process than the liver, differential expression of transcription factors induces pancreatic progenitor cells to become specific endocrine, acinar, or ductal cells (see Fig. 70.3) (Zaret and Grompe, 2008; Jennings et al., 2015). The first transition in organogenesis is defined by proliferation of pancreatic progenitor cells, followed by differentiation, growth, branching, and cell lineage delineation into endocrine and exocrine subtypes. The final transition involves remodeling, apoptosis, neogenesis, and growth to produce a mature organ (Dassaye et al., 2016).

From an organogenesis aspect, rotation and fusion of the dorsal and ventral pancreatic buds occur by 7 weeks' gestation. By 14 weeks, immunoreactive insulin can be detected, and pancreatic zymogen granules are present in the acinar cells. By 16 weeks, amylase is present. Trypsin, lipase, and amylase are secreted into the duodenum by 31 weeks (Zoppi et al., 1972). As mentioned earlier, the liver is derived as an outbudding from the foregut. The cranial portion of the bud differentiates into hepatic parenchyma and the caudal portion into the gallbladder. Specific hepatic lobules and bile canaliculi can be detected by 6 weeks. Bile acids are

present in the liver by 12 weeks and are actively secreted into the small intestine by 22 weeks' gestation.

Midgut and Hindgut

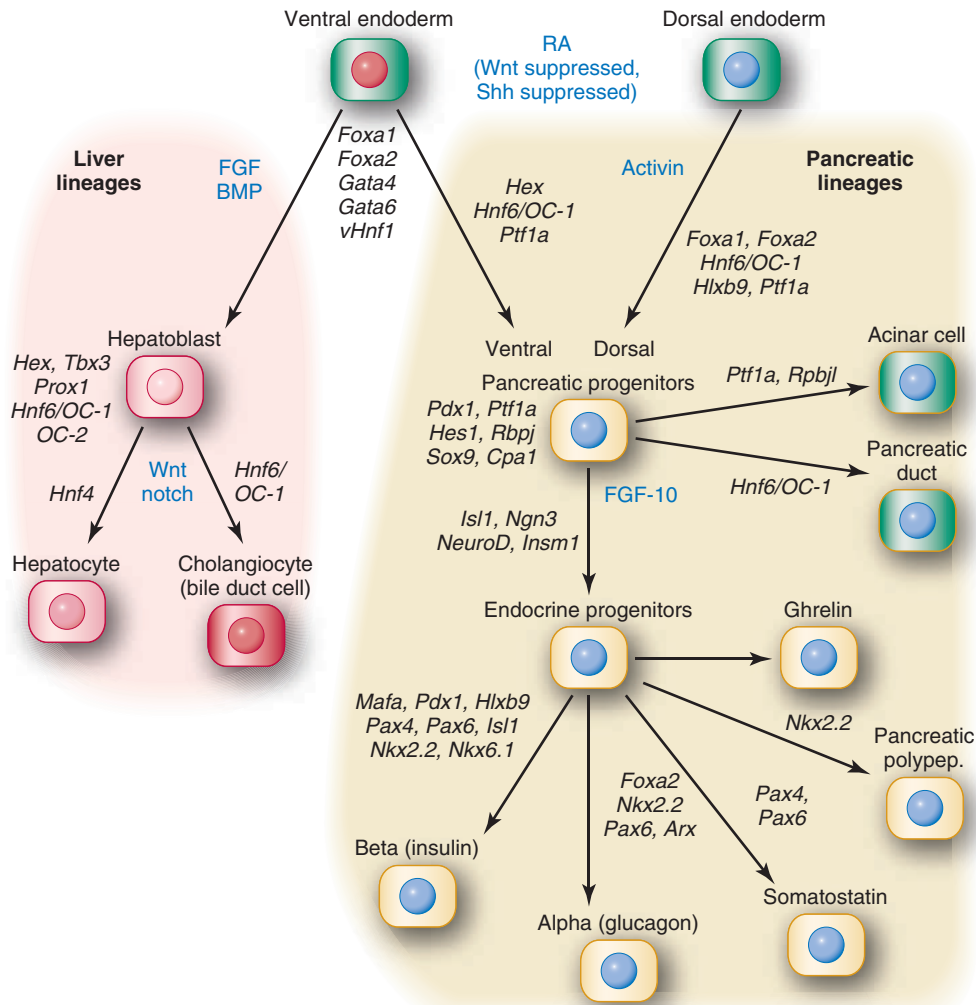
Although portions of the small intestine are derived from the foregut and midgut, and portions of the colon are derived from the midgut and hindgut, we discuss these portions of the GI tract from an end-organ perspective.

Small Intestine

The ultimate digestive function of the small intestine requires intestinal epithelium to secrete digestive enzymes and to provide sufficient surface area to absorb nutrients. Therefore it is important to understand the development of the cellular differentiation as well as overall intestinal length.

As previously mentioned, the hedgehog signaling pathway is also important in endodermal and mesodermal differentiation in small-intestinal development. In addition, the genes *Sox9*, *Sox17*, and *SRY* that encode the corresponding transcription factors have been shown to be essential in endoderm differentiation, whereas the *Hox* family of transcription factor genes are involved in mesodermal differentiation (de Santa Barbara et al., 2003).

After differentiation, the intestinal villous and crypt development is under the control of several growth factors that are secreted in autocrine, paracrine, endocrine, and exocrine pathways (Ménard,



• **Fig. 70.3** Regulatory Factors Controlling Cell Type Lineages Within the Liver and Pancreas. Transcription factor genes involved in differentiation are noted in italics. (From Zaret KS, Grompe M. Generation and regeneration of cells of the liver and pancreas. *Science*. 2008;322:1490–1494.)

2004). Glucagon-like peptide 1 and 2 are secreted by intestinal neurons and L cells, respectively, and are associated with increased intestinal length (Sigalet et al., 2004). Clinical trials in adults are under way administering glucagon-like peptide 2 in patients with short bowel syndrome and may eventually show promise in pediatric patients with intestinal failure. Preclinical experiments show promise that trophic factors, including glucagon-like peptide 2, insulin-like growth factor, and epidermal growth factor, may be beneficial as therapies in neonatal and pediatric short bowel syndrome (Lim et al., 2016). New advances in the generation of intestinal organoids have brought a new dimension to understanding the molecules responsible for intestinal development and in vitro tissue engineering (Clevers et al., 2014).

The small intestine is well developed after its extracorporeal migration into the umbilical cord. Rapid epithelial proliferation occludes the small-intestinal lumen early in development, but the lumen becomes patent at 12 weeks' gestation. There is a gradual development of digestive function that is not fully complete until 34 weeks' gestation, thus posing additional problems regarding the administration of enteral nutrition to premature infants.

Colon

The hindgut is formed by an early dilatation of the fetal cecum at 4 weeks' gestation. By 12 weeks, this primitive structure takes on the gross anatomic features of the colon. At this same time, the midgut rotation is completed, resulting in the cecum being located in the right lower abdominal space. The hallmark of colonic function is coordinated motility, especially in the development of the rectum. The rectum forms by 8 weeks, and formation of complete muscle layers and neural migration of neural crest cells are accomplished by 24 weeks. By 22 weeks, the premature colon maintains some aspects of the small intestine, including villi and disaccharidase function. With developmental maturation, the colonic crypt structures dominate the mucosal surface, and the intestinal characteristics diminish (Raul et al., 1986).

From a cellular developmental aspect, several trophic factors have been linked to colonic differentiation. Elf-3, a member of the Ets transcription factor family, controls intestinal epithelial differentiation during development by regulating the expression of growth factor receptors in epithelial cells (Jedlicka and Gutierrez-Hartmann, 2008). MicroRNAs have been shown to regulate cell

differentiation and maintenance of the pluripotent stem cells (Monzo et al., 2008).

The developing enteric nervous system is derived in a large part from the vagal neural crest cells, with additional contribution from migration of the precursor cells from the sacral region. Molecules that have been shown to control the migration of ganglion cells include the RET/GFRa1/GDNF and endothelin receptor-B/endothelin-3 signaling pathways, transcription factors such as Phox2b, Sox10, Pax3, Mash1, Hox11L1 and Sip1, the hedgehog signaling system, neurotrophins, and bone morphogenetic proteins (Burns and Thapar, 2006; Monzo et al., 2008; McKenna et al., 2010). Defects in these molecules may be related to congenital colonic disease, including Hirschsprung disease.

Mucosal Immune System Development

The GI tract is the largest immune organ in the body and thus warrants review of its development. As with other aspects of GI tract development, the mucosal immune system undergoes both fetal and postnatal changes. The dramatic changes following exposure to food and bacterially derived molecules after birth have been shown to be a major determinant in ongoing mucosal immune modification.

Mucosal immunity is composed of innate and adaptive arms. The innate immune system includes a chemical/noncellular component (e.g., gastric acid, intestinal mucous layer, epithelial barrier function, and defensins), as well as a cellular component (e.g., neutrophils, macrophages, and antigen-presenting cells). The adaptive immune system is made up of T-cell lymphocytes and B-cell-mediated humoral immunity.

Although primarily considered to be involved in digestion, gastric acid, bile salts, and pancreatic secretions function to inhibit potential pathogenic bacterial growth. Clinical investigators have demonstrated that delaying feedings in premature infants results in decreased gastric acid secretion, possibly the mechanism whereby trophic feedings prevent necrotizing enterocolitis (NEC) (Hyman et al., 1983; Berseth et al., 2003). In addition, administering histamine H₂ blockers to premature infants is associated with increased sepsis (Beck-Sague et al., 1994) and NEC (Guillet et al., 2006).

Mucins, glycoproteins, immunoglobulins, glycolipids, trefoil factors, and albumin are all constituents of the luminal mucus layer. Previously, it was believed that mucus functions in a non-specific manner to prevent bacterial adhesion and expel potential pathogens. It is now clear that certain components of the mucus layer are involved in mucosal healing. Specifically, the trefoil factor family has proangiogenic and antiapoptotic qualities, as well as modulating cell-to-cell contacts and potentiating epidermal growth factor (Hoffmann, 2005).

The epithelial barrier function is accomplished by intracellular proteins that anchor the cells to each other, preventing large molecules from penetrating. Recently, it has been shown that these proteins are not just “spot welds” but are dynamic in nature and respond to physiologic and pathologic stimuli (Graham et al., 2009). In addition, dendritic cells, a type of antigen-presenting cell, can sample the microbiota of the intestinal lumen by sending “periscopes” through the tight junctions, suggesting an epithelial-immune cell interaction.

Defensins are antimicrobial proteins secreted by specialized intestinal epithelium called Paneth cells. It appears that defensins are uniformly expressed in the GI tract at 14 weeks’ gestation but are restricted to the small intestine by 17 weeks. The number of

Paneth cells and the expression of defensins in 24-week infants are significantly lower than in term infants and may be associated with increased sepsis and NEC in premature infants (Mallow et al., 1996).

The cellular components of innate immunity include macrophages and granulocytes. Macrophages are present in the fetal intestine as early as week 11 of gestation. A recent study has demonstrated that chemerin is a potent recruiter of intestinal macrophages with peak level production at 20 to 24 weeks (Maheshwari et al., 2009).

Previously, the cells of the innate immune system were believed to act in a nonspecific manner in response to pathogenic stimuli. It is now clear that specific bacterial components signal through membrane-associated receptors termed toll-like receptors (TLRs). For example, lipopolysaccharide from gram-negative bacteria is the ligand for TLR-4, which results in a downstream inflammatory response. TLRs are expressed by both immune cells and GI epithelial cells. TLR4 is expressed on fetal enterocytes as early as 18 weeks’ gestation (Fusunyan et al., 2001) and may be induced following birth with the acquisition of the intestinal microbiota.

The adaptive immune system of host defense is composed of T cells and humoral immunity. Lymphocytes have been shown to proliferate in response to mitogenic stimuli at 12 weeks’ gestation. Peyer patches are lymphoid tissue that process lumen antigens. M cells are differentiated intestinal epithelia that function to process antigens to lymphocytes and other cells in Peyer patches and can be observed at 17 weeks’ gestation. Peyer patches are essential for the production of plasma cells that secrete mucosal associated immunoglobulins.

Once naive T cells interact with antigen, they develop into specific phenotypes based on their cytokine production. Currently, T-cell subsets include T-helper (Th) cell 1, Th2, Th17 (producing IL-17), and regulator T cells (Weaver et al., 2006). In the sterile in utero environment, T cells are predominantly Th2, promoting a symbiosis between fetus and mother. On presentation with antigen, there is a switch to the more mature cells. The gestational age at which this T-cell commitment can occur is unknown and is a fertile area for future research.

Gastrointestinal Microbiota

It is unclear when microbes begin to colonize the human gut, but there is growing evidence that it occurs earlier than previously appreciated. Newborns born vaginally have a different GI microbiota from newborns born by cesarean section, with microbial sequencing on full-term newborns within the first few days of life showing an enrichment of *Bacteroides*, *Bifidobacterium*, *Parabacteroides*, and *Escherichia/Shigella* species in vaginally born newborns. This is in contrast to the *Enterobacter*, *Haemophilus*, *Staphylococcus*, *Streptococcus*, and *Veillonella* species found in cesarean-born newborns (Bäckhed et al., 2015). These differences appear to be related to the initial exposure to the skin and the environment of the surgical field or the vagina, as vaginal flora bacteria can be partially restored by exposing cesarean-born newborns to vaginal secretions (Dominguez-Bello et al., 2016). However, data showing that premature newborns have different microbiota from full-term newborns suggest that the gut may not be sterile before birth. Gram-positive and gram-negative bacteria reside within the basal plate of the placenta, with the placental microbiome having a taxonomic profile similar to the adult oral microbiome (Aagaard et al., 2014).

GI microbiota play important roles in intestinal development, health, and disease. In mice, intestinal microbiota affect villus

height, crypt depth, epithelial cell proliferation, numbers of goblet and Paneth cells, and tight junctions (Yu et al., 2016). Microbiota are involved in the early assembly of intestinal neural circuits and modulate neurotransmitter release via TLR ligands and generation of short-chain fatty acids (Obata et al., 2016). Through luminal antigen sampling, intestinal microbes regulate the immune system. Commensal organisms stimulate the generation of tolerogenic macrophages and dendritic cells, with subsequent generation of regulatory T cells, thereby creating an antiinflammatory environment (Maranduba et al., 2015). The creation of both a local and systemic proinflammatory immune state has been implicated in a growing number of autoimmune diseases, including but not limited to inflammatory bowel disease (Myoshi et al., 2016), type 1 diabetes (Needell et al., 2016), and psoriasis (Zakostelska et al., 2016).

Digestive Physiology

The human GI tract functions to digest and absorb nutrients consumed in food. Nutrient digestion consists of breaking down carbohydrates, proteins, and fats to smaller component molecules (monosaccharides, oligopeptides, and amino acids and free fatty acids and monoglycerides) that can be transported into absorptive intestinal epithelial cells and then into the portal circulation. Human digestive function matures throughout embryogenesis as the GI organs develop and acquire different digestive capacities.

Carbohydrate Digestion

Carbohydrates supply approximately 40% of ingested calories in term newborns. Dietary carbohydrates consist of sugars and starches. Lactose is the predominant sugar in human breast milk and most milk-based infant formulas. Before absorption of its constituent glucose and galactose monosaccharides, the lactose disaccharide must be hydrolyzed by the enzymatic action of the intestinal lactase enzyme. Lactase is a membrane-bound protein present on the apical surface of enterocytes, the intestinal absorptive cells. During early fetal life, the lactase gene is expressed in the colon and small intestine. By term, however, a spatial gradient of lactase gene expression is established, with peak expression in the proximal intestine (Raul et al., 1986). Lactase activity increases during fetal maturation, with the greatest increase, about fourfold, occurring during the third trimester (Weaver et al., 1986). In the absence of sufficient lactase hydrolysis, undigested lactose can result in an osmotic diarrhea. Although preterm infants have relatively low levels of lactase activity, they are often able to sustain normal growth with little diarrhea when fed breast milk or lactose-containing formula (MacLean and Fink, 1980). Some formulas also contain complex carbohydrates in the form of glucose polymers. Glucose polymers require amylase for hydrolysis. The process of complex carbohydrate digestion is initiated in the lumen of the GI tract by the action of alpha-amylases secreted by the salivary gland and the pancreas. Because pancreatic amylase levels are low in neonates, salivary amylase supports a significant amount of glucose polymer digestion along with the mucosal glucoamylase enzyme (Hodge et al., 1983). Glucoamylase is a membrane-bound hydrolase synthesized by the enterocyte that removes single glucose residues from alpha (1–4) chains of glucose polymers. Glucoamylase activity is detectable as early as 13 weeks' gestation and increases twofold to threefold in the near-term newborn (Ménard, 1994).

The monosaccharide products of luminal and membrane-bound hydrolysis of carbohydrates are transported across both the apical and basolateral enterocyte membranes and into the portal circulation. Active glucose and galactose transport into enterocytes are

carried out predominantly by the apical sodium-glucose cotransport protein, SGLT1. Glucose and galactose are transported out of the enterocyte by the basolateral GLUT2 transport protein. Congenital defects of the SGLT1 protein result in rare cases of glucose–galactose malabsorption characterized by severe, watery, acidic diarrhea in the newborn period (Martín et al., 1996). Treatment consists of a glucose-free and galactose-free diet, initially given as a fructose-based formula.

Protein Digestion

Proteins contribute less than 10% of ingested calories in infants. Many of the amino acids used for protein synthesis are produced within the body. Essential amino acids, however, must be provided through dietary protein. Similar to digestion of complex carbohydrates, protein digestion involves both luminal and mucosal enzymatic hydrolysis. Proteins are initially digested within the intestinal lumen by proteases secreted by the stomach (pepsin) and pancreas (trypsin, chymotrypsin, carboxypeptidase, and elastase). The final products of luminal digestion are amino acids and oligopeptides composed of two to six amino acid residues. Digestion of oligopeptides greater than two residues is performed by mucosal membrane-bound brush border peptidases. Bipeptides and tripeptides can be transported through the enterocyte membrane via the proton-coupled PEPT1 transporter and may be further hydrolyzed by cytosolic peptidases that exist within the cell or transported out of the cell and into the portal system as peptides (Liang et al., 1995). Amino acids are transported into enterocytes by a great variety of amino acid transporters. The activities of most of the brush border and cytosolic peptidases are well developed in the preterm infant. There is evidence, however, that some milk proteins may be absorbed intact into the circulation (Kuitunen et al., 1994).

Fat Digestion

Fat provides 40%–50% of the caloric intake of the newborn. Similar to carbohydrate and protein digestion, fat processing is initiated in the lumen before mucosal absorption. By means of enzymatic action, lingual, gastric, and pancreatic lipases function to hydrolyze fat to its constituent free fatty acids and monoglycerides. Pancreatic lipases are in relatively low concentration at birth. Lingual lipases and gastric lipases, however, are present by 26 weeks' gestation and thus contribute significantly to neonatal fat digestion (Hamosh et al., 1981). In addition, a bile-salt-dependent lipase present in human milk is capable of hydrolyzing fats (Freed et al., 1987). Bile salts secreted by the liver and gallbladder are necessary for efficient absorption of fats and function to emulsify fat in the intestinal lumen. Bile flow to the intestine increases rapidly after birth and initiation of feeding. After luminal hydrolysis of fat, mixed micelles of fatty acids and monoglycerides diffuse directly across the cell membrane of mucosal intestinal cells.

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Structural Anomalies of the Gastrointestinal Tract

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KEY POINTS

- Small inclusion cysts in the oral cavity are common in newborns and almost always resolve without treatment.
- Suspect the diagnosis of esophageal atresia when there is feeding difficulty with an inability to pass a tube from the nose (or mouth) into the stomach.
- In the most common type of esophageal atresia, there is a fistula from the trachea to distal esophagus (tracheoesophageal fistula [TEF]) that accounts for the air in the gastrointestinal tract (GI) tract seen on radiographs. When no air is seen it suggests that there is only esophageal atresia and no TEF.
- The most important risk factors for mortality in esophageal atresia anomalies are associated prematurity and major congenital heart disease.
- Infants with pyloric stenosis present with nonbilious vomiting of feedings, intravascular volume depletion, and hypochloremic, hypokalemic metabolic alkalosis in the first few weeks of life.
- Bilious vomiting in newborns indicates mechanical obstruction of the GI tract and, specifically, malrotation with midgut volvulus until proven otherwise.
- Suspected midgut volvulus requires urgent surgical evaluation. It is best to make the diagnosis of volvulus when there are signs of intestinal obstruction and not to wait for signs of intestinal ischemia.
- If there is a concern that there may be intestinal malrotation and midgut volvulus, then an urgent upper GI contrast study should be done to evaluate the position of the ligament of Treitz (the transition point between duodenum and jejunum).
- Failure to pass meconium in the first day of life should raise concerns about distal bowel obstruction.
- The way to rule out the diagnosis of Hirschsprung disease in a newborn is by suction rectal biopsy.

This chapter will provide an overview of structural anomalies of the gastrointestinal (GI) tract seen in newborns. Most of the conditions are congenital; however, some are acquired. Although there are an almost limitless number and variety of structural anomalies, we will concentrate on those that are relatively common and those that have important clinical neonatal presentations. The topics will be discussed in anatomic progression, starting from the mouth and ending at the anus.

Several important conditions of the GI tract and abdomen such as biliary atresia, necrotizing enterocolitis (NEC), abdominal wall defects, and abdominal tumors are addressed in other chapters.

Disorders of the Oral Cavity

This section reviews disorders of the epithelial surfaces of the mouth, including cysts and tumors, and disorders of the tongue and salivary glands.

Mouth

Newborns commonly have transient inclusion cysts in the mouth that may be classified into three types: Epstein pearls, Bohn nodules, and dental lamina cysts (Singh et al., 2012; Cizmeci et al., 2014). Epstein pearls are small keratin-filled nodules found in the midline of the palate. They are thought to be epithelial inclusion cysts located at the developmental fusion line of the palate (Singh et al., 2012). Bohn nodules are usually found along the alveolar (dental) ridges and are remnants of developing salivary glands (Singh et al., 2012). Dental lamina cysts are small, raised, white papules in the midline of the palate or on the alveolar (dental) ridges. These are inclusion cysts of the dental lamina, the keratin-producing ectoderm of tooth development. Dental lamina cysts may be isolated or may occur in clusters. They are very common in term newborns with the palatal subtype having a prevalence as high as 65%, and the alveolar subtype having a prevalence of 25%–53%. Although at times concerning in appearance, all three types of oral inclusion cysts are benign and usually asymptomatic. Their natural history is to spontaneously rupture and fuse with the oral epithelium within 5 months of birth, thus conservative management is appropriate and treatment rarely necessary (Singh et al., 2012). Oral inclusion cysts may sometimes be confused with an oral cavity tumor such as a small congenital epulis or with the eruption of a neonatal tooth.

Oral cavity tumors are rare in newborns. The two most common oral cavity tumors are the granular cell tumor and the oropharyngeal teratoma. A granular cell tumor is also known as a *congenital epulis* (“epulis” is a name for any gingival growth). It is a benign tumor that presents as a smooth, red, or pink mass arising from the alveolar (dental) ridge (Kumar et al., 2015).

An oral cavity teratoma arises from the upper jaw or palate and is also known as an *epignathus*. *Teratomas* are tumors that arise from fetal germ cells during development and consist of cells and tissues derived from all three germ cell layers (ectoderm, mesoderm, and endoderm). In the newborn, they are commonly found in the sacrococcygeal region but may occur in the gonads or other midline, or near-midline, locations. Symptoms are typically caused by compression of normal structures, although malignant degeneration may occur.

Larger oral cavity teratomas may be seen on prenatal imaging, but granular cell tumors are typically smaller and discovered on physical examination after birth (Kim et al., 2008). Granular cell tumors and oral cavity teratomas vary in size and, when large, their mass effect may cause feeding difficulty and even upper airway obstruction (Kumar and Sharma, 2008; Kumar et al., 2015). Most oral cavity tumors require surgical resection. The timing of surgery depends upon the severity or potential severity of the symptoms (Kumar and Sharma, 2008; Kumar et al., 2015).

Tongue

The tongue is critical for the important newborn activities of sucking and swallowing, so congenital disorders such as aglossia, ankyloglossia, and macroglossia are usually noted early in life. *Aglossia*, or congenital absence of part or all of the tongue, is a very rare condition caused by failure of tongue embryogenesis in weeks 4 to 8 of gestation. It is usually associated with craniofacial and limb anomalies (Bommarito and Zanato, 2016).

Ankyloglossia is commonly known as *tongue-tie*. By far the most common form of tongue-tie is *ankyloglossia inferior*, which is an abnormally short, thickened, and/or tight inferior frenulum that limits tongue mobility and can interfere with swallowing and speech (Chinnadurai et al., 2015; Francis et al., 2015). Most newborns with ankyloglossia inferior have no difficulty breastfeeding. However, if they do have difficulty with breastfeeding then lactation evaluation and counseling are the next steps, as most will improve without intervention. If feeding difficulties persist then frenotomy, which is the surgical division of the frenulum, may improve feeding. Ankyloglossia inferior may also impact the articulation of speech, but it does not delay overall speech and language development (Chinnadurai et al., 2015). *Ankyloglossia superior* is a rare but more serious anomaly that refers to a fibrous or osseous connection between the tongue and the hard palate. Ankyloglossia superior is associated with other significant anomalies, such as cleft palate, microglossia, micrognathia, GI malformations, and deformed limbs (Bolling et al., 2007; Wieker and Sieg, 2014). Ankyloglossia superior can cause difficulty in feeding and even airway obstruction, making early recognition and treatment necessary (Bolling et al., 2007).

Macroglossia is defined as enlargement of the tongue and may be classified as “true macroglossia” or “pseudomacroglossia” (Perkins, 2009; Klosterman and Tatum, 2015). True macroglossia can be due to muscle hypertrophy of the tongue, which is usually the result of genetic abnormality, such as Beckwith–Weidemann syndrome (Perkins, 2009). Tissue infiltration from amyloidosis, infection, hemangiomas/lymphangiomas, or systemic diseases such as hypothyroidism and diabetes can also lead to macroglossia (Perkins, 2009; Klosterman and Tatum, 2015). *Pseudomacroglossia* occurs when the tongue appears large because of a relatively small oral cavity such as in babies with mandibular hypoplasia (micrognathia) or when the tongue is displaced anteriorly (Klosterman and Tatum, 2015). Surgical treatment is needed if there is airway compromise, dysphagia, or dysarthria. Operations for macroglossia

aim to reduce the size of the tongue and preserve its function (Perkins, 2009; Klosterman and Tatum, 2015).

Salivary Glands

The salivary glands (the parotid, submandibular, and sublingual glands) may be the source of several newborn problems. A *ranula* is a mucus-filled cyst arising from the sublingual gland. In newborns, a ranula is caused by congenital obstruction of a sublingual gland duct (George et al., 2015). Ranulas that are entirely intraoral are known as *simple ranulas*, while those that extend through the floor of the mouth beyond the mylohyoid muscle are known as *plunging ranulas* and present as a submandibular or neck mass (Clyburn et al., 2009). A ranula may be suspected on physical examination, and diagnosis can be confirmed by ultrasound, computed tomography (CT), or magnetic resonance imaging (MRI). If physical examination and imaging are not definitive then aspiration of the cyst showing yellow fluid containing amylase and mucin confirms the diagnosis (Zhi et al., 2014). Ranulas may spontaneously resolve and thus may be observed for up to 6 months before excision or sclerotherapy (Zhi et al., 2014).

Neonatal suppurative sialadenitis and *parotitis* are rare infections of the salivary and parotid glands, respectively, but should be considered in any newborns presenting with unilateral inflammatory submandibular or parotid swelling (Weibel et al., 2005; Ismail et al., 2013; Ryan and Padmakumar, 2015). Bacterial contamination of the glands is from the oral cavity via the draining ducts or from hematologic spread from transient bacteremia (Spiegel et al., 2004; Miranda and Pereira, 2010; Ismail et al., 2013). Risk factors for these infections include prematurity, prolonged nasogastric feeding, mechanical ventilation, dehydration, and maternal mastitis (Ryan and Padmakumar, 2015). *Staphylococcus aureus* is the most common causative organism, though *Streptococcus* species, *Escherichia coli*, and *Haemophilus influenzae* have also been reported. Treatment with broad-spectrum intravenous antibiotics is recommended, including an antistaphylococcal β -lactam and an aminoglycoside or third-generation cephalosporin (Weibel et al., 2005; Ismail et al., 2013; Ryan and Padmakumar, 2015).

Parotid hemangiomas are the most common salivary gland tumors in newborns. They present as a rapidly growing mass in the preauricular area (Weiss et al., 2011). They may be segmental, involving structures in the distribution of the V3 (mandibular) branch of the trigeminal nerve, including the parotid gland, the overlying skin, and the airway. Alternatively, they may be focal, located within and involving only the parotid gland (Weiss et al., 2011). Hemangiomas traditionally have a rapid growth phase in the first months of life followed by spontaneous involution over the next decade. Diagnosis is by physical examination and is confirmed by imaging with Doppler ultrasound and sometimes MRI (Kollipara et al., 2013). Observation alone is appropriate for small, nondisfiguring lesions with no systemic manifestations such as congestive heart failure. Treatment options include intralesional corticosteroids, systemic nonselective β -blocker (i.e., propranolol), endovascular sclerotherapy, and surgical resection with intraoperative facial nerve mapping (Weiss et al., 2011).

Disorders of the Neck

Although most disorders of the neck are not structural anomalies of the GI tract, abnormalities of the developing oropharynx, including branchial cleft and thyroid anomalies, may present as neck masses. The branchial cleft anomalies usually present as lateral

neck masses and thyroid anomalies usually present as midline neck masses. We will consider these topics and other lateral and midline neonatal neck masses briefly before covering disorders of the esophagus in the next section.

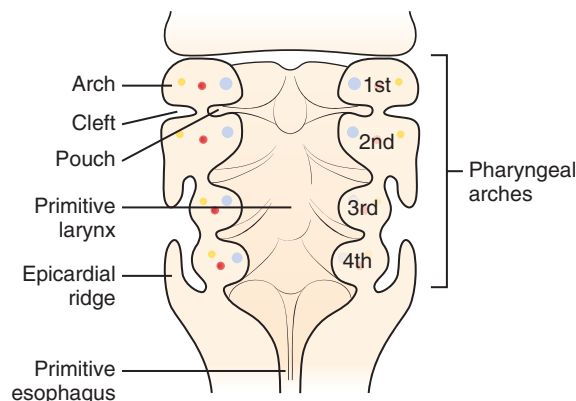
Branchial Anomalies

During early development the flat, trilaminar embryonic disc forms into a cylindrical body, and in the head, neck, and upper chest this process involves the pharyngeal or branchial (Greek for *gills*) apparatus. (*Branchial* is the term most commonly used in clinical medicine to describe these developmental structures so we will use it here.) The branchial apparatus consists of a series of arches that fold and meet in what will be the ventral midline. The arches contain mesoderm and are separated by indentations that on the outer ectodermal surface are called *clefts* and on the inner endodermal surface are called *pouches*. The arches and separating clefts and pouches are numbered based on their cranial to caudal embryonic positioning (Prosser and Myer, 2015) (Fig. 71.1).

Branchial cleft anomalies arise from incomplete obliteration of the embryonic clefts and pouches and result in an epithelial-lined cyst, sinus, or fistula. A cyst has no direct connection from its epithelial inner lining to the normal epithelium of the skin or pharynx. In contrast, a sinus is an abnormal epithelial extension from either the skin or pharynx while a fistula is an abnormal epithelial connection between skin and pharynx. Most branchial cleft anomalies are cysts, and 95% of the cysts arise from the second branchial cleft. Although they are congenital anomalies present since birth, they often present in older children and adolescents or even adults. They usually present as neck masses on the anterior border of the sternocleidomastoid muscle (Prosser and Myer, 2015). These neck masses may be asymptomatic, or they may produce symptoms because of mass effect or infection. Diagnosis of the cyst is made by the characteristic position on physical examination and may be supported by ultrasound, CT, or MRI appearance. A true fistula may be demonstrated by contrast esophagram (Prosser and Myer, 2015; Adams et al., 2016). Treatment of branchial cleft cysts includes initial treatment of any complicating infection and then complete surgical excision when the infection has resolved.

Thyroid

The thyroid may be the source of a neck mass when remnants of its embryologic origin persist and enlarge, when the gland is in an abnormal location, or when it is enlarged in the normal location.



• Fig. 71.1 Branchial Cleft Anomalies.

During weeks 5 to 7 of gestation the rudimentary thyroid migrates from the foramen cecum (i.e., the base of the tongue) to its final position in the lower neck anterior to the trachea (Guerra et al., 2014). At the onset of normal migration the endoderm of the primitive pharynx invaginates and forms the thyroglossal duct with the developing thyroid gland at its inferior aspect. The duct descends to the lower neck and is usually obliterated by week 10 of gestation. Incomplete obliteration of the duct may lead to a thyroglossal duct cyst that presents as a midline neck mass between the hyoid bone and thyroid gland. Thyroglossal duct cysts have a propensity to become infected, and surgical excision is indicated (Simon and Magit, 2012; Eriksi and Hosgor, 2014).

Simple excision of a thyroglossal duct cyst results in a high rate of recurrence, presumably because of small residual bits of thyroglossal duct tissue not in continuity with the main cyst. Therefore the operation of choice is the *Sistrunk procedure*: an en bloc excision of the cyst and its tract, including the middle part of the hyoid bone. Previous infections increase the risk for cyst recurrence after surgery (Simon and Magit, 2012).

Disordered thyroid development may result in abnormally located or ectopic thyroid. In general, functional thyroid tissue not located anterior to the second, third, or fourth tracheal rings is considered to be ectopic. Ectopic thyroid tissue can be found anywhere between the foramen cecum and the mediastinum (Santangelo et al., 2016). The most common location for ectopic thyroid is the tongue. Lingual thyroid results from the failure of normal migration of the thyroid during development and presents as a mass in the posterior midline of the tongue. Most patients with a lingual thyroid do not have additional thyroid tissue in the normal position. A large lingual thyroid may cause symptoms of airway obstruction.

A diffusely enlarged, nontender thyroid gland at birth is known as a *congenital goiter*. It may be associated with hyperthyroidism, hypothyroidism, or a euthyroid state. The diagnosis of congenital goiter can be confirmed with ultrasound. Thyroid function should be assessed to determine the need for thyroid hormone replacement.

Other Neck Masses

Neck masses are often distinguished by their location. As noted, the branchial anomalies usually present as lateral neck masses. The differential for lateral neck masses presenting in the first few weeks of life also includes cystic hygromas and those associated with torticollis.

The “sternocleidomastoid pseudotumor of infancy,” or the neck mass associated with congenital muscular torticollis, is a firm, nontender thickening in the lower half of the sternocleidomastoid muscle. The mass is associated with tightness and shortening of the sternocleidomastoid muscle that leads to a characteristic positioning of the patient’s head—the face turned away from the side of the lesion with the chin tilted up. Range of motion is reduced. The etiology is unknown, but it is associated with difficult deliveries. The condition is more common in males, more frequent on the right, and generally presents in the first few weeks of life (Skelton and Howlett, 2014). Diagnosis is by characteristic examination findings. Treatment is directed at improving the range of motion and posture by physical therapy. Surgery is not usually required.

Cystic hygromas are congenital lymphatic malformations. Lymphatic malformations may be defined by the size of the cystic spaces (<2 cm is microcystic, >2 cm is macrocystic), and location

is based on the de Serres staging system or the Mulliken/McGill system (Adams et al., 2012). Cystic hygromas in the neck are classically macrocystic and located in the posterior triangle, more commonly on the left side. They are present at birth and typically grow with the child but may enlarge suddenly with internal hemorrhage or infection. Treatment is by surgical excision or sclerotherapy. A systematic review in 2012 did not find conclusive evidence to support superiority of one treatment modality over the other (Adams et al., 2012).

In addition to thyroid anomalies, midline neck masses in newborns include dermoid cysts, teratomas, and, rarely, an undescended thymus. Dermoid cysts are subcutaneous cysts lined by keratinized, stratified, squamous epithelium with appendages such as hair follicles, sweat glands, and sebaceous glands (Bloom et al., 2002). They commonly occur on the face and scalp at lines of embryonic fusion and can also occur in the midline neck near the hyoid bone and be confused with thyroglossal duct cysts. They are prone to infection and should be excised. Imaging is not needed for straightforward cases. Teratomas in the neck are often found in the midline and are biologically the same tumor as oropharyngeal teratomas, described in the earlier section. They usually originate near the larynx and trachea and may cause airway obstruction. Almost all midline neck masses require surgical resection.

Disorders of the Esophagus

Newborns with disorders of the esophagus are challenging patients to manage, particularly if they have associated prematurity or congenital heart disease. This section will focus on esophageal atresia and its common variants and then briefly review related congenital conditions such as laryngotracheal cleft, esophageal stenosis, esophageal duplication cyst, and acquired esophageal perforation.

Esophageal Atresia

Esophageal atresia is the most common congenital anomaly of the esophagus and exists when the esophagus is congenitally separated into pieces that are not in continuity. Esophageal atresia is often associated with a tracheoesophageal fistula (TEF).

Epidemiology

The incidence of esophageal atresia is estimated at approximately 1 in 2500 to 1 in 4500 live births with some variation between regions of the world (Kyyronen and Hemminki, 1988; Pedersen et al., 2012). A population-based study from Italy carried out between 1981 and 2012 found 407 cases in 1,417,724 live births for an incidence of 1 in 3500 (Cassina et al., 2016). Esophageal atresia is usually a sporadic disorder, and the recurrence risk for a future sibling is less than 1% (Choinitzki et al., 2013).

Etiology and Associated Anomalies

The basic embryology of the foregut and the variants of esophageal atresia and TEF are not understood completely (Merei and Hutson, 2002; Metzger et al., 2011). Esophageal atresia may be associated with genetic syndromes such as Down syndrome, Edwards syndrome, CHARGE syndrome (coloboma of the eye, heart defects, atresia of the choanae, retardation of growth and development, and ear abnormalities and deafness), Feingold syndrome, and Fanconi anemia (Felix et al., 2007). However, rather than any specific syndrome, esophageal atresia is most often accompanied by additional anomalies in the absence of a known genetic defect

or syndrome. These additional anomalies are commonly in the distribution of VACTERL association (Vertebral defects, Anorectal malformations, Cardiac defects, TracheoEsophageal anomalies, Renal anomalies and Limb abnormalities) (Solomon, 2011). Among 2689 children with esophageal atresia in the United States, 59.1% were found to have associated cardiac defects (most commonly atrial septal defect 46.6% and ventricular septal defect 21.2%). Vertebral, spine, or rib defects were seen in 25.4% of children. Additionally, renal anomalies were associated in 21.8% of children, anorectal malformations in 11.6%, and limb deformities in 6.4%. Approximately one-third of patients had three defects including esophageal atresia, thus qualifying them for a formal VACTERL diagnosis. Duodenal atresia, while not part of VACTERL, was also seen in 4.7% of children with esophageal atresia included in the study (Lautz et al., 2015).

Classification

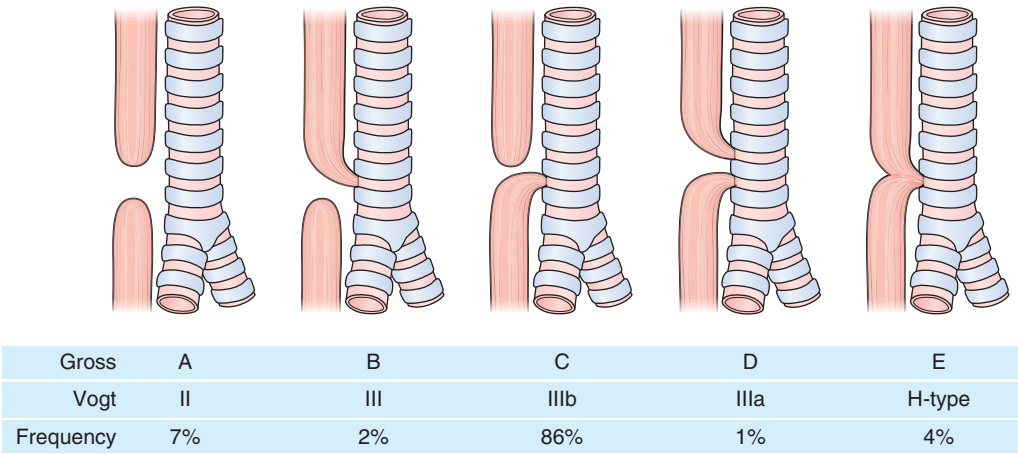
Esophageal atresias with tracheoesophageal malformations occur as a spectrum of anomalies. Several classification systems have been proposed to name the various arrangements (Vogt, 1929; Gross, 1953); however, in a clinical setting it is best to simply describe the anatomic abnormalities. The most important variants are seen in Fig. 71.2.

Diagnosis

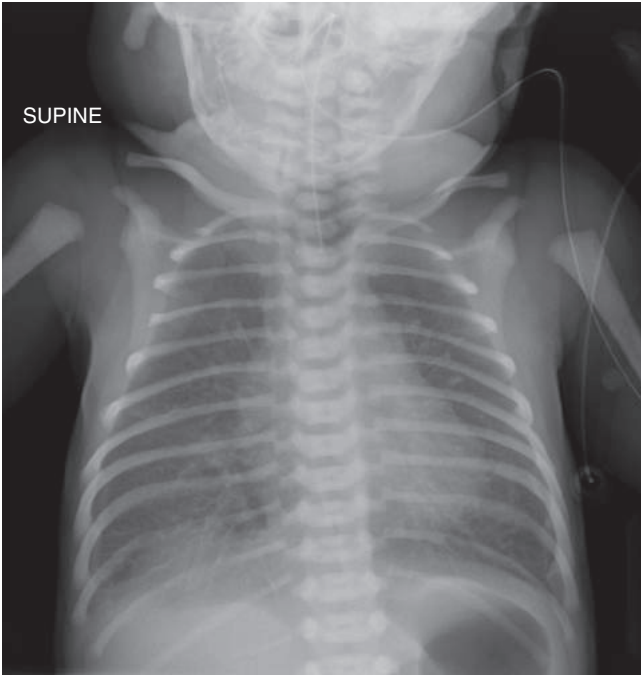
Although the diagnosis of esophageal atresia may be suspected prenatally, the diagnosis is usually made after birth. Prenatal ultrasound showing polyhydramnios and an abnormally small stomach may suggest esophageal atresia; however, both findings are nonspecific (Stringer et al., 1995). If, in addition to polyhydramnios and a small stomach, a dilated esophagus is seen in the neck, a diagnosis of esophageal atresia is more likely (Shulman et al., 2002). Fetal MRI should be considered if prenatal ultrasound suggests esophageal atresia (Langer et al., 2001). Prenatal diagnosis allows opportunity to counsel parents about the diagnosis, postnatal management, and prognosis.

When a neonate is suspected to have esophageal atresia, because of prenatal findings or postnatal clinical symptoms such as excessive drooling, choking, or coughing with feeding, the best way to rule out the diagnosis is to pass a tube into the stomach. A nasogastric (NG) tube that does not pass easily into the stomach, and by X-ray stops or coils in the mid to upper thorax at the 2nd to 4th vertebral body level, is diagnostic of esophageal atresia (Fig. 71.3). A lateral film may demonstrate the tube posterior to the airway, and an air-filled TEF may sometimes be seen. A formal contrast study to outline the pouch is rarely necessary and carries a risk of aspiration.

When esophageal atresia is found, the presence of a distal TEF is confirmed by air in the stomach and intestinal tract (see Fig. 71.3). Absence of air in the abdomen indicates a pure esophageal atresia or an esophageal atresia with an isolated upper pouch TEF (Fig. 71.4). The diagnosis of an isolated TEF without esophageal atresia (i.e., H-type) is often not made at birth because presentation may be subtle. Symptoms can include choking and coughing with feeds and recurrent respiratory infections (caused by aspiration). The abdomen may also appear distended because of excessive air entering the GI tract from the fistula (Karnak et al., 1997). Since the esophagus is intact, a tube will pass normally into the stomach. The diagnosis may be made by bronchoscopy (Fig. 71.5) or specialized imaging studies such as a “pull-back esophagram” (Fig. 71.6) in which contrast is injected as the NG is slowly withdrawn from the stomach to better demonstrate the fistula (Karnak et al., 1997).



• **Fig. 71.2** Esophageal Atresia With and Without Tracheoesophageal Fistula, Classified by Vogt and Gross Classification and Associated Frequency (Vogt, 1929; Gross, 1953; Spitz, 2007).



• **Fig. 71.3** Radiograph of Esophageal Atresia With Distal Tracheoesophageal Fistula.



• **Fig. 71.4** Radiograph of Pure Esophageal Atresia Without Fistula.

Preoperative Management

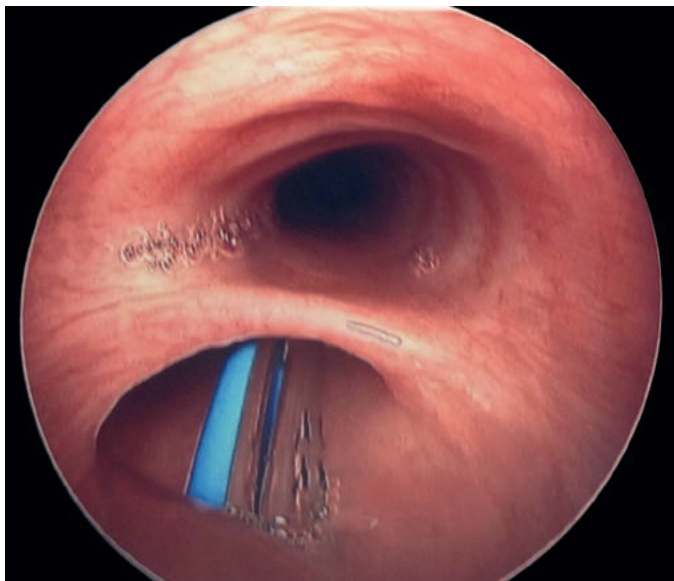
When esophageal atresia is diagnosed or suspected, the infant should be promptly evaluated by a pediatric surgeon. In the most common situation, when a TEF to the distal esophagus is present, respiratory distress may result from aspiration of contents of the proximal pouch, aspiration of gastric contents into the trachea, loss of ventilation through the TEF, and compression of the lungs by a distended abdomen. Therefore positive pressure ventilation should be avoided if possible, the patient positioned with the head elevated, and the proximal pouch contents evacuated with an NG tube.

Most commonly, the infant is stable, and there is time before surgery to confirm the diagnosis and to evaluate for associated VACTERL anomalies. The most important investigation is an echocardiogram looking for cardiac defects or great vessel anomalies,

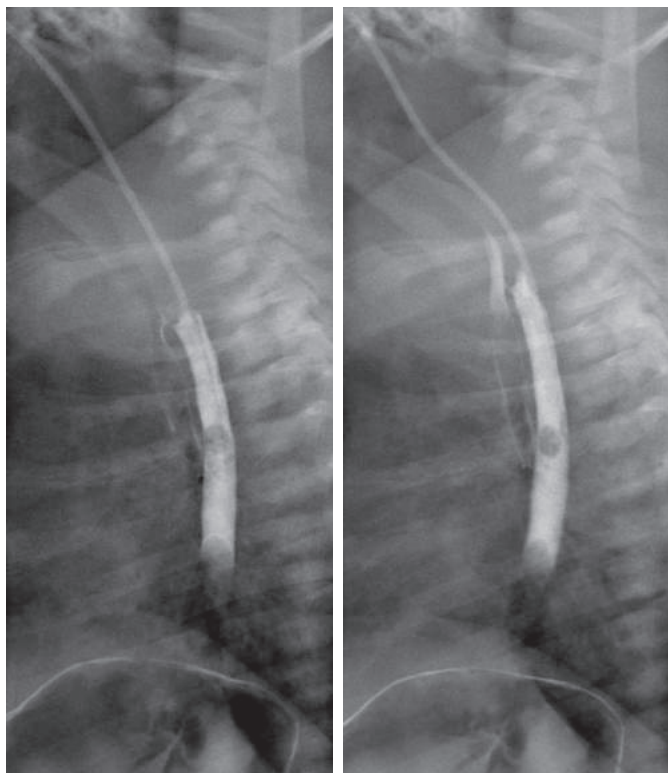
which are present in approximately 18% of cases (Berthet et al., 2015) and can influence the surgical approach. The remainder of the VACTERL work-up including renal ultrasound and spinal imaging may be performed after the operation. Occasionally, inability to ventilate the infant forces an urgent operation to ligate the fistula or decompress the abdomen.

Operation

The primary goal of the initial operation is to divide and close the TEF. Simple ligation (tying off) of the TEF is not recommended because of the high risk of recurrence. Once the TEF is divided an end-to-end esophageal anastomosis should be performed. In unstable or premature infants, it is often safer to perform a staged



• **Fig. 71.5** Bronchoscopy of H-type Tracheoesophageal Fistula.



• **Fig. 71.6** Pull-back Esophagram.

repair by performing the esophageal anastomosis at a later date, after the patient has stabilized and grown (Petrosyan et al., 2009). A long distance between the proximal and distal esophageal segments may also preclude anastomosis at the initial operation. If no anastomosis is performed, a gastrostomy tube should be placed at the initial operation for feeding access until the atresia can be repaired. A chest tube is often placed to evacuate any leakage from the esophageal anastomosis.

Postoperative Management

The infant should be extubated as soon as safely possible to reduce the risk of endotracheal tube and positive pressure trauma to the tracheal suture line. Similarly, deep suctioning should not be done, to avoid injury to the tracheal and esophageal repairs. Likewise, reintubation, if needed, should be performed with *extreme* care. Enteral feedings may be given by gastrostomy or by an NG tube placed across the anastomosis during surgery, or they may be delayed until the anastomosis is judged to be healed and ready for oral feeding or tube placement. We usually perform a contrast study of the esophagus 7 days postoperatively and if the anastomosis has healed, remove the chest tube, although this is not uniform practice (Yanchar et al., 2001).

Outcomes

Overall survival is reported to be 93%, with low birth weight and the presence of major cardiac disease the most important predictors of mortality (Spitz et al., 1994). Ongoing improvements in neonatal critical care have led to greatly improved outcomes in even the smallest premature infants (Lopez et al., 2006; Malakounides et al., 2015).

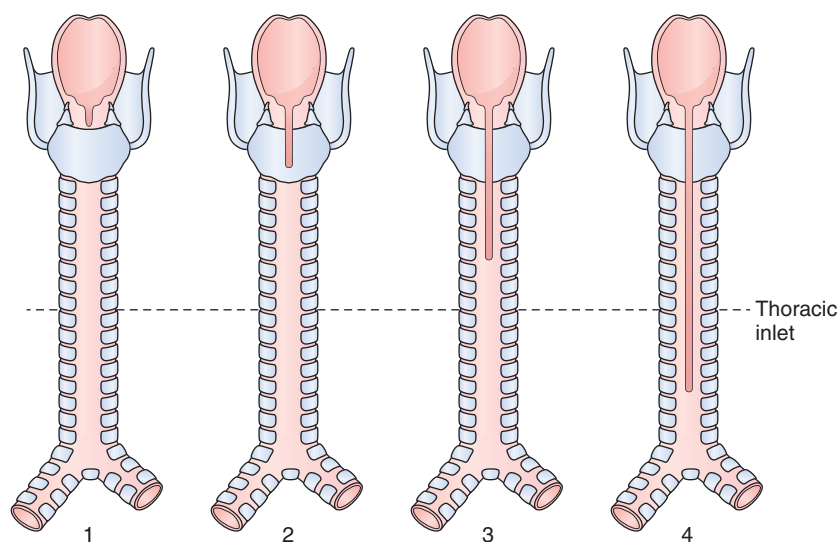
Early complications after repair of esophageal atresia include anastomotic leak and stricture. Leaks occur in up to 20% of cases although most are small, contained, and heal without another operation (Chittmittrapap et al., 1992). Leaks may be detected by observing saliva in the chest drain but are usually discovered on routine postoperative contrast studies. Stricture at the anastomosis is seen in up to one-third of cases and is more common when the anastomosis was created under tension or was complicated by a postoperative leak (Chittmittrapap et al., 1990). Clinically significant strictures usually manifest with feeding difficulty and can usually be successfully treated with serial dilations (Serhal et al., 2010; Raitio et al., 2016).

Patients who have esophageal atresia commonly suffer from gastroesophageal reflux (GER) and tracheomalacia (Tovar and Fragoso, 2013). GER may be more problematic in patients with esophageal atresia because of inherent dysmotility in the distal esophagus (Kawahara et al., 2007). Routine use of gastric acid blockade is recommended to improve symptoms and to decrease risk of stricture formation because of acid exposure. Many patients go on to require surgical treatment for reflux with fundoplication (Bergmeijer et al., 2000).

Tracheomalacia is a focal or generalized structural weakness of the trachea leading to airway narrowing or collapse with increasing intrathoracic pressure (e.g., expiration). Patients may have expiratory stridor, a “barking cough,” recurrent respiratory illnesses, or acute life-threatening events. Diagnosis is by bronchoscopy, which shows airway collapse during spontaneous breathing. In severe cases, an aortopexy can effectively treat the condition by suspending the aorta (and thus the attached anterior wall of the trachea) to the sternum and thus pulling it away from the posterior wall of the trachea (Corbally et al., 1993; Dave and Currie, 2006).

Laryngotracheoesophageal Cleft

A *laryngotracheoesophageal cleft* (LC) is a rare congenital connection of variable length between the posterior larynx and trachea and the anterior esophagus resulting from failed midline fusion during development. The cleft starts proximally at the larynx and extends distally. The length of the communication can be as short as a



• **Fig. 71.7** Diagram of Laryngotracheoesophageal Cleft.

superficial mucosal defect between the arytenoid cartilages or as long as the entire length of the trachea and even into the mainstem bronchi (Fig. 71.7) (Ryan et al., 1991; Ryan and Doody, 2014). An LC can be associated with Pallister–Hall syndrome, CHARGE syndrome, Opitz G syndrome, or VACTERL association (Leboulanger and Garabedian, 2011). Approximately 12% of infants with LC also have a more distal TEF (Fraga et al., 2015). Symptoms are related to the length of the connection between the esophagus and trachea. Minor defects may cause subtle recurrent aspiration and recurrent respiratory infections. In long defects, there may be an inability to ventilate with severe respiratory distress. It can be difficult to treat the respiratory distress because the endotracheal tube can migrate (“fall”) into the esophagus after placement through the vocal cords. Diagnosis is best made by rigid bronchoscopy, which may also assist in placing the endotracheal tube beyond the distal aspect of the defect. Minor defects are often successfully treated with thickened feeds alone though may be repaired endoscopically (Sandu and Monnier, 2006). Repairs of long defects are major, complex operations of the chest and neck that may require cardiopulmonary bypass.

Congenital Esophageal Stenosis

Congenital esophageal stenosis is rare, with an incidence of 1 in 25,000 to 1 in 50,000 (Vasudevan et al., 2002). It is usually associated with other anomalies, and up to 50% will also have esophageal atresia (McCann et al., 2015). There are three histologic types: (1) fibromuscular thickening, (2) cartilage from ectopic tracheobronchial remnants in the esophageal wall, and (3) membranous web. The majority of defects are found in the distal esophagus, most commonly 1–2 cm above the gastroesophageal junction. More than half of cases are diagnosed after the neonatal period. Dysphagia and recurrent vomiting are common symptoms. Diagnosis can be reliably made with a contrast study and confirmed by endoscopy. Diagnosis is also possible in cases associated with esophageal atresia by evaluation of the distal esophagus during and after surgical repair (Michaud et al., 2013). Treatment is dilation or surgical resection (Vasudevan et al., 2002).

Esophageal Duplication Cyst

Duplications are congenital malformations of unknown etiology that may form anywhere along the length of the developing GI tract but are most common in the small intestine. They may be cystic or tubular. *Esophageal duplication cysts*, also known as *foregut duplication cysts*, are typically covered by a layer of smooth muscle and contain an epithelial lining that can comprise a hybrid histology with elements of respiratory and alimentary tissue within the same lesion (Nobuhara et al., 1997). The lumen of the cyst typically does not communicate with the normal foregut lumen (Michaud et al., 2013).

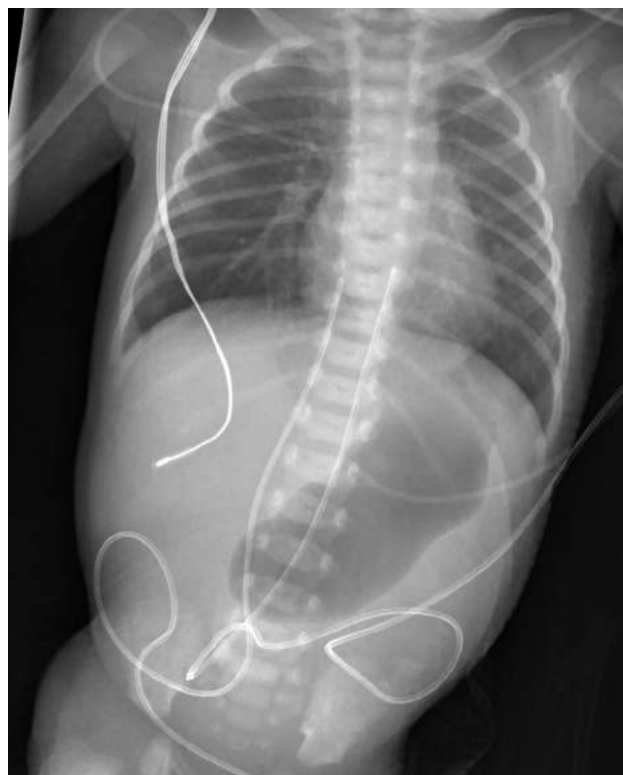
Clinical presentation is often due to compression of adjacent structures in the chest, and symptoms may include cough, wheezing, and dysphagia. Diagnosis is often made later in childhood (Azzie and Beasley, 2003). The majority of cases have an abnormal chest X-ray because of mass effect, and contrast studies will demonstrate compression on the esophageal lumen. CT often offers better visualization of the lesions (Fig. 71.8) (Shamberger et al., 1995). Treatment is surgical resection (Holcomb et al., 1989).

Esophageal Perforation

Low birth weight neonates are at higher risk for iatrogenic perforation of the esophagus (Hesketh et al., 2015). In a newborn, the presentation of esophageal perforation can mimic esophageal atresia; in both there is inability to pass an orogastric or NG tube into the stomach (Krasna et al., 1987). But when the tube passes beyond the level of the carina but does not enter the stomach it should raise the possibility of perforation because atresias are almost always at or above the carina (see Fig. 71.8). Treatment depends on the location and severity of perforation. When the perforation is small and the leakage of esophageal contents is contained by surrounding structures in the mediastinum, then it may heal with antibiotics alone without surgical repair. Follow-up contrast study to document healing is important before the initiation of feeds. When the perforation and leak of esophageal contents is large, especially with contamination of the pleural space, then urgent surgical intervention is indicated (Mollitt et al., 1981).



• **Fig. 71.8** Esophagram of Esophageal Duplication Cyst.



• **Fig. 71.9** Radiograph of Pyloric Atresia in the Setting of Epidermolysis Bullosa.

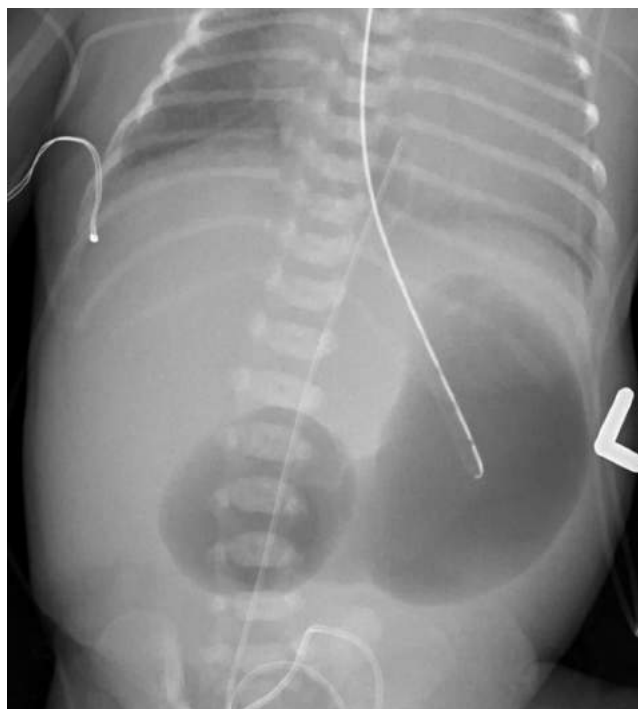
Disorders of the Stomach

The two congenital malformations of the stomach discussed, pyloric atresia and gastric duplication, are quite rare and present with vomiting because of partial or complete obstruction. An acquired gastric obstruction, pyloric stenosis, is much more common and also presents with vomiting later. The last condition reviewed, neonatal gastric perforation, is an acquired condition that presents with peritonitis and even sepsis.

Pyloric Atresia

Pyloric atresia is a congenital, intrinsic, complete obstruction of the pylorus. It is rare, occurring in 1 in 100,000 live births. There are three distinct anatomic variants of pyloric atresia: (1) a simple membrane (57% of cases), (2) replacement by solid tissue (34% of cases), and (3) a complete separation of the stomach and duodenum (9% of cases) (Okoye et al., 2000).

Prenatally, pyloric atresia leads to polyhydramnios and a dilated stomach, thus prenatal diagnosis is common. Nonbilious vomiting occurs with feeds after birth. Diagnosis is confirmed by abdominal radiographs showing air in a dilated stomach but no air in the GI tract distal to the stomach (Fig. 71.9). The lack of a dilated proximal duodenum on imaging studies distinguishes pyloric atresia from the more common duodenal atresia (Fig. 71.10). Babies with pyloric atresia should be evaluated for epidermolysis bullosa (EB) because of the common association of these two rare conditions (Pfendner and Lucky, 2013). The prognosis of patients with EB and pyloric atresia is poor although survivors have been reported (Hayashi et al., 1991). Surgical treatment for all forms of pyloric atresia consists of either removing or bypassing the obstruction.



• **Fig. 71.10** Radiograph of Duodenal Atresia Presenting With a "Double Bubble" Sign.

Gastric Duplication

Gastric duplications are similar to esophageal duplication cysts in that they are surrounded by smooth muscle and usually do not communicate with the lumen of the stomach. They are uncommon and comprise only 5% of abdominal duplications.

They are usually located in the distal stomach, and as they enlarge, they cause symptoms of progressively severe gastric outlet obstruction, with vomiting that is usually nonbilious (Azzie and Beasley, 2003). Approximately one-third of gastric duplications are found in the neonatal period. The diagnosis may be suspected by palpating an upper abdominal mass, by extrinsic compression of the distal stomach on gastrointestinal series (UGI), or by visualizing the mass on ultrasound or CT. Treatment is surgical excision and is recommended even in cases discovered incidentally, since most will eventually cause symptoms of gastric outlet obstruction (Azzie and Beasley, 2003).

Pyloric Stenosis

Pyloric stenosis (or *hypertrophic pyloric stenosis* [HPS]) is an acquired disorder of hypertrophy of the pyloric muscle at the distal end of the stomach. This hypertrophy narrows the gastric outlet and leads to progressively severe, nonbilious vomiting.

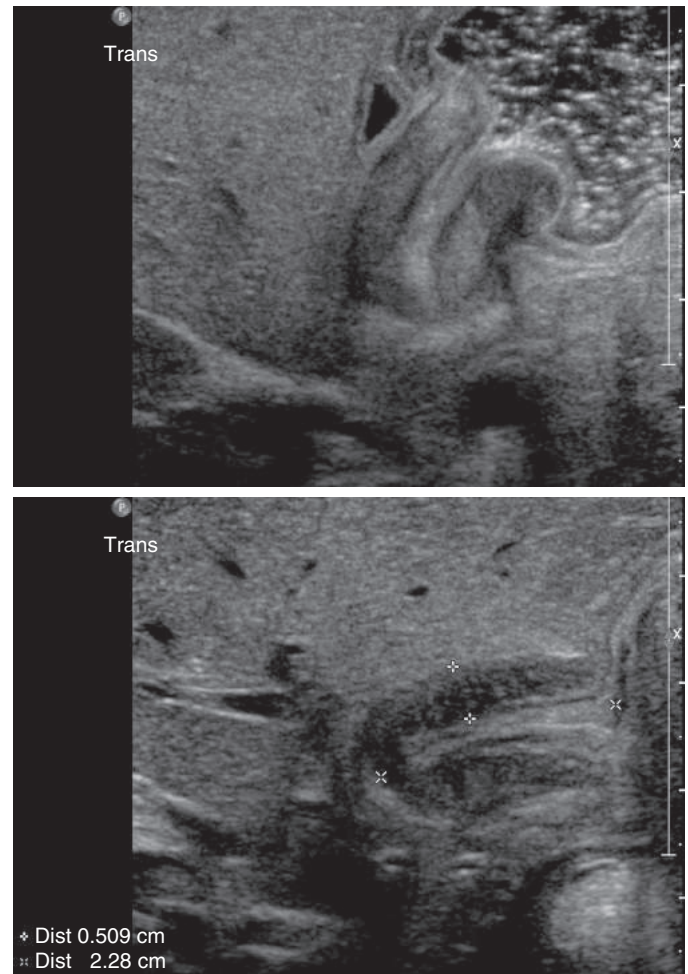
Pyloric stenosis is one of the most common structural problems of the GI tract in infants, with an incidence of 2 to 5 in 1000 live births in the Western population. It occurs almost exclusively between weeks 3 to 8 of life. It is four to five times more common in males than in females, and white infants are more commonly affected than black or Asian infants. There is decline in risk with increasing birth order (MacMahon, 2006).

The etiology of HPS is unknown. Genetic factors must have a role since there is an increased risk within families: for example, if a baby has pyloric stenosis then the identical twin has an almost 25% chance of having the condition while a nontwin sibling has a 5.8% chance (MacMahon, 2006). HPS also occurs more frequently than expected in infants with certain syndromes (Panteli, 2009). Exposure to erythromycin and prostaglandins may also increase the risk of developing HPS (Pandya and Heiss, 2012).

As noted, infants with HPS present with a history of vomiting feedings and gastric contents, similar to patients with GER or milk protein allergy. Because the pyloric hypertrophy and degree of gastric outlet obstruction are progressive, the vomiting is also progressively more severe. Unlike intestinal obstructions that are distal to the bile duct insertion in the duodenum, the vomiting in HPS is nonbilious, and the loss of gastric contents undiluted by bile and pancreatic secretions leads to hypochloremic, hypokalemic metabolic alkalosis.

The physical examination of a baby with pyloric stenosis may be notable for dehydration, and in some cases the hypertrophied pylorus may be palpable as a firm, mobile upper abdominal mass known as the pyloric “olive.” The diagnosis is usually confirmed with imaging, most commonly an abdominal ultrasound. Ultrasound findings of pyloric stenosis include a pyloric muscle thickness greater than 3 mm and length greater than 15 mm (Fig. 71.11) (Hernanz-Schulman, 2009). Upper GI contrast radiographs can also confirm the diagnosis of pyloric stenosis if ultrasound is not available.

After diagnosis, the first step in treatment is to correct intravascular volume depletion as this will permit the correction of any associated hypokalemia and alkalosis. The metabolic alkalosis should be corrected before surgery to prevent alkalosis-induced apnea after general anesthesia. It may take a day or more to optimize



• Fig. 71.11 Ultrasound of Hypertrophic Pyloric Stenosis.

intravascular volume, acid–base balance, and serum electrolytes. To limit the risk of aspiration, the stomach should be evacuated with an NG tube from the time of admission and with a larger red rubber catheter immediately before induction of anesthesia.

The operative treatment of pyloric stenosis is to incise and split the pyloric muscle layer completely so that the submucosa is exposed from the duodenum to the gastric antrum. This pyloromyotomy can be achieved by open or laparoscopic approach. The operation is remarkably effective so that feedings can be resumed soon after surgery, and discharge is usually possible within a day or two.

The outcomes of modern management of pyloric stenosis are excellent. Mortality is extremely rare, and there is only a small risk of surgical site infection or wound problems. Other uncommon problems after pyloromyotomy are (1) peritonitis and sepsis from an unrecognized full-thickness mucosal injury and (2) an incomplete myotomy, which can lead to recurrent vomiting (diagnosed by UGI as repeat ultrasound will show postoperative changes). After pyloromyotomy the pylorus eventually heals, remodels, and appears normal. There are no functional abnormalities of the stomach in the long term.

Gastric Perforation

Similar to esophageal perforations, low birth weight infants are thought to be at higher risk for catheter-associated gastric

perforations than term babies. In the absence of obvious trauma, rupture of the stomach is called “spontaneous,” although possible risk factors such as prematurity, duodenal atresia, mechanical ventilation, or neonatal hypoxia are often identified (Leone and Krasna, 2000; Duran et al., 2007). The mechanism of spontaneous gastric perforation is not known, but it appears to be distinct from NEC as NEC typically spares the stomach, and the gross and microscopic appearance seen in NEC and spontaneous intestinal perforation are not seen in spontaneous perforations of the stomach (Holgersen, 1981). Gastric perforations usually present with large amounts of free air on abdominal X-rays. Emergency operation and urgent surgical repair are indicated.

Disorders of the Intestine

Disorders of the intestine reviewed include the potentially life-threatening intestinal malrotation and midgut volvulus, intestinal atresias, enteric duplications, intussusception, and meconium ileus. These conditions present with intestinal obstruction that may be complicated by intestinal ischemia, necrosis, bleeding, perforation, and/or sepsis. The clinical presentations of these conditions and many other GI tract disorders are usually nonspecific and consist of some combination of signs and symptoms such as pain, feeding intolerance, vomiting, lack of stooling, tenderness, distention, or mass.

The history and physical examination often provide clues to the diagnosis. Pain and tenderness may be caused by visceral distention and/or peritoneal irritation. Typically, nonbilious vomiting is seen with obstruction proximal to the ampulla of Vater (the site of bile drainage into the duodenum) while bilious vomiting results from a more distal obstruction. Abdominal distention is usually the result of diffusely dilated loops of bowel caused by either a functional ileus (intestinal stasis with accumulation of swallowed air, feedings, and GI secretions) or a distal obstruction. More-proximal obstructions of the stomach and duodenum usually do not cause abdominal distention. Failure to pass meconium in the first 24 hours of life should raise concern for distal GI obstruction.

A clinical concern for intestinal obstruction requires simultaneous supportive care and diagnostic evaluation. Feedings should be stopped, and if there is abdominal distention or signs of intestinal distention on imaging studies, then gastric decompression via an NG or orogastric tube should be done. Intravenous fluids should be given to meet ongoing maintenance needs, replace any deficits, and account for any ongoing, abnormal losses. Antibiotics should be given when there is concern for infection.

A directed abdominal examination should be done looking for distention, tenderness, localized abdominal edema and erythema, hernias, and masses. Abdominal radiographs can help with diagnosis but are rarely definitive. The characteristic imaging findings of specific disorders will be reviewed. If there are significant concerns for intestinal obstruction then surgical consultation is advised.

Malrotation and Volvulus

Normal intestinal rotation occurs when the midgut that is herniated outside the abdominal cavity into the umbilical cord early in development returns to the abdominal cavity during weeks 10 to 12 of gestation and fixes to the retroperitoneum in a precise pattern. Normal intestinal rotation results in: (1) the duodenum being fixed in the retroperitoneum around the pancreas and transitioning to the intraperitoneal jejunum (visible externally as the ligament

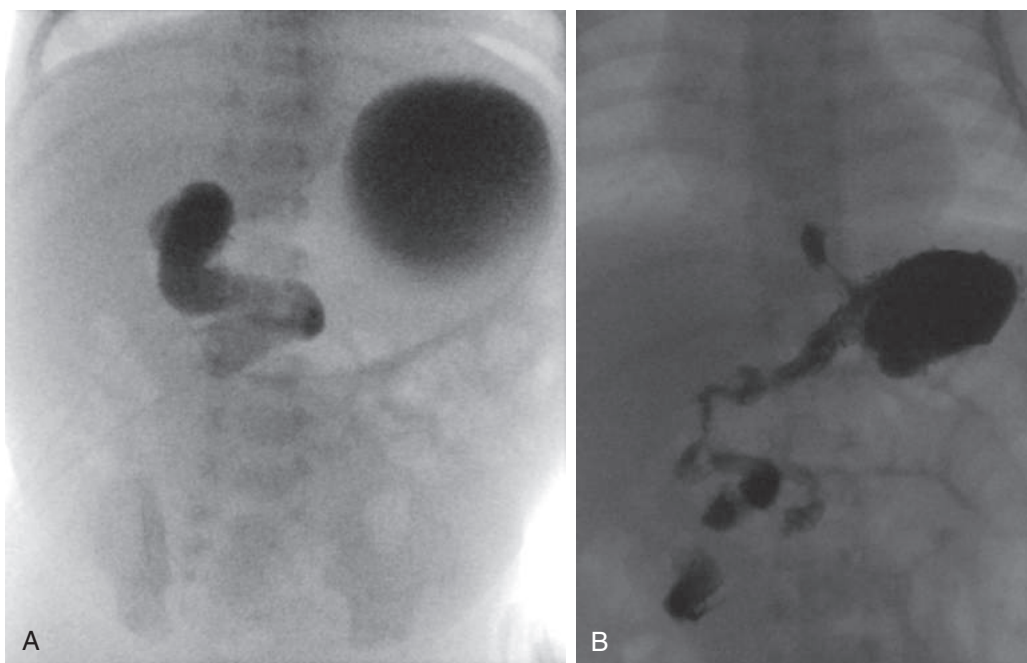
of Treitz) at a point to the left of the spine and above the level of the duodenal bulb, (2) all the small bowel loops floating free in the abdomen, (3) the cecum and ascending colon being attached to the right-lateral posterior body wall up to the hepatic flexure, (4) the descending colon attached to the left-lateral body wall from the splenic flexure to the sigmoid colon, and (5) the blood supply to the entire midgut (jejunum, ileum, and right and transverse colon) attached to the retroperitoneum in a wide pedicle from the ligament of Treitz in the left upper quadrant of the abdomen to the cecum in the right lower quadrant. While a wide spectrum of anatomic abnormalities exists (as a group known as *intestinal rotation anomalies*) (Graziano et al., 2015), we will focus on classic intestinal malrotation where the duodenum does not cross over the spine, and the other normal attachments to the retroperitoneum do not form, resulting in a narrow mesenteric vascular pedicle. This narrow vascular pedicle supports most of the small and large bowel and is prone to twist and cause midgut volvulus. Classic malrotation may be accompanied by abnormal retroperitoneal attachments, known as *Ladd bands*, which cross the second portion of the duodenum.

The true prevalence of malrotation is difficult to determine for several reasons including symptomatic presentation throughout life and a number of children (and even adults) discovered to have asymptomatic variants during imaging studies or operations. Presumably the more severe anatomic abnormalities are more prone to volvulus and present earlier in life, although life-threatening volvulus can occur in adults. The majority of cases, however, present in infancy. Malrotation may occur alone, but it is a regular component of gastroschisis, omphalocele, and congenital diaphragmatic hernia and also may accompany intestinal atresia, intussusception (i.e., Waugh syndrome) (Al Momani, 2014), heterotaxy, and various cardiac defects.

Newborns and infants with midgut volvulus initially present with bilious vomiting, since the twist and obstruction are distal to the ampulla of Vater. Early in the course there may be abdominal pain, but abdominal tenderness and distention may also be absent. As the twist of the mesentery progresses, the blood supply to the bowel is compromised, and intestinal ischemia and eventually intestinal necrosis and peritonitis set in with resulting pain, tenderness, guarding, abdominal distention, and, eventually, signs of sepsis. Abdominal X-rays may be normal or may show a gasless abdomen or distended bowel loops. Laboratory studies may be normal until intestinal ischemia peritonitis and sepsis develop.

Since the entire midgut is potentially at risk for vascular compromise with volvulus, it is best to make the diagnosis at the onset of symptoms and signs of obstruction, before symptoms and signs of intestinal ischemia develop. Since early diagnosis is critical, any newborn or infant with bilious vomiting should be considered to have malrotation and volvulus until proven otherwise, and an urgent upper GI series should be strongly considered. In malrotation, the ligament of Treitz will fail to cross midline to its normal position at the left-sided vertebral body pedicle and will be lower (in the cephalad–caudal axis) than the level of the duodenal bulb (Fig. 71.12).

Other imaging findings suggesting malrotation and volvulus include an abnormal position of the cecum by contrast enema and abnormal relationship of the mesenteric vessels by ultrasound. Malrotation can be also diagnosed by CT, but CT is rarely indicated in newborns. It is important to recognize that volvulus in this setting can be associated with a relatively normal-appearing plain film of the abdomen, and thus a high degree of suspicion must be maintained when clinical findings are suggestive.



• **Fig. 71.12** (A) and (B) Upper Gastrointestinal Series Showing Malrotation With and Without Obstruction/Volvulus.

Midgut volvulus is a surgical emergency. While laparoscopic approaches have been described and may be useful for asymptomatic malrotation, in the setting of acute symptoms an open laparotomy is recommended (Lodwick et al., 2015). The operation of choice is a Ladd procedure that consists of (1) untwisting the volvulized bowel, (2) dividing abnormal bands, (3) broadening of the mesentery, and (4) arranging the bowel so that the midgut mesentery is flat in the retroperitoneum, with the proximal small bowel on the right side of the abdomen and the distal ileum and cecum in the left upper quadrant. The appendix is usually removed since the operation makes its final location unpredictable and diagnosis of appendicitis later in life very difficult. When there is intestinal necrosis, nonviable bowel is excised. When bowel viability is questionable, “2nd look” procedures may be performed. Prophylactic treatment of asymptomatic malrotation may be laparoscopic; however, there is debate as to whether a laparoscopic Ladd procedure is as effective as an open procedure (Graziano et al., 2015; Lodwick et al., 2015). In neonates with congenital heart defects and asymptomatic malrotation, the American Pediatric Surgery Association recommends either observation or postponing the Ladd procedure until after palliation of the congenital heart defect (Graziano et al., 2015).

Intestinal Atresia

Intestinal atresia is a congenital complete obstruction of the bowel lumen. Atresia is an intrinsic problem of the bowel and is not an extrinsic compression. Esophageal and pyloric atresias are discussed in other sections of this chapter. Duodenal atresia and anorectal atresia are considered separately since each has unique embryology, risk factors, and associated conditions. Jejunal, ileal, and colon atresias have many common features and are considered together. Depending upon the anatomic location, some forms of atresia are incomplete and more correctly called intestinal “stenosis,” but the presentation, evaluation, and management are similar, so we will consolidate the discussion of atresias and stenosis together.

Duodenal Atresia

There are several anatomic variations of duodenal atresia including membranous webs, simple occlusions, complete separations of proximal and distal segments, and annular pancreas, in which abnormal fusion of the ventral and dorsal pancreas lead to encirclement of the second portion of duodenum and complete or incomplete obstruction. The exact nature of the developmental abnormality is unknown but may be due to a failure of recanalization (Fairbanks et al., 2006).

The prevalence of duodenal atresia is probably around 1 in 5000 births, but it is not completely clear since many studies evaluate all intestinal atresias rather than duodenal atresia specifically (Sinha et al., 2010). Duodenal atresia is frequently associated with other anomalies including Trisomy 21, VACTERL association, cardiac anomalies, malrotation, anorectal malformations, and biliary tract anomalies, which has important implications for evaluation and management (Escobar et al., 2004; Choudhry et al., 2009).

Duodenal atresia may be suspected prenatally when other associated anomalies or polyhydramnios are present. The diagnosis can be confirmed prenatally by an ultrasound showing a “double bubble” (Choudhry et al., 2009). If the diagnosis is not suspected prenatally, then the typical newborn presentation is vomiting. The vomiting is usually, but not always, bilious since the obstruction is typically distal to the ampulla of Vater. An abdominal X-ray showing a double bubble with no air in the distal GI tract confirms the diagnosis of duodenal atresia (see Fig. 71.10). An X-ray with a double bubble and air present in the distal GI tract indicates the infant may have duodenal stenosis, but malrotation and volvulus must remain a concern.

After the diagnosis, initial management includes gastric decompression with an NG tube, fluid resuscitation, and correction of electrolyte abnormalities. Surgical treatment is bypass of the obstruction, usually with a duodenoduodenostomy. The entire bowel should be evaluated for other intestinal atresias. An open approach is typical; however, a laparoscopic approach has been

described (Kay et al., 2009). Outcomes are generally good, but possible early complications include anastomotic leak and stricture. Potential long-term problems include poor motility of the stomach and duodenum with or without associated megaduodenum (Escobar et al., 2004).

Jejunioleal and Colon Atresia

Jejunioleal and colonic atresias are classified based upon the anatomic abnormality (Fig. 71.13). The etiology of jejunioleal and colon atresia is thought to be due to a prenatal mesenteric vascular compromise. Genetic factors and defective fibroblast growth factor signaling may also play a role in some cases (Fairbanks et al., 2006).

The prevalence of intestinal atresias is 1.6 to 2.8 per 10,000 live births (Cragan et al., 1993; Hemming and Rankin, 2007; Best et al., 2012). Important clinical features of patients with jejunioleal atresia are that about one-third are premature and associated anomalies are less likely, especially compared with patients with duodenal atresia (Adams and Stanton, 2014).

Prenatal ultrasound may show dilated loops of bowel. After birth, neonates with jejunioleal and colon atresia present with bilious vomiting, abdominal distention, and failure or delayed passage of meconium; the more proximal the atresia the more rapid the onset of signs and symptoms. Abdominal radiographs show dilated loops of bowel with airfluid levels and an absence of gas in the rectum. After abdominal X-rays the next step in evaluation of possible intestinal atresia is a contrast enema, which will help differentiate intestinal atresia from uncomplicated meconium ileus, Hirschsprung disease (HD), and small left colon syndrome (Adams

and Stanton, 2014). Contrast enema will also demonstrate that the distal colon is patent, which is important for intraoperative decision making.

Initial management is gastric decompression and fluid resuscitation, followed by operation, which, in addition to repairing the bowel, often involves resection or tapering of large dilated segments that are prone to severe dysmotility.

Early results and long-term outcomes of the operative repair of jejunioleal and colon atresia are good. There is a small risk of surgical site infection and anastomotic leak and stricture. Short bowel syndrome is a potential long-term problem if the atresias lead to loss of a large amount of small-intestinal length.

Newborns with colonic atresia act similarly to those with more proximal atresia; however, because the obstruction is more distal in the GI tract, the presentation is often delayed until the 2nd or 3rd day of life. Abdominal radiographs show dilated distal bowel and may show a very dilated colon if the ileocecal valve prevents air from refluxing back into the distal ileum. Treatment is similar to cases of jejunioleal atresia, with laparotomy, resection of the dilated colon, and anastomosis. Timely diagnosis and treatment are essential and are associated with a generally excellent outcome, while late diagnosis and treatment (over 72 hours from birth) increase the risk of morbidity and mortality (Haxhija et al., 2011).

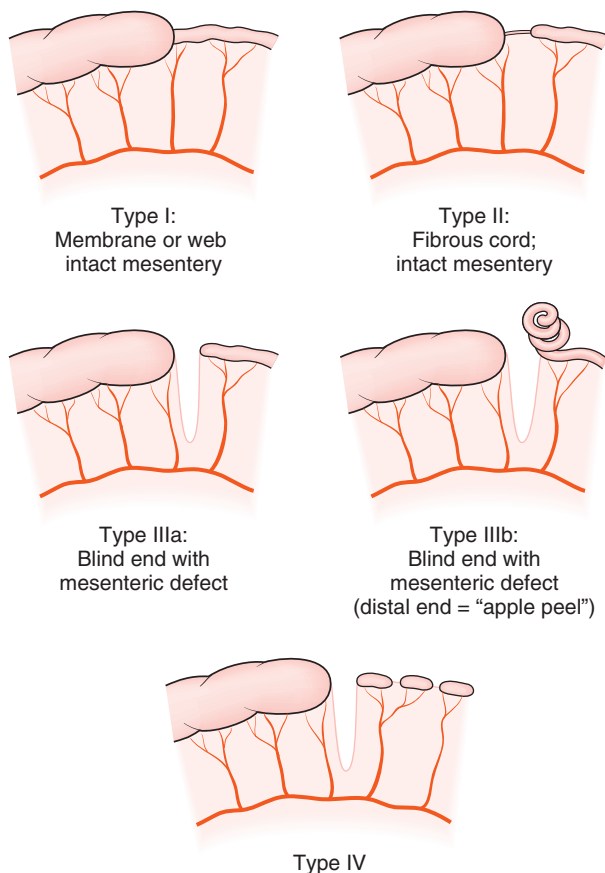
Meconium Ileus

Meconium ileus is an intraluminal obstruction of the terminal ileum by abnormal meconium. Cystic fibrosis (CF) accounts for 90% of patients presenting with meconium ileus, while up to 20% of patients with CF present as newborns with meconium ileus. CF is caused by mutations in the CF transmembrane regulator (CFTR) that alter bicarbonate and chloride transport. These intraluminal fluid changes may result in the characteristic meconium that adheres to the bowel wall and causes the obstruction. Meconium ileus may be “uncomplicated” and result only in bowel obstruction, or it may be “complicated” when the obstruction leads to prenatal perforation or twisting and ischemia that may cause ileal atresia (Rescorla and Grosfeld, 1993). Note that *meconium peritonitis* is a description of the result of any in utero perforation, whether that perforation is caused by meconium ileus or another problem.

Prenatal diagnosis of CF may be suspected when there is a family history of CF or when the parents are carriers of CFTR mutations. CF diagnosis can be confirmed by fetal DNA analysis and, while neither sensitive nor specific, prenatal ultrasound findings of hyperechoic bowel, bowel dilation, and inability to identify the gallbladder have all been associated with meconium ileus (Carlyle et al., 2012).

The newborn clinical presentation of meconium ileus is similar to a jejunioleal atresia with abdominal distention 12 to 24 hours after birth, vomiting, and failure to pass meconium (Rescorla and Grosfeld, 1993). Abdominal X-rays show dilated loops of bowel suggesting distal intestinal obstruction. If intrauterine perforation has occurred, abdominal distention immediately at birth (rather than several hours later) and abdominal X-rays may show intra-abdominal calcifications.

Initial management of babies with meconium ileus is the same as any neonate with bowel obstruction and includes intravenous fluids, correction of electrolyte abnormalities, and gastric decompression. A contrast enema may confirm the diagnosis and offers the chance of nonoperative management of uncomplicated meconium ileus by instilling water soluble contrast around and into the abnormal meconium, softening it, and encouraging it to pass out



• Fig. 71.13 Types of Intestinal Atresias.

per rectum, thus relieving the obstruction (Carlyle et al., 2012). Hyperosmolar solutions have been advocated to draw fluid into the bowel lumen, but they carry the risk of potentially dangerous intravascular fluid shifts. The administration of *N*-acetylcysteine into the proximal or distal bowel lumen has also been used to help break up the abnormal meconium and relieve the obstruction. Failure of nonoperative measures or the presence of a complicated meconium ileus are indications for surgical intervention.

Operative management of uncomplicated meconium ileus involves laparotomy, opening the small bowel proximal to the obstruction, and irrigation with saline or *N*-acetylcysteine until the obstructing meconium is cleared. The bowel can usually be closed primarily, although sometimes tubes are placed to allow additional irrigation after surgery. By definition, complicated meconium ileus requires an operation. The operation involves resection of compromised bowel, repair of atresias, and, usually, primary anastomosis, although at times temporary diverting stomas are used.

Long-term GI outcomes after nonoperative or operative management of uncomplicated meconium ileus are generally quite good from a surgical perspective, although pancreatic insufficiency requires lifelong medical management.

Enteric Duplication Cysts

Enteric duplication cysts, often simply referred to as *duplications*, can occur anywhere in the alimentary tract from mouth to anus (Gross et al., 1952; Holcomb et al., 1989). Duplications can be cystic or tubular and consist of an inner lining of GI epithelium and an outer layer of smooth muscle. Most duplications occur in the small intestine, especially the distal ileum, and are often on the mesenteric side of the lumen (Fig. 71.14) (Narlawar et al., 2002).

The overall prevalence of duplications is poorly defined, but a cumulative incidence for all sites and all types is probably around 1 in 4000 to 5000, although any specific duplication type or location is quite rare. Various theories exist to explain the embryologic etiology for enteric duplications; however, the split notochord theory is generally accepted (Sharma et al., 2009).

The clinical presentation of an enteric duplication depends on three factors: (1) its location, (2) its relative mass effect, and (3) complications relating to secretions of the involved epithelium

(Azzie and Beasley, 2003). Esophageal and gastric duplication cysts are described in other sections, so this section will focus on small and large bowel duplications. Only about 4%–5% of enteric duplications are located in the duodenum (Macpherson, 1993; Lopez-Fernandez et al., 2013). Duodenal duplications may be connected with biliary or pancreatic ducts. Clinical presentations include asymptomatic masses, GI obstruction, and pancreatitis (Chen et al., 2010; Lopez-Fernandez et al., 2013). Jejunal and ileal duplications often present with bowel obstruction or intussusception. Colonic and rectal duplications are rare but may present with obstruction or volvulus (Holcomb et al., 1989).

Diagnosis is made with ultrasound and/or contrast studies, which may show a filling defect. Ultrasound can be used prenatally, leading to early treatment postnatally (Laje et al., 2010). CT and MRI may be used to define the location and size of the duplication. Treatment is usually surgical excision; however, endoscopic excision has been described for proximal duplications (Meier and Mellinger, 2012).

Intussusception

Intussusception is the telescoping of one portion of bowel into another, usually involving the distal ileum and ascending colon. It usually occurs in older infants and toddlers, but it is also a rare cause of intestinal obstruction in neonates (Aboagye et al., 2014). The presenting symptoms of feeding intolerance, vomiting, abdominal distention, and blood in the stool are nonspecific and can delay diagnosis and treatment (Taskinlar et al., 2014). The diagnosis may be made by ultrasound or contrast enema or may be made at the time of operation for intestinal obstruction. Treatment is surgical reduction with resection if needed.

Disorders of the Colon

Neonatal Appendicitis

Neonatal appendicitis is rare (Karaman et al., 2003). Some cases are associated with HD. The clinical presentation usually includes vomiting, abdominal distention and tenderness, and fever (Raveenthiran, 2015). It is rare to make the diagnosis before an operation is performed for peritonitis because of appendiceal perforation. A possible association with HD has been discussed in the literature, and consideration should be given to a suction rectal biopsy in infants who present with appendicitis.

Hirschsprung Disease

HD is characterized by absence of intrinsic parasympathetic ganglion cells in the submucosal and myenteric plexuses caused by premature arrest of the normal craniocaudal migration of neural crest cells into the bowel wall. HD most commonly involves the rectum and sigmoid colon; however, up to 10% of cases involve the entire colon (i.e., total colon aganglionosis). Rarely, the small intestine is involved (Kessmann, 2006; Amiel et al., 2008). The aganglionic colon has impaired motility and results in a functional distal bowel obstruction of variable severity. It may present as intestinal obstruction in the newborn or constipation in older infants and children. HD may be complicated by enterocolitis, which can cause a clinical picture of marked abdominal distention, pain, tenderness, and systemic manifestations of sepsis. The cause of enterocolitis is unknown; however, it can occur before or after HD is surgically corrected.



• Fig. 71.14 Intraoperative Photo of a Duplication Cyst.

The prevalence of HD is estimated to be 1 per 5000 live births (Amiel et al., 2008). Males are more frequently affected than females (4:1) and Asians, African-Americans, and Caucasians (2.8, 2.1, and 1.5 per 10,000 live births respectively) are more commonly affected than Hispanics (1.0 per 10,000 live births) (Amiel et al., 2008). HD may be an isolated problem; however, almost 20% of cases have chromosomal abnormalities. Although, abnormalities of many genes have been implicated in HD, in the majority of patients the genetic abnormalities have not been defined (Amiel et al., 2008).

When HD presents in a newborn the clinical picture is usually of a distal bowel obstruction with feeding intolerance, vomiting, and abdominal distention. The diagnosis may be suspected when this there is passage of meconium later than 24 hours after birth. Abdominal radiograph may show dilated loops of bowel and paucity of rectal gas. Contrast enema will show normal caliber rectum and distal bowel extending some distance proximally and then a “transition zone” to the dilated, more proximal bowel (Fig. 71.15). Diagnosis is confirmed by rectal biopsies, which show hypertrophic nerve trunks and a lack of ganglion cells (Kessmann, 2006; Amiel et al., 2008).

The surgical treatment of HD involves excision of the abnormally innervated distal colon and a “pull-through” of the normally innervated proximal bowel that is connected to the preserved distal rectum, just above the anal canal, and several different techniques have been described. The procedures may be done in multiple stages, with an initial colostomy and then a definitive pull-through done later, or the procedure may be done in a single stage. In addition, the procedures may be done laparoscopically, open, completely via a transanal approach, or with some combination of approaches.

Potential operative complications include anastomotic leak or stricture or damage to nearby structures, including components of the anal sphincter. The functional outcome for any one patient will not be known for years, when the child reaches the age of

toilet training, but in general, most patients can be expected to achieve social continence and have a good quality of life (Lane et al., 2016). It is important to remember that HD is not cured by surgery (every operation leaves behind an abnormally innervated anal sphincter), but a well-done operation can provide excellent palliation, especially with long-term follow-up and management by appropriate specialists.

Meconium Plug

Meconium plug syndrome is the intraluminal obstruction of the colon by abnormal meconium. This contrasts to the intraluminal obstruction of the terminal ileum in meconium ileus. Infants with meconium plug present with delayed passage of meconium, abdominal distention, and perhaps vomiting (Cuenca et al., 2012). Meconium plug is diagnosed by recognition of the passage of abnormal meconium after rectal stimulation, irrigation, or contrast enema (Fig. 71.16). Contrast enema usually resolves the obstruction, and few infants need surgical intervention, although they may require further evaluation for CF or HD.

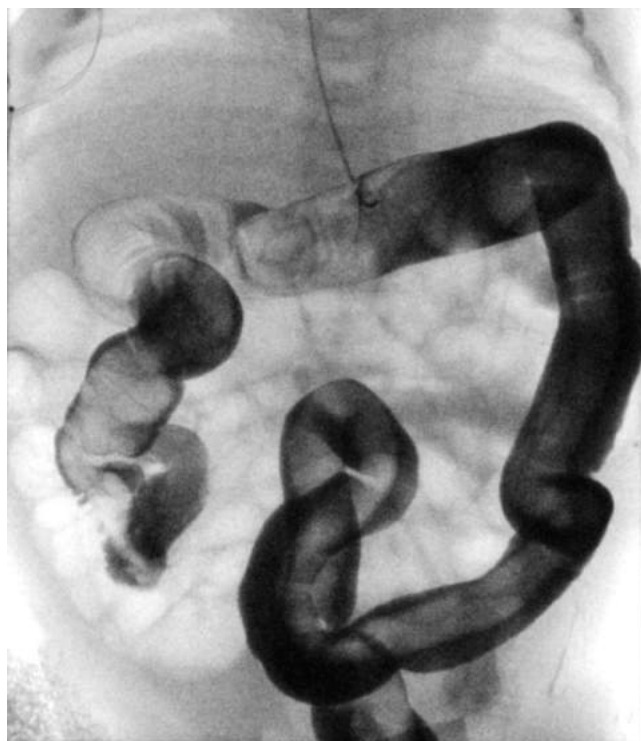
Anorectal Disorders

Anorectal malformations (ARMs) are a spectrum of congenital anomalies in which the anus is either absent or abnormally located outside the normal sphincter muscles. ARMs are often described as “high” or “low” depending on whether the rectum ends above or below the levator muscle component of the anal sphincter muscle complex. In this classification of high versus low, those lesions that have fistulae to the urinary tract or vagina are high.

The embryology of ARMs is incompletely understood because they result from a disturbance of the complex coordination of the distal GI tract (the hindgut), distal genitourinary structures



• Fig. 71.15 Contrast Enema of Hirschsprung Disease.



• Fig. 71.16 Contrast Enema of Meconium Plug.

(especially the bladder, urethra, and vagina), and the musculoskeletal components of the pelvis and perineum (Kluth, 2010).

The prevalence of ARMs is about 1 in 5000 live births. Associated anomalies are common. Most notable are those of the VACTERL association. In a large multicenter review, of the 4962 children who underwent ARM repair, 31% had vertebral anomalies, 40% had congenital heart disease, 7% had esophageal atresia (EA)/TEF, 34% had genitourinary anomalies, and 7% had limb defects. Thirty-six percent had three or more defined anomalies and met criteria for the formal VACTERL diagnosis (Lautz et al., 2015). ARMs are also associated with malrotation (Chesley et al., 2015), HD, (Hofmann and Puri, 2013), and Trisomy 21.

Prenatal diagnosis of ARMs is uncommon. After birth the diagnosis of an ARM is dependent upon a good physical examination. Imaging studies are not necessary; however, they can assist in determining the level of defect and the existence of fistula. The normal anus is typically halfway between the coccyx and the scrotum or vaginal orifice, and fistulae associated with ARMs are anterior to the normal position of the anus, with more severe cases entering the urethra, bladder, or vagina (which are not visible on the skin). The absence of an anus is fairly straightforward to determine; however, when there is a fistula on the perineum, especially when the details of its position are obscured by meconium or stool, the diagnosis may be delayed.

The management of patients with ARMs includes supportive care for bowel obstruction, evaluation for possible associated anomalies, and relief of the obstruction. Gastric decompression and intravenous fluids are needed until the bowel obstruction is relieved and enteral feedings are possible. The evaluation for associated anomalies includes a detailed physical examination and imaging studies including echocardiogram, spine radiographs, and renal and spinal ultrasound (Solomon et al., 2014).

Relief of the obstruction usually requires an operation, although some ARMs have a low resistance fistula that allows for spontaneous passage of meconium and stool. ARMs in which the end of the rectum is close to the middle of the anal sphincter complex on the perineum (usually less than 1 cm) can often be corrected with a single, neonatal operation known as an anoplasty. ARMs in which the rectum ends further away from the anal sphincter complex often require a staged approach with an initial colostomy and a later anoplasty and colostomy closure. An anoplasty can usually be performed completely from the perineum, although very high or complex ARMs may require a combined abdominal and perineal approach. The goal of surgical repair of ARMs is to place the rectum into the middle of the anal sphincter complex. The posterior sagittal anorectoplasty, first described by De Vries and Pena in 1982 (De Vries and Pena, 1982), is the most common procedure performed.

The outcomes of ARMs are related to the severity of the anatomic abnormality and to the presence and severity of associated anomalies. Similar to HD, the surgery for ARMs is more palliative than curative, and functional outcomes take years to accurately assess.

A good prognostic sign is the presence of a normal sacrum, presumably because an abnormal sacrum usually means abnormal pelvic innervation. A child with a low ARM and normal sacrum has a good chance of voluntary continence; however, in a child with a high ARM and abnormal sacrum voluntary continence is much less likely. However, many children with relatively severe anatomic anomalies can achieve social continence after a good operation and long-term medical (bowel) management, and many children with less severe anatomic abnormalities also require medical (bowel) management to obtain a good result. Besides incontinence and constipation, other long-term complications may include urinary incontinence, ejaculatory dysfunction, and erectile dysfunction (Rigueros Springford et al., 2016).

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Innate and Mucosal Immunity in the Developing Gastrointestinal Tract

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KEY POINTS

- Development of the human intestinal tract is a complex process that begins in early embryogenesis and continues until the introduction of solid foods in late infancy to early childhood.
- An effective barrier requires the presence of both innate and adaptive immune systems.
- The development, maturation, and maintenance of the intestinal tract's complex functions require interaction with environmental exposures such as amniotic fluid and intestinal commensal bacteria.
- The preterm infant possesses and experiences multiple perturbations to postnatal intestinal and immune development, all of which probably work in concert with one another to increase the vulnerability of the preterm infant to necrotizing enterocolitis.

The fully developed human gastrointestinal (GI) tract reaches a total length of approximately 22 to 30 feet (Hounnou et al., 2002). It has a mucosal surface area of 300 to 400 m² (DeWitt and Kudsk, 1999; Helander and Fandriks, 2014), equivalent to the size of a singles tennis court. Coexistent within the intestinal tract is a diverse microbial community with an abundance of organisms that well exceeds the number of cells in the entire human body (Bjorksten, 2004). During development, the interaction between this microbiome and the intestinal mucosal epithelium is an essential component in the education of the host's immune and inflammatory responses against foreign antigens.

The anatomic and functional development of the GI tract begins during early embryogenesis and continues well into childhood. In addition to the essential digestive and absorptive capacities provided by this organ system, the intestinal tract will also become the largest defense barrier and immune organ in the body. Its complex anatomic structures and dynamic functions protect the host from an onslaught of dietary and environmental antigens, beginning immediately after birth. These multiple layers of intestinal defenses are elegantly coordinated and tightly regulated, as immune and inflammatory responses must constantly balance between aggressive inflammatory attack against potential pathogens and eliciting a tolerant response to environmental antigens that facilitate development. Abnormal development and regulation in the balance between

immune tolerance and inflammatory responsiveness result in an inappropriate host response to antigenic challenges. This increases the vulnerability of the host to diseases of chronic, unregulated inflammation and dysregulated immunity.

Development of the Gastrointestinal Tract

The development of the GI tract spans from early embryogenesis until the introduction of solid foods in late infancy to early childhood. This period of ontogeny can be separated into five developmental phases (Table 72.1, Fig. 72.1) (Wagner et al., 2008). Embryonic organogenesis and primitive gut formation occur in phase I. In phase II, the GI tract becomes a tubular structure, anatomic development (elongation and formation of villus structures) continues, and the functional role of the intestinal epithelium is initiated. Phase III is characterized by rapid linear growth, continued anatomic development of the villus architecture, and ongoing cellular differentiation with maturation of specific cellular physiologic functions. Phase IV begins immediately after birth when postnatal structural and immune development are mediated by the interaction between the host and exogenous, environmental exposures. This developmental phase is mostly directed by the establishment of the intestinal microbiome and the response to dietary factors present in human milk and/or formula. Weaning from human milk or formula and the introduction of solid foods signal the beginning of phase V. During this period, structural development of the intestines and maturation of mucosal immunity are refined (Cummins and Thompson, 2002; Cummins et al., 2006).

Fetal Development

Phases I–III of intestinal development occur in utero. By the end of the first trimester many of the epithelial cellular elements, including specialized cells, have made their appearance (Table 72.2). During this same period, development of the anatomic structures necessary for optimal function of the GI tract proceeds, including formation of crypts and villi. Key microscopic features such as tight junctions (TJs) also occur in this phase. Also in the first trimester and into the second trimester, specialized cells such as goblet, Paneth, enteroendocrine, and the microfold (M) cells appear

TABLE 72.1 Stages of Gut Development

| Stage | Time Period | Development |
|--|-----------------------------------|---|
| Stage I: Embryonic | 0–5 weeks' gestation | Embryonic phase of organogenesis Primitive gut forms Entrance and exit sites of gastrointestinal tract form |
| Stage II: Tubular development | 6–20 weeks | Swallowing begins. Development of villi (8–12 weeks) and crypts (12–19 weeks) Specialized epithelial cells develop; mucin, antimicrobial peptides appear Peyer's patches begin to develop. |
| Stage III: Linear growth and differentiation | 20–40 weeks | Characterized by active differentiation Crypts increase in cell number, causing cells to migrate up villi. Rapid increase in intestinal length (nearly 10 cm/week in the 24–40-week period) Appearance of intestinal enzymes Macrophages, lamina propria lymphocyte populations are established Growth accompanied by selective apoptosis; occurs not only at the tips of villi but also in crypts |
| Stage IV: Mucosal expansion-I | Birth to 5 months, before weaning | Begins after birth with exposure to enteral nutrition Human milk feedings bring about more rapid mucosal differentiation and development than artificial feedings. Mucosal growth continues during infancy: deepening of crypts, increasing villus width and number, and the appearance of submucosal folds. Gastrointestinal tract is confronted with largest antigenic load to body in form of dietary proteins, commensal organisms, and pathogens: greater antigenic load with artificial feedings Mucosal immune system of gut develops extraordinary ability to distinguish between foreign pathogens and safe nutrient proteins and commensal organisms: process facilitated by human milk, leads to effective oral tolerance; success of such local interactions between local innate and specific immunity becomes the prerequisite for lifelong health. |
| Stage V: Mucosal expansion-II | >5–6 months, following weaning | Occurs during weaning phase in late infancy/early childhood during transition from milk feedings to complementary, solid foods Second phase of mucosal expansion associated with epithelial hyperplasia that renders the gut similar in function to that of older children and adults. Larger, lanceolate villi, crypt fissures seen |

Modified from Wagner CL, Taylor SN, Johnson D. Host factors in amniotic fluid and breast milk that contribute to gut maturation. *Clin Rev Allerg Immunol*. 2008;34:191–204.

(Henning, 1985; Buisine et al., 1998; Sanderson et al., 1999; Fusunyan et al., 2001; Rumbo and Schiffrin, 2005; Lin et al., 2008; McElroy and Weitkamp, 2011; Heida et al., 2016).

Role of Amniotic Fluid in Early Gastrointestinal Development

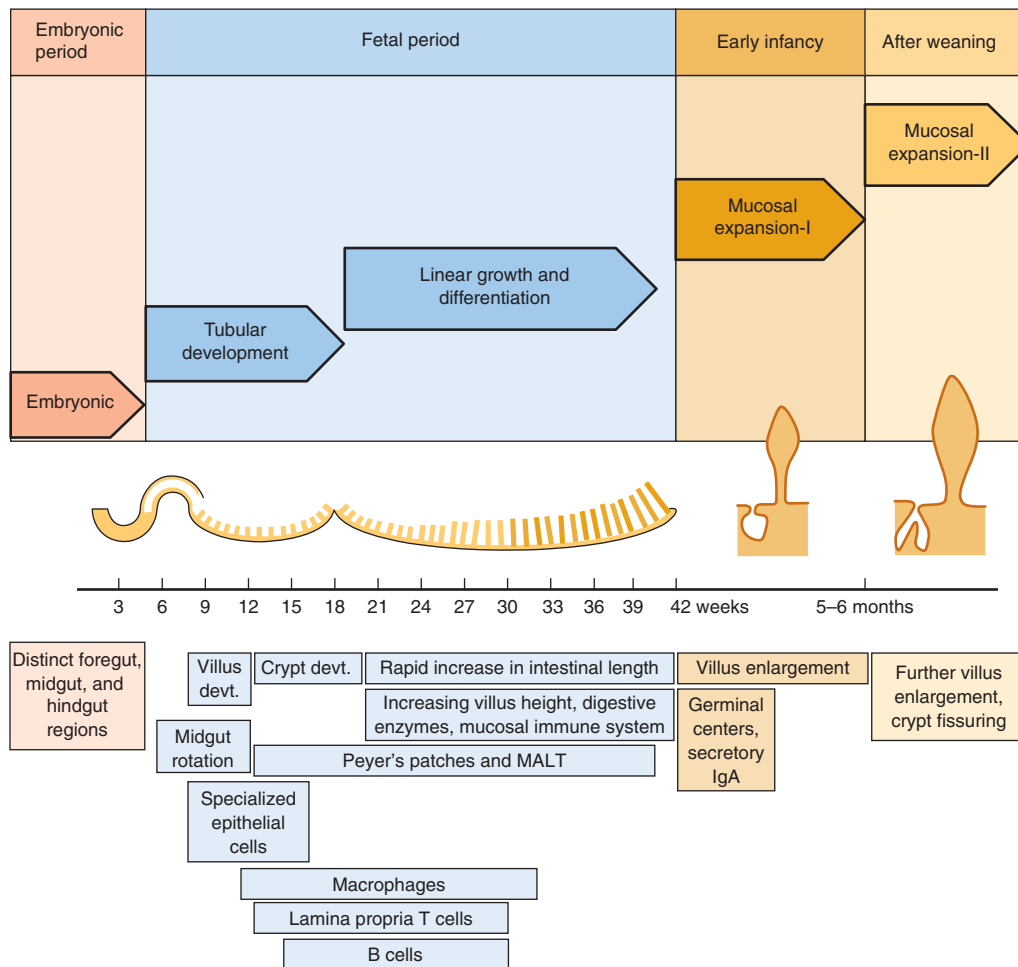
The development, maturation, and maintenance of the intestinal tract's complex functions require interaction with environmental exposures. This process will repeat itself with each changing environment and antigenic exposure. Different periods of new exposure include intrauterine life (fetus), postnatal introduction to the environment and to human milk or formula as the sole dietary source (early infancy), transition to solid foods (late infancy), and expansion of the dietary repertoire and thus the diversity of antigenic exposure (early childhood).

Amniotic fluid is arguably the first “environmental exposure” contributing to GI development. The amniotic fluid cavity is identified early in embryogenesis, and amniotic fluid rapidly accumulates during early gestation (Underwood et al., 2005). Initially, it is predominantly composed of water and solute from maternal plasma that is delivered to the fetus via the placenta and diffuses from the nonkeratinized fetal tissues into the amniotic space. As gestation lengthens, there are other contributors to the contents of amniotic fluid, including the placenta, amniotic

membranes, and the fetus. It is not until the second half of pregnancy that the fetus actively and significantly contributes to the volume and composition of the amniotic fluid, mainly through swallowing and urination (Underwood et al., 2005).

Amniotic fluid is a complex, dynamic mixture. Its composition varies over the gestational period. Proteomic analysis at 16 to 18 weeks' gestation identified more than 500 proteins, although this is likely an underrepresentation (Cho et al., 2007). Amniotic fluid is enriched with hormones, cytokines and growth factors, nutrients and other plasma proteins, modulators of coagulation, modulators of immunity and inflammation, and mediators of cell growth and differentiation, which together facilitate the development of the GI tract and the associated immune system (Box 72.1) (Underwood et al., 2005; Cho et al., 2007; Wagner et al., 2008).

Starting at 8 to 11 weeks' gestation, the fetus ingests increasing amounts of amniotic fluid that may reach 500 mL/day in the third trimester (Pritchard, 1966; Brace et al., 1994; Mann et al., 1996; Montgomery et al., 1999). Thus early in the second trimester until parturition, the intestinal tract of the fetus is continuously bathed in amniotic fluid. In a fetal sheep model (Trahair et al., 1986; Trahair and Harding, 1992), interruption of amniotic fluid ingestion by esophageal ligation during mid-gestation caused mucosal atrophy, villus blunting, and enterocyte abnormalities such as effacement of microvilli, glycogen accumulation, cellular extrusion, and



• **Fig. 72.1** Gastrointestinal Development. The development of the gastrointestinal tract can be separated into five stages, spanning from tubular development in early embryogenesis to late infancy to early childhood. *devt.*, Development; *IgA*, immunoglobulin A; *MALT*, mucosa-associated lymphoid tissue.

lysosomal dysgenesis. Consistent with these findings, human neonates with congenital intestinal obstruction also show villus blunting and shallow, poorly organized crypts distal to the site of obstruction (Condino et al., 2004). In the fetal sheep model described above, the effects of interrupted amniotic fluid swallowing on the intestinal mucosa were gradually reversed following the removal of esophageal ligatures and restitution of amniotic fluid ingestion but not by the infusion of Ringer's lactate. These findings indicated that the "trophic" effects of amniotic fluid were secondary to the bioactive molecules present in amniotic fluid and not merely because of the flow of fluid through the gut lumen (Trahair and Harding, 1995).

A large number of cytokines and growth factors can be detected in amniotic fluid, including epidermal growth factor (EGF), heparin binding-EGF, transforming growth factor (TGF)- β (isoforms TGF- β_1 and TGF- β_2), insulin-like growth factors, hepatocyte growth factor, fibroblast growth factor, chemokines, erythropoietin, and granulocyte colony-stimulating factor (MohanKumar et al., 2017). These cytokines have a complex, multifaceted role in intestinal development. In addition to the cytokines and growth factors delivered to the intestinal mucosa via amniotic fluid, and then after birth, in mother's milk, the fetal/neonatal intestine constitutively expresses many so-called "inflammatory" cytokines and growth factors at levels much higher than in the adult intestine. Intestinal

epithelial cells express cognate receptors for most of these cytokines, and evidence from in vitro and animal models suggests that many of these agents can survive GI digestion and can increase enterocyte proliferation, migration, and differentiation, prevent apoptosis, and promote mucosal restitution (Underwood et al., 2005; Jain et al., 2014; Good et al., 2015; MohanKumar et al., 2017).

Mucosal Immunity in the Developing Gastrointestinal Tract

Soon after delivery, phase IV of GI development begins. The newborn is rapidly presented with environmental, microbial, and dietary stimuli that influence intestinal development and maturation and mucosal immunity. The successful interaction between these antigenic exposures and the host's cellular responses is essential for the development of the innate and adaptive mucosal immunity of the GI tract and ultimately long-term protection of the host. An inappropriate intestinal response to environmental challenges may lead to altered immune and inflammatory regulation with local (GI) and systemic consequences.

However, before understanding the potential influences of environmental exposures on intestinal development and mucosal immunity, the specific structures that exist to coordinate this

TABLE 72.2 Fetal Development of the Intestinal Tract

| Developmental Feature | Gestational Age, Weeks |
|--|------------------------|
| Specialized Cells | |
| Intraepithelial lymphocytes | 8 |
| Intestinal absorptive epithelium | 9 |
| Goblet cells | 8–10 |
| Enteroendocrine cells | 9–11 |
| Paneth cells | 20–24 |
| Microfold cells (M cells) | 17 |
| Dendritic cells | 19 |
| Advanced Structural Components | |
| Tight junctions | 10 |
| Crypt-villus architecture | 12 |
| Peyer's patches | 19 |
| Elements of Innate Mucosal Immunity | |
| Mucin | 8–10 ^a |
| Defensins | 13 |
| Lysozyme | 20 |
| Toll-like receptors: TLR2, TLR4 | 20 |

^aSome investigators note the presence of mucin as early as 6–7 weeks' gestation (Buisine MP, Devisme L, Savidge TC, et al. Mucin gene expression in human embryonic and fetal intestine. *Gut*. 1998;43:519–524).
Data from Buisine MP, Devisme L, Savidge TC, et al. Mucin gene expression in human embryonic and fetal intestine. *Gut*. 1998;43:519–524; Fusunyan RD, Nanthakumar NN, Baldeon ME, Walker WA. Evidence for an innate immune response in the immature human intestine: toll-like receptors on fetal enterocytes. *Pediatr Res*. 2001;49:589–593; Louis NA, Lin PW. The intestinal immune barrier. *NeoReviews*. 2009;10:e180–e190; Mallow EB, Harris A, Salzman N, et al. Human enteric defensins. Gene structure and developmental expression. *J Biol Chem*. 1996;271:4038–4045; Neu J, Li N. The neonatal gastrointestinal tract: developmental anatomy, physiology, and clinical implications. *NeoReviews*. 2003;4:e7–e13; Rumbo M, Schiffrin EJ. Ontogeny of intestinal epithelium immune functions: developmental and environmental regulation. *Cell Mol Life Sci*. 2005;62:1288–1296; Heida FH, Beydüz G, Bulthuis ML, et al. Paneth cells in the developing gut: when do they arise and when are they immune competent? *Pediatr Res*. 2016; 80:306–310.

interaction must be reviewed. The intestinal components that make up the layers of defense and mediate appropriate oral tolerance or aggressive responsiveness can be found within the epithelial layer, leukocytes in the *lamina propria* and the submucosa, and the structures that comprise the gut-associated lymphoid tissue (GALT).

Physical and Chemical Barriers

On entering the intestinal lumen, a foreign antigen (e.g., bacterial pathogen, food antigen, or xenobiotic) encounters many tightly coordinated layers of mucosal defenses. The first layer of defense is a series of physical and chemical barriers designed to provide constant surveillance and prevent epithelial adherence and translocation of the potential pathogens or passage of antigens between the paracellular spaces. Within this first line of defense are the acidic environment of the stomach, the numerous digestive enzymes that exist along the entire GI route, and bile salts (Martin and Walker, 2008). These factors serve to digest dietary nutrients, but a side

• BOX 72.1 Amniotic Fluid Composition

Hormones

GH
GRP
Prolactin

Trophic or Growth Factors

EGF
TGF- α
TGF- β_1
IGF-1
Erythropoietin
G-CSF
HGF
Vasoactive endothelial growth factor

Nutrients and Other Proteins

Water
Electrolytes
Carbohydrates
Amino acids
Lipids
Albumin
Serotransferrin
Ceruloplasmin
Alpha-fetoprotein
Vitamin D-binding protein
Apolipoprotein A-1

Modulators of Immunity and Inflammation

Immunoglobulins
Interleukins
Complement
 α -Defensins
Lactoferrin
Lysozyme
Calprotectin
Cathelicidin
 α_1 -Antitrypsin
 α_1 -Microglobulin

Cell Growth and Differentiation

Fibronectin
Periostin
TGF- β -induced protein Ig-h3 precursor
Polyamines

Modulators of Coagulation

Antithrombin III
Plasminogen

EGF, Epidermal growth factor; G-CSF, granulocyte colony-stimulating factor; GH, growth hormone; GRP, gastrin-releasing peptide; HGF, hepatocyte growth factor; IGF-1, insulin-like growth factor 1; TGF- α , transforming growth factor- α ; TGF- β_1 , transforming growth factor- β_1 .

benefit of this digestive process is the destruction of ingested pathogens and other potentially immunogenic proteins into small, nonimmunogenic molecules of less than approximately 10 amino acids in length (Mayer, 2003). Additional mechanisms of the initial mucosal defense include production of mucus by goblet cells to inhibit microbial adherence, presence of polymeric secretory immunoglobulin A (sIgA) within the mucus layer to bind luminal antigens, peristalsis to facilitate removal of antigen-antibody complexes (McElroy and Weitkamp, 2011; Johansson et al., 2013),

secretion of antimicrobial peptides (AMPs) by Paneth cells (Clevers and Bevins, 2013), and maintenance of TJs to prevent paracellular passage (Groschwitz and Hogan, 2009; Marchiando et al., 2011).

Epithelial Cell Layer and Tight Junctions

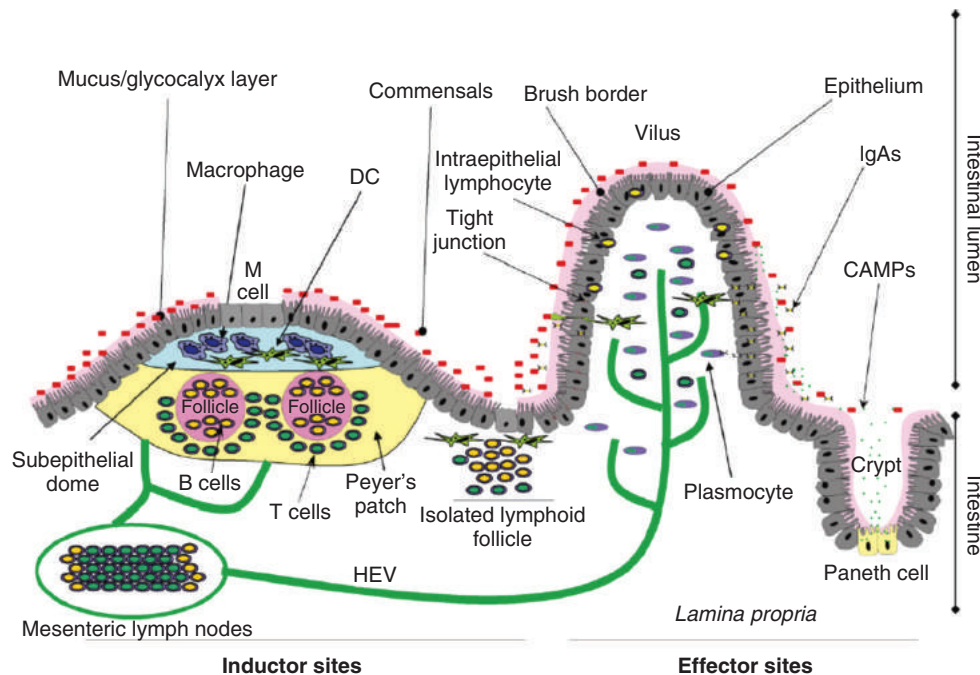
The intestinal epithelium is a single layer of cells composed of multiple cell types, including enterocytes, enteroendocrine cells, intraepithelial lymphocytes, goblet cells, Paneth cells, tuft cells, and M cells (Fig. 72.2) (Crosnier et al., 2006; Lievin-Le Moal and Servin, 2006; van der Flier and Clevers, 2009). Each of these lineages has individualized specialized functions (discussed in further detail later); together their cell-to-cell interactions and adherence form the basis of the intestinal cell barrier and the first-line defense against the potential penetration of pathogenic bacteria and antigens into the underlying mucosa.

The intestinal barrier is maintained by regulation and maintenance of two pathways: the transcellular pathway and the paracellular pathway (Groschwitz and Hogan, 2009). The paracellular pathway regulates selective transfer of fluids, electrolytes, and small peptides through the intercellular space. It is regulated by three intercellular junctions: TJs, which bind intestinal epithelial cells together near their apical surfaces and regulate the permeability of the monolayer, and the adherens junctions (AJs) and desmosomes, which anchor cells at the lateral surfaces. These junctions are multi-protein complexes that anchor the plasma membrane to the actin cytoskeleton, cluster and organize transmembrane proteins, regulate selective permeability of the epithelium, and promote regulated

extrusion of cells from the intestinal surface (Guan et al., 2011; Marchiando et al., 2011).

TJs may contain up to 40 different transmembrane proteins, including occludin, the claudins, the junctional adhesion molecules, and tricellulin (Ikenouchi et al., 2005; Suzuki, 2013). TJs connect the cell to the intracellular scaffolding through the *zonula occludens* family of proteins (ZO 1–3). In addition, they form pores that contain charged loops of amino acids, which help to regulate the size, strength, and specificity of ions that can pass across the junction (Al-Sadi et al., 2011). The AJs consist primarily of cadherin–catenin complexes (Lievin-Le Moal and Servin, 2006). While TJ proteins control selective porosity of the epithelial barrier, AJs anchor adjacent cells to one another through cytoskeletal interactions (Desai et al., 2013), coordinate cortical actin rings, and serve as a site of intracellular signaling. The AJ also seems to act as a mechanical stress sensor, allowing the plasma membrane to resist tensile forces as cells rearrange during development or homeostasis (Grashoff et al., 2010; Yonemura et al., 2010).

Disruption of junctional proteins is common in inflammatory intestinal diseases (Clark et al., 2006; Bergmann et al., 2013; Hogberg et al., 2013). Tumor necrosis factor (TNF), interferon (IFN)- γ , and nitric oxide can promote the reorganization of junctional proteins and cause barrier dysfunction, such as during necrotizing enterocolitis (NEC) (Elding et al., 2011; Hunter and De Plaen, 2014). In contrast, signaling mediators that enhance or protect TJ formation, such as the short-chain fatty acid butyrate and the amino acid glutamine (Fasano and Shea-Donohue, 2005), tend to be protective against intestinal disorders.



• **Fig. 72.2** Structural Components of the Intestinal Epithelial Barrier and Immune System. Epithelial cell layer: enterocytes, enteroendocrine cells, intraepithelial lymphocytes, goblet cells, Paneth cells, and microfold cells. Gut-associated lymphoid tissue: *inductor sites*: mesenteric lymph nodes, Peyer's patches, isolated lymphoid follicles; *effector sites*: epithelium (intraepithelial lymphocyte) and lamina propria immunoglobulin A-producing plasma cells, primed T cells, monocytes, and mast cells. *CAMPs*, Cationic antimicrobial peptides; *HEV*, high endothelial vessels; *IgAs*, immunoglobulins A; *M cell*, microfold cell. (From Magalhaes JG, Tattoli I, Girardin SE. The intestinal epithelial barrier: how to distinguish between the microbial flora and pathogens. *Semin Immunol.* 2007;19:106–115.)

Specialized Epithelial Immune Cells

Goblet cells are a major secretory cellular lineage in the intestinal epithelium that produce mucus, which is composed chiefly of mucins and inorganic salts suspended in water (Lievin-Le Moal and Servin, 2006; Johansson et al., 2013; Kandasamy et al., 2014). The mucus layer adsorbs other glycoproteins, glycolipids, and albumin, and this complex admixture forms a functional, yet highly dynamic barrier between the mucosa and the luminal microbes (Johansson et al., 2013). It also sequesters/stabilizes various bioactive molecules critical for intestinal development, homeostasis, and restitution (Lievin-Le Moal and Servin 2006; Lin et al., 2008; Johansson and Hansson, 2011; Johansson et al., 2013).

More than 20 mucin genes have been identified so far, with *MUC2* being the predominant form produced by intestinal goblet cells (Andrianifahanana et al., 2006; Kandasamy et al., 2014). In the mature intestine, goblet cells increase mucus production upon exposure to inflammatory cytokines such as TNF or bacterial products such as lipopolysaccharides, flagellin A, and lipoteichoic acids (Dharmani et al., 2009). However, in the developing intestine, many of the same inflammatory mediators have a paradoxical, inhibitory effect on mucus production (Levine et al., 1995; McElroy et al., 2011). Consistent with these data, surgically resected tissue samples of human NEC and animal models of NEC-like injury also show a reduction in mucin-positive goblet cells and decreased levels of mucin 2 (Clark et al., 2006; Khailova et al., 2009; Martin et al., 2011; McElroy et al., 2011).

In addition to mucins, intestinal goblet cells also produce intestinal trefoil factor (TFF), specifically TFF3, and resistin-like molecule- β (RELM- β) (Dharmani et al., 2009), which can augment and stabilize the mucus layer (Suemori et al., 1991; Krime et al., 2008). In the adult intestine, goblet cells are capable of presenting low-molecular-weight antigens to dendritic cells (DCs) in the lamina propria (McDole et al., 2012). While the physiologic significance and developmental regulation of this activity are not yet fully understood, it is clear that these cells may have a more complex function in the GI tract than previously appreciated.

Paneth cells are located in the crypt bases and, unlike goblet cells, do not migrate up the crypt-villus axis but remain in the crypt base (Clevers, 2013; Clevers and Bevins, 2013). These cells produce AMPs both constitutively and in response to enteric pathogen exposures (Ouellette, 2010; Salzman, 2010; Bevins and Salzman, 2011). Their position in the crypt base near stem cells allows them to protect these nondifferentiated cells from microbes and thus preserve the regenerative ability of the intestinal epithelium. Paneth cell-derived AMPs are currently categorized into two main families: defensins (α -defensins and β -defensins) and cathelicidins. The antimicrobial activity of these AMPs results from incorporation into the cell membrane and pore formation, causing solute and water influx (Ouellette et al., 1994; Salzman, 2010; Bevins and Salzman, 2011). The α -defensins, also known as cryptdins, are activated by matrix metalloprotease-7 (MMP-7) to acquire microbicidal activity (Schenk and Mueller, 2008). Other bactericidal compounds found in Paneth cells include secretory phospholipase A₂, regenerating islet-derived protein 3 gamma (RegIII- γ), MMP-7, interleukin (IL)-1, TNF- α , IL-1 β , and lysozyme (Salzman et al., 2007; Ouellette, 2010; Bevins and Salzman, 2011; Underwood, 2012; Clevers and Bevins, 2013; McElroy et al., 2013).

Paneth cell disruption can have significant consequences in the intestine, such as impaired clearance of bacterial pathogens (Sherman et al., 2005) or the disruption of normal stem cell function (Clevers, 2013; Clevers and Bevins, 2013) and has been associated with

intestinal disorders such as inflammatory bowel disease and NEC (Wehkamp 2005a, 2005b; McElroy et al., 2011). In addition to the production of AMPs, Paneth cells play important roles in epithelial cell homeostasis (Vaishnava et al., 2011), regulation of splanchnic microvasculature (Stappenbeck et al., 2002), and in the maintenance of the stem cell niche (Sato et al., 2011).

M cells reside within the epithelial cell layer, overlying organized foci of lymphoid tissue. Similar to goblet cells, M cells originate from the crypt, differentiate, and migrate up the crypt-villus axis. However, unlike their neighboring cells, M cells lack microvilli on their luminal surfaces. Rather, the luminal surface has microfolds, giving rise to its name (Mabbott et al., 2013). In addition, the basolateral membrane is invaginated to form a cytoplasmic “pocket” that typically contains lymphocytes and occasionally macrophages or other cells (Schmedtje, 1980; Jarry et al., 1989; Ermak et al., 1994). M cells play an important role in presenting luminal antigens to the cells of the immune system and have been demonstrated in the human fetus as early as 17 weeks (Jakobovits et al., 1972; Bockman and Cooper, 1975; Moxey and Trier, 1978; Braegger et al., 1992; Daya, 1993). The M-cell population expands rapidly in the first postnatal week in animal models, but these changes have not been studied in the human neonate, and their functional role in the human is not well defined (Torres-Medina, 1981; Roy and Ruiz, 1986; Wolf et al., 1987).

Monocytes, Macrophages, Dendritic Cells, and Mast Cells

Macrophages appear in the submucosa and the lamina propria as early as 10 weeks' gestation, and a sizable macrophage population can be seen during mid-gestation (Braegger et al., 1992; Maheshwari et al., 2009, 2011) that develops both from proliferation of resident macrophages and through the recruitment of circulating monocytes (Bain et al., 2014). The expansion of the macrophage population in the intestine contrasts with the lung, where the alveolar macrophage populations remain small in the fetus and expand mainly after birth (Alenghat and Esterly, 1984; Jacobs et al., 1985; Kurland et al., 1988).

In the intestine, resident macrophages are the first cells of the innate immune system to encounter bacteria that breach the epithelium and gain access to the lamina propria (Schenk and Mueller 2008; Farache et al., 2013). In the adult intestine macrophages show a unique functional dichotomy where these cells display avid phagocytic and bactericidal activities but do not show signs of activation or produce cytokines upon exposure to bacterial products (Smythies et al., 2005; Schenk and Mueller, 2008). This “inflammatory anergy” of intestinal macrophages is a result of TGF- β -mediated differentiation and serves to minimize inflammation in the intestinal mucosa despite the close physical proximity to luminal bacteria. In contrast to the adult intestine, the fetal/preterm intestine is developmentally deficient in TGF- β bioactivity (Maheshwari et al., 2011; Namachivayam et al., 2013). Consequently, macrophages newly recruited to the developing intestine tend to retain their inflammatory characteristics and the capacity to produce an exaggerated inflammatory response during bacterial translocation and NEC (Maheshwari et al., 2011).

DCs are antigen-presenting cells (APCs) that are thought to act as an intestinal sentry system by constantly surveying the intestinal lumen. In the adult intestine, DCs use their long projections to sense inflammatory signals and capture luminal antigens and then migrate to secondary lymphoid organs to interact with T-cell lymphocytes (Johansson-Lindbom et al., 2005; Jaensson et al., 2008; Bogunovic et al., 2009; Schulz et al., 2009; Farache et al., 2013). However, there are very limited data on fetal/neonatal

intestinal DCs. Human leukocyte antigen D-related (HLA-DR⁺) DC-like cells can be detected in both lamina propria as well as in the Peyer's patches after 14 weeks' gestation, but these cells may have some overlap with lamina propria macrophages (Spencer et al., 1987; MacDonald, 1996).

Mast cells appear in the intestine mainly after the postnatal microbial colonization. These cells contain large stores of preformed cytokines and chemokines and serve as sentinels that inform about exposure to bacteria or bacterial products in the mucosa (McIlwain et al., 2010). Mast cells also bind IgE via fragment crystallizable region (Fc) epsilon receptors and release histamine and serotonin. These mediators upregulate mucin production, increase intestinal permeability, contract smooth muscle cells, and promote leukocyte recruitment by releasing stored chemokines.

Gut-Associated Lymphoid Tissue and Adaptive Immunity

GALT has two main components: inductor sites and effector sites (see Fig. 72.2) (Magalhaes et al., 2007). The inductor sites represent the structures where immune responses are initiated, namely antigen uptake and processing (Neurath et al., 2002; Magalhaes et al., 2007; Bogunovic et al., 2009). It is within these structures that APCs activate CD4 T cells, CD8 T cells, and B cells. The inductor sites consist of mesenteric lymph nodes, Peyer's patches, isolated lymphoid follicles (ILFs), and cryptopatches, which are clusters of immature lymphocytes that give rise to ILFs.

Rudimentary Peyer's patches become identifiable in fetal ileum at 11 weeks as aggregates of HLA-DR⁺ CD4⁺ lymphoid cells (Spencer et al., 1985; Finke et al., 2002). T and B cells appear at 18 to 20 weeks, and distinct B-cell and T-cell zones can be identified. At 24 weeks, Peyer's patches can be identified macroscopically (Spencer et al., 1985; MacDonald and Spencer, 1994; Husband and Gleeson, 1996). Germinal centers develop after birth (Husband and Gleeson, 1996).

Effector cells are the immune cells, which, once activated, modulate downstream immune and inflammatory signaling. Effector sites are the locations where these effector cells reside; these include the epithelium and the lamina propria. The primary effector cell within the epithelium is the intraepithelial lymphocyte (IEL). IELs are T lymphocytes situated along the basolateral side of the epithelial cell layer. The IEL population is limited in the fetal intestine and develops mainly after birth (Cerf-Bensussan and Guy-Grand, 1991; MacDonald and Spencer, 1994). In contrast, lamina propria lymphocytes reach term levels by 19 to 27 weeks' gestation (Spencer et al., 1986). Functionally, intestinal T cells may take several years to mature; the T-cell receptor β -chain repertoire shows a polyclonal pattern during fetal period and infancy and undergoes gradual restriction to the oligoclonal pattern typical of adults (Williams et al., 2004). Approximately 10%–30% of the IELs may express the $\gamma\delta$ -T-cell receptor (Spencer et al., 1985). These $\gamma\delta$ -T cells may play a role in enterocyte function and antimicrobial immunity and may display cytotoxic activity (Boismenu and Havran, 1994; Komano et al., 1995; Kagnoff, 1998).

In the fetal intestine, B cells are first observed in the lamina propria at 14 weeks' gestation (Spencer et al., 1985). Most of the intestinal B cells show a mature B-cell phenotype (CD20⁺IgM⁺IgD⁺light chain⁺). Although B cells in the fetal intestine include both IgM⁺ and IgG⁺ populations (Rognum et al., 1992), microbial colonization after birth drives the expansion of the IgM⁺ (Fagarasan et al., 2001) and IgA⁺ subsets (Crabbe et al., 1970a, 1970b; Shroff et al., 1995).

Antigen Sampling and Presentation

Bacterial, dietary, and/or xenobiotic antigens that are present in the intestinal lumen are internalized and processed by several pathways involving M cells, intraepithelial DCs, and the enterocytes, which may take up antigens by nonspecific pinocytosis, receptor-mediated endocytosis, or via toll-like receptor (TLR) or nucleotide-binding oligomerization domain (NOD) receptor recognition (Fig. 72.3).

Microfold Cell and Dendritic Cell Pathway

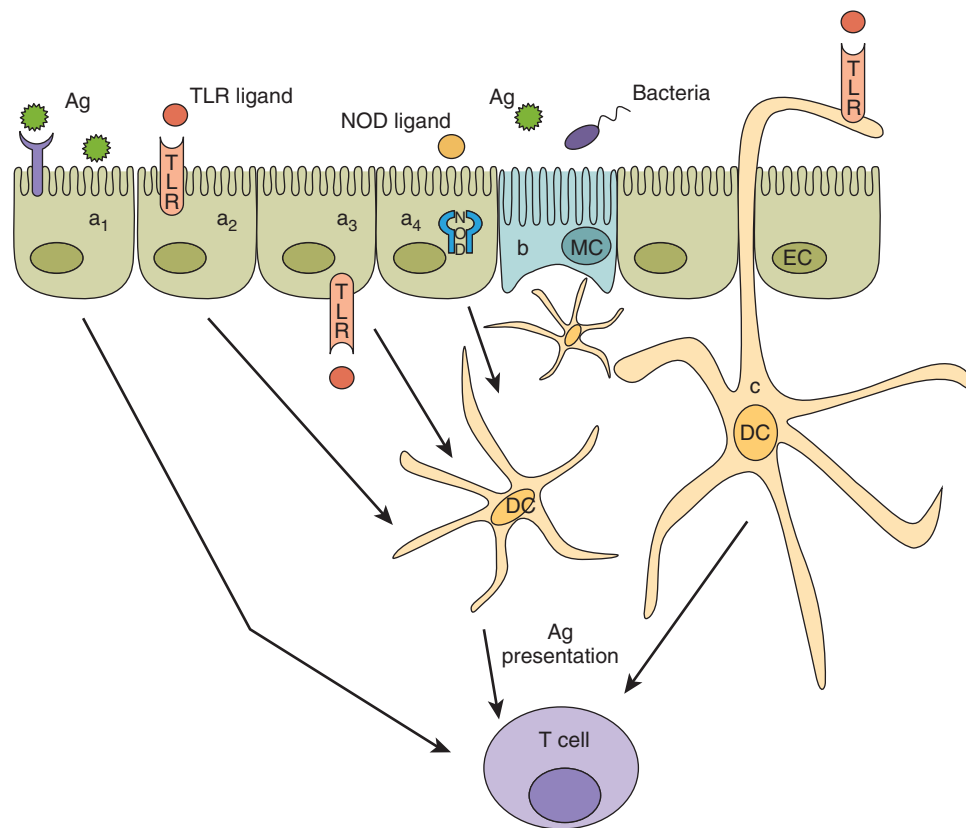
M cells reside within the single-cell epithelial layer and are uniquely positioned over organized sections of lymphoid tissue and Peyer's patches. M cells sample antigens, actively transport them into the submucosa, and present them to effector cells such as DCs and lymphocytes. This process leads to T-cell differentiation and B-cell activation. In the adult intestine, DCs are known to interdigitate their dendritic extensions across the paracellular junctions to sample luminal antigens and then migrate to lymphoid structures to participate in T-cell and B-cell activation. Similar pathways may also be at work in the neonatal intestine.

Receptor-Mediated Endocytosis and Processing: Toll-Like and Nucleotide-Binding Oligomerization Domain Receptors

Pattern recognition receptors (PRRs) such as TLRs, formylated peptide receptors, and NOD receptors recognize and bind to highly conserved specific pathogen regions or molecular motifs, commonly referred to as pathogen-associated molecular patterns (PAMPs) or microbe-associated molecular patterns. The conservation of these molecular motifs among microorganisms of the same class allows the mucosal cell to recognize most microorganisms by using a select group of PRRs. General classes of PAMPs include nucleic acids (e.g., ds ribonucleic acid in viral organisms), polypeptides (e.g., flagellin), and macromolecules (e.g., lipopolysaccharide in the bacterial cell wall of gram-negative organisms and peptidoglycan in the bacterial cell wall of gram-positive organisms) (Louis and Lin, 2009; Tanner et al., 2015).

TLRs are transmembrane proteins that are expressed on the luminal or basolateral surfaces of host defense cells. The extracellular component of leucine-rich repeat recognition domain binds to the specific PAMP, while the intracellular IL-1 receptor-like domain activates cytoplasmic proteins responsible for downstream signaling (Macdonald and Monteleone, 2005; Gribar et al., 2008; Louis and Lin, 2009). The TLR family in mammals consists of TLR1–13 proteins. TLR1–11 are conserved between human and mouse, but TLR10 is not functional in mice (Joosten et al., 2016). Specifically, the human intestine expresses TLR1–9 (Yu and Gao, 2015). Each TLR recognizes and binds with specific PAMPs, some unique to a particular TLR, and some overlapping with other TLRs. TLR4 is preferentially expressed by enterocytes within crypts, whereas TLR2, TLR3, and TLR5 are preferentially expressed on the villus enterocytes (Fusunyan et al., 2001; Winkler et al., 2007; Gribar et al., 2008). NODs are distinct from TLRs in that they reside within the cell's cytoplasm. Of special interest to the intestinal tract, NOD1 and NOD2 (also known as caspase recruitment domain 4 [CARD4] and CARD15) are expressed within intestinal APCs and epithelial cells (Winkler et al., 2007).

The PAMP–PRR complex results in the activation of several well-described cytoplasmic signaling pathways. These pathways include activation of nuclear factor kappa B (NF- κ B), mitogen-activated protein kinase (MAPK), and interferon regulatory factor



• **Fig. 72.3** Antigen Sampling. a_1 , Pinocytosis, receptor-mediated endocytosis; a_2 , apical toll-like receptor (TLR) recognition; a_3 , basolateral TLR recognition; a_4 , nucleotide-binding oligomerization domain receptor recognition; b , microfold cell pathway; c , intraepithelial dendritic cell pathway. Ag, Antigen; DC, dendritic cell; EC, enterocyte; MC, M cell; NOD, nucleotide-binding oligomerization domain; TLR, toll-like receptor. (From Winkler P, Ghadimi D, Schrezenmeier J, Kraehenbuhl JP. Molecular and cellular basis of microflora-host interactions. *J Nutr.* 2007;137:756S–772S.)

(IRF) (Louis and Lin, 2009; Neish, 2009). Modulation of these pathways involves a regulated series of phosphorylation and ubiquitination. With ubiquitination, NF- κ B, MAPK, and IRF are free to translocate into the cell's nucleus and upregulate the production of downstream molecules that combine to initiate an inflammatory response to effectively rid the body of the invading pathogen. This diverse group of molecules includes cytokines, AMPs, chemotactic messengers, adhesion molecules, and other acute-phase reactants. Concomitant with activation of these proinflammatory pathways is the stimulation of apoptotic pathways (via caspases) to assist in the removal of infected or injured cells (Fig. 72.4) (Louis and Lin, 2009; Sharma and Tepas, 2010; Gunther et al., 2013, 2014).

T-Helper 1/T-Helper 2 Polarization

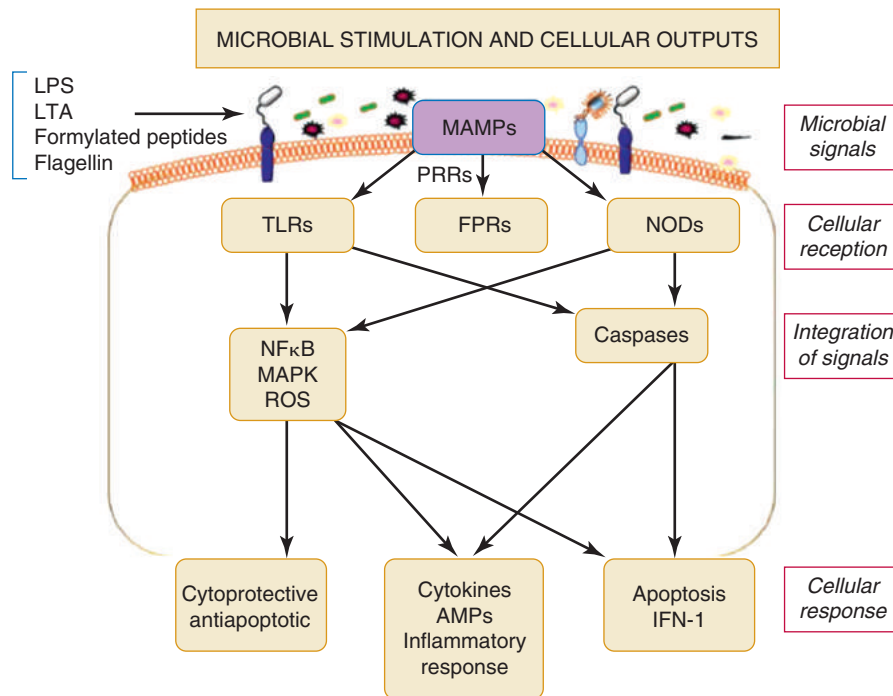
Downstream signaling mediated by PRR–PAMP complexes activates APC differentiation, which in turn modulates the differentiation of T-helper (Th) cells. The specific class of APC mediates the type of differentiation that the Th cells will undergo. For example, the CD8⁺ DC regulates the maturation of the Th cell to a Th1 cell, whereas the CD8-DC activates Th2 cells. Th1 cells increase cytokine production of IFN- γ and lymphotoxin, activate macrophages, and are associated with delayed-type hypersensitivity. Th2 cells increase the production of IL-4, IL-5, IL-9, and IL-13. Th2 cells mediate allergic responses and facilitate antibody production, mast-cell

degranulation, and eosinophil activation (Neurath et al., 2002) (Fig. 72.5).

Exogenous Exposures and Their Impact on Innate Intestinal Immune Defenses

Bacterial Colonization

The average adult human has an intestinal microbiota that consists of 10^{13} to 10^{14} microorganisms representing 800 to 1000 species of bacteria that encode millions of genes (Qin et al., 2010). The abundance of microorganisms increases distally along the intestinal tract (Martin and Walker, 2008). Interestingly, colonization profiles are unique to the location within the intestinal tract, and this is true not just across the major divisions (e.g., ileum vs colon) but also across the microstructures within the gut (e.g., microbiota within the lumen vs within the mucus layer vs within the crypts vs colonization and adherence on the intestinal epithelial cells) (Lievin-Le Moal and Servin, 2006; Romano-Keeler and Weitkamp, 2015). To effectively colonize the intestinal epithelium, bacteria adhere in a lectin-like manner to carbohydrate receptors or glycoconjugates. Glycoconjugate expression is controlled by glycosyltransferase enzymes, which are developmentally regulated, and ongoing maturation of this process is stimulated by colonizing bacteria (Walker, 2002; Forchielli and Walker, 2005).



• **Fig. 72.4** Pattern-Recognition Receptors and Pathogen-Associated Molecular Patterns (Microbial-Associated Molecular Patterns) Complex Activation and Downstream Signaling. Microbial components such as lipopolysaccharide (LPS), lipoteichoic acid (LTA), formylated peptides, and flagellin serve as microbial-associated molecular patterns (MAMPs) and signal pattern-recognition receptors (PRRs) including toll-like receptors (TLRs), formylated peptide receptors (FPRs), or nucleotide-binding oligomerization domain-like receptors (NODs). Integration of these signals evokes cellular outputs based on the initial perception of the triggering organism. Output can be a protective response to commensal microbiota or an inflammatory response to pathogenic organism(s), or it can trigger apoptosis. AMPs, Antimicrobial peptides; IFN-1, interferon-1; MAPK, mitogen-activated protein kinase; NF- κ B, nuclear factor kappa B; ROS, reactive oxygen species. (Reproduced with permission from Sharma R, Young C, Neu J. Molecular modulation of intestinal epithelial barrier: contribution of microbiota. *Jrnl of Biomedicine and Biotechnology*, vol. 2010, Article ID 305879, 15 pages, 2010. doi:10.1155/2010/305879.)

During the first week of life, the bacterial colonization profile of a healthy, full-term newborn is unstable and constitutes a simple array of organisms. This is quickly followed by a persistent, more stable colonization density in the range of 10^9 to 10^{10} per gram of stool (Favier et al., 2002; Palmer et al., 2007; Jost et al., 2012). Once the intestinal flora pattern is established, there is relative stability in this microbial population, with very few shifts in colonization profiles over time. However, shifts do occur, mainly in response to major changes in dietary intake such as introduction of solid foods or with medication use (antibiotics) (Stark and Lee, 1982; Penders et al., 2006; Palmer et al., 2007; Bezirtzoglou et al., 2011; Jost et al., 2012). Within a short period of time after the initiation of a microbial shift, stability is reestablished, and by the end of the first year of life, colonization patterns begin to mirror those seen in adults (Underwood et al., 2015). The timing of microbial colonization and the specific composition of the established microbiome vary with mode of delivery, diet, and other environmental exposures (hospital environment, antibiotic use) (Collado et al., 2015).

Mode of Delivery

At birth, the human newborn has a paucity of bacteria in its intestinal tract. After birth, however, the newborn's intestinal flora rapidly expands (Elgin et al., 2016). Newborns who are born by vaginal delivery have an initial intestinal flora that resembles

maternal vaginal flora, including *Lactobacillus* and *Prevotella* species, whereas newborns born by cesarean delivery have an initial intestinal composition that is more similar to maternal skin flora, including *Staphylococcus* and *Corynebacterium* (Biasucci et al., 2008; Dominguez-Bello et al., 2010). Once colonization does occur, patterns tend to reflect the indigenous microbial population that resides in the infant's environment, with low levels of strict anaerobes such as *Bifidobacterium* and *Bacteroides* and a higher proportion of *Clostridium difficile* (Penders et al., 2006; Adlerberth and Wold, 2009). While it has recently been suggested that the adult microbiome is relatively insensitive to some early-life events, including mode of delivery (Falony et al., 2016), differences in these populations may still play a role in susceptibility to pediatric diseases through altered developmental dynamics.

Diet

Infants fed human milk tend to demonstrate *Bifidobacterium*, a commensal organism with multiple immune benefits, as the predominant organism within their intestinal microbiota (Underwood et al., 2015). Other organisms that may be present in lesser quantities include staphylococci, streptococci, and lactobacilli and other rare anaerobes (Penders et al., 2006; Palmer et al., 2007). In contrast, the intestinal flora of formula-fed infants is more likely to have a greater number of pathogenic species such as Enterobacteriaceae, *Bacteroides* species, and *Clostridium difficile* in their

• BOX 72.2 Nutritional and Bioactive Compounds Found in Human Milk

Proteins

α -Casein
 β -Casein
 κ -Casein
 α -Lactalbumin

Lipids

Long-chain polyunsaturated fatty acid (LCPUFAs); arachidonic acid (AA); docosahexaenoic acid (DHA); eicosapentaenoic acid (EPA)
 Conjugated linoleic acid
 Cholesterol
 Milk fat globule membrane

Carbohydrates

Lactose
 Oligosaccharides
 Mucins

Enzymes

α -Amylase
 α_1 -Antitrypsin
 Bile salt–stimulated lipase
 Lactoperoxidase and myeloperoxidase
 Haptocorrin
 Folate-binding protein
 Glycoconjugates

Immune Factors

Immunoglobulins (sIgA, IgA, IgG, IgM)
 Lactoferrin
 Lysozyme
 Lactoadherin
 Nucleotides
 Toll-like receptors
 Cytokines, interleukins
 Macrophages
 Lymphocytes
 Neutrophils
 Commensal microorganisms

Growth Factors

Insulin-like growth factors (IGF-1, IGF-2)
 Epidermal growth factor (EGF)
 IGF-binding protein
 Transforming growth factor (TGF)- α , TGF- β
 Nerve growth factor
 Human growth factor
 Granulocyte colony-stimulating factor (G-CSF)
 Vascular endothelial growth factor (VEGF)
 Erythropoietin (Epo)

and to appropriately provide the essential nutrients to optimize the infant's developmental stage. For example, growth and immune factors tend to be higher in preterm milk than in term milk (Walker, 2010; Donovan et al., 2012).

Factors present in breast milk have been shown, in piglet and rodent models, to contribute to the ongoing structural development of the intestinal tract. When provided breast milk (especially colostrum) versus formula or in the absence of any enteral substrate, animals receiving breast milk demonstrate an increase in mucosal

mass and villus height (Sangild, 2006). Morphologic studies of human infants to examine the potential benefits of breast milk are more difficult to perform for obvious reasons. However, studies evaluating diet and intestinal barrier function have been performed in human infants and can be used as an indirect measure for morphologic development and integrity. Indeed, infants fed predominantly formula versus breast milk demonstrated increased intestinal permeability, suggesting compromise in intestinal barrier function (Taylor et al., 2009).

Over time, the composition of formula has been iteratively modified; however, formula continues to fall short of being able to provide the multitude of factors that are present in breast milk, and thus infants fed formula fail to share the same health benefits seen in breastfed infants. Infants fed predominantly breast milk have a reduced incidence of infections (e.g., diarrhea, upper respiratory illness, otitis media, urinary tract infections, bacteremia) (Quigley et al., 2009; Hengstermann et al., 2010), atopic illnesses such as eczema and asthma (Bener et al., 2007), and autoimmune diseases such as diabetes mellitus (Pereira et al., 2014). In preterm infants, those fed breast milk are less likely to develop infections (Hylander et al., 1998) and 10 times less likely to develop NEC compared with infants fed formula (Lucas and Cole, 1990).

Impact of Altered Mucosal Immunity on Early and Later Disease

Developmental immaturity of the immune system, hospital environmental exposures, and medical interventions combine to hamper postnatal development of innate and mucosal immunity in the preterm infant (Table 72.3) (Martin and Walker, 2006). These factors pose potential threats to the preterm infant, particularly to conditions mediated by altered mucosal immunity and intestinal bacterial colonization.

Prematurity and Necrotizing Enterocolitis

Inadequate Physical and Chemical Barriers

The first line of defense of nonspecific physical and chemical barriers is often disrupted because of inadequate production of gastric acidity, digestive enzymes, mucus, Igs, and decreased peristalsis. In addition, common medication exposures (antibiotics, histamine H₂ receptor blockers, vasoconstrictors, sedatives, and paralytics) decrease these inherent defenses by decreasing expression of antibacterial peptides altering natural acidity and decreasing peristalsis (Schumann et al., 2005; Louis and Lin, 2009). As a result, there is a reduced ability to eliminate pathogenic organisms, allowing for increased epithelial adherence and bacterial translocation. Inadequate barrier development is further exacerbated by induced mucosal and villus atrophy that probably develops as a consequence of prolonged absence of enteral feedings (Hartl and Alpers, 2011).

Abnormal Colonization of the Preterm Gut

Although it was once thought that the in utero environment was sterile, bacteria have been identified in early meconium samples in preterm infants, suggesting a potential prenatal influence on early intestinal bacterial colonization (Elgin et al., 2016). Once a broader microbial community has been established, the fecal organisms commonly observed in preterm infants include enterococci, members of Enterobacteriaceae, *Escherichia coli*, staphylococci, streptococci, *Clostridium* species, and *Bacteroides* species (Stark

TABLE 72.3 Neonatal Intensive Care Unit Exposures and Potential Consequences on Intestinal Barrier Defense and Bacterial Colonization in Premature Infants

| | Exposure | Potential Consequences |
|-----------------------------|--|---|
| Nonspecific barrier defense | Prematurity | ↓ immunoglobulin levels ↓ production of digestive enzymes ↓ production of mucus Dysfunctional peristalsis |
| | Delayed feeding | Villous atrophy ↓ production of digestive enzymes ↓ production of mucus ↓ peristalsis |
| | Medications | |
| | Histamine H ₂ receptor blockers | ↓ gastric acidity |
| | Vasopressors and Indocin | ↑ risk for intestinal ischemia and enterocyte injury |
| | Sedatives and paralytic agents | ↓ peristalsis |
| Bacterial colonization | Prematurity | Accentuated inflammatory response Abnormal glycosylation pattern |
| | Delayed feedings | Delay in bacterial colonization |
| | Broad-spectrum antibiotics | Prolonged sterilization of gut Delayed colonization of beneficial, commensal bacteria Preferred bacterial colonization of pathogenic bacteria |
| | Formula feeding and hospitalization | Preferred bacterial colonization of pathogenic bacteria |

From Martin CR, Walker WA. Intestinal immune defences and the inflammatory response in necrotising enterocolitis. *Semin Fetal Neonatal Med.* 2006;11:369–377.

and Lee, 1982; Palmer et al., 2007; Jost et al., 2012; La Rosa et al., 2014; Elgin et al., 2016). This bacterial profile is distinctly different from those seen in breastfed term infants, yet similar to patterns exhibited by formula-fed full-term infants (Schwartz et al., 2003; Arboleya et al., 2012a). However, in contrast to formula-fed term infants, the colonization by these pathogenic bacteria persists longer (Arboleya et al., 2012a, 2012b, 2015), and there is slow development of increased species diversity with a nonrandom progression toward a *Clostridium*-dominated biome (Schwartz et al., 2003; La Rosa et al., 2014).

The differences in bacterial colonization patterns between healthy, full-term infants and preterm infants can be partially explained by the inadequate physical and chemical barriers described above, which allow for greater penetration and adherence by pathogenic organisms. Additional key factors include immaturity of epithelial glycoconjugate expression (an important modulator of bacterial

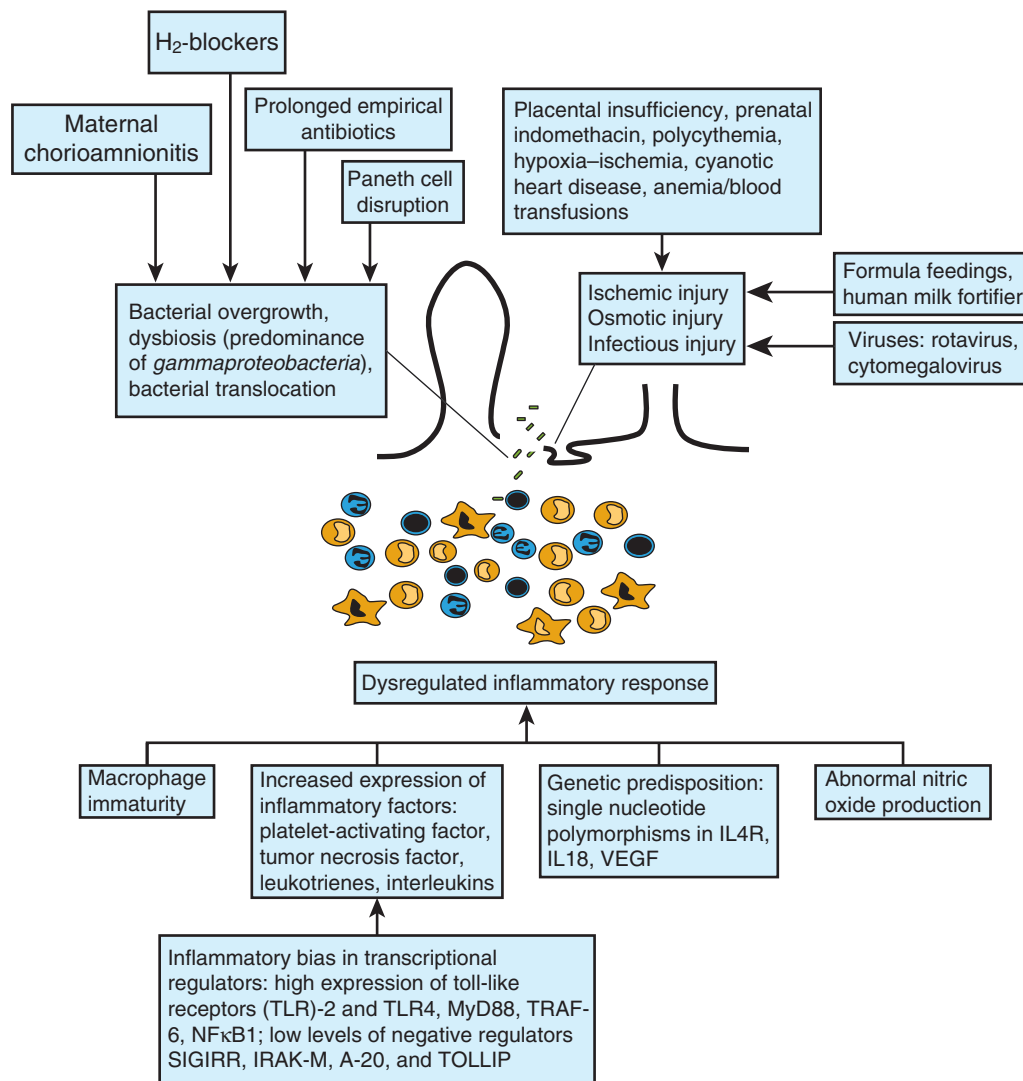
adherence) and the unique environmental exposures experienced by the preterm infant. Almost universally, preterm infants experience delayed enteral feedings, are exposed to early and prolonged broad-spectrum antibiotics, and are introduced to residential hospital flora (Elgin et al., 2016). Each of these factors contributes to delayed intestinal colonization by commensal, nonpathogenic bacteria, a predominance of pathogenic bacteria, and a lack of microbial diversity.

Necrotizing Enterocolitis

NEC is a devastating GI disease predominantly observed in preterm infants. The pathogenesis is complex and not well-understood. Current evidence suggests that NEC occurs in the preterm intestine when a dysfunctional or anatomically disrupted epithelial barrier allows bacterial translocation into the bowel wall, where these bacteria and their products induce a highly exaggerated and dysregulated inflammatory response. Fig. 72.6 links some of the clinical antecedents and plausible pathophysiologic events in NEC. In this schematic, critical host factors (prematurity), environment (ischemic/osmotic/infectious factors/agents that can produce mucosal injury), and agent factors (bacterial overgrowth, dysbiosis with proteobacterial blooms) are required. As discussed, the preterm infant possesses and experiences multiple perturbations to postnatal intestinal and immune development, all of which probably work in concert with one another to increase the vulnerability of the preterm infant to NEC. Just a few examples that illustrate this are the increased prevalence in formula-fed infants (lack of exposure to the bioactive compounds found in breast milk), the association of increased proteobacterial blooms observed in the microbiome composition in infants who developed NEC versus control infants (Claud et al., 2013; Morrow et al., 2013; Gritz and Bhandari, 2015; Lu and Ni, 2015; Warner et al., 2016), the reduction of NEC with the use of probiotics (Neu, 2014; Patel and Denning, 2015), and the increased incidence of NEC after exposure to prolonged antibiotics (delayed bacterial colonization with preference for pathogenic microorganisms) and to H₂ blockers (decrease gastric acidity, thus dampening one component of the first line of defense against pathogenic antigens provided by the intestinal tract) (Guillet et al., 2006; Cotten et al., 2009).

Atopic Diseases

The incidence of allergic diseases has dramatically increased over the past several decades in developed countries. A potential mechanism for this phenomenon has been termed the *hygiene hypothesis* (Bach, 2002; von Mutius, 2007). In this model, interaction with microbes and subsequent colonization by these organisms are essential in training mucosal immunity. With colonization of a diverse microbial population, the newborn shifts from a predominant Th2 cellular response (conditioned during fetal development to prevent maternal rejection of the fetus) (Morein et al., 2007) to a Th1 cellular response. However, in developed countries with improved hygiene and sanitation, the diversity of microbes in the intestinal ecosystem is lacking, and Th2 cellular responses continue to dominate (Tlaskalova-Hogenova et al., 2002). Supporting the vital contribution that the intestinal ecosystem plays in development of allergic responses are the observations of different colonization patterns observed in atopic subjects versus healthy controls (Bjorksten, 2004; Vaarala et al., 2008) and the reduction of atopic diseases with probiotic supplementation (Bjorksten, 2005). Other defects in mucosal immunity observed are that allergic



• **Fig. 72.6** Clinical Antecedents and Likely Pathophysiologic Hits in Necrotizing Enterocolitis. *IL*, Interleukin; *MyD88*, myeloid differentiation primary-response gene 88; *NF-κB1*, nuclear factor kappa B1; *VEGF*, vascular endothelial growth factor. (From Kasivajula H, Maheshwari A. Pathophysiology and current management of necrotizing enterocolitis, *Indian J Pediatr*. 2014;81:489–497.)

individuals have increased intestinal permeability and a deficiency of IgA.

Inflammatory Bowel Disease, Celiac Disease, and Type 1 Diabetes

Alterations in several areas of innate mucosal immunity play a significant role in the pathogenesis of Crohn's disease, celiac disease, and type 1 diabetes mellitus. Individuals with Crohn's disease have impaired intestinal barrier function, differential expression in TLR3 and TLR4, and Th1-dominated cellular responses. Differential expression in TLRs and Th1-dominated cellular responses probably contribute to the inappropriate, excessive inflammatory response to antigenic stimuli (toward *both* commensal and pathogenic bacteria), which is one of the hallmarks of this disease (Neurath et al., 2002; Macdonald and Monteleone, 2005; Sartor, 2008). An imbalance in Th1/Th2 cellular responses in Crohn's patients is further suggested by the finding that a genetic mutation in the

NOD2 gene, produces a protein that in its native form inhibits TLR2 activation, and is more common in patients with Crohn's disease than in controls (O'Neill, 2004; Strober et al., 2014). Impaired intestinal barrier function has been well described in Crohn's disease, though whether barrier defects are initiating events or amplifiers of the pathogenesis remains controversial.

The presence of a "leaky gut" may be important in the pathogenesis of autoimmune diseases such as celiac disease and type 1 diabetes mellitus. The process by which a breach in intestinal barrier function leads to celiac disease has become the generic disease model of intestinally mediated autoimmune disorders. Gliadin (the protein in wheat, barley, and rye) activates myeloid differentiation primary-response gene 88, which, in turn, upregulates the release of zonulin. Zonulin upregulation opens the TJCS, allowing for intraepithelial passage of gliadin into the intestinal submucosa to interact with effector immune cells and increase the production of proinflammatory cytokines, ultimately leading to intestinal inflammation and cell damage. Removal of gluten from the diet

halts this process, allowing for restoration of intestinal barrier function and intestinal recovery (Stein and Schuppan, 2014). The interplay among altered intestinal bacterial colonization, impaired barrier function, and a dysregulated proinflammatory response has also been implicated in the pathogenesis of type 1 diabetes mellitus (Mejia-Leon and Barca, 2015). Similar to subjects with Crohn's disease, compared with controls, individuals with type 1 diabetes mellitus have impaired intestinal barrier function (Li and Atkinson, 2015). Altered barrier function has also been shown in animal models that eventually develop diabetes. Diabetic animals have decreased expression of claudin, an important protein in TJ complexes, before the onset of disease. Finally, examination of intestinal biopsies from children with type 1 diabetes mellitus demonstrated an increased presence and expression of inflammatory cells and biomarkers (e.g., HLA class II molecules, intercellular adhesion molecule-1, IL-4, IL-1 α) within the villus architecture.

Obesity

Altered bacterial colonization patterns are evident in obesity. In animal models of genetic obesity, obese mice have distinctly different microbial colonization patterns compared with their lean littermates, with bacteria counts higher in Firmicutes and lower in Bacteroidetes. These microbial shifts are also seen in diet-induced animal models of obesity by placing the mice on a prolonged Western diet (Reinhardt et al., 2009). In humans, childhood obesity is increased in formula versus breastfed infants. In addition, as discussed earlier, different microbial colonization patterns exist between these two groups, suggesting that their ecosystem may play a role in the propensity for obesity. In a longitudinal study of childhood obesity, infants who later developed obesity, compared with infants who maintained a normal weight in childhood, had a fecal microbial pattern that contained lower concentrations of *Bifidobacterium* and higher concentrations of *Staphylococcus aureus*. Obesity has been associated with low-grade chronic inflammation; thus the reduction of the commensal organism *Bifidobacterium* and the increased presence of the pathogenic organism *Staphylococcus aureus* may, in part, contribute to the pathogenesis of chronic inflammation (Kalliomaki et al., 2008).

The intestinal ecosystem is an important modulator of the host's metabolic activities, some of which are important to the pathogenesis of obesity. Metagenomic studies of the microbiota in obese patients demonstrate an increased presence of genes that are involved in energy harvest. Other metabolic pathways mediated by the microbiota include increased hepatic lipogenesis, for example, decreased fatty acid oxidation in skeletal muscle and lipopolysaccharide-mediated chronic inflammation in adipose tissue (Reinhardt et al., 2009).

Conclusion

The GI tract serves many complex nutritional and immune functions critical for survival. The morphologic development of the intestinal tract to serve these specialized functions begins in early embryogenesis and continues throughout childhood. Exposure to amniotic fluid followed by postnatal exposures to human milk, formula, and environmental microorganisms is essential for the continued anatomic differentiation of the intestinal tract and development of mucosal immunity. Ultimately, multiple layers of immunologic defenses and a balance between oral tolerance and inflammatory responsiveness are established and maintained throughout the life of the host. Perturbations to this balance or to any component of the multiple layers of mucosal defenses increase the vulnerability of the host to atopic diseases, autoimmune disorders, or conditions mediated by chronic, unregulated inflammation.

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Suggested Readings

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Abdominal Wall Defects

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KEY POINTS

- Omphalocele is a congenital midline abdominal wall defect of the umbilical region. The bowel, liver, and other organs that herniate out of the abdomen are covered by a membrane. The umbilical cord inserts into the omphalocele membrane.
- Gastroschisis is a congenital abdominal wall defect located just to the right of the umbilicus. The bowel that herniates out of the abdomen is not covered by a membrane.
- Fetuses and newborns with an omphalocele have a high risk of associated anomalies, especially chromosomal abnormalities and cardiac malformations.
- Fetuses and newborns with a gastroschisis have a low risk of associated anomalies, except for a 10%–25% risk of intestinal atresia.
- The clinical outcomes of patients with omphalocele are mainly determined by their associated anomalies.
- The clinical outcomes of patients with gastroschisis are mainly determined by their intestinal function.

This chapter discusses some of the congenital and acquired abdominal wall problems seen in newborns. The chapter begins with a brief review of abnormalities of the umbilical cord seen on prenatal imaging; this is followed by a discussion of abnormalities of the umbilicus seen after birth. The remainder of the chapter is a review of congenital abdominal wall defects and hernias. It concentrates on gastroschisis and omphalocele, which are the two most common congenital abdominal wall defects seen in the neonatal intensive care unit (NICU). This discussion is followed by a brief review of less common congenital abdominal wall defects. The chapter finishes with a brief consideration of abdominal wall hernias.

Umbilical Cord Abnormalities

The umbilical cord is the direct connection between the placenta and the fetus. It normally consists of an umbilical vein and two umbilical arteries encased in a gelatinous matrix known as Wharton jelly that is covered with a layer of amnion. Abnormalities of the umbilical cord have become more frequently recognized as prenatal imaging has become more common and more detailed. Even when umbilical cord abnormalities are not problematic, they are often associated with other pathologic conditions that result in poor fetal and neonatal outcomes. Therefore when abnormalities of the

umbilical cord are found, it usually prompts further investigation of the fetus for chromosomal abnormalities and structural malformations. In addition, many fetuses with umbilical cord abnormalities will need more frequent evaluations of fetal well-being ([Moshiri et al., 2014](#)). Several umbilical cord abnormalities are reviewed later, although many more umbilical abnormalities have been described.

Noncoiled Umbilical Cord

Almost 5% of umbilical cords are noncoiled or hypocoiled, making it one of the most common umbilical cord abnormalities. The umbilical coiling index (UCI) was developed and gestational age standards determined to quantify the degree of umbilical cord coiling ([de Laat et al., 2005](#)). A fetus with a UCI less than the fifth percentile for gestational age has an increased risk of intrauterine growth restriction (IUGR), preterm labor, fetal distress during labor and delivery, meconium-stained amniotic fluid, stillbirth, and chromosomal aberrations ([Mittal et al., 2015](#)), although these relationships are controversial ([Khong, 2010](#); [Jessop et al., 2014](#)).

Single Umbilical Artery

A single umbilical artery is also a relatively common anomaly seen in 0.5%–1% of pregnancies ([Pomeranz, 2004](#); [Granese et al., 2007](#); [Jessop et al., 2014](#)). It is associated with stillbirth, IUGR, and multiple gestation ([Voskamp et al., 2013](#)). Although a single umbilical artery is not diagnostically specific or sensitive for any specific congenital anomaly, fetuses with a single umbilical artery are at an increased risk of congenital malformations, especially genitourinary malformations and chromosomal anomalies ([Voskamp et al., 2013](#)). When a single umbilical artery is discovered after birth, it is not clear if the newborns need specific evaluation beyond a routine physical examination ([Deshpande et al., 2009](#)).

Umbilical Vessel Dilatations

Focal dilatations of the umbilical vessels are much less common than a single umbilical artery. Focal dilatations of the umbilical vein are known as umbilical vein varices, and similar focal dilatations of the umbilical artery are known as umbilical artery aneurysms. Both conditions are rare but seem to be associated with fetal and chromosomal anomalies and also with an increased risk of adverse fetal outcomes ([Mankuta et al., 2011](#); [Doehrman et al., 2014](#)).

Umbilical Cord Cysts

Umbilical cord cysts can be true cysts that are epithelial-lined remnants of the vitelline duct, also known as the omphalomesenteric duct (Heifetz and Rueda-Pedraza, 1983) or allantois, which forms the urachus. Alternatively, umbilical cord cysts may be pseudocysts that are fluid collections in the cord not surrounded by epithelium (Sepulveda et al., 1999). Pseudocysts are more common than true cysts and their presumptive cause is either degeneration of Wharton jelly, pooling of cord edema, or liquefaction of an umbilical cord hematoma. Umbilical cord pseudocysts discovered during the first trimester are relatively common and often spontaneously resolve, but pseudocysts that persist into the second trimester are associated with fetal aneuploidy and malformations (Ross et al., 1997). Umbilical cord cysts are usually small but if they are large they may lead to compression of umbilical cord blood flow and require aspiration.

Umbilical Cord Hematomas

Umbilical cord hematomas may be a result of intentional or unintentional umbilical cord puncture during amniocentesis or less commonly occur spontaneously. Most umbilical cord hematomas do not cause any problems but some are of a size and position to impair blood flow through umbilical vessels (Sepulveda et al., 1995).

Umbilical Abnormalities

Umbilical abnormalities seen in newborns usually present with an umbilical mass or with umbilical drainage. The most common umbilical abnormality is acquired granulation/granuloma tissue. Other abnormalities include delayed separation of the umbilical cord, umbilical and periumbilical infections, and congenital remnants of the urachus and omphalomesenteric duct.

Delayed Separation of the Umbilical Cord

After birth the umbilical cord usually dries out and falls off within a week, but there is considerable variability in the timing of this separation. Some of the variability in umbilical cord separation depends upon how the cord is cared for after birth since bacterial colonization seems to play an important role in the process. This explains why cords that are treated with more intensive antiseptic regimens typically fall off later than cords that are simply kept clean and dry (Mullany et al., 2013).

There is no standard definition of “delayed separation of the umbilical cord,” but in the United States if cord separation takes more than 3 weeks then it raises concerns for immune deficiency, urachal anomalies, or infection (Razvi et al., 2001). Leukocyte adhesion deficiency should be suspected when a newborn presents with delayed cord separation and soft tissue infection around the umbilical stalk (van de Vijver et al., 2013). However, it is important to remember that most infections associated with the umbilical cord are not associated with immune deficiencies.

Umbilical and Periumbilical Infections

The lack of an epithelial barrier at the unhealed umbilicus, normal bacterial colonization of the skin, and potential contamination of the area during cord care make the umbilicus vulnerable to bacterial invasion. Newborns with unimmunized mothers and improper cord care are at risk for neonatal tetanus, which can be a significant

problem in less developed countries (Razvi et al., 2001; Khan et al., 2013).

More typical bacterial soft tissue infections of the periumbilical region are known as omphalitis. Omphalitis ranges in severity from simple cellulitis to necrotizing soft tissue infection of the abdominal wall. The severity of infection depends upon host defenses, the virulence of the infecting organism, and the depth of invasion. In developed countries the incidence of omphalitis is less than 1%, but in less developed countries the incidence is much higher (Sinha et al., 2015). Omphalitis is often a polymicrobial infection with *Staphylococcus*, *Streptococcus*, aerobic gram-negative rods, and anaerobes (Fraser et al., 2006). Treatment of omphalitis includes broad-spectrum antibiotics and, for invasive infections, surgical debridement of involved tissues. Omphalitis can be a serious infection causing life-threatening systemic sepsis. The infection can also spread up the umbilical vein and cause inflammation and thrombosis of the portal vein that may evolve into symptomatic portal hypertension years later (Fraser et al., 2006).

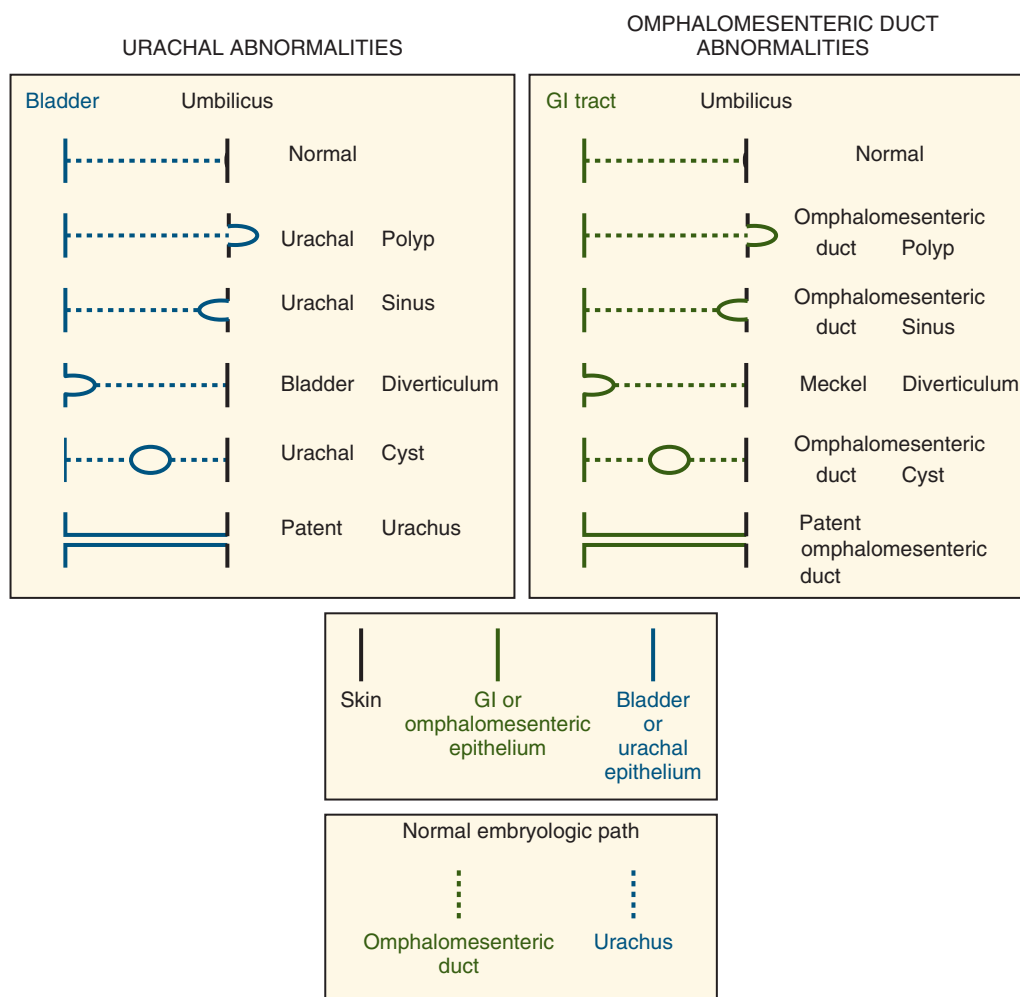
Umbilical Granulomas

Umbilical granulomas are the result of persistence and hypertrophy of the normal granulation tissue present at the base of the umbilicus after the umbilical cord separates away from the body wall. Histologically, granulomas are composed of fibroblasts and capillaries. Umbilical granulomas are the most common cause of an umbilical mass and umbilical drainage in an infant. Granulomas usually present as small (<1 cm in diameter) moist, pink, or red masses at the umbilicus. They may have serosanguineous drainage or fibrinous exudate that can be confused with the drainage of a soft tissue infection. Most small granulomas will spontaneously dry out, and the surrounding skin will contract and heal the umbilicus (Pomeranz, 2004; Snyder, 2007). Sometimes skin will grow over the granuloma and, as the inflammatory tissue shrinks, eventually result in a skin tag. These skin tags usually become smaller over time. Silver nitrate cauterization of the granulation tissue will eliminate or decrease the size of the umbilical granuloma and allow the skin to heal. Surgical excision is usually not necessary unless the lesions persists (Snyder, 2007).

Persistent Remnants of Urachus and Omphalomesenteric Duct

The urachus and omphalomesenteric duct are embryologic structures of the umbilical cord that normally regress or obliterate. When they persist, the persistent remnants may cause problems. The urachus is the remnant of allantois, which in the first weeks of development is a protrusion of the inner or endodermal surface of the embryo that connects to the yolk sac (which is in the body stalk), which later becomes the umbilical cord. The connection point of the allantois to the developing embryo is part of the hindgut that eventually becomes the bladder. The omphalomesenteric duct (also known as the vitelline duct) is also a protrusion of the inner endodermal surface of the embryo that connects to the yolk sac, but the connection point of the omphalomesenteric duct is to the midgut. The omphalomesenteric duct usually disappears by the ninth week of gestation.

Persistent remnants of the urachus and omphalomesenteric duct may present with drainage from the umbilicus, with an umbilical or periumbilical mass, or with both. The size and location of the persistent remnant often determine the clinical presentation more than the remnant's embryologic origin. The individual



• **Fig. 73.1** Schematic of the Types of Urachal and Omphalomesenteric Duct Abnormalities.

pathologic types of remnants are schematically represented in [Fig. 73.1](#) and include umbilical polyps, sinuses, fistulae, cysts, and bands. These types may exist alone or in combination.

Umbilical polyps are much less common than umbilical granulomas as a cause of an umbilical mass or umbilical drainage. An umbilical polyp is an epithelial remnant of the omphalomesenteric duct or the urachus that persists at the skin level and presents as a moist, bright red, round mass. The serous exudate or mucous secretions of the epithelium account for the umbilical drainage. Unlike the granulation tissue of an umbilical granuloma, the epithelium of an umbilical polyp will not respond to silver nitrate cauterization, and surgical excision is required.

A partial, persistent remnant of the urachus or omphalomesenteric duct that is open to the skin at the umbilicus is known as an urachal sinus or an umbilical (or omphalomesenteric) sinus (see [Fig. 73.1](#)). Similar to umbilical polyps, sinuses present with umbilical drainage and require surgical excision. A partial, persistent remnant of the urachus or omphalomesenteric duct that is open to the lumen of the bladder or gastrointestinal (GI) tract is known as a bladder diverticulum or Meckel diverticulum, respectively (see [Fig. 73.1](#)). A partial, persistent remnant of the urachus or omphalomesenteric duct that does not connect to the skin or the underlying bladder or bowel lumen is known as a urachal or omphalomesenteric cyst, respectively (see [Fig. 73.1](#)) and presents as a midline mass in the abdominal wall at or near the umbilicus. Cysts may also present



with increasing size, pain, tenderness, and fever if they become infected. Cysts require excision, although if infected they may first require incision and drainage and then interval excision.

A complete persistent remnant of the urachus or omphalomesenteric duct is a fistula between the umbilicus and bladder or bowel, respectively (see [Fig. 73.1](#)). When the fistula is connected to the bladder, it is known as a patent urachus, and it may drain urine. When the fistula is connected to the GI tract, it is known as an omphalomesenteric (or vitelline) duct fistula and may drain enteric contents. Treatment for fistulae includes surgical excision and closure of the bladder or bowel, respectively ([Snyder, 2007](#)).

Abdominal Wall Defects

This section reviews gastroschisis and omphalocele, which are the two most common abdominal wall defects seen in the NICU, and briefly discusses some less common abdominal wall defects.

Gastroschisis and omphalocele are similar in that both are congenital abdominal wall defects that present with herniation of abdominal contents outside of the body. Both entities require reduction of the herniated contents back into the abdominal cavity and closure of the abdominal wall defect. However, gastroschisis and omphalocele are distinct conditions with important differences in anatomy and associated conditions that account for their unique management and outcomes ([Fig. 73.2](#)).

| | GASTROSCHISIS | OMPHALOCELE |
|---------------------------------------|---|--|
| |  |  |
| Location of abdominal wall defect | Right of umbilical cord | Midline, includes umbilical cord |
| Covering membrane | No | Yes |
| Umbilical cord insertion | Normal | Into omphalocele membrane |
| Herniated organs | | |
| Bowel | Always | Common |
| Liver | Uncommon for more than an edge to be out | Common for large amount of liver to be out |
| Associated anomalies | | |
| Chromosomal | Rare | Common |
| Syndromes | Rare | Common |
| Cardiac | Uncommon, 2%–5% | Common |
| Bowel atresia | 10%–25% | Rare |
| Major determinant of clinical outcome | Condition and function of bowel | Associated anomalies |

• **Fig. 73.2** Gastroschisis Versus Omphalocele.

Gastroschisis

Gastroschisis is a circular abdominal wall defect just to the right of a normally inserted umbilical cord with herniation of a variable amount of intestine and possibly parts of other organs outside the abdominal cavity. There is no covering membrane, and the intestine is exposed directly to amniotic fluid prenatally and to the air after birth (see Fig. 73.2). It is unusual for a significant amount of liver to herniate out of a gastroschisis defect. Rarely, the gastroschisis defect is in a mirror-image position on the left side of the umbilicus.

Gastroschisis may be classified as either “simple” or “complex.” Gastroschisis is “complex” when there is an associated intestinal atresia, segmental or midgut volvulus, ischemic bowel, intestinal perforation, or necrotizing enterocolitis. Vanishing gastroschisis is a form of complex gastroschisis where the abdominal wall muscle around the gastroschisis defect closes in utero and strangulates the bowel (Fig. 73.3). Babies with vanishing gastroschisis may have

little or no viable bowel outside the abdomen and suffer from short bowel syndrome.

The prevalence of gastroschisis is 2.3 to 4.4 cases in 10,000 live births (Kirby et al., 2013). There is no gender predilection in gastroschisis, but the incidence is higher in Hispanic and non-Hispanic white families (Jones et al., 2016). For unknown reasons, the incidence of gastroschisis around the world is increasing. In the United States, the incidence nearly doubled from 1995 to 2005 (2.3 to 4.4 per 10,000 live births) (Parker et al., 2010), and there was a 30% increase in incidence from 1995 to 2005 to 2006 to 2012 (Jones et al., 2016).

Gastroschisis is usually an isolated defect that occurs sporadically. The etiology is probably multifactorial. The most consistent risk factor for gastroschisis is young maternal age. Mothers under the age of 20 years have a severalfold increased risk of carrying a baby with gastroschisis (Loane et al., 2007; Chabra et al., 2011; Skarsgard et al., 2015). Numerous other maternal factors have been linked with gastroschisis including recreational drugs, low body mass



• **Fig. 73.3** Vanishing Gastroschisis. Note that the gastroschisis defect has closed completely, leading to severely constricted mesenteric blood supply and intestinal atresia and a tiny, isolated blind loop on the outside of the abdominal wall. Inside the abdomen there is a proximal small bowel atresia that is completely separated away from the closed end of the remaining distal bowel.

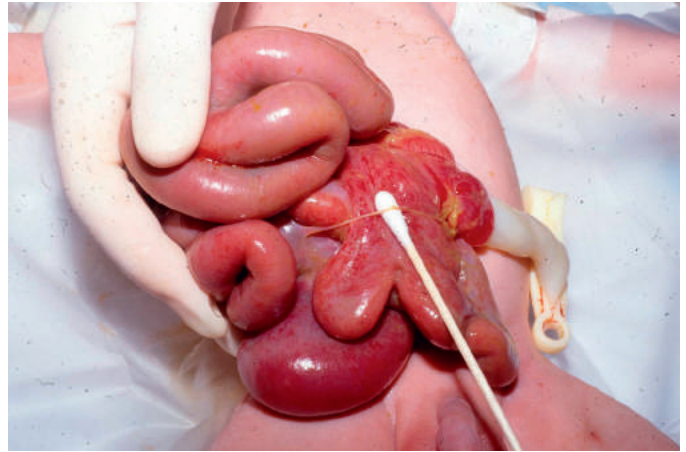
index, cigarette smoking, antidepressants, unmarried status, and genitourinary infections during gestation (Centers for Disease Control and Prevention, 2007; Parker et al., 2010; Hook-Dufresne et al., 2015). The tendency of gastroschisis to occur in clusters suggests the influence of teratogens, and population-based studies have shown a higher incidence of gastroschisis in areas where a common agricultural chemical (atrazine) is used and surface water concentrations of atrazine are elevated (Mattix et al., 2007; Waller et al., 2010).

The embryology of gastroschisis is not completely defined; however, several theories have been postulated, including (1) failure of the lateral right ventral fold to unite with other body wall folds at the umbilicus or (2) failure of development of the umbilical coelom as the early gut elongates (Feldkamp et al., 2007; Sadler, 2010). There is no known specific genetic etiology for gastroschisis, although there are rare case reports of families with multiple affected offspring.

Unlike omphalocele, extraintestinal anomalies are not commonly associated with gastroschisis. In a large international study 4.5% of the participants had central nervous system anomalies, 2.5% had cardiovascular anomalies, 2.2% had limb anomalies, and 1.9% had kidney anomalies (Mastroiacovo et al., 2007). Anomalies of the intestine are the most common associated malformations in patients with gastroschisis. Nearly all babies with gastroschisis have intestinal malrotation, since the bowel did not return to the abdominal cavity normally during fetal development and had no chance for normal rotation and fixation to the retroperitoneum. Intestinal atresia occurs in 10%–25% of babies with gastroschisis (Kronfli et al., 2010; Ghionzoli et al., 2012) and is probably caused by the contracting abdominal wall (see Fig. 73.3) or associated bands that can impair blood flow to the bowel (Fig. 73.4).

With routine prenatal care in the developed world, about 90% of gastroschisis cases are diagnosed prenatally. The maternal alpha fetoprotein (AFP) level is usually elevated with fetal gastroschisis (Saller et al., 1994), and the diagnosis can be confirmed early in the second trimester by prenatal ultrasound. Diagnostic prenatal ultrasound findings of gastroschisis are extraabdominal loops of bowel without a covering sac.

Gastroschisis can be difficult to differentiate from a ruptured omphalocele. Findings that favor the diagnosis of gastroschisis rather than a ruptured omphalocele are a relatively small abdominal



• **Fig. 73.4** Gastroschisis. Gastroschisis with a band from mesentery to the umbilicus. The cotton-tipped applicator stick is beneath the band.

wall defect (usually <4 cm in diameter), the absence of liver protruding outside the body wall, and an umbilical cord that is normally inserted into the body wall just to the left of the defect.

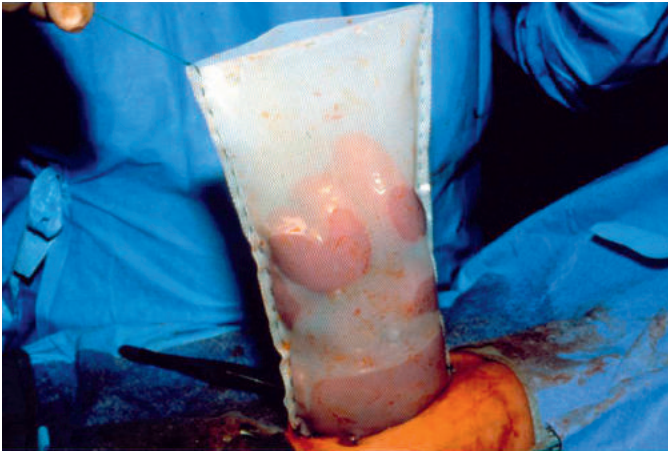
Patients with gastroschisis are best cared for by a multidisciplinary team of maternal fetal medicine specialists, neonatologists, and pediatric surgeons. Prenatal consultation with this multidisciplinary group is strongly recommended. Fetuses with gastroschisis have a significant risk of IUGR, spontaneous preterm labor, and fetal demise (Carpenter et al., 1984; Netta et al., 2007; Santiago-Munoz et al., 2007; Barseghyan et al., 2012). Karyotype of the fetus is not routinely performed since there seems to be no specific genetic etiology of gastroschisis and the chromosomes are usually normal. Fetuses with gastroschisis are followed closely with ultrasounds every 2 to 3 weeks to evaluate the bowel anatomy and fetal growth.

The timing of delivery in gastroschisis remains controversial. Planned preterm delivery is believed by some to reduce postnatal intestinal complications and the incidence of intrauterine fetal demise; however, there are no high-quality data, and retrospective studies have conflicting results. A Cochrane review included only one randomized prospective study, which was too underpowered to resolve the issue, hence, additional research was recommended (Grant et al., 2013).

The argument against planned preterm delivery is bolstered by contemporary outcome studies that consistently demonstrate that preterm delivery is a major source of adverse outcome in infants with gastroschisis (Boutros et al., 2009; Maramreddy et al., 2009; South et al., 2013; Carnaghan et al., 2014; Overcash et al., 2014). In addition, the weekly prevalence of intrauterine fetal demise does not increase after 35 weeks' gestation (South et al., 2013). Therefore many centers, including our own, now advocate for delivery close to term.

There is a stronger consensus regarding the route of delivery for patients with gastroschisis, since cesarean section seems to offer no benefit to the baby or the mother (Segel et al., 2001; Salihu et al., 2004; Abdel-Latif et al., 2008). Cesarean section is reserved for standard obstetric indications.

After delivery, the perfusion of the herniated contents should be carefully evaluated. If bowel ischemia or infarction is suspected, then immediate surgical consultation is indicated for bowel detorsion or even emergency enlargement of the gastroschisis defect. If the viscera are well perfused, it is important to next place a clear plastic bag over the exposed bowel as a temporary covering to minimize evaporative heat and fluid loss. The bag may be placed over the



• **Fig. 73.5** Gastroschisis. Gastroschisis with herniated bowel in a Silastic (Dow Corning) silo that has been sewn into a surgically enlarged abdominal wall defect.

entire lower body from the nipples to the feet. A peripheral intravenous line is placed to start intravenous fluids and broad-spectrum antibiotics. An orogastric or nasogastric tube is placed to suction and empty the stomach. The baby should be kept warm and dry throughout the initial resuscitation.

After initial resuscitation and stabilization, the patient is then transported to a NICU with pediatric surgical services. Endotracheal intubation is not required for transport unless indicated for respiratory support. The baby should be placed with the right side angled slightly down to prevent kinking of the mesentery and maximize blood flow to the bowel. The orogastric or nasogastric tube should remain on low intermittent suction during transport. Bowel perfusion should be monitored.

The goal of surgical repair in gastroschisis is safe reduction of the eviscerated contents and eventual closure of the abdominal wall. Multiple surgical options exist to accomplish this goal, and both primary repair and staged repair are acceptable alternatives.

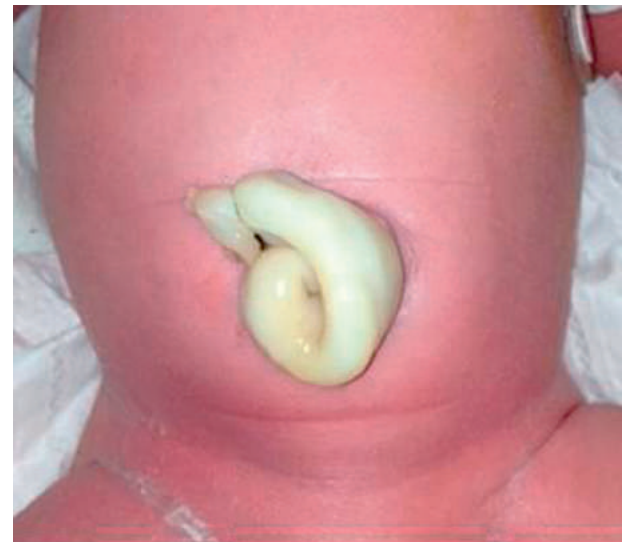
Traditional primary repair of gastroschisis involves closing the fascial defect with sutures in the operating room under general anesthesia. This is performed while monitoring intragastric or bladder pressure to make sure that the closure does not cause abdominal compartment syndrome. In some cases, a prosthetic patch is required to close the defect.

Staged repairs with initial silo coverage of gastroschisis are also an accepted procedure that requires later reduction of the herniated bowel and closure of the abdominal wall. Silos may be made from Silastic (Dow Corning) or other plastic sheeting and sewn circumferentially to the fascial edges of the original or extended gastroschisis defect (Fig. 73.5). Another technique is to use a premade, spring-loaded Silastic silo that is manufactured with different diameters and can be inserted into the gastroschisis defect at the bedside (Fig. 73.6). Once a silo is placed, the bowel gradually reduces over several days into the abdominal cavity by either gravity alone or external pressure. When the reduction takes more than 7–10 days the risk of the silo dehiscence from the abdominal wall increases. Delayed primary closure of the fascia is performed when the abdominal contents have almost completely reduced into the abdominal cavity.

Primary umbilical cord closure is a relatively new technique that uses the patient's own umbilical cord stump as a biologic dressing to seal the gastroschisis defect without attempting a primary fascial closure (Sandler et al., 2004; Choi et al., 2012). In this



• **Fig. 73.6** Gastroschisis. Gastroschisis with herniated bowel in pre-formed spring-loaded Silastic (Dow Corning) silo that has been placed into the gastroschisis defect.



• **Fig. 73.7** Gastroschisis. Gastroschisis after reduction of herniated bowel into the abdomen and suture-less coverage of the gastroschisis abdominal wall defect with the coiled umbilical cord.

technique, the cord is left relatively long at delivery, and the eviscerated contents are reduced into the abdominal cavity while monitoring intraabdominal pressure. The fascial and skin defect are then covered with the coiled umbilical cord that is secured into place with a bandage (Fig. 73.7). In most cases, the gastroschisis defect contracts, and the skin heals beneath and around the cord within 14 days. Using this technique, some children will have a persistent umbilical hernia that can be repaired at 3 to 4 years of age.

Despite many retrospective studies, database analyses, and metaanalyses, it is unclear which surgical approach is best for any individual baby with gastroschisis (Mills et al., 2010; Alali et al., 2011; Bradnock et al., 2011; McNamara et al., 2011; Murthy et al., 2014; Stanger et al., 2014; Emami et al., 2015; Ross et al., 2015). Many studies suffer from significant selection bias and do not separately analyze patients with complex gastroschisis, thereby

making outcome comparisons difficult. The only randomized, prospective study to evaluate primary versus delayed fascial closure was published in 2008 and did not find significant outcome differences between techniques (Pastor et al., 2008). A recently published metaanalysis suggests that delayed primary repair may be associated with improved outcome measures when studies with selection bias are eliminated from the analysis (Kunz et al., 2013). The outcomes from contemporary literature suggest that both primary and delayed closure strategies are safe and effective.

The choice of primary fascial closure versus delayed repair using a silo may be less relevant in the era of the primary umbilical cord closure. Primary umbilical cord closure is successful in most babies with gastroschisis, may be performed at the bedside, does not always require general anesthesia, and reduces duration of mechanical ventilation (Riboh et al., 2009; Emami et al., 2015). In our experience, the technique is associated with shorter NICU stays and shorter time to initiation of enteral nutrition (Chesley et al., 2015).

Babies with uncomplicated gastroschisis have average hospital stays of 4 to 6 weeks, which is largely due to their inability to tolerate full enteral feedings. Babies with complicated gastroschisis often have much longer hospital stays.

Outcomes for children born with gastroschisis in the developed world are excellent. Survival of greater than 95% can be expected in cases of uncomplicated gastroschisis. Infants with complex gastroschisis are at higher risk for overall mortality, short bowel syndrome, bowel obstruction, necrotizing enterocolitis, and the need for home parenteral nutrition after discharge (Bergholz et al., 2014). While only a small minority of babies with gastroschisis will need extensive bowel resection, gastroschisis remains a leading etiology of short bowel syndrome in most series of children with intestinal failure (Modi et al., 2007; Javid et al., 2010). Despite the excellent overall outcomes for gastroschisis in the developed world, mortality remains very high in the developing world (Ford et al., 2016).

Long-term outcomes in gastroschisis are favorable. Most patients will have normal GI function and normal neurodevelopmental outcomes (Gorra et al., 2012; Harris et al., 2016). Even though gastroschisis is almost always associated with intestinal malrotation, the risk of midgut volvulus later in life is low, probably because intraabdominal adhesions that result from the newborn reduction and closure limit the ability of the bowel to twist. These patients may develop hernias at the site of repair. Finally, although boys with gastroschisis commonly have undescended testicles, about 50% of patients will undergo spontaneous testicular descent and not require an operation.

Omphalocele

An omphalocele (known as exomphalos in the United Kingdom) is a midline abdominal wall defect with herniation of bowel and possibly liver and other organs outside the abdomen. The herniated contents are covered with a membrane consisting of peritoneum on the inside, amnion on the outside, and Wharton jelly between those two layers. The umbilical cord inserts into the membrane rather than the abdominal wall (see Fig. 73.3). The omphalocele membrane is usually intact, but occasionally it ruptures and the herniated visceral contents are not covered. Cases of ruptured omphalocele can be confused with gastroschisis, but there are distinct differences (see the section on [Gastroschisis](#)).

Some authors make a distinction between an omphalocele and a hernia of the umbilical cord. In a hernia of the umbilical cord,

the umbilical ring is reportedly normal. Since there is only an open umbilical ring and no deficiency of abdominal wall, the surgical repair is much easier. More importantly, unlike newborns with omphaloceles, newborns with a hernia of the umbilical cord reportedly have a low risk of associated anomalies. However, the clinical differentiation can be difficult, and some physicians do not make a distinction between the two conditions (Raju et al., 2015).

The incidence of omphalocele is estimated to be between 1.5 and 3 per 10,000 births (Marshall et al., 2015). The incidence of omphalocele has been stable over time, unlike the incidence of gastroschisis, which is increasing (Kirby et al., 2013; Marshall et al., 2015). Most cases of omphalocele are sporadic, but there are rare familial occurrences (Frolov et al., 2010). Risk factors for omphalocele include a maternal age of less than 20 years or greater than 40 years (Byron-Scott et al., 1998).

Normal development of the abdomen involves the flat, three-layered embryonic disk folding from top, bottom, and both sides to meet at the umbilical ring surrounding the umbilical cord to form the cylindrical torso during the fourth to fifth week of gestation. The GI tract also begins to grow very rapidly early in the first trimester and, because there is not enough room in the abdominal cavity, there is a normal herniation of bowel through the umbilical ring into the umbilical cord. By 10 to 12 weeks' gestation, the bowel normally returns to the abdominal cavity in a precise pattern of rotation and then fixation to assume its final position. The embryology of omphalocele is not completely understood but is thought to involve incomplete abdominal wall folding and failure of the intestinal tract to return from the umbilical cord (Sadler, 2010; Torres et al., 2015).

In contrast to the relatively low risk of associated anomalies in babies with gastroschisis, babies with omphalocele commonly have associated anomalies that play a major role in how these patients are managed and in their eventual outcome (Lakshminarayanan and Lakhoo, 2014). Chromosomal anomalies such as trisomy 13 and 18 are common, especially in fetuses diagnosed early in gestation (Prefumo and Izzi, 2014; Marshall et al., 2015). In addition, other congenital malformations are common, especially congenital heart disease, which may occur in up to 50% of newborns with omphalocele (Marshall et al., 2015). Finally, omphalocele is a component of many different syndromes of congenital malformations, including Beckwith–Wiedemann syndrome, which occurs in 6% of newborns with an omphalocele and in up to 10%–20% of those newborns thought to have an isolated omphalocele on prenatal evaluation (Corey et al., 2014).

The prenatal diagnosis of omphalocele may be suspected when maternal serum AFP levels are elevated or when there is fetal aneuploidy, but the definitive diagnosis is made by prenatal ultrasound. The prenatal ultrasound findings of omphalocele are abdominal organs herniated outside the abdominal cavity that are covered with a membrane and an abnormal insertion of the umbilical cord into the membrane rather than into the abdominal wall (Prefumo and Izzi, 2014). The prenatal ultrasound diagnosis of omphalocele can be reliably made after the first trimester when the bowel that is normally herniated into the umbilical cord returns to the abdominal cavity. Since the liver is never normally outside the abdominal cavity, the finding of liver outside the abdomen potentially allows an earlier and more accurate diagnosis of omphalocele (Tassin and Benachi, 2014). It is also important to note the presence or absence of liver within the omphalocele, since the absence of liver is associated with a higher risk of other anomalies (Prefumo and Izzi, 2014).

The prenatal management of omphalocele includes evaluation for associated anomalies and monitoring of fetal growth. All fetuses with suspected omphalocele should have an imaging evaluation for structural anomalies. Because of the high risk of congenital heart disease, fetal echocardiography is indicated. Prenatal monitoring of fetal growth is done because of the risk of IUGR. Other specific evaluations for associated pulmonary hypoplasia and the relative size of the defect are advocated to provide improved prenatal counseling about the expected hospital course and the long-term prognosis (Danzer et al., 2012b).

Other obstetric care, including the timing and method of delivery, is usually determined by traditional maternal and fetal factors rather than by the presence of the omphalocele. Specifically, there is usually no benefit (and there is potential harm) with preterm delivery (Porter et al., 2009). Cesarean section is not indicated for most fetuses with an omphalocele, although for fetuses with large defects containing liver (i.e., giant omphaloceles) it has been advocated and is commonly performed (Segel et al., 2001; Biard et al., 2004) to avoid rupture of the sac.

After delivery, the initial evaluation and resuscitation of a baby with an omphalocele follow the same priorities and sequence as for all newborns. During the initial resuscitation, a newborn with an omphalocele should be handled carefully to prevent the omphalocele membrane from tearing. After the initial resuscitation, the omphalocele should be inspected to confirm that it is intact and then covered with a nonadherent dressing to protect the sac.

The membrane of a ruptured omphalocele can sometimes be repaired (Akakpo-Numado et al., 2012), but that is not always possible. When a ruptured omphalocele cannot be repaired, the care of the patient and the abdominal wall defect follows a pathway more similar to the care of newborns with gastroschisis, although surgical closure is usually more complicated, and both mortality and morbidity are high (Yamagishi et al., 2007).

Newborns with omphaloceles, especially large omphaloceles, may have respiratory insufficiency caused by pulmonary hypoplasia and pulmonary hypertension (Baerg et al., 2015), and they may require respiratory support (Panitch, 2015). In addition, those babies with associated Beckwith–Wiedemann syndrome may have hypoglycemia and require supplemental glucose. An early evaluation for possible associated anomalies, especially congenital heart disease, is required to diagnose conditions that may need further treatment.

The goal of operative treatment of omphalocele is to reduce the herniated organs back into abdomen and close the abdominal wall. Surgical closure of an omphalocele is not an emergency as long as the omphalocele membrane remains intact, so there is time for an evaluation for associated anomalies and supportive treatment of other significant problems.

When the omphalocele is relatively small (Fig. 73.8), and the baby is otherwise stable, an early operation with excision of the omphalocele membrane, reduction of the herniated organs, and closure of the abdominal wall muscles is usually done. When the defect is too large to safely reduce the organs and close the abdominal wall in a single operation, the repair can be staged with an initial silo placement, followed by serial reduction of the silo, and finally closure of the abdominal wall. This strategy is almost identical to the staged approach often used for gastroschisis (Ledbetter, 2012). The early, staged approach is not always successful if the abdominal defect is large and there is a significant amount of herniated contents that need to be reduced into a relatively small abdominal cavity. In addition, reducing the abdominal contents may impair ventilation, which can be a critical problem in the setting of underlying pulmonary insufficiency (Tsakayannis et al., 1996).



• **Fig. 73.8** Small Omphalocele. Note the umbilical cord inserting into the intact omphalocele membrane.

To avoid the stress of neonatal surgery, the repair of an omphalocele can be delayed until the baby is bigger and more able to tolerate a major operation. This delayed approach can be accomplished when the sac remains intact and is best accomplished when the membrane is treated with topical agents that allow the sac to epithelialize (Ledbetter, 2012). A variety of topical agents have been used to treat the omphalocele sac (Whitehouse et al., 2010; Ein and Langer, 2012; Nicoara et al., 2014). As the sac is healing, the baby is growing. Growth of the abdominal cavity allows the herniated abdominal organs to reduce back into the abdomen with gravity alone or with additional external compression. In addition, lung growth will improve the baby's ability to tolerate an operation. The eventual repair of the abdominal wall can be done months or even years later (Lee et al., 2006).

Although there is no consensus definition of giant omphalocele, it is often described as omphaloceles with fascial defects greater than 5 cm in diameter or those containing large amounts of liver (Whitehouse et al., 2010). Topical treatment and delayed repair are important tools in the management of giant omphaloceles when there is no reasonable chance for early reduction and primary closure. We recommend topical silver sulfadiazine, as it is easy to use, safe, and effective (Lee et al., 2006). Any topical agent used has the potential of being systemically absorbed and potentially harmful to the baby. For example, mercurochrome can lead to mercury poisoning, and iodine has been associated with hypothyroidism and hyperthyroidism. Silver toxicity is a theoretical concern of using silver sulfadiazine or other silver-containing wound care products; however, clinical signs and symptoms have not been reported.

The survival and long-term outcomes of fetuses or newborns with omphaloceles are mainly determined by the severity of associated anomalies (Gamba and Midrio, 2014; Marshall et al., 2015). Babies with giant omphaloceles have increased mortality and morbidity because of the large abdominal wall defect and associated pulmonary hypoplasia and pulmonary hypertension (Biard et al., 2004; Danzer et al., 2012a, 2015).

Other Abdominal Wall Defects

Body Stalk Anomaly

Body stalk anomaly, which is also known as the limb–body wall complex, is a rare, severe body wall defect of the abdomen and

chest, which is almost always lethal. Prenatal ultrasound can visualize the herniated organs from the chest and abdomen that are covered by a membrane. In addition, a fetus with a body stalk anomaly will usually have scoliosis, and other malformations are common. The umbilical cord is either very short or absent. If the umbilical cord is absent, there are direct vascular connections between the fetus and placenta. It is important to recognize body stalk anomaly on prenatal imaging so that families can receive appropriate prenatal counseling (Pakdaman et al., 2015).

Bladder Exstrophy

Bladder exstrophy is an anomaly of the lower abdominal wall, pelvis, and pelvic organs in which an open bladder makes up the lower part of the anterior abdomen. The open bladder is accompanied by a separation of the pubic symphysis. It is a rare anomaly, with an incidence of 3 to 5 cases in 100,000 births, and it occurs at least twice as often in boys compared with girls (Jayachandran et al., 2011). The etiology is unknown, and it is grouped into the exstrophy–epispadias complex of anomalies. Surgical treatment is required but is not an emergency, so patients can be transferred to specialized centers for definitive care. Early management consists of protecting the exposed bladder mucosa from injury by applying a nonadherent dressing. Evaluation for associated anomalies includes ultrasound for associated upper urinary tract anomalies and an abdominal radiograph to assess the degree of pelvic bone separation.

Cloacal Exstrophy

Cloacal exstrophy is another anomaly grouped into the exstrophy–epispadias complex of anomalies. It is also known as OEIS complex—Omphalocele, Exstrophy, Imperforate anus, Spinal dysraphism—that describes its usual features. It is a rare anomaly, occurring in about 1 in 100,000 births, and is associated with the 1p36 deletion (Feldkamp et al., 2011). Most patients present with an abdominal wall defect that at its cephalic aspect consists of an omphalocele. The omphalocele is in continuity with a more caudal complex exstrophy of two lateral bladder halves joined to a central bowel exstrophy called a cecal plate. This exstrophy complex is accompanied by pubic symphysis diastasis (similar to that seen in bladder exstrophy) and imperforate anus. It may be associated with spinal dysraphism.

Bowel peristalsis and abdominal pressure often lead to prolapse of the terminal ileum and the distal colon/hindgut lumens that are normally attached to the cecal plate. Early management consists of a nonadherent bandage to the exposed bladder and bowel mucosa. Surgical reconstruction is complex and often done in stages. Overall survival is high, but there is substantial long-term morbidity, including abnormal anorectal, urinary tract, and neurologic function (Versteegh et al., 2013).

Prune Belly Syndrome

Prune belly syndrome (PBS), also known as Eagle–Barrett syndrome, is a rare condition notable for complete or partial lack of abdominal wall muscle and severe genitourinary abnormalities. A major cause of mortality in newborns with PBS is pulmonary hypoplasia, while comprising a major component of long-term morbidity are urinary tract abnormalities that often result in end-stage renal disease (Seidel et al., 2015; Arlen et al., 2016). A variety of reconstruction procedures of the abdominal wall have been described that reportedly improve bladder and GI function as well as improve the appearance (Hassett et al., 2012).

Abdominal Wall Hernias

A hernia exists when the contents of a body cavity extend through the normal wall of that cavity. Abdominal wall hernias are conditions where intraabdominal contents protrude through the normal muscle and fascial layers of the abdominal wall. The congenital abdominal wall defects discussed in the previous section are types of abdominal wall hernias. In contrast to those conditions that present prenatally or immediately after birth, this section will briefly discuss other abdominal wall hernias that usually present after birth, although their anatomic cause may be due to an abnormality of development.

Diastasis Recti

Although diastasis recti is a normal variant and not a hernia, it is often mistaken for an abdominal wall hernia, and so it is discussed here. The central section of a normal anterior abdominal wall consists of two rectus abdominus muscles that extend from the ribs to the groin. Between the rectus muscles there is an extension of the rectus muscle fascia, known as the linea alba, that joins the two rectus muscles and creates a solid abdominal wall. A visible separation of the rectus muscles with an intact linea alba fascia is known as diastasis recti or rectus abdominis diastasis. In this condition, the body wall musculofascial layer is intact, but the midline bulge of the linea alba fascia when the abdominal muscles contract is often mistaken for a hernia.

Diastasis recti is readily apparent on physical examination as midline bulging of the abdominal wall between the xiphoid process and the umbilicus. Imaging studies are not indicated. Although diastasis recti may be a cosmetic problem in a postpartum woman, the condition is not a pathologic problem in an infant and will typically be less noticeable after normal growth. Surgical treatments have been advocated for adults to improve the appearance of the abdominal wall but are not indicated in infants and children (Akram and Matzen, 2014).

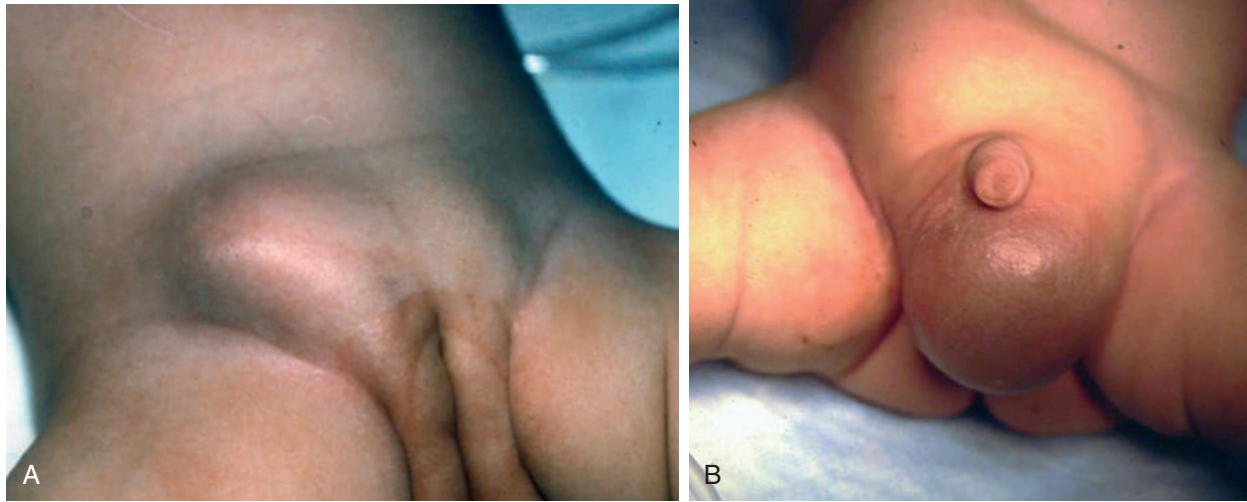
Epigastric Hernia

Epigastric hernias are small fascial defects in the linea alba, the midline fascia between the rectus abdominis muscles, through which preperitoneal fat can herniate. Because they are typically only a few millimeters in diameter, the fascial defects are usually too small to palpate, but preperitoneal fat can herniate out like a mushroom with only a small stalk traversing the defect and a larger cap in the subcutaneous tissue that may be visible and palpable (Coats et al., 2000). Epigastric hernias are usually asymptomatic, and surgical repair can be delayed until later in life.

Umbilical Hernia

The umbilical ring is the normal fascial layer that surrounds the umbilical cord going through the abdominal wall. It represents the junction of superior, inferior, and lateral body wall folds. An umbilical hernia is a protrusion of bowel or omentum through an open umbilical ring, producing a bulge at the umbilicus. The umbilical ring normally closes after birth, but it may take months or years to become too small for bowel to herniate through the abdominal wall.

Umbilical hernias are usually not present at birth or in the first few days of life but may become noticeable after a few weeks. They are more common in infants born prematurely and much more common in African-American infants than in Caucasian infants. Most umbilical hernias are sporadic, but for unknown reasons some families have an increased risk. Umbilical hernias are more noticeable when the intraabdominal pressure



• **Fig. 73.9** Inguinal Hernia in an Infant. Right inguinal hernias in a female (A) and male (B) infant. Note the asymmetry in the groin area with the prominent bulge indicating the hernia.

increases with crying or straining, but the hernias usually do not cause pain or distress. The great majority of umbilical hernias close spontaneously before school age, and symptoms are uncommon early in life so surgical repair is usually delayed until 3 to 5 years of age or until symptoms develop (Kelly and Ponsky, 2013).

Inguinal Hernia and Hydrocele

Inguinal hernias are the bulging of intraabdominal contents through the abdominal wall in the groin (Fig. 73.9). In adults this may be the result of abdominal wall muscular injury or weakness, but in infants the hernia is a result of a persistent congenital protrusion of the peritoneum through the normal internal and external ring openings of the abdominal wall muscles. This peritoneal protrusion is known as a patent processus vaginalis, and as it crosses the abdominal wall it is immediately adjacent to the spermatic cord structures in boys and the round ligament of the uterus in girls. In boys the processus vaginalis is the path of normal descent of the testis, and it normally closes except for a small pouch around the testis. When it remains open and is large enough, then pressure in the abdomen may allow intraabdominal contents through the abdominal wall, perhaps all the way into the scrotum. When organs or parts of organs protrude, it is called a hernia, and when only fluid goes into the processus vaginalis, it is known as a hydrocele (Kelly and Ponsky, 2013).

Roughly 1% of children will have an inguinal hernia. Hernias are much more common in boys than in girls (Grosfeld, 1989). Hernias are also much more common in premature babies compared to term babies (Peevy et al., 1986). Unlike umbilical hernias, inguinal hernias do not spontaneously heal, and they tend to get larger with time. The major risk of an inguinal hernia is the possibility of strangulation of the hernia contents with life-threatening intestinal ischemia. An additional complication would be compression of blood supply to a herniated gonad by the protruding hernia with subsequent loss of testicle or ovary. Strangulation only occurs

when hernias are incarcerated, i.e., when the contents are not reducible back into the abdomen. Therefore a nonreducible or incarcerated inguinal hernia requires urgent surgical evaluation. In otherwise healthy infants, inguinal hernias are repaired electively, soon after their diagnosis, since the operation is well tolerated and the risk of anesthesia is low (Davidson et al., 2016). Premature infants are prone to apnea after general anesthesia, so the ideal timing of operation in this group is controversial (Duggan et al., 2015; Sulkowski et al., 2015). A baby who has an inguinal hernia has an increased risk of developing a contralateral hernia later in life, but the risk may not be high enough to justify routine “prophylactic” hernia repair (Wenk et al., 2015).

Hernias present with groin bulges that may extend into the scrotum or labia. When fluid alone is present in a persistent processus vaginalis, it is known as a hydrocele. Hydroceles may be simple collections present at birth in the normally present pouch around the testis, or they may develop after birth along any portion of the path of the processus vaginalis. Hydroceles present at birth may spontaneously resolve, although it may take up to a year. Hydroceles that develop after birth presumably have an open connection into the abdomen and are unlikely to resolve, so they are treated as inguinal hernias, although their repair is not urgent. Larger hydroceles can be difficult to differentiate from inguinal hernias, especially when hydrocele fluid extends up into the inguinal canal.

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Neonatal Gastroesophageal Reflux

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KEY POINTS

- Gastroesophageal reflux is physiologic in newborns, but when associated with complications it is classified as gastroesophageal reflux disease (GERD) and may warrant intervention.
- The symptoms of gastroesophageal reflux disease overlap with many other common conditions.
- Pathophysiologic symptoms in infants often attributed to GERD may be divided into three categories: benign esophageal, serious esophageal, and extraesophageal.
- There is no gold standard test for the diagnosis of GERD in infants; thus the choice of intervention and measure of its success must be guided by the presenting complaints.
- No histamine antagonists or proton pump inhibitors have US Food and Drug Administration approval for use in infants.
- There is significant geographic and institutional variation in the frequency and type of antireflux operative intervention.

Background

Gastroesophageal reflux (GER) is the physiologic passage of gastric contents into the esophagus. It is a naturally occurring phenomenon in infants, children, and adults that typically lasts fewer than 3 minutes, has minimal or no associated symptoms, and usually occurs in the postprandial period. It may occur several times per day and on occasion may be associated with spit-ups or vomiting. Normal physiologic GER in infants is common, occurring in as many as 65% of infants younger than 4 months, naturally decreasing in frequency over time, affecting only 1% by 12 months (Henry, 2004). The trajectory of this natural history is likely related to lengthening of the esophagus and a decrease in episodes of transient relaxation of the lower esophageal sphincter (Henry, 2004).

Normal Anatomy and Physiology

The normal swallowing mechanism consists of a food or saliva bolus traveling from the mouth to the pharynx through the esophagus to the stomach and is propelled forward with peristaltic muscle contractions of the esophagus. The lower esophageal sphincter, located at the gastroesophageal junction, has a resting tone that increases with increasing gastric distention, which acts as an anatomic barrier for refluxing gastric contents into the esophageal lumen. Decreased lower esophageal sphincter pressure occurs with swallowing and release of gas from the stomach in the form of eructation (Henry, 2004).

Abnormal Physiology: Gastroesophageal Reflux Disease

In contrast, gastroesophageal reflux disease (GERD) is the pathophysiologic passage of gastric material into the esophagus that is associated with a variety of significant symptoms and/or complications (Milla et al., 2002; Gold, 2004; Hassall, 2005; Vandenplas et al., 2009). GERD is uncommon in infants, affecting only 1 in 300, though certain populations such as premature or critically ill infants are believed to be at greater risk (Henry, 2004).

The complexity in distinguishing GER from GERD, however, lies in defining significant symptoms and complications and clearly proving that they are directly caused by the passage of gastric content into the esophagus. Several symptoms in infants such as frequent vomiting, irritability, distress, and unexplained crying are common. While these symptoms can be attributed to GER and GERD, they can also be due to many other underlying problems such as cow's milk protein allergy, neurologic disorders, urinary tract infection, and constipation (Vandenplas et al., 2009). In general, symptoms that are not causing physiologic or behavioral impact may need no additional evaluation. For example, the "happy spitter" is an infant who regurgitates a small amount after a few feeds each day but continues to grow and gain weight along a normal trajectory. When recurrent emesis is coupled with poor weight gain, however, a complete work-up for GERD and other diagnoses in the differential should be considered (Hassall 2005; Vandenplas et al., 2009).

Pathophysiologic symptoms in infants that are often attributed to GERD may be divided into three categories: benign esophageal, serious esophageal, and extraesophageal. Benign esophageal symptoms include recurrent emesis, feeding refusal, and arching. Serious esophageal symptoms include stricture, erosive esophagitis, and Barrett esophagus. Extraesophageal symptoms include poor weight gain, apnea with associated bradycardia, wheezing, asthma, aspiration, otitis media, sinusitis, hoarseness, dental erosions, chronic sore throat, chronic stridor, blood-tinged emesis, anemia, chronic cough, and recurrent pneumonia (Gold, 2004; Henry, 2004).

There are a number of etiologic factors that increase an infant's propensity to higher frequency of GER (Milla et al., 2002; Hassall, 2005). These factors can be divided into those associated with normal infant physiology, abnormal underlying physiology, and iatrogenic causes. Term and preterm neonates normally have anatomic and physiologic features that increase their risk of GERD, including a shorter and more narrow esophagus, delayed gastric emptying, and a shorter lower esophageal sphincter that is both

frequently superior to the diaphragm and periodically relaxes independent of swallowing (Henry, 2004). Beyond the anatomic considerations, infant diets consist primarily of liquids (breast milk or formula), which are provided frequently and in larger relative volume because of their feeding schedules and high caloric requirements (Henry, 2004). Examples of factors that are associated with abnormal underlying physiology include inhibition of esophageal body peristalsis with relaxation of the lower esophageal sphincter, decreased lower esophageal sphincter resting tone, gastric distention, delayed esophageal clearing, hiatal hernia, and neurologic impairment (Gold, 2004). Examples of iatrogenic factors that increase risk for GERD include supine positioning during feeds and adjuvant treatments of neonates such as caffeine for apnea.

Symptoms of older children and adults are distinct from those in infants. Older children may experience abdominal discomfort or pain, early morning nausea, eructation that burns, substernal chest pain, heartburn, recurrent emesis, and extraesophageal symptoms including apnea/bradycardia, wheezing/asthma, otitis media, hoarseness, dental erosions, chronic sore throat, chronic cough, esophagitis, esophageal stricture, Barrett esophagus, and recurrent pneumonia (Gold, 2004; Hassall, 2005). A natural history study of children aged 2 to 17 years (mean age 6.8 years) by Ashorn et al. found that in children the most common symptom was recurrent abdominal pain and was reported in 63% of patients (Ashorn et al., 2002).

Diagnosis

Objective Measures

A variety of diagnostic tests have been employed to augment clinical history and physical examination in the diagnosis of GERD, though none has sufficient accuracy to act as the reference standard. Moreover, data are lacking to determine the sensitivity, specificity, positive predictive value, or negative predictive value of these tests. These tests include 24-hour pH monitoring, combined 24-hour pH multichannel intraluminal impedance (pH-MII), manometry, endoscopy, gastric scintigraphy (gastric emptying study), and barium swallow (Mauritz et al., 2011).

24-Hour pH Monitoring

24-hour pH monitoring is frequently used in the diagnosis of GERD. This is seen as an objective measure of acid exposure in the esophagus that includes four elements: the total time the esophagus experiences a pH higher than 4, the number of episodes of pH higher than 4, the percentage of time at a pH higher than 4, and the longest period of pH higher than 4. Often, the studies are performed with at least two channels—one in the distal esophagus, and one in the proximal—to ascertain the level of esophageal acid exposure as well. Studies of the usefulness of this test in children and infants are mixed. A study by Ashorn et al. found that correlation between abnormal pH-monitoring results and histologic inflammation found on endoscopic examination with biopsy was poor (Ashorn et al., 2002). Tovar et al. examined patients who underwent antireflux operations in the setting of normal pH studies (Tovar et al., 1991). The authors studied 14 children with a diagnosis of GERD, based on clinical symptoms such as vomiting, dysphagia/pain, and respiratory tract disease who underwent an antireflux operation for GERD, but had normal manometry. These patients were compared with 14 controls who also had normal manometry and who also had a workup for GERD but were ultimately found not to have indications for an antireflux

operation. The authors found that those children who underwent an antireflux operation had symptom resolution, thus concluding the possibility of false-negative results of manometry. A study by Gorenstein et al. examined findings on pH monitoring between children with respiratory, gastrointestinal, and mixed symptoms of GERD and found only that children with gastrointestinal (GI) symptoms had a slightly higher percentage of time with pH higher than 4 and that the longest episode was on average 4 minutes longer than the comparison groups (Gorenstein et al., 2003). These findings suggest that while pH monitoring may measure esophageal acid exposure, this metric alone in children is poorly correlated with symptoms and signs thought to be associated with the diagnosis of GERD.

24-Hour Combined pH and Multichannel Intraluminal Impedance

The pH-MII exam combines abnormal pH detection in the esophagus with impedance and, specifically, measurement of lower esophageal sphincter pressure. Multichannel intraluminal impedance adds the ability to detect bolus movement through the esophagus. It is important in evaluation of GERD because it can examine for acid and nonacidic reflux. In a direct comparison of the 24-hour pH probe and the pH-MII, Wu et al. determined that the pH-MII detected significantly more reflux episodes than the pH probe alone, with better symptom correlation (Wu et al., 2013). Despite the theoretical benefit of pH-MII, Rosen et al. noted that findings on pH-MII did not correlate with postantireflux procedure outcomes (Rosen et al., 2010). In contrast, Berquist et al. found that the mean frequency of GERD episodes prior to the antireflux procedure was significantly higher than for controls, and following the operation it was significantly lower (Berquist et al., 1981).

Manometry

Manometry measures lower esophageal sphincter pressure and length. Manometry may be especially useful for the diagnosis of esophageal motility disorders. The use of high-resolution esophageal manometry, while common in adults, has been more slowly adopted in pediatric patients (Goldani et al., 2010).

Gastric Scintigraphy

Historically, scintigraphy of the stomach (gastric emptying) was an essential component of the work-up and diagnosis of GERD for some providers, specifically to identify the presence of delayed gastric emptying as an etiology for reflux. Scintigraphy is a nuclear medicine radiologic study in which patients ingest technetium formula, with serial images captured over time measuring movement of contrast either out of the stomach or retrograde into the esophagus. In one study, the authors performed gastric scintigraphy on 51 patients with documented GER and compared the results with those of 24 controls without GER and found a statistically significant difference in gastric emptying times (Knatten et al., 2013). Other studies have had mixed results, and a well-designed prospective study of the use of gastric scintigraphy and gastric emptying procedure in the setting of delayed gastric emptying is lacking. Perhaps the best use of this examination in patients suspected of GERD is those with aspiration pneumonia in which identification of technetium in the lungs during the examination is fairly sensitive and specific for aspiration (Sherman et al., 2009).

Upper Gastrointestinal Series

Upper GI series may be used to define luminal anatomy by following a bolus of contrast through the esophagus, stomach, and duodenum.

It is optimally utilized to evaluate for diagnoses such as achalasia, nutcracker esophagus, and other anatomic anomalies that may be associated with symptoms related to GERD. In the neonatal population, a common diagnosis with a significant symptom overlap with GERD is an anomaly of malrotation, which is best diagnosed with an upper GI series. For the evaluation of classic reflux, the upper GI series performs poorly compared with other tests such as pH monitoring. In their retrospective cohort study of 656 patients with a mean age of 17.4 months (range 0.15–228 months) who underwent an antireflux procedure and had a preoperative upper GI series, Valusek et al. found that an upper GI series altered clinical management in only 4% of cases when attempting to delineate pathologic reflux (Valusek et al., 2010). The utility of the study is likely higher if considering patients who had a work-up for GERD and were found on upper GI series to have malrotation, requiring a Ladd procedure rather than an antireflux procedure.

Indications for Medical and Surgical Management of Gastroesophageal Reflux Disease

Medical or surgical intervention should be targeted only to those infants with troublesome symptoms or complications. Measurement and success of treatment must be resolution of the initial indication. Consensus definition of pediatric GERD was performed by a panel of experts and published in 2009 (Sherman et al., 2009). The experts defined GERD as the following: GERD in pediatric patients is present when reflux of gastric contents is the cause of troublesome symptoms and/or complications. Importantly, regurgitation was defined as the passage of refluxed contents into the pharynx, mouth, or from the mouth, and while it is a characteristic symptom of GERD, it is neither necessary nor sufficient for the diagnosis of GERD (Sherman et al., 2009). The panel also noted that bilious emesis should not be diagnosed as GERD.

Options for Interventions

Pharmacologic Agents

The two main classes of pharmacologic agents used for the treatment of GERD are acid buffering or suppressant and prokinetic medications. Acid buffering and suppressant medications include antacids, histamine receptor antagonists, and proton pump inhibitors. Antacids decrease the acidic gastric environment by acting as a buffer. In theory, the elevation in pH allows for mucosal healing and relief of symptoms. In general, however, these medications are not recommended in infants or children with GERD since they have been shown to cause aluminum toxicity and milk-alkali syndrome (Drug and Therapeutics Bulletin, 2010; Lightdale et al., 2013).

Histamine receptor antagonists block the histamine receptor on parietal cells in the stomach thus reducing acid release. While a randomized controlled study has found significant improvement in erosive esophagitis and acid exposure with nizatidine compared with placebo in children, tachyphylaxis is common (Simeone et al., 1997; Lightdale et al., 2013). A nonrandomized, multicenter observational prospective study of consecutively admitted very low birth weight infants compared those who did and did not receive ranitidine (Terrin et al., 2011). The authors found that infants who received ranitidine were five times more likely to have an infection and had an over sixfold higher risk of necrotizing

enterocolitis and significantly higher mortality compared with controls.

Proton pump inhibitors decrease acid secretion by blocking the hydrogen/potassium (H^+ / K^+) ATPase on the gastric parietal cell. Compared with histamine receptor antagonists, proton pump inhibitors are more potent by directly blocking acid secretion and have a resultant increase in pH higher than 4 for a longer period of time (Lightdale et al., 2013). In a prospective study of 78 consecutive children (mean age 40.6 months, standard deviation 36.4 months, range 1–181 months) referred to the pediatric department of GI endoscopy and motility at the University of Naples in Italy, proton pump inhibitors and histamine H_2 -receptor antagonists were compared (Ummarino et al., 2012). Proton pump inhibitors were found to be superior in extraesophageal symptom resolution. Although proton pump inhibitors may be more effective, none has been approved for use in infants (aged less than 1 year), and the indication for treatment must be specific since this class of medication has not been shown to reduce symptoms of irritability (Moore et al., 2003; Griebel, 2010).

Four proton pump inhibitors have been studied with prospective, randomized controlled trials. A multicenter randomized, double-blind, placebo-controlled trial of the proton pump inhibitor lansoprazole in infants aged 1–12 months weighing over 2 kg, with symptomatic GERD (defined as documentation of crying within 1 hour of at least 25% of feeds) and at least 1 week of nonpharmacologic management of symptoms, found no statistically significant differences in symptom improvement (Orenstein et al., 2009). A randomized, double-blind, placebo-controlled, crossover design trial of omeprazole of 10 preterm infants with symptoms such as feeding problems, vomiting, irritability, and weight loss thought to be related to GERD found that while gastric and esophageal acidity decreased significantly in the omeprazole group, there were no significant differences in symptom frequency (Omari et al., 2007). A multicenter, randomized double-blind, placebo-controlled parallel-group study of esomeprazole saw no difference in the time to medication discontinuation related to symptom-worsening between groups (Winter et al., 2015). A study by Winter et al. initially treated 128 infants aged 1 to 11 months with pantoprazole then randomized 106 of those patients in a double-blind, placebo-controlled evaluation of pantoprazole (Winter et al., 2010). The authors found that while symptoms decreased significantly during the open label phase of the trial when all patients received pantoprazole, there was no significant increase in mean weekly symptom scores when the patients were randomized to receive pantoprazole or placebo. Additional risks potentially associated with acid suppression medications include community-acquired pneumonia, gastroenteritis, candidemia, and necrotizing enterocolitis in preterm infants (Van der Pol et al., 2011; Lightdale et al., 2013). No proton pump inhibitor has US Food and Drug Administration approval for infants younger than 1 year of age with GERD, though off-label use is likely common (Drug and Therapeutics Bulletin, 2010; Griebel 2010).

Prokinetic agents are another class of medications used to treat symptoms of GERD. The proposed mechanism of action for prokinetic agents in the treatment of GERD includes increased lower esophageal sphincter pressure and increased gastric emptying. The most common prokinetic agent used is metoclopramide, although significant adverse reactions include drowsiness, extrapyramidal reactions, and restlessness. Although medical management is recommended as a first-line treatment for GERD, there are no medications that have US Food and Drug Administration approval for use in infants, and definitive prospective data in infants are

lacking (Rudolph, 2003; *Drug and Therapeutics Bulletin*, 2010; Greene and Moeny, 2010). Despite the lack of approval, prescriptions have been rising (Greene and Moeny, 2010).

Surgical Intervention

A multitude of surgical interventions have been described for the treatment of GERD. Various technical approaches include, but are not limited to, open, laparoscopic, and robotic antireflux procedures. Technical variations range from partial and full fundoplication. For children with significant neurologic impairment and severe refractory GERD, some surgeons have advocated for an esophagogastric separation (Islam et al., 2004; Boubnova et al., 2009; Zaidi et al., 2010; Cundy et al., 2013). More recently, newer operations, that are not yet mainstream, have been attempted such as lower esophageal sphincter buttressing via magnets, endoluminal fundoplication, and sclerosis. There appears to be geographic and institutional variation in the type and approach of antireflux operative intervention (Goldin et al., 2009; Nusrat and Bielefeldt, 2014). The multitude of approaches and technical variations of antireflux procedures speaks to the fact that there is no single operation that has been shown to be consistently superior. In general, antireflux operations are designed to correct and improve the anatomy of the lower esophageal sphincter and diaphragm. The most commonly used procedure, called a *Nissen fundoplication*, includes several components. It increases the intra-abdominal esophageal length, thereby increasing external pressure and augmenting the function of the lower esophageal sphincter. Wrapping the fundus of the stomach around the lower esophageal sphincter further strengthens the lower esophageal sphincter since they share nerve supply and contract simultaneously to recreate the angle of His, thus diverting gastric content away from the esophagus, effectively repairing the diaphragmatic crura.

All operations that address symptoms of GERD, regardless of approach, have been noted to be associated with both short-term and long-term complications. A study of laparoscopic Nissen fundoplication in infants and children weighing less than 5 kilograms found that 71% required a postoperative intensive care unit stay for an average of 2 weeks, and 31% required postoperative mechanical ventilation for an average of 12 days (Shah et al., 2010). Complications from the procedure in this cohort were noted to include recurrent reflux, gastric perforation, and hemorrhage (Shah et al., 2010). A multiinstitutional review found that younger age at antireflux procedure was significantly associated with the need for a redo fundoplication (Baerg et al., 2013). Young age has also been associated with a lower rate of hospitalization after antireflux procedure (Goldin et al., 2006).

Comparison of laparoscopic and open Nissen fundoplication is limited though the current literature indicates that the laparoscopic approach is likely a safe alternative (Blewett et al. 2002; Rangel et al., 2003). Comparison of partial and complete fundoplication shows a lack of well-designed studies, so currently the two approaches appear to have comparable effectiveness for control of the symptoms of GERD in neurologically normal children (Mauritz et al., 2013).

Conclusion

Despite the frequency with which children, and especially neonates, are diagnosed with GERD, there is significant complexity in

obtaining objective proof that GERD is responsible for any presenting symptom. It is important to remember that many of the symptoms associated with GERD are neither sensitive nor specific to the diagnosis. It is therefore critical to understand that one's goal is to treat the symptom or sign attributed with GERD and not necessarily the GERD itself.

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Complete references used in this text can be found online at www.expertconsult.com

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The Intestinal Microbiome

JOSEF NEU

KEY POINTS

- Evidence is accumulating that the fetal environment is not sterile, nor is human milk, and that the early exposure to microbes may play an important role in subsequent health.
- Manipulation of the early microbiome with antibiotics (given prenatally or postnatally), mode of delivery, and other environmental conditions may alter the delicate balance between the microbes and the human host and result in disease.
- Metabolites produced by microbes as well as by the host–microbial interactions may play a role in the immunologic development of the host.

Since the initiation of the Human Microbiome Project in 2007 (Turnbaugh et al., 2007), there have been marked advances in our knowledge of the interactions between humans and their microbial environment. It is becoming clear that the vast majority of bacterial microbes, the “microbiota,” that interact with humans are not pathogens but rather commensals and symbionts, many of which play a major role in health. The interaction of microbes with the pregnant mother, the fetus, neonate, and infant appears to be especially important for future development, health, and disease. This chapter provides an overview of current knowledge of the microbiome, defined as the combined genetic material of the microorganisms in a particular environment, and how it relates to the fetal–maternal unit, the neonate, and subsequent health. Included will be a brief description of new and developing technologies that are rapidly advancing this field. Also described are various aspects of the microbiome during intrauterine and fetal life as it relates to premature labor, premature and term infant disease entities such as necrotizing enterocolitis (NEC) and sepsis, basic interactions between the microbiome and the intestinal immune system, and its bioreactor/nutritional function. Perturbations that affect the human–microbial ecosystem—the “holobiont” (Gilbert, 2014a)—such as antibiotics and other drugs, mode of delivery, and diet are also discussed.

Historical Perspectives

Beyond Culture

New technologies using nonculture-based methods have shown that the majority of microbes in the intestine have not yet been

cultured. Advances in nonculture DNA sequencing–based technologies that evaluate taxa of microbes residing in various niches of the human body are providing us with the knowledge that when one evaluates the number of genes or cells in the average human, only 10% are mammalian, and the rest are microbial (Xu and Gordon, 2003; Gill et al., 2006). We now know that there are more than 2000 taxa of bacterial microorganisms within our bodies, most of them residing in our intestines. The recent development of nonculture-based techniques to evaluate microbial DNA is providing new insights into the relationship that exists between microbes and their mammalian hosts, especially the microbes that reside in the gastrointestinal (GI) tract (Gill et al., 2006), where they have a profound effect on digestive physiology, development, and health of the host (Stappenbeck et al., 2002; Xu and Gordon, 2003; Hooper, 2004; Dethlefsen et al., 2007). During evolution, the host has developed protective responses against foreign microbes but at the same time has developed a mechanism that provides a welcome and mutually beneficial environment for microbes and the host (Hooper and Gordon, 2001; Hooper et al., 2002; Bäckhed et al., 2004, 2005; Hooper, 2004).

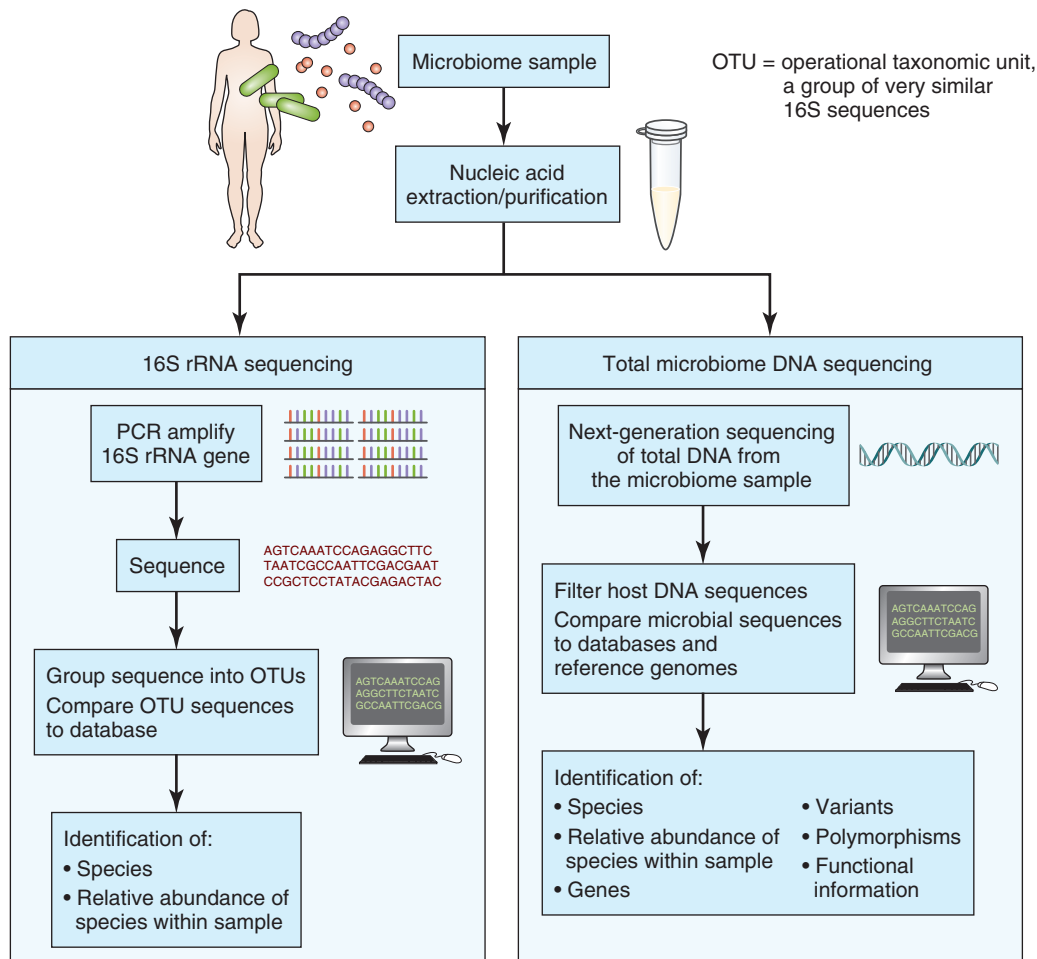
Technologies

Currently, there are two nonculture-based methods (Fig. 75.1) commonly used for characterization of the microbiome (the collection of genes in the microbiota):

- Small-subunit ribosomal ribonucleic acid (rRNA) studies, in which the 16S rRNA variable gene sequences are used as phylogenetic markers to define which lineages are present in a sample
- Metagenomic sequencing studies, in which community DNA is subject to shotgun sequencing (Fodor, 2014)

The 16S rRNA is a part of the ribosomal RNA that is used for phylogenetic studies as it is highly conserved between different species of bacteria and archaea. In addition to highly conserved primer binding sites, 16S rRNA gene sequences contain hypervariable regions that can provide species-specific signature sequences useful for bacterial identification. As a result, 16S rRNA gene sequencing has become prevalent in medical microbiology as a rapid, accurate alternative to phenotypic methods of bacterial identification.

In this technique, DNA is extracted from fecal samples; the targeted 16S rRNA genes (usually the V1–3 or V4 region) are amplified using universal polymerase chain reaction primers capable of efficient annealing to rRNA genes from most bacteria. Based on the degree of nucleotide similarity (usually between 95% and



• **Fig. 75.1** Two Commonly Used Methods of Microbiome Analysis. 16S rRNA sequencing is the faster and less expensive method. It provides information about the relative abundance of taxa present in a sample (“who is there”). Total microbiome DNA sequencing analyzes the total DNA from a sample, but in addition to providing taxonomic information, it can also provide functional information (“what the microbes are doing”). PCR, Polymerase chain reaction; rRNA, ribosomal ribonucleic acid. (Adapted from: <https://www.neb.com/tools-and-resources/feature-articles/addressing-challenges-in-microbiome-dna-analysis>.)

99%) sequences are then separated into operational taxonomic units that form the basis for comparisons between samples. This technique is primarily used to provide taxonomic information (“who’s there”) rather than function data (“what are they doing”) that can better be provided by metagenomic shotgun sequencing (Fodor, 2014).

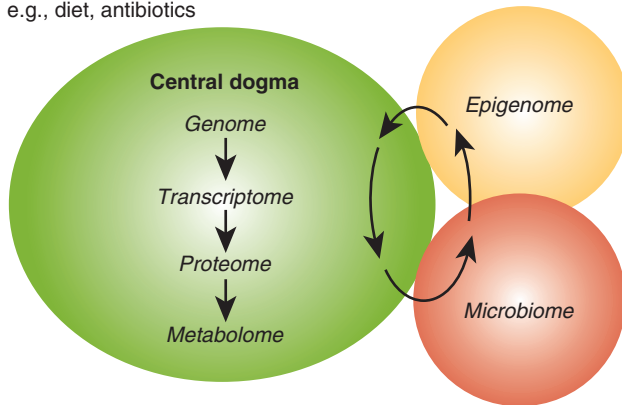
Metagenomics is the study of genetic material recovered directly from environmental samples that enables studies of organisms that are not easily cultured in a laboratory as well as studies of organisms in their natural environment. Studies use shotgun sequencing to get (mostly) unbiased samples of all genes from all members of sampled communities (Fodor, 2014). This technique provides much more information with regard to total microbial gene function but is also much more expensive than the 16S rRNA-based techniques. However, these techniques are rapidly evolving, and the costs are becoming much less prohibitive.

Other “Omics”

The aforementioned discussion underlines that fact that the human organism is composed and controlled not only by its mammalian

genetic complement (human genome comprising approximately 23,000 genes) but also the microbial genetic component (the microbiome—sometimes termed the “second genome” comprising at least 100 times as many genes as those derived from the human sperm and egg). These synergize with environmental factors such as diet to comprise the epigenome, proteome, transcriptome, metabolome, and epigenome (Fig. 75.2). These correspond to various microbial functions, and the technologies for their analysis are rapidly evolving. Transcriptomics will allow for the detection of more specific microbial gene products as will metabolomics and proteomics. It is believed that the microbiome plays a prominent role in human metabolism because microbes produce harmful and beneficial small molecules for humans such as vitamins, polyphenols, cholesterol, and short-chain fatty acids (SCFAs) (Nicholson et al., 2012). A recent comparison of plasma profiles in conventional and germ-free mice showed a strong correlation between the plasma metabolite levels and the microbes present (Wikoff et al., 2009). Future studies may find significantly different metabolite patterns in urine and fecal samples from the mothers who deliver prematurely compared with those delivering at term when we employ systems biology pathway mapping.

Environmental influences:
e.g., diet, antibiotics



• **Fig. 75.2** The Central Dogma of the Human Genome. DNA is transcribed to RNA, which codes for protein, which in turn produces metabolites. The microbiome undergoes similar transformations, but metabolic products from the microbiome may be epigenetically active and modulate the human genomic pathway.

Epigenomics will allow for the integration of the techniques for microRNA, DNA methylation, and histone modification analysis in the amplification and silencing of host genes and gene products. In addition, analysis of microbe–host gene interactions is an area that is receiving considerable attention (Duca et al., 2014; Sanchez et al., 2015; Sun and Hu, 2016).

Alterations in the intestinal microbiome have been linked to obesity, autoimmune diseases, allergy asthma, eczema, and even the induction of premature labor, some of which are described in this chapter (Chang and Neu, 2015). The role of intestinal microbes, especially during early development in these highly interrelated components, is clearly related to human health and disease and is an exciting area for future investigation (Turnbaugh and Gordon, 2008). Use of these developing multiomic technologies and their interpretation using sophisticated “big data” approaches will be keys to progress in these areas.

Actions of the Intestinal Microbiome

The human metaorganism has been described as a holobiont, which has been defined as a “super or meta-organism that adjusts and transforms itself according to environmental changes causing evolution of the entire entity” (Gilbert, 2014a, 2014b). It contains a prokaryotic component in the intestine that comprises nearly 10^{14} cells that weigh over 1 kg in the human adult. There is a marked aboral gradient in the number and types of microbes in different regions of the intestine, with 10^{11} in the ascending colon and 10^{7-8} in the distal ileum and 10^{2-3} in the jejunum. A better understanding of many of the actions of the microbiota in the GI tract (Box 75.1) is emerging. Although there is overlap with function between intestinal regions, the small intestine is primarily involved with immunoreactivity and immune functions, and the large intestine plays more of a bioreactor/metabolic function (Neish, 2009).

Intestinal Inflammation and Immune Function

The intestinal microbes represent a key regulatory check point for the development of the adaptive immune system and also for the innate inflammatory response. The intestinal epithelium relies on

• BOX 75.1 Functions of Microbiota

1. Metabolic/nutritional
 - a. Vitamins
 - b. Short-chain fatty acids
2. Intestinal development
 - a. Epithelial proliferation, restitution
 - b. Angiogenesis
3. Exclusion of pathogens
4. Innate immune regulation
 - a. Modulation of inflammatory response
 - b. Recognition and destruction of pathogens
5. Adaptive immune regulation
 - a. Development of tolerance

toll-like receptors (TLRs) to act as an interface between the luminal microbiota and signal transduction pathways. TLRs are cell-surface receptors that recognize specific microbial ligands, from both pathogens and commensals, enabling the innate immune system to recognize nonself and activate both innate and adaptive immune responses (Takeda et al., 2003). The epithelium and resident immune cells are dependent on commensal bacteria, which secrete molecules such as lipopolysaccharides and lipoteichoic acid. These, in turn, interact with a population of surface TLRs, wherein signaling enhances the ability of the epithelial surface to withstand injury while at the same time priming the surface for enhanced repair responses (Madara, 2004; Strober, 2004). Therefore disrupting TLR or removing the TLR ligands (bacteria) will decrease the ability of the intestinal surface to protect and repair itself after occurrence of an inflammatory or infectious insult (Rakoff-Nahoum et al., 2004).

In addition to the effects of the microbiota on the innate immune system, there are significant effects on the adaptive responses as well. Regulatory T cells (T_{regs}) are able to dampen inflammation after microbial infection. Subsets of T_{regs} are generated in the gut from naïve T cells (“inducible” T_{regs}). This can be important because these produce the anti-inflammatory cytokine interleukin-10 (IL-10) (Lee and Mazmanian, 2010). Several bacteria have been shown to induce T_{regs} and IL-10 production in the gut (O’Mahony et al., 2008). Members of the genus *Bacteroides* contain the bacterial molecule polysaccharide A, which directs the cellular and physical development of the immune system via T_{regs} and is thereby considered protective because of its ability to suppress inflammation-driven host pathology. This is an area of considerable current investigation because of its likely role in numerous disease entities such as type 1 diabetes and other autoimmune diseases.

The Bioreactor Function

Complex carbohydrates are poorly digested by the human digestive system and require microbiota for breakdown via fermentation. In the distal intestine, primarily the colon, a bioreactor function of the intestinal microbes occurs and is highly amenable to study using metabolomic methodologies (Nyangale et al., 2012). The end products of fermentation include SCFAs such as acetate, propionate, and butyrate. The SCFAs influence various aspects of intestinal physiology beyond being a caloric source (Rios-Covian et al., 2016). They possess differentiating and growth-promoting activities that are thought to be related to their effects on histone deacetylase activity and also have epigenetic effects. They have

immunomodulatory effects and major effects on the interepithelial tight junctions. Examples of processes that can be mediated via this bioreactor process include how changes in the microbial population affect obesity, noninsulin-dependent diabetes, and atherosclerosis (Ley et al., 2006). In the distal intestine, primarily the colon, undigested complex carbohydrates in postweaned individuals are primarily vegetable fibers, whereas in the infant, they are primarily undigested human milk oligosaccharide (Bode, 2015) and, in some cases, primarily very preterm infants, nonabsorbed lactose (Kien et al., 2002). This is of importance for preterm infants, who are thought to have lower activities of lactase than term infants (Antonowicz and Lebenthal, 1977). Even if there is a deficiency of lactase activity in the small intestine of very premature neonates, a healthy distal intestinal flora is able to “salvage” some of the undigested lactose into absorbable and usable 2–4 carbon energy sources (Kien, 1996).

There is a large effect of the microbiota on mammalian plasma biochemistry. Mass spectrometry-based profiling of serum from germ-free and nongerm-free mice demonstrates that a significantly large number of chemical species found in systemic circulation arise because of the presence of the microbiota. These microbe-related metabolites can be beneficial or toxic to the host (Wikoff et al., 2009).

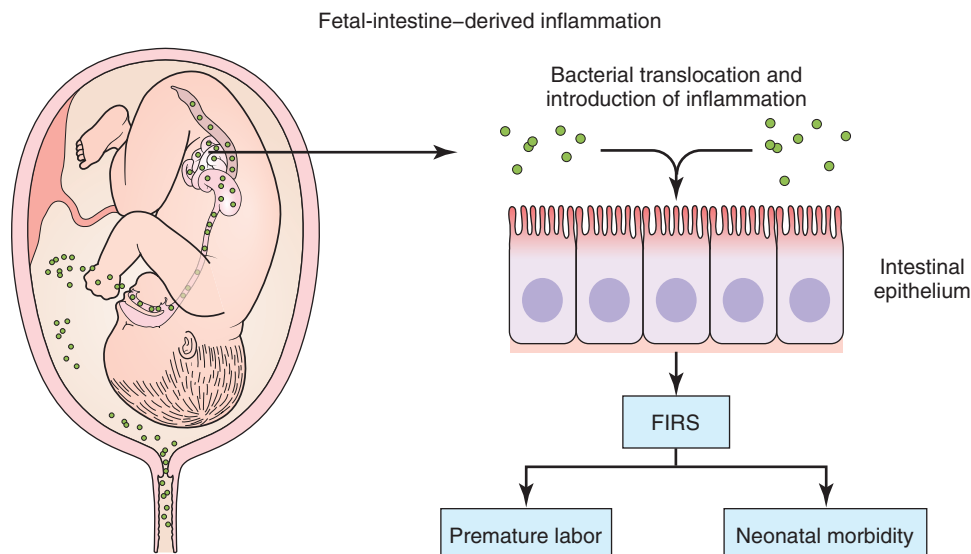
Development of the Intestinal Microbiome

The Nonsterile Fetal Environment

The dogma that the fetus resides in a sterile environment and that the newborn only attains its microbiota after exposure to the extrauterine environment is being effectively challenged (Funkhouser and Bordenstein, 2013; Reid et al., 2015; Romano-Keeler and Weitkamp, 2015; Rautava, 2016). It has been known for decades that even without a ruptured amnion, amniotic fluid frequently contains significant levels of bacteria (Bobitt and Ledger, 1977).

Additionally, studies using nonculture-based techniques further reveal the presence of microbes in placenta, amniotic fluid, and meconium (Han et al., 2009; DiGiulio, 2012; Moles et al., 2013; Aagaard et al., 2014; Ardisson et al., 2014; Payne and Bayatibojakhi, 2014), and the taxonomy of these microbes differs, depending on the stage of fetal maturity (Aagaard et al., 2014; Ardisson et al., 2014). The finding that a greater degree of prematurity is directly related to the bacterial DNA load in amniotic fluid suggests a relationship between preterm delivery and microbial load (DiGiulio et al., 2008; DiGiulio, 2012). This relationship is further causally implicated by microbial DNA concentrations that directly correlate with levels of white blood cells and IL-6 in the amniotic fluid. This suggests a pathophysiologic sequence of increased microbial load, inflammation, and preterm birth (DiGiulio et al., 2008; Han et al., 2009; Combs et al., 2014). Intrauterine “infection” and a subsequent fetal inflammatory response have been linked to prematurity, brain, lung, and eye disease after preterm birth (Dammann et al., 2002; Dammann and Leviton, 2006). One hypothesis relates swallowed amniotic fluid microbes to an intestine-derived inflammatory response and preterm labor and neonatal morbidities (Fig. 75.3). In this hypothesis, amniotic fluid microbes (presumably derived from ascending vaginal microbes) are swallowed by the fetus. These microbes subsequently reach the highly immunoreactive fetal GI tract, where a fetal-derived inflammatory response syndrome (FIRS) incites an inflammatory response that leads to premature labor and neonatal morbidity.

Studies interrogating the placental microbiome show a relationship between different taxa of microbes and level of prematurity (Aagaard et al., 2014). Of interest, a similarity between placental microbes and oral microbial DNA sequences from a database of nonpregnant women was found. It was also found in these studies that *Escherichia coli* was one of the largest taxa represented in the placental samples (Aagaard et al., 2014). It is of interest that this is a common resident of the intestine and thus suggests a maternal intestinal origin.



• **Fig. 75.3** Hypothetical Pathway Through Which Amniotic Fluid Microbes Are Swallowed by the Fetus. These microbes (presumably derived from ascending vaginal microbes) subsequently reach the highly immunoreactive fetal gastrointestinal tract, where a fetal-derived inflammatory response syndrome incites an inflammatory response that leads to premature labor and neonatal morbidity. *FIRS*, Fetal-derived inflammatory response syndrome.

The mechanisms of how the maternal–fetal microbes relate to preterm birth are unclear, but evidence suggests that microbes in the fetal intestine may play a role (see Fig. 75.3). This is supported by (1) the well-known relationship of amniotic fluid microbial colonization, FIRS, and premature labor (Bobitt and Ledger, 1977; Romero et al., 1988, 1998, 2007; Andrews et al., 1995; Goldenberg et al., 2008), (2) the fact that the fetus swallows large quantities of amniotic fluid during the late second and third trimesters of pregnancy (Brace and Wolf, 1989; Gilbert and Brace, 1993), and (3) the capability of systemic inflammation to be derived from the GI tract (Carrico et al., 1986), especially by the fetus (Nanthakumar et al., 2000).

Maternal Intestinal Host–Microbial Interactions

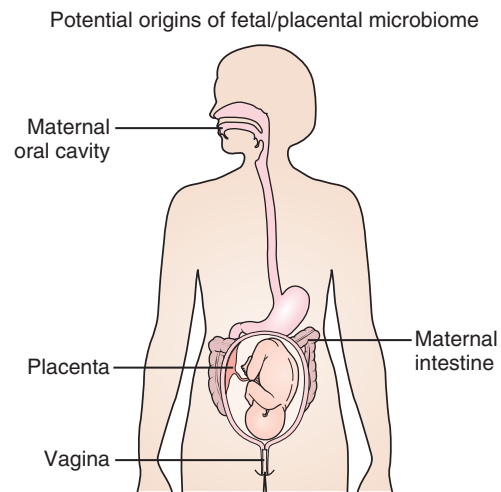
Maternal intestinal host–microbial interactions contribute to maternal metabolism during pregnancy and suggest that “dysbiosis” (defined as a microbial imbalance on or inside the body) in the maternal GI tract may influence the fetal inflammatory mediators, T-cell effector, and tolerizing regulatory cells and metabolic patterns that lead to preterm delivery. The mother immunologically recognizes the fetus as self via a balance of these T cells and maintains tolerance, but dysbiosis may affect this balance, leading to a response that leads to prematurely eliminating the fetus from the intrauterine environment (Trowsdale and Betz, 2006). The effects of the pregnant woman’s diet on the microbiome, epigenome, and metabolome affecting subsequent disease in the offspring have been recently shown in studies where feeding mice a high-fiber diet yielded a distinctive gut microbiota, which increased the levels of the SCFA acetate and markedly decreased allergic disease in the offspring (Thorburn et al., 2015). Thus an evaluation of the mother’s diet and how it affects the microbiota may reveal approaches to decrease morbidity and preterm birth by modulation of the maternal microbiome by dietary means during pregnancy. This could be via altered metabolites and/or inflammatory mediators, which should be detectable in the maternal stools, blood, and/or urine.

Accumulating evidence supports that innate immune cells such as neutrophils, macrophages, and mast cells mediate the process of labor by releasing proinflammatory factors such as cytokines, chemokines, and matrix metalloproteinases (Lusyati et al., 2013). T-cell subsets and B cells (adaptive immune cells) participate in the maintenance of feto–maternal tolerance during pregnancy, and an alteration in their function or abundance may lead to preterm labor (Challis et al., 2009; Gomez-Lopez et al., 2014). Marked elevations of peripheral blood CD4⁺CD25⁺ Forkhead Box P3 (Foxp3⁺) T_{regs} are found during early pregnancy but decline during labor, either at term or preterm, suggesting their involvement in maintenance of pregnancy and initiation of labor (Xiong et al., 2010). Also, immune cells that bridge the innate and adaptive immune systems such as dendritic cells may also participate in the pathophysiology of preterm labor. A balance between innate and adaptive immune cells is required in order to sustain pregnancy; an alteration of this balance will lead to labor (Gomez et al., 1998; Gomez-Lopez et al., 2014).

The Fetal–Maternal Microbiome Source

There is a gap in our knowledge of the source of the placental/fetal microbiome. Potential sources are illustrated in Fig. 75.4.

Vaginal microbes have been implicated in induction of preterm labor (McGregor et al., 1994; White et al., 2011; DiGiulio, 2015). However, another study contradicts the notion that microbes



• **Fig. 75.4** Potential Origins of the Fetal/Placental Microbiome. These include a hematogenous route from the oral cavity or intestinal tract or an ascending route via translocation through the chorio–decidual membranes.

originating from the vagina are the origin of preterm labor (Romero et al., 2014). Another potential source is from the maternal mouth (periodontal tissue) (Madianos et al., 2013). One study interrogated the placental microbiome from infants born at various gestational ages and found associations between placental microbial taxa, preterm birth, and infections early in pregnancy (Aagaard et al., 2014). Intriguingly, a similarity between the placental microbes and those found in the mouth from a human microbiome database was also found, but the samples were not derived from the same individuals; thus direct comparisons between microbial sources are not possible. In addition, the placental taxa that were most highly represented in that study were *E. coli*, which are intestinal microbes. More recent studies (Shiozaki et al., 2014) evaluated maternal stool and vaginal samples from three groups of women who (1) delivered term babies without preterm labor, (2) delivered term babies but with preterm labor, and (3) had preterm birth. Significant differences in microbial taxa were found in fecal samples between these three groups: *Clostridium*, *Lactobacillus*, and *Bacteroidetes* clusters differed significantly. As in a study of Romero et al. (2014), no differences were found in the vaginal microbiota between these three groups, suggesting the maternal intestinal tract is the source of fetal microbes linked to preterm birth. Importantly, it is known that microbes can translocate via the maternal GI tract in rodents (Jimenez et al., 2008) and humans (Rautava et al., 2012) and that food-borne *Listeria* can translocate from the maternal intestine to the fetus (Vazquez-Boland et al., 2001). Thus finding the major sources of the microbes that relate to preterm labor and other morbidities related to inflammation will be a major challenge.

Knowledge of where the fetal–maternal microbes are derived from is critical if we are to propose specific microbial-based therapeutic interventions to prevent spontaneous preterm birth. The origin of these bacteria may differ, depending on the bacterial taxa being considered. One could conjecture that the maternal intestine is a likely source of most of these microbes, but, as previously mentioned, ascending microbes from the vaginal tract and/or hematogenously derived oral microbes may also be involved. This requires more rigorous investigation by evaluating microbiota from various sources in the same mother–infant pair (placenta, vagina,

mouth, blood, maternal stool, and infant meconium) using 16S rRNA and metagenomic sequencing and newly developed bioinformatics source tracking tools.

The Infant Microbiome

Mode of Delivery

There is an association between mode of delivery—vaginal versus cesarean section (C-section)—and the subsequent composition of the microbiome of the developing infant (Salminen et al., 2004; Biasucci et al., 2008; Dominguez-Bello et al., 2010; Cabrera-Rubio et al., 2012; Azad et al., 2013; Jakobsson et al., 2014). This could have significant public health implications for areas where the rate of C-section delivery without strong medical indications is very high, since C-section delivery is associated with greater risks of various adverse outcomes (Cardwell et al., 2008; Decker et al., 2010; Blustein et al., 2013; Bernardi et al., 2015; Miettinen et al., 2015; Mueller et al., 2015). These epidemiologic studies are fraught with confounding factors, but such associations need to be seriously considered. The causal relationship between C-section delivery and some of these adverse outcomes remains to be rigorously established. Whether breastfeeding, as suggested by small studies (Azad et al., 2013; Madan et al., 2016), may reverse the microbiome toward one that is similar in composition and function as that resulting from vaginal delivery remains unclear. Studies showing partial restoration of the microbiota of C-section-born infants via vaginal microbial transfer using vaginal swabs are intriguing in that they do suggest that the vaginal microbiota can be restored in C-section-delivered infants (Dominguez-Bello et al., 2016). However, the safety of inoculating the newborn with unknown vaginal microbes has not been adequately investigated, and thus this method is not ready for routine use.

Intestine-Derived Inflammation

A major role has been ascribed to systemic (usually intestine-derived) inflammation on several neonatal diseases, including NEC, chronic lung disease, intraventricular hemorrhage, periventricular leukomalacia, and hematopoietic abnormalities (Dammann and Leviton, 1999; Dammann et al., 2002).

Total Parenteral Nutrition

Practices that are common in the neonatal intensive care unit (NICU) include prolonged use of total parenteral nutrition (TPN), antibiotics, and H₂ blockers. In the premature infant, the reliance on parenteral nutrition may be highly significant in the promotion of intestinal inflammation (Kudsk, 2002). Whether this is partially due to stimulation of commensal bacterial growth is speculative, but studies in animals suggest an overgrowth of potentially pathogenic Proteobacteria when TPN is provided.

Summary

In this chapter, the newly emerging science of the microbiome as it relates to pregnancy and the neonate has been briefly discussed. Interactions between microbes and the host as well as microbial products and the host, especially early in life, may have

Effect of Diet

Human milk contains a wide array of biologically active components. Breastfed infants, unlike those who are formula-fed, have an intestinal ecosystem characterized by a strong prevalence of bifidobacteria and lactobacilli that appear to protect the preterm infant (Saavedra, 2001; Bourlioux et al., 2003). Several studies have shown the presence of microbes in human milk (Hunt et al., 2011; Collado et al., 2015). *Streptococcus* and *Staphylococcus* are highly represented, but other taxa are found as well. These studies suggest that microbes in an individual mother's milk are stable over time but differ markedly from one mother to the next, suggesting “personalization” of milk microbes. Among the numerous substances present in human milk, oligosaccharides are also present (Bode, 2012). These are thought to stimulate the development of bifidogenic microbes in the colon; hence human milk can be considered a symbiotic (Coppa et al., 2004) substance that has the properties of both a probiotic and prebiotic.

Effects of Antibiotics on Intestinal Microbiota

Administration of antibiotics to preterm infants is a common practice in NICUs due to the concern for sepsis. Several studies have shown a dysbiosis that occurs before the onset of NEC (Claud and Walker, 2001; Mai et al., 2011, 2013; Claud et al., 2013; Warner et al., 2016). More recently, it has been shown that the odds of developing NEC and mortality associated with NEC correlate with length of antibiotic exposure (Cotten et al., 2009; Alexander et al., 2011). However, in these studies it is unclear whether the initial degree of illness led to increased antibiotic usage or whether increased antibiotic usage led to increased degree of illness. The effects of antibiotic exposure on the microbial diversity of the intestine have been shown to last months after treatment is discontinued and have been shown to have long-lasting effects. Therefore it is critical that the routine overuse of antibiotics in the preterm be reconsidered.

Effects of H₂ Blockers

Premature infants have limited gastric acid secretion, which can be reduced even further with the use of H₂ blockers that have been linked to NEC in preterm neonates (Guillet et al., 2006; Terrin et al., 2012). H₂ antagonists have been used in the preterm population historically for treatment of apnea and bradycardia putatively because of gastroesophageal reflux, as well as in TPN while a patient is nil per os. It is thought that H₂ antagonists raise the gastric pH and reduce the bactericidal action in the stomach, which increases the total number of bacteria, promoting an overgrowth of potentially pathogenic bacteria (Gupta et al., 2013). These are among the most widely misused drugs in the NICU, and their routine use is discouraged (Ho et al., 2015).

transgenerational effects via epigenetic mechanisms. It should be clear that this is an area that is ripe for scientific and clinically relevant breakthroughs in the prevention and treatment of disease and maintenance of health, even beyond infancy.

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Necrotizing Enterocolitis and Short Bowel Syndrome

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KEY POINTS

- Necrotizing enterocolitis (NEC) is the most common gastrointestinal emergency in the neonatal intensive care unit and a leading cause of mortality among very low birth weight infants.
- Pathogenesis is multifactorial with the combination of impaired intestinal perfusion, abnormal bacterial colonization, impaired gut barrier function, and an immature, overactive immune response leading to intestinal inflammation, ischemia, and eventual necrosis.
- Medical management includes bowel rest, broad-spectrum antibiotics, and supportive care.
- Surgical options include primary peritoneal drainage or laparotomy with bowel resection.
- Mortality from NEC reaches 50% in patients that require surgery, hence preventative measures such as breast-milk feeding and probiotic supplementation are important.

Necrotizing Enterocolitis

Necrotizing enterocolitis (NEC) is the most common gastrointestinal emergency in the neonatal intensive care unit (NICU) and is a leading cause of mortality among preterm and low birth weight infants. The incidence of NEC is inversely proportional to gestational age and birthweight, with NEC affecting between 7% and 10% of infants weighing more than 1500 g. Unfortunately, despite decades of research and improvements to NICU care, the mortality associated with NEC remains between 15% and 30% and is even higher for infants with NEC that require surgical intervention. NEC survivors may suffer from long-term complications such as intestinal stricture, short bowel syndrome (SBS), and neurodevelopmental impairment (NDI). Preventative strategies such as enteral feeding with human milk and probiotic supplementation are paramount for combating this deadly disease.

Epidemiology

NEC is primarily a disease of premature, low birth weight infants that develops days to weeks following the initiation of enteral feedings. In fact, over 90% of reported NEC cases are in premature infants (Uauy et al., 1991; Lemons et al. 2001). In term infants, the incidence of NEC is 0.5 per 1000 live births (Lemons et al.,

2001), and it is typically associated with other risk factors that decrease mesenteric perfusion such as congenital heart disease (CHD), hypoxemic perinatal events, intrauterine growth restriction, sepsis, or respiratory disease (Holman et al., 2006). Premature infants that have not been enterally fed may also develop intestinal perforation, but this disease process tends to be a different pathologic entity termed *spontaneous intestinal perforation* (SIP). SIP is an isolated intestinal perforation without additional signs of intestinal ischemia or necrosis typically seen in NEC. Unlike NEC, SIP most often presents before the initiation of enteral feeding in the first 2 weeks of life (Pumberger et al., 2002). Infants with SIP have much lower morbidity and mortality compared with those with NEC (Fisher et al., 2014). However, SIP and NEC can be difficult to differentiate before operative exploration, and many studies of clinical outcomes in NEC may include babies with both NEC and SIP.

Interestingly, the incidence of NEC varies greatly throughout the world. In the United States, the incidence of NEC in very low birth weight (VLBW) (<1500 g) infants ranges between 3% and 10% (Llanos et al., 2002; Guillet et al., 2006; Holman et al., 2006) compared with 28% in Hong Kong (Siu et al., 1998), 14% in Argentina (Halac et al., 1990), 7% in Austria (Eibl et al., 1988), 7% in Canada (Sankaran et al., 2004), 4% in Wales (Luig et al., 2005), and 1%–2% in Japan (Okuyama et al., 2002). There has been no appreciable decline in the incidence of NEC over the past two decades.

Risk Factors

Prematurity and Low Birth Weight

A number of risk factors have been found to be consistently associated with NEC, the most convincing being prematurity and low birth weight. Lemons et al. (2001) first reported the inverse relationship between birthweight and NEC. Recently, Jaksic and colleagues have elegantly demonstrated this relationship and how it relates to NEC-associated mortality, using data from the Vermont Oxford Network (Fitzgibbons et al., 2009). The incidence of NEC increases steadily from 3% in infants between 1250 g and 1500 g to 12% in infants between 500 g and 750 g. Concordantly, the mortality of those two groups is 16% and 42%, respectively.

Formula Feeding

Formula feeding is associated with an increased risk of NEC development, compared with feeding with human milk. Human milk has long been known to be the optimal nutritional source for premature infants and protective against the development of NEC (Lucas and Cole, 1990). There is now a large body of evidence to support these findings, including a recent Cochrane review that reports a 2.8-fold increase in the risk of NEC in premature infants fed with formula, compared with those fed with human milk (Cristofalo et al., 2013; Quigley and McGuire, 2014).

Congenital Heart Disease

Additional risk factors that have been associated with an increased rate of NEC include CHD and indomethacin use.

Prophylactic patent ductus arteriosus (PDA) closure was previously thought to carry up to a 30% risk of NEC; however, further study has indicated that it is indomethacin treatment, rather than the PDA itself, that increases the risk of NEC. Subsequent randomized controlled trials and Cochrane reviews have indeed demonstrated an increased rate of NEC in premature infants treated with indomethacin. Furthermore, recent data have found that PDA closure with ibuprofen was as effective as indomethacin and resulted in a greater than 50% risk reduction of NEC (Ohlsson et al., 2015).

More serious forms of CHD (excluding atrial septal defects, PDA, and ventricular septal defects) are associated with an increased incidence of NEC. The Vermont Oxford Network recently reported a 13% risk of NEC in VLBW infants with CHD compared with 9% in VLBW infants without CHD. In addition, the combination of CHD and NEC increased the overall mortality rate to 55% (Fisher et al., 2015).

Blood Transfusions

There is a growing body of evidence that suggests an association between packed red blood cell (PRBC) transfusion and NEC. It is hypothesized that intestinal vascular autoregulation occurs after correction of anemia leading to transfusion-related acute gut injury (or “TRAGI”) (Marin and Strickland, 2013; Nickel and Josephson, 2015). More data are needed to better understand this pattern as all studies to date are retrospective, and there is at least one cohort study showing no temporal association of NEC and PRBC transfusions.

Infectious Agents

Though NEC typically presents sporadically, there are numerous reports of NEC outbreaks in the NICU setting and occasional epidemics that seem to be associated with a single, presumably causative, bacterial pathogen (Boccia et al., 2001). Organisms that have been associated with NEC include *Klebsiella pneumoniae*, *Escherichia coli*, *Clostridium perfringens*, coagulase-negative staphylococci, and rotavirus. Although it has been widely hypothesized that NEC is caused by a single infectious agent, no single pathogen has fulfilled all of Koch postulates, and it is now presumed that abnormal colonization of the premature infant gut with pathogenic bacteria is a key contribution to but not the absolute cause of NEC.

Pathogenesis

The exact mechanism of NEC pathogenesis has long been the scourge of many research laboratories; despite decades of dedicated

research, a complete understanding of NEC pathogenesis remains elusive. The current understanding of the disease is that it is multifactorial, with the combination of impaired intestinal perfusion, abnormal bacterial colonization, impaired gut barrier function, and an immature, overactive immune response leading to intestinal inflammation, ischemia, and eventual necrosis.

Bowel Ischemia

Early hypotheses regarding NEC pathogenesis revolved around the idea that perinatal hypoxemic/ischemic events led to splanchnic hypoperfusion and resultant bowel injury (Touloukian et al., 1972). Supporting this hypothesis, histologic specimens of NEC demonstrate ischemic features such as mucosal ulceration and coagulative necrosis (Nowicki, 2005). Epidemiologic findings, including the increased incidence of NEC in infants with severe CHD, maternal cocaine use, and, potentially, an association with blood transfusion, also support this mechanism. However, the vast majority of infants with NEC have no antecedent hypoxemic event (Stoll et al., 1980). In addition, many NEC animal models use intermittent hypoxia to induce intestinal injury similar to human NEC, though often an additional factor such as a pathologic bacterium or lipopolysaccharide is required to induce NEC, suggesting hypoxia alone is insufficient to cause NEC (Sodhi et al., 2008). More recent evidence would suggest that an exaggerated inflammatory response in the premature intestine leads to alteration of intestinal microcirculatory homeostasis, regulated by a balance between vasodilatory nitric oxide and the vasoconstricting mediator endothelin-1 (Watkins and Besner, 2013). Mucosal inflammation alters the balance to favor vasoconstriction, which leads to intramural microvascular ischemia and represents a critical secondary event in NEC pathogenesis but most likely not the initiating factor.

Abnormal Bacterial Colonization

Compared with full-term neonates, premature neonates have decreased overall bacterial diversity as well as a decreased quantity of commensal organisms such as *Bifidobacteria* species (spp.) and *Lactobacillus* spp. that protect against inflammation and promote intestinal health (Gewolb et al., 1999). In addition, there have been several reports of local NEC outbreaks suggesting that bacteria may play a causative role in NEC pathogenesis (Boccia et al., 2001). Although there have been numerous studies attempting to identify specific bacteria associated with NEC, there is little consensus, and no consistent bacterial species has been identified that meets criteria as a cause of NEC. Even so, germ-free animals do not develop NEC (Museumche et al., 1986), and it is accepted that abnormal colonization of the intestine with potentially pathogenic gram-negative bacteria such as *Escherichia*, *Salmonella*, *Shigella*, *Klebsiella*, and/or *Pseudomonas* plays a key role in NEC pathogenesis (Grishin et al., 2013).

Impaired Gut Barrier Function and Proinflammatory Response

Regardless of the etiology of the initial insult, these events lead to breakdown of the intestinal epithelial barrier coupled with an exaggerated and immature immune response. This, in turn, results in a proinflammatory response that drives the progression of NEC.

The centerpiece of the proinflammatory response appears to be toll-like receptor 4 (TLR4) (Hackam et al., 2013). TLR4 is a

highly conserved pattern-recognition receptor that is activated by lipopolysaccharide, a virulent cell wall protein found on gram-negative bacteria. Full-term infants express low levels of TLR4 on the intestinal mucosa. In contrast, the premature gut is characterized by high levels of TLR4. When the premature gut is colonized with pathogenic, gram-negative bacteria, there is an exaggerated downstream inflammatory response mediated by increased production of nuclear factor kappa B, which drives transcription of proinflammatory cytokines (e.g., interleukin (IL)-1 and tumor necrosis factor- α), inducible nitric oxide synthase, and phospholipase A2 (Gribar et al., 2009). Premature infants have also shown a propensity to synthesize the proinflammatory cytokine IL-8 in response to pathogenic bacteria (Claud et al., 2004). In addition, premature infants have decreased platelet-activating factor (PAF)-acetylhydrolase activity, which predisposes to increased levels of PAF, a potent proinflammatory mediator that contributes to NEC (Caplan et al., 1990). This exaggerated proinflammatory response leads to gut barrier breakdown, further translocation of pathogenic bacteria, systemic sepsis, and continued overactivation of the proinflammatory cascade.

In addition to the enhanced proinflammatory pathways, antiinflammatory mediators such as epidermal growth factor (EGF) are depleted in premature neonates (Shin et al., 2000). In particular, heparin-binding EGF (HB-EGF), a member of the EGF family, is a growth factor that functions to maintain gut barrier function and facilitates intestinal epithelial repair following injury. HB-EGF also functions as a potent antiinflammatory mediator and has shown benefit in animal models as a treatment for intestinal injury and prevention of NEC. Furthermore, HB-EGF is present in breast milk and is decreased in infants with NEC, suggesting a significant role in NEC pathogenesis (Besner, 2015).

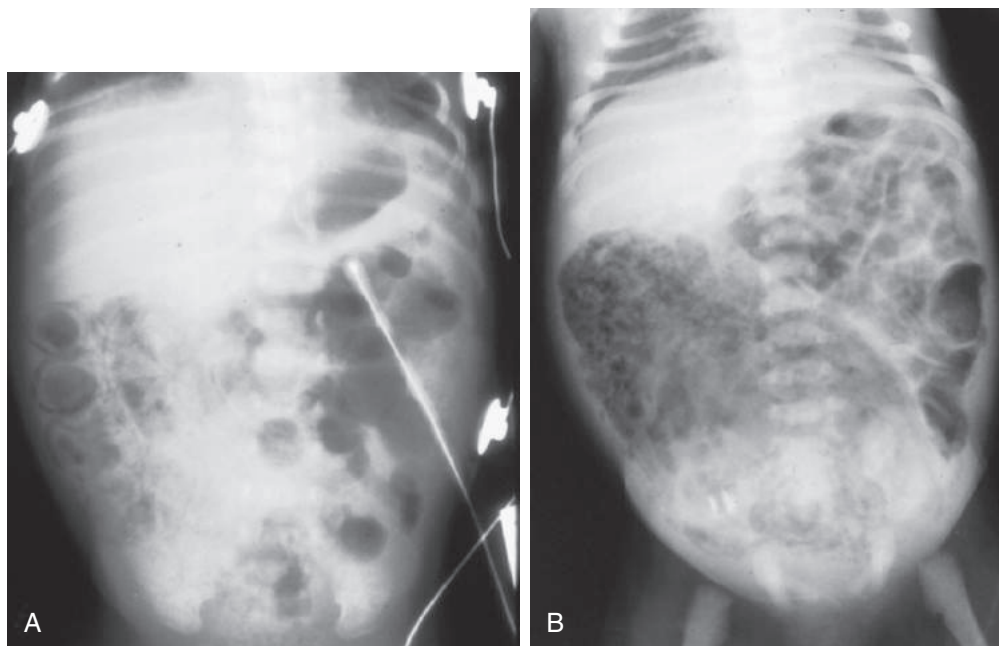
This combination of an overzealous proinflammatory response without counterregulation by key antiinflammatory mediators, coupled with impairment of intestinal epithelial barrier, results in

severe intestinal inflammation and necrosis, the clinical hallmarks of NEC.

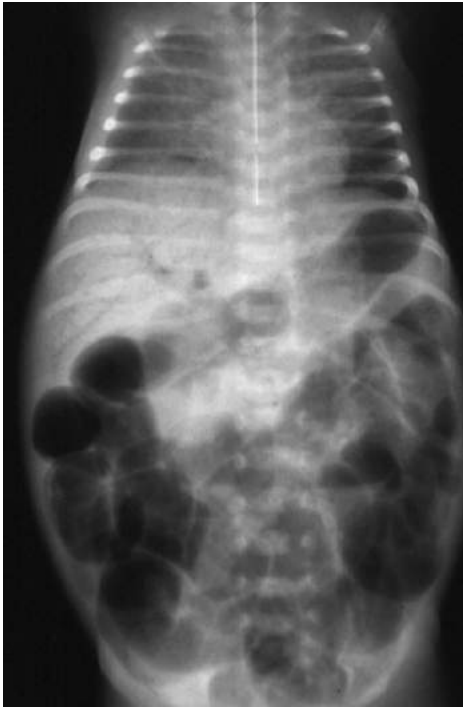
Clinical Presentation

The clinical presentation of NEC can include a wide range of findings. In mild cases, the only signs may include slight hematochezia and high gastric residuals. More involved cases of NEC may present with abdominal distention and tenderness with frank intolerance of feedings. In the most severe cases, signs of significant inflammatory response and sepsis are present, which may include thrombocytopenia, leukopenia, lethargy, acidosis, hypotension, apnea, and death. Additionally, the time course for progression from first symptoms to significant hemodynamic compromise can be extremely variable, with the potential for rapid progression and arrest if unrecognized.

A common finding with new onset NEC is pneumatosis intestinalis (Fig. 76.1) on abdominal radiograph, and this can be seen with or without the presence of portal venous gas (Fig. 76.2). The **Bell criteria**, published first in 1978 (Bell et al., 1978) and then further modified in 1986 (Walsh and Kliegman, 1986), is a classification system that was developed with the goal of standardizing the therapeutic options for NEC based on the precise stage of the disease at diagnosis (Table 76.1). The presence of portal venous gas with or without pneumatosis intestinalis is associated with a worse prognosis (Molik et al., 2001). Although it has been debated whether the presence of portal venous gas on abdominal radiograph is a relative indication for an operation, it is widely regarded as an indicator of poor clinical outcome. It is important to obtain multiple view abdominal films in order to identify the presence of pneumoperitoneum, and this is most easily identified on a cross-table lateral or right lateral decubitus film (Fig. 76.3A). Other more occult findings of free air that may be seen include a periumbilical collection or so-called “football sign,” seen on an



• **Fig. 76.1** (A) Typical abdominal radiographic appearance of intestinal pneumatosis seen in necrotizing enterocolitis: dark concentric rings around the bowel loops in the right upper quadrant. (B) Radiograph displaying the *bubbly gas* pattern occasionally seen in necrotizing enterocolitis. (Courtesy Dr. Lalo Cabrera-Meza, Baylor College of Medicine, Houston, Texas.)



• **Fig. 76.2** Radiograph displaying the presence of portal gas, which is seen as linear dark streaks within the hepatic density. (Courtesy Dr. Lalo Cabrera-Meza, Baylor College of Medicine, Houston, Texas.)

anterior–posterior film (see Fig. 76.3B). More recently, ultrasonographic findings of portal venous gas and pneumatosis have been used to diagnose NEC. Bohnhorst et al. (2003) and Dördelmann et al. (2009) have shown that these sonographic findings may allow for earlier, more precise diagnosis of NEC. Ultrasound finding of portal venous gas, however, should be used with caution when deciding on the need for operative intervention, as this is found in up to 10% of patients with mild cases of NEC that resolve with medical management alone (Silva et al., 2007; Garbi-Goutel et al., 2014).

Despite the development of scoring systems, it is still difficult to predict which infants will progress rapidly to fulminant NEC requiring operative intervention and which will have a more benign clinical course. Importantly, some infants who require surgery will remain stable enough to transport to the operating room for surgery while others will progress so rapidly that transport to the operating room for intervention is unsafe, requiring operative intervention in the NICU or resulting in the infant succumbing to his or her disease. Ng et al. (2013) described the LIT score, using a combination of biomarkers to attempt to identify the most severely affected surgical NEC patients. A combination of gut barrier biomarkers, *L-FABP*, *I-FABP*, and *7FF3* (LIT), was measured, and infants who went on to develop surgical NEC had significantly higher levels of biomarkers when compared with infants who only exhibited signs of septicemia or medical NEC. Although this study has shown promise in identifying the most high-risk patients, it is currently not used at most centers.

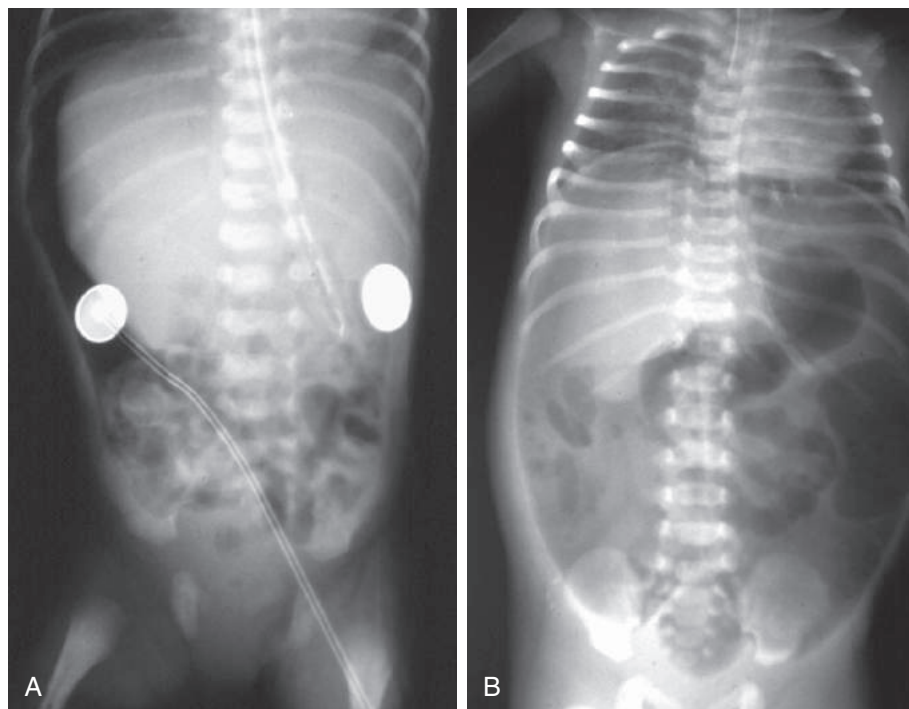
TABLE 76.1

Modified Bell Staging Criteria for Necrotizing Enterocolitis

| Stage | Systemic Signs | Intestinal Signs | Radiologic Signs | Treatment |
|-----------------------------------|---|---|--|--|
| I: Suspected | | | | |
| A | Temperature instability, apnea, bradycardia | Elevated pregavage residuals, mild abdominal distention, occult blood in stool | Normal or mild ileus | NPO antibiotics × 3 days |
| B | Same as for IA | Same as for IA, plus gross blood in stool | Same as for IA | Same as for IA |
| II: Definite | | | | |
| A: Mildly ill | Same as for IA | Same as for I, plus absent bowel sounds, abdominal tenderness | Ileus, intestinal pneumatosis | NPO, antibiotics × 7–10 days |
| B: Moderately ill | Same as for I, plus mild metabolic acidosis, mild thrombocytopenia | Same as for I, plus absent bowel sounds, definite abdominal tenderness, abdominal cellulitis, right lower quadrant mass | Same as for IIA, plus portal vein gas, with or without ascites | NPO, antibiotics × 14 days |
| III: Advanced | | | | |
| A: Severely ill, bowel intact | Same as for IIB, plus hypotension, bradycardia, respiratory acidosis, metabolic acidosis, disseminated intravascular coagulation, neutropenia | Same as for I and II, plus signs of generalized peritonitis, marked tenderness, and distention of abdomen | Same as for IIB, plus definite ascites | NPO, antibiotics × 14 days, fluid resuscitation, inotropic support, ventilator therapy, paracentesis |
| B: Severely ill, bowel perforated | Same as for IIIA | Same as for IIIA | Same as for IIB, plus pneumoperitoneum | Same as for IIA, plus surgery |

NPO, Nil per os.

(From Walsh MC, Kliegman RM, Fanaroff AA. Necrotizing enterocolitis: a practitioner's perspective. *Pediatr Rev*. 1988;9:219–226. Reproduced with permission from *Pediatrics*.)



• **Fig. 76.3** (A) Radiograph demonstrating intestinal perforation, displayed by the presence of a gas lucency lying between the hepatic density and the outer abdominal wall. (B) Radiograph from another infant whose bowel is perforated. Air lies anterior to the loops of intestine as well as along the right abdominal wall, where it is displacing loops of bowel medially. (Courtesy Dr. Lalo Cabrera-Meza, Baylor College of Medicine, Houston, Texas.)

Treatment

Medical Management

Treatment for NEC is generally geared toward supportive care, since no particular therapy or intervention has been identified to slow or stop the progression of this disease process. Prompt initiation of broad-spectrum antibiotics, cessation of feedings, and gastric decompression are the keys to treatment. There is no data-driven consensus on the specific antibiotic regimen for treating NEC, as highlighted by an international survey and Cochrane review ([Shah and Sinn, 2012](#); [Zani et al., 2015](#)). Therefore antibiotics are often prescribed by institutional protocol depending on local antibiotic sensitivities. In general, broad-spectrum antibiotics are initiated early to cover typical enteric flora, including gram-negative and anaerobic coverage. In centers with frequent staphylococcal colonization, vancomycin should also be considered. Conservative treatment in this fashion is usually continued for a minimum of 7 to 10 days depending on the severity of clinical and radiographic findings, although no studies have specifically investigated the optimal course for antibiotic therapy. In patients who require surgical intervention, intraoperative cultures may help guide antibiotic therapy.

Generally, as long as the clinical status of the patient is stable or improving with these measures, intermittent radiographs monitoring pneumatosis and medical management are continued until resolution of the pneumatosis on films and improvement in the abdominal examination. Any deviation from a stable clinical course exhibited by hemodynamic instability, respiratory compromise from abdominal distention, thrombocytopenia, anemia, or disseminated intravascular coagulation may obligate surgical intervention. Once the antibiotic course has been completed and

the abdominal examination normalized, enteral feeds are slowly introduced and advanced carefully.

Surgical Management

Despite optimal neonatal intensive care, up to 50% of infants with NEC will require surgical intervention. The one truly absolute indication for operative intervention is pneumoperitoneum. Relative indications include diffuse pneumatosis intestinalis, portal venous gas, a fixed, dilated loop of intestine, or clinical deterioration despite maximal medical therapy. When the severity of NEC reaches the point of surgical intervention, there is an associated increase in mortality: 35% for VLBW infants with surgical NEC compared with 21% for VLBW infants with medical NEC ([Fitzgibbons et al., 2009](#)). In addition, surgical NEC survivors are at increased risk for SBS, intestinal strictures ([Heida et al., 2015](#)), and NDI compared with infants with medical NEC ([Murthy et al., 2014](#)).

Laparotomy Versus Peritoneal Drain Placement

Historically, surgical intervention for NEC was predicated on exploratory laparotomy, resection of all segments of necrotic bowel, and creation of enterostomies ([Walsh and Kliegman, 1986](#)). More recently, however, there has been a movement toward use of primary peritoneal drainage (PPD) as a definitive therapy for NEC. PPD was first used as a temporizing measure in 1977 ([Ein et al., 1977](#)) and was described as definitive therapy in 1992 ([Takamatsu et al., 1992](#)). Since that time there have been many studies comparing the effectiveness of PPD with laparotomy. In 2001, a metaanalysis was unable to determine a difference in outcomes between PPD and laparotomy in infants with NEC who required surgical

intervention (Moss et al., 2001). This was followed by an observational study using the National Institute of Child Health and Human Development database that also found no difference in mortality between PPD and laparotomy for extremely low birth weight (ELBW) infants with surgical NEC (Blakely et al., 2006). However, this study also showed a trend toward improved neurodevelopmental outcomes in the laparotomy group. In contrast, a study using the Kids' Inpatient Database found that PPD was associated with a 5.7-fold increased odds of death compared with laparotomy; this has been confirmed in additional cohort studies (Choo et al., 2011). All of these studies are confounded by selection bias, with the most critically ill and most premature infants tending to be treated with PPD.

These conflicting data led to two randomized controlled trials that compared PPD and laparotomy, the NEC Steps and NET trials. The NEC Steps trial found that in VLBW infants there was no statistical difference in 90-day mortality or duration of parenteral nutrition between PPD as definitive therapy and laparotomy (Moss et al., 2006). The NET trial studied PPD as a temporizing measure versus initial laparotomy in ELBW infants and also found no difference in mortality between the two groups. There was, however, a trend toward prolonged time to full enteral feeds in the PPD group (Rees et al., 2008). Of note, 50% of infants in the PPD groups required eventual laparotomy in both trials. Unfortunately, both of these studies were underpowered and did not look at NDI as an outcome. As a result, both a Cochrane review (Rao et al., 2011) and a recent systematic review by the American Pediatric Surgical Association outcomes committee (Downard et al., 2012) have concluded that there is still insufficient evidence to recommend PPD as a definitive therapy for surgical NEC. We will hopefully have an answer to this debate after the completion of the NEST trial (NCT 01029353), which has randomized infants weighing less than 1000 g to either PPD or laparotomy and will assess outcomes, including NDI, at 18 to 22 months.

Surgical Options

It is important to know that several surgical options exist when the decision is made to perform a laparotomy for NEC. The traditional approach of resection of necrotic bowel and ostomy creation is effective but carries the risks of stoma complications as well as the need for a second operation for ostomy takedown (O'Connor and Sawin, 1998). Resection with primary anastomosis in an appropriately sized and hemodynamically stable premature infant avoids the risks of stomas and has the advantage of requiring only one operation (Griffiths et al., 1989). However, the risk of leak and stricture may be greater with this treatment, and more data are needed before a definitive recommendation for primary anastomosis can be made.

For patients with more extensive necrosis or multiple segments of compromised bowel, additional surgical options such as the "clip and drop" technique (Vaughan et al., 1996) or the "patch, drain, and wait" approach (Moore, 2000) require multiple, staged operations but may result in improved preservation of bowel length in those infants at high risk of developing SBS. Data for these techniques are limited to small case series in the surgical literature.

Outcomes

NEC-associated mortality remains high, with the poorest prognosis in VLBW infants who develop surgical NEC. Overall, the mortality for VLBW infants with medical NEC is 21%, ranging between

6% in infants weighing 1500 g to nearly 50% in infants less than 500 g. Similarly, infants with NEC who weigh less than 500 g and require surgical intervention have an associated mortality of nearly 50%. Unlike medical NEC, mortality in surgical NEC remains at 30%, even for infants weighing 1500 g (Fitzgibbons et al., 2009).

One of the most common delayed complications in survivors of both medical and surgical NEC is the formation of intestinal strictures, leading to feeding intolerance. Strictures occur in up to 35% of NEC survivors after recovery and reinitiation of enteral feeds (Horwitz et al., 1995). Although the typical presentation is feeding intolerance 2 to 3 weeks after reinitiation of feeds, NEC-associated strictures have been reported as late as 6 weeks following a course of NEC (Valla et al., 1985). Most strictures associated with NEC will require surgical intervention and are diagnosed using fluoroscopic imaging studies.

Other complications associated with NEC include SBS, line infections from chronic central venous access, and parenteral nutrition-associated liver disease (PNALD). Premature infants are at increased risk for PNALD in general, and therefore PNALD is frequently observed in infants who suffer from NEC. Additionally, recurrent NEC occurs in up to 6% of patients, with medical management being successful for most recurrent cases (Stringer et al., 1993).

Long-term NDI is common in survivors of surgical NEC. Previous studies have demonstrated NDI in up to 50% of NEC survivors at 12 to 20 months of age (Sonntag et al., 2000). A study using the NICHD database found that ELBW infants with surgical, but not medical, NEC had significantly increased odds of NDI compared with infants without NEC (Hintz et al., 2005).

Prevention

Because of the acute presentation of NEC, its rapid progression, and the lack of readily available screening tests, the most promising method of reducing NEC morbidity and mortality lies with prevention. Furthermore, preventative measures are desperately needed as improved neonatal care has resulted in the increased survival of extremely premature infants, who are at the highest risk for developing NEC. In animal studies, a lower incidence of NEC has been associated with breast-milk feeding (Dvorak et al., 2004), supplementation with immunoglobulin A (IgA) (Barlow et al., 1974), probiotics (Caplan et al., 1999), PAF antagonists (Caplan et al., 1997), polyunsaturated fatty acids (PUFAs) (Lu et al., 2007), EGF (Dvorak et al., 2008), intestinal alkaline phosphatase (Whitehouse et al., 2010), or oxygen radical scavengers (Cueva and Hsueh, 1988). Human studies have shown limited success in the prevention of NEC by using IgA (Eibl et al., 1988), steroids (Halac et al., 1990), PUFAs (Carlson et al., 1998), glutamine (El-Shimi et al., 2015), arginine (Mitchell et al., 2014), and lactoferrin (Manzoni et al., 2014). To date, none of these strategies have shown a significant reduction in the incidence of NEC in large-scale randomized trials, and limited evidence exists to argue for widespread implementation. Current evidence suggests that the two most promising strategies for NEC prevention are exclusive use of human milk and probiotic supplementation.

Exclusive Use of Human Milk

Human milk is the ideal nutritional source for the preterm infant for a myriad of reasons. Importantly for NEC prevention, human milk provides an array of antiinfectious factors such as IgA and

probiotics, as well as essential nutrients that promote intestinal health. There are now numerous randomized controlled trials that have demonstrated a decreased incidence of NEC when premature infants are fed exclusively with human milk, when compared with formula feeding (Schanler et al., 2005; Sullivan et al., 2010; Cristofalo et al., 2013). In addition, there is now a growing body of evidence, including results from a recent randomized controlled trial, of a decreased incidence of NEC in premature infants fed exclusively with donor breast milk, when compared with formula feeding (Cristofalo et al., 2013). Recent Cochrane reviews have concluded that there is compelling evidence to suggest that all premature infants should be fed with human milk when available (Quigley and McGuire, 2014). Future studies will need to evaluate the risk of NEC in babies fed human milk fortified with formula, as this is frequently used for VLBW infants, with that of feeding with standard human milk or formula alone.

In addition to the practice of using human milk, many have posited that carefully regimented feeding regimens may decrease the incidence of NEC. Despite the theoretical benefits, several randomized controlled trials and two recent Cochrane reviews have found no difference in NEC incidence with fast versus slow feeding advancement (Morgan et al., 2015), delayed versus early initiation of enteral feeds (Morgan et al., 2014), and continuous versus bolus enteral feeding (Downard et al., 2012).

Probiotics

Another promising method to prevent NEC is supplementation with probiotics. As discussed earlier, there is abnormal colonization of the premature infant's intestine, and supplementation with commensal, nonpathologic probiotics may blunt the proinflammatory response that drives NEC. Indeed, there are several randomized controlled trials showing a decrease in NEC incidence in VLBW infants after supplementation with probiotics (Wang et al., 2012). In fact, a recent Cochrane review concluded that probiotic supplementation in premature infants is associated with a decrease in both overall mortality and incidence of NEC (Alfaleh and Anabrees, 2014). This group has updated its stance to "strongly encourage" a change in practice to provide routine supplementation with probiotics in the premature infant population. One issue that must still be addressed, however, is the type, timing, and length of treatment, as many of the trials have used different probiotic regimens. In addition, care must be taken when choosing probiotic formulas as these preparations are not carefully regulated, and there have been reports of increased asthma in childhood related to probiotics (Kalliomäki et al., 2007) as well as adverse outcomes in critically ill adults with pancreatitis who were treated with probiotics (Besselink et al., 2008). However, to date, the overwhelming body of evidence suggests that probiotics are safe and effective in preventing NEC.

Short Bowel Syndrome

Intestinal failure is defined as the inability of the intestine to meet energy, fluid, and electrolyte requirements because of inadequate length or function. SBS is the most common form of intestinal failure in children. Frequent etiologies of pediatric SBS include NEC, gastroschisis, intestinal atresia, and midgut volvulus. In this way, SBS in children is often the result of massive intestinal resection in young infants.

The management of children with SBS requires complex and multidisciplinary care. Recently, select tertiary pediatric institutions

have implemented dedicated intestinal rehabilitation programs that incorporate a multidisciplinary team of providers to facilitate long-term care of intestinal failure. Data have demonstrated a survival benefit when children with SBS are treated at dedicated, multidisciplinary pediatric intestinal rehabilitation centers (Modi et al., 2008).

The goal of intestinal rehabilitation is to slowly advance enteral feeds and wean parenteral nutrition as the remaining bowel undergoes the process of intestinal adaptation. Following massive intestinal resection, it is well established that the remnant bowel undergoes significant structural alterations. From a macroscopic standpoint, the proximal bowel dilates to increase the mucosal surface area for nutrient absorption. Any increase in bowel length beyond the overall growth of the patient is negligible with the exception of the extremely preterm infant in whom the bowel may continue to lengthen until term. A recent study, using intraoperative bowel length measurements, demonstrated that small bowel length can double, when the extremely premature neonate is compared with a full-term young infant (Struijs et al., 2009). Microscopic changes in intestinal adaptation include increased crypt depth, greater villous height, and an increased enterocyte number to allow for mucosal hyperplasia. The process of intestinal adaptation likely begins soon after resection but may continue for years in children (Squires et al., 2012). It is thought that the ileum adapts better than the jejunum, and this may explain why patients with an intact ileocecal valve have a better chance of weaning from parenteral nutrition (Khan et al., 2015).

Over the past decade, significant improvement has been observed in the long-term survival of children with SBS. The improved outcomes are likely multifactorial and related to multidisciplinary care, safer delivery of parenteral nutrition, improved prevention and treatment of central line-associated sepsis, and innovative bowel lengthening techniques. Long-term survival of children with SBS now routinely reaches 80%–90% in dedicated intestinal failure centers (Javid et al., 2010; Khalil et al., 2012; Infantino et al., 2013). It is now a reasonable expectation that, if children survive their neonatal critical illness and barring severe extraintestinal comorbidities, children with intestinal failure can survive to school age and beyond. Even children with the shortest bowel lengths can have reasonable long-term outcomes, although they require ongoing advanced care (Infantino et al., 2013; Sanchez et al., 2013). In addition, preliminary studies on the neurodevelopment of children with intestinal failure have shown encouraging results (Javid et al., 2015). Controversy exists as to the minimum length of small bowel required for survival, and this issue poses both physiologic and ethical challenges. Infants with any length of remaining viable bowel distal to the ligament of Treitz have shown reasonable survival in contemporary series, although infants with the shortest bowel remnants may ultimately require intestinal transplantation as older children.

In particular, modification of intravenous lipid in infants and toddlers with intestinal failure has led to a dramatic reduction in the incidence and severity of parenteral nutrition-associated cholestatic liver disease, now more appropriately termed *intestinal failure–associated liver disease (IFALD)*. While the etiology of IFALD is not fully understood, it has been demonstrated that replacement of the standard soybean-based lipid emulsion in parenteral nutrition with fish oil–based lipids rich in omega-3 can lead to biochemical reversal of IFALD (Puder et al., 2009). In addition, emerging data have shown that restriction of intravenous soy-based lipid to a total allotment of 1 g/kg per day can both prevent and reverse IFALD in infants with SBS (Rollins et al., 2013; Sanchez et al.,

2013). Many centers now routinely treat infants at risk for IFALD with lipid restriction to avoid the complications of IFALD. The reduction in the incidence of IFALD has allowed providers to focus on long-term intestinal rehabilitation in these patients rather than strive for early intestinal and liver transplantation.

The Pediatric Intestinal Failure Consortium (PIFCon) was established in 2006 and consists of 18 institutions throughout North America with multidisciplinary pediatric intestinal rehabilitation programs. In a multicenter retrospective cohort study of patients treated between 2000 and 2007 at PIFCon centers, 27% of patients died, and 26% underwent intestinal transplantation (Squires et al., 2012). Nearly one-half of patients enrolled in the retrospective study attained enteral autonomy. Interestingly, children continued to wean off parenteral nutrition several years after referral to an intestinal rehabilitation center indicating that the process of intestinal adaptation in children may take years. Additional data from the PIFCon database demonstrate that infants who suffered from NEC had a greater chance of reaching enteral autonomy than children with alternative etiologies of SBS (Khan et al., 2015).

The role of surgical bowel lengthening in the management of pediatric SBS continues to evolve. Common techniques for bowel lengthening include the longitudinal intestinal lengthening and tapering procedure designed by Bianchi (Bianchi, 1999) and the serial transverse enteroplasty (STEP) introduced in 2002 (Kim et al., 2003). Both procedures are designed to prolong intestinal transit time so that nutrient absorption can be optimized. Children with short bowel anatomy who plateau in their enteral feeding advancement and develop significant bowel dilation are the ideal candidates for a bowel lengthening procedure. Outcomes from lengthening procedure vary by center, although a recent publication from the International STEP Data Registry reported that 55% of children reached enteral autonomy after STEP (Jones et al., 2013).

Intestinal transplantation remains an option for children with severe SBS who cannot attain enteral autonomy over time. Most centers also consider loss of the majority of central venous access sites because of thrombosis as an indication for transplantation. With the recent success of intestinal rehabilitation, however, the utilization of intestinal transplantation in young children with

SBS has decreased. Long-term patient and graft survival in pediatric intestinal transplantation are improving and approaching the outcomes seen in liver and kidney transplantation (Sudan et al., 2014).

Suggested Readings

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Disorders of the Liver

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KEY POINTS

- Early onset (<24 hours of age) or new jaundice is never normal and should be investigated.
- Initial evaluation of a jaundiced infant should always include conjugated and unconjugated bilirubin levels.
- Infants presenting with jaundice secondary to conjugated hyperbilirubinemia should undergo expedient evaluation for potentially life-threatening and treatable causes of cholestasis and then for other causes.

Introduction to Neonatal Liver Disease

The liver is the largest abdominal organ and serves as the body's main location of energy production, metabolism, protein synthesis, and detoxification. Embryologically, the liver is derived from an endodermal outgrowth from the foregut and septum transversum. There are multiple cell types within the liver parenchyma, including hepatocytes, cholangiocytes, stellate cells, endothelial cells, and cells of the innate immune system. The key roles of the hepatocyte include protein synthesis, fatty acid synthesis and oxidation, formation of lipids, cholesterol and bile salts, bilirubin metabolism, gluconeogenesis, glycogen synthesis, urea cycle and production of ammonia, and detoxification. The hepatocyte is the only cell in the body that manufactures albumin, fibrinogen, and prothrombin clotting factors. In the setting of significant liver injury, loss of normal hepatocyte synthetic function often results in the development of coagulopathy and hypoalbuminemia.

Bile is primarily composed of bile acids, bilirubin, and fats, which are manufactured in the hepatocyte, secreted into the canaliculus, transported into the biliary ducts, and, ultimately, secreted into the intestine or stored within the gallbladder. Bile is critical for solubilizing dietary fats and fat-soluble vitamins (A, D, E, and K) to make them available for absorption. Disruption of this process at any level results in cholestasis. Cholestasis refers to obstruction of the normal excretion of bile from the liver resulting in the abnormal accumulation of bile components within the liver and serum. While cholestasis is not synonymous with jaundice from conjugated hyperbilirubinemia, serum conjugated bilirubin level is the most clinically useful marker of cholestasis.

Like the lungs, the liver is unique in that it has a dual blood supply: nutrient-rich venous blood from the portal vein and oxygen-rich arterial blood from the hepatic artery. Venous drainage of the liver occurs through the hepatic veins. In the fetus, the

umbilical vein delivers oxygenated blood to the liver via the left portal vein and the ductus venosus, which then joins the left hepatic vein as it drains into the inferior vena cava. The ductus venosus closes spontaneously at birth. Congenital anomalies of the portal vein or thrombosis associated with umbilical vein catheter placement may lead to portal vein obstruction and portal hypertension. Portal hypertension describes a pathologic increase in venous pressure in the portal venous system and occurs when the portal pressure rises above 10 mmHg. Portal hypertension may be pre-hepatic (portal vein), intrahepatic (cirrhosis), or posthepatic (hepatic venous) in etiology. Signs and symptoms of portal hypertension include splenomegaly, ascites, and gastrointestinal bleeding secondary to bleeding varices.

Neonates with liver disease may present with an array of clinical phenotypes ranging from asymptomatic jaundice to acute liver failure. Clinical evaluation and management differ depending on underlying etiology. Clinical signs suggestive of underlying liver disease include ascites, hepatomegaly with or without splenomegaly, coagulopathy, elevated transaminases, hyperammonemia, hypoglycemia, and cholestasis. The initial evaluation of suspected liver disease must include a careful physical examination, laboratory evaluation, and imaging. The initial laboratory evaluation for suspected liver disease comprises an assessment of hepatocellular injury and function, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), conjugated and unconjugated bilirubin levels, prothrombin time/international normalized ratio (INR), and albumin levels. Elevations in serum ALT and AST indicate hepatocellular injury; however, they are not specific to the liver and may be found in other tissues such as red blood cells and skeletal muscle. An elevation in ALP and GGT may indicate biliary obstruction or inflammation; however, care must be taken in children, as elevated ALP is also indicative of normal bone growth and metabolism. As stated previously, an elevation in transaminases (AST, ALT) indicates hepatocyte injury, whereas an elevation in prothrombin time/INR and hypoalbuminemia indicates a loss of normal hepatocyte function and most likely a greater degree of hepatocyte injury. The most appropriate initial imaging modality is abdominal ultrasound (US), which can detect anatomic or vascular anomalies, thrombosis, or suggest underlying portal hypertension.

Liver disease in the neonate frequently presents with new or persistent jaundice. Infants presenting with new jaundice deserve an urgent evaluation, as this is never normal. Term infants with jaundice persisting beyond 14 days or preterm infants with jaundice

beyond 21 days of life deserve expedient evaluation (Fawaz et al., 2017; Murray, Horslen, 2014). In any jaundiced infant, it is necessary to determine whether jaundice is due to conjugated versus unconjugated hyperbilirubinemia. Although the differential of potential causes will vary by the type of predominant bilirubin, prioritizing the evaluation to identify those conditions where early intervention is associated with improved outcome, such as sepsis, is prudent.

Elevated unconjugated hyperbilirubinemia is often seen secondary to breast milk jaundice, sepsis, hemolysis secondary to blood group incompatibility (ABO and rhesus) or red blood cell dyscrasia, and, more rarely, Crigler–Najjar syndrome. While physiologic jaundice and breast milk jaundice are common, jaundice secondary to unconjugated hyperbilirubinemia should still be investigated if it is of very early onset (within 24 hours of life), prolonged beyond 14 days, or at high levels. Breast milk jaundice is the most likely etiology of unconjugated hyperbilirubinemia if the serum level is downtrending; there is no evidence of hemolysis, infection, abnormal thyroid function, or elevated serum aminotransferases; and the child is clinically well. However, if unconjugated hyperbilirubinemia persists or is rising, or the child is ill, disorders influencing bilirubin conjugation and hemoglobin metabolism should be investigated.

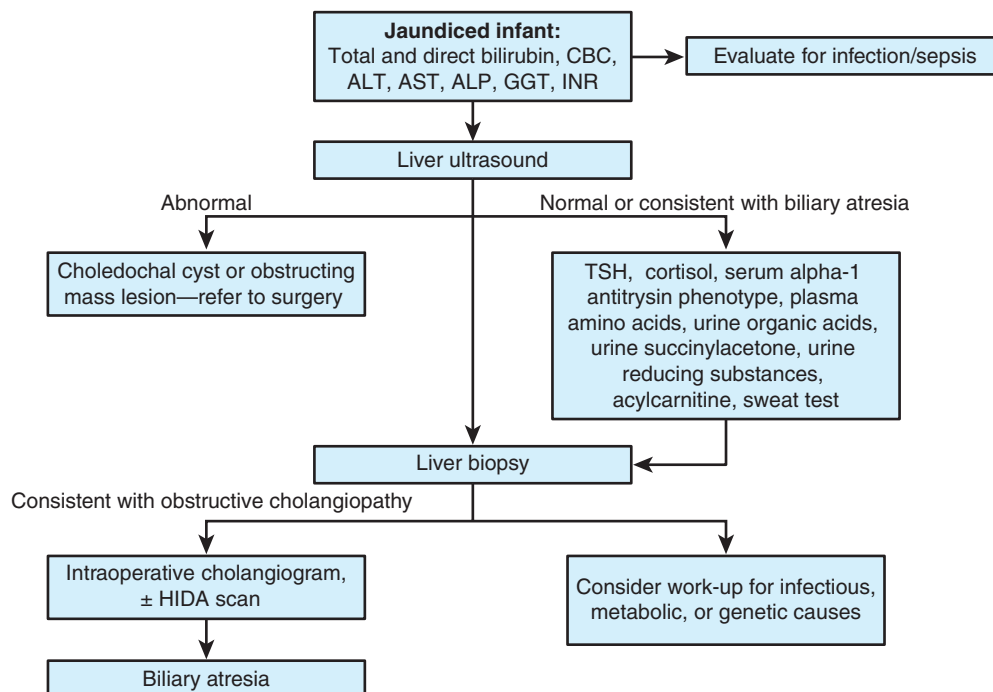
The presence of conjugated hyperbilirubinemia (defined as conjugated bilirubin level $\geq 15\%$ of the total) should raise concern for underlying liver disease. An initial approach to the evaluation of neonatal conjugated hyperbilirubinemia is shown in Fig. 77.1. Specific etiologies of neonatal cholestasis are reviewed below and summarized in Table 77.1. Idiopathic neonatal hepatitis is a term historically applied to infants presenting with neonatal cholestasis or hepatitis in whom no specific etiology could be identified. Liver biopsies in these infants often demonstrate nonspecific intrahepatic cholestasis and giant cell transformation of hepatocytes (Balistreri,

Bezerra, 2006) (Fig. 77.2). Currently, it is recognized that multinucleated giant cells represent a stereotypical response by the immature liver to many etiologies of hepatocellular injury, including infection, biliary obstruction, and metabolic disease. Today, with advancements in next generation DNA sequencing, the number of identifiable etiologies of neonatal cholestasis and hepatitis has increased dramatically, further reducing the frequency and utility of this diagnosis.

Cholestatic Liver Disease

Cholestasis refers to obstruction of the normal excretion of bile from the liver, resulting in abnormal accumulation of bile salts, bilirubin, and lipids in the blood. In infants, cholestasis may present as asymptomatic jaundice, pruritus, unexplained fat-soluble-vitamin deficiency, or acute liver failure. The presence of acholic stools suggests functional or anatomic biliary obstruction. The following sections review the most common etiologies of neonatal cholestasis and discuss the corresponding disease-specific evaluation and clinical management.

Nutritional management is critical and central to the care of infants with chronic cholestasis. Growth failure commonly occurs in time, secondary to malabsorption from inadequate bile flow, intestinal congestion from portal hypertension, and increased caloric needs in the setting of chronic liver inflammation. The estimated daily caloric intake for infants with chronic cholestasis may approach 150% of that of healthy infants (Hsu, Chang, 2014). Additionally, malabsorption of fat-soluble vitamins (A, D, E, and K) can result in progressive coagulopathy and pathologic fractures. While enteral nutrition is preferable, some infants go on to require nutritional optimization with total parenteral nutrition. The general principles of nutritional management in cholestatic infants include assurance



• **Fig. 77.1** Algorithmic approach to the evaluation of an infant with conjugated hyperbilirubinemia. ALP, Alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CBC, complete blood count; GGT, gamma-glutamyl transpeptidase; HIDA, hepatobiliary iminodiacetic acid scan; INR, international normalized ratio; TSH, thyroid-stimulating hormone.

TABLE 77.1 Causes of Neonatal Cholestasis

| | |
|--------------------------|--|
| Infection | Herpes simplex virus Cytomegalovirus Adenovirus Hepatitis B Sepsis/urinary tract infection Cholecystitis Cholangitis |
| Endocrine | Hypothyroidism Panhypopituitarism Adrenal insufficiency |
| Metabolic/Genetic | Galactosemia Tyrosinemia type 1 Dubin–Johnson syndrome Rotor syndrome Bile acid synthesis defects α -1 Antitrypsin deficiency Cystic fibrosis Defects of bile transport (progressive familial intrahepatic cholestasis) Peroxisome biogenesis disorders |
| Cardiovascular | Heart failure Shock Hepatic ischemia |
| Syndromic | Trisomy 21 Trisomy 13 Trisomy 18 Joubert syndrome Ivemark syndrome Beckwith–Wiedemann syndrome Bardet–Biedl syndrome |
| Biliary | Biliary atresia Choledochal cyst Alagille syndrome Choledocholithiasis Neonatal sclerosing cholangitis Caroli disease Obstruction from mass or stricture |
| Nutritional | Total parenteral nutrition |

of adequate absorbable calories and nutrients, monitoring levels of and supplementation with fat-soluble vitamins, and preparation to escalate the nutritional support and supplementation in infants who cannot sustain adequate intake for growth orally, with supplemental enteral feeds via nasogastric tube or initiation of parenteral nutrition in situations of failure to grow or gain weight adequately with maximal enteral nutrition. The selection of formula should consider medium-chain triglyceride (MCT) content, as this fat source is directly absorbed into the portal venous system and does not require emulsification by bile acids or active transport that is

disrupted in cholestasis. Children with portal hypertension and ascites also benefit from sodium restriction.

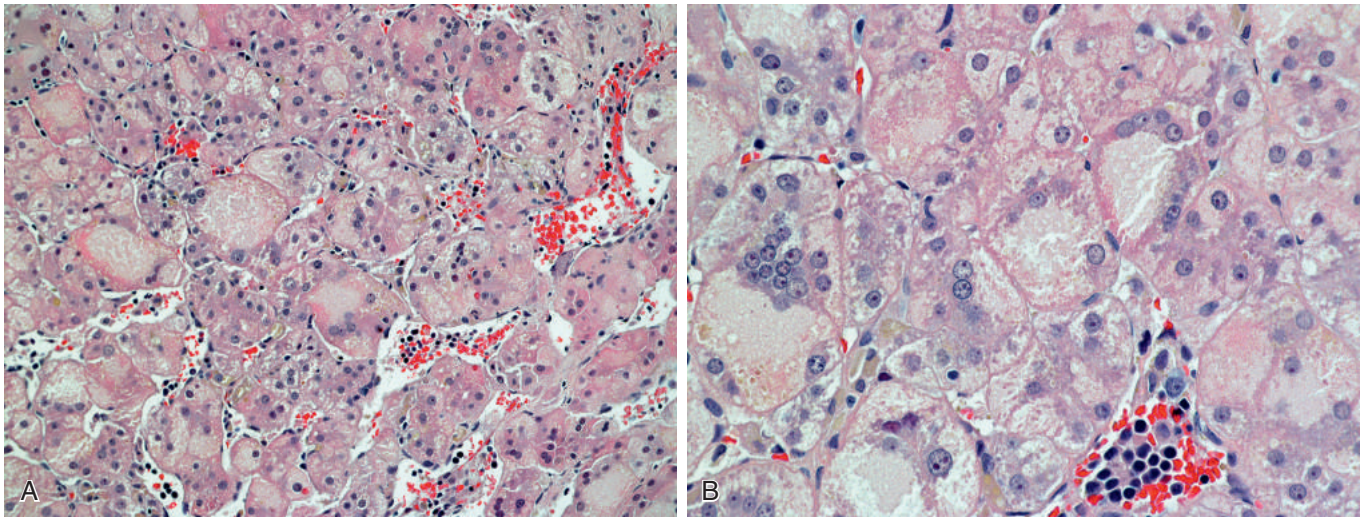
Biliary Atresia

Biliary atresia (BA) is an idiopathic hepatobiliary disorder of infancy characterized by inflammation and progressive fibrosis resulting in the obliteration of the extrahepatic biliary ducts (Balistreri et al., 1996; Hartley et al., 2009). As the disease progresses, the end result is variable destruction and obliteration of the intrahepatic bile ducts. BA is the most common cause of infantile chronic liver disease and most frequent indication for liver transplantation in the pediatric population. The reported incidence of BA is 0.5 to 3.2 per 10,000 live births but varies based on geography and ethnicity. Babies with BA generally present between 2 and 5 weeks of age, but without rapid intervention the natural history of BA is uniform fatality by 2 years of age.

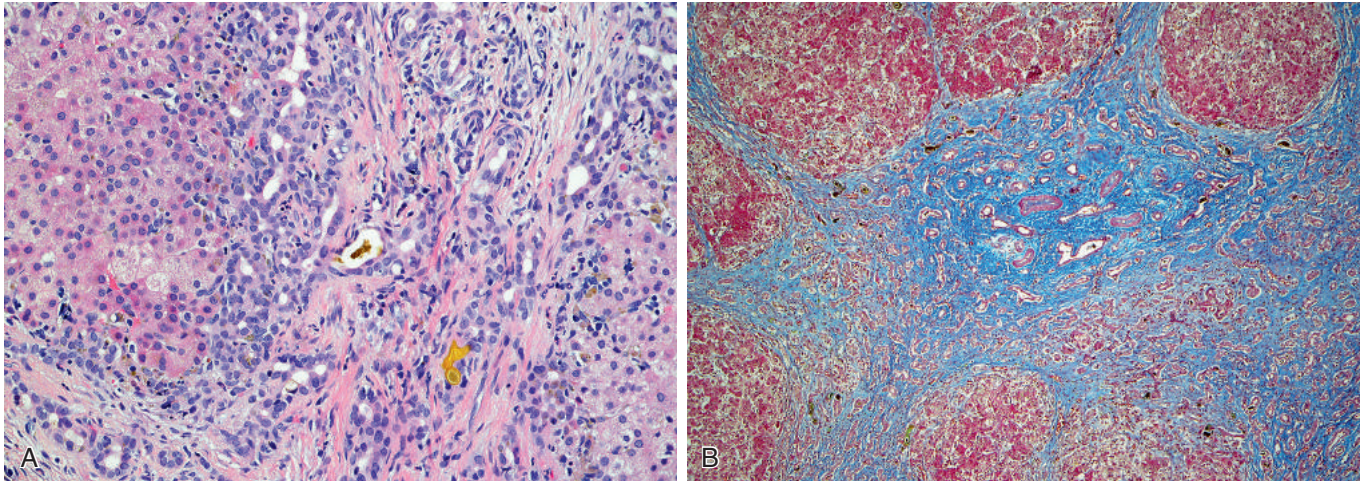
BA is characterized by the anatomy of extrahepatic biliary obstruction (Karrer et al., 1990). Two clinical phenotypes exist: “classical” BA, which is not associated with extrahepatic congenital anomalies, and “BA with splenic malformation,” which presents with other congenital anomalies such as situs inversus, polysplenia or asplenia, vascular and cardiac malformations, and intestinal malrotation.

BA typically presents with cholestatic jaundice and hepato-splenomegaly between 2 and 5 weeks of age. Acholic stools suggest biliary obstruction and are frequently present, but onset is commonly well after the onset of jaundice. If the infant also had a preceding history of physiologic jaundice, the cholestatic jaundice may not be recognized as new; this highlights the importance of evaluating any prolonged or new jaundice in infants. Although splenomegaly is commonly present at diagnosis, other signs of portal hypertension such as ascites generally occur later in the course of disease. As chronic inflammation and cholestasis lead to malabsorption, many infants with BA present with inadequate weight gain.

Expedient differentiation of BA from other causes of neonatal cholestasis is critical, as surgical intervention before 2 months of age has been shown to improve surgical success and outcome (Ohi, 2001; Shneider et al., 2006). If early laboratory evaluations are suggestive of BA, consultation with a pediatric hepatologist is mandatory. Laboratory evaluation early in the course of disease typically demonstrates conjugated hyperbilirubinemia between 2 and 7 mg/dL and total bilirubin levels between 5 and 12 mg/dL (Hsu, Chang, 2014). Elevations in ALT, ALP, and GGT are generally seen (Hsu, Chang, 2014). Abdominal US is recommended as the first-line imaging modality and may demonstrate absence of the gallbladder after adequate fasting or a fibrotic remnant of extrahepatic bile duct. In the porta hepatis a triangular or tubular echogenic cord of fibrous tissue representing the biliary remnant may be described as “triangular cord sign.” The reported sensitivity of this sonographic finding is 73% (Lee et al., 2003). As a follow-up to abdominal US, hepatobiliary scintigraphy with technetium-labeled iminodiacetic acid derivatives (HIDA scan) may be used to assist in the differentiation between obstructive and nonobstructive causes of neonatal cholestasis. In BA, radionuclide scans demonstrate rapid uptake of tracer but absence of excretion into the bowel at 24 hours. The sensitivity of the HIDA scans may be increased by pretreatment with oral phenobarbital (5 mg/kg per day) for 5 days. Care must be taken, however, to not delay definitive diagnosis by awaiting scan results and recognition that functional causes of cholestasis (such as hypothyroidism) can also result in a nonexcreting HIDA scan. Percutaneous liver biopsy is helpful in excluding



• **Fig. 77.2** Neonatal Hepatitis Histology. (A) Low power of a hematoxylin and eosin stain from a liver biopsy demonstrating hepatocytes with giant cell transformation and ballooning. Some cholestasis is evident. (B) Higher power of same biopsy shows the hepatocytes with giant cell transformation as well as a focus of extramedullary hematopoiesis.



• **Fig. 77.3** Biliary Atresia Histology. (A) Hematoxylin and eosin stain of a liver biopsy from a 3-month-old girl demonstrating a proliferation of bile ductules. Bile plugs are present. (B) Masson trichrome stain from a liver transplant specimen from the same girl at 8 months of age. This specimen has diffuse cirrhosis with fibrous expansion of portal tracts. The portal triads lack bile ducts, but there is a marked bile ductule reaction, many containing bile plugs.

alternate causes of cholestasis. Histopathologic findings supportive of a diagnosis of BA demonstrate bile ductular proliferation and bile duct plugging (Fig. 77.3). Given the progressive nature of BA, the histologic findings will vary with the point in progression.

Failure to exclude BA after the above evaluation is complete necessitates surgical exploration with intraoperative cholangiogram. The diagnosis of BA may be made or confirmed at the time of laparotomy with the observation of an atretic biliary tree and intraoperative cholangiogram demonstrating lack of patency in the biliary ductal system. If BA is confirmed, surgical intervention with a Kasai hepatic portoenterostomy is recommended. The Kasai is a surgical procedure that works to restore the normal flow of bile by excising the obstructed bile ducts and creating an anastomosis of a jejunal limb of a Roux-en-Y with the liver at the porta hepatis, the area of the liver from which the bile ducts become extrahepatic. Restoration of bile flow may prevent or delay progression of disease,

worsening of fibrosis, and development of end-stage liver disease. However, despite Kasai portoenterostomy, the majority of children progress to cirrhosis and portal hypertension and ultimately require liver transplantation.

Alagille Syndrome (Arteriohepatic Dysplasia)

Alagille syndrome (ALGS) is an autosomal dominant or sporadic de novo genetic disorder characterized by chronic, progressive cholestasis secondary to a paucity of intralobular bile ducts. The estimated prevalence is 1 in 30,000 live births (Murray and Horslen, 2014). The majority of children with ALGS carry a mutation in the gene *JAG1*, located on chromosome 20 (Li et al., 1997; Oda et al., 1997; Warthen et al., 2006). The product of *JAG1* is a ligand in the Notch signaling pathway that plays a key role in embryogenesis and the pathogenesis of the disorder. A small number of infants

with ALGS have mutations in *NOTCH2* (McDaniell et al., 2006; Kamath et al., 2012).

ALGS is a multisystem syndrome characterized by cholestatic liver disease, stereotypical facial features, congenital heart disease, posterior embryotoxin, butterfly vertebrae, and renal disease. Most infants with ALGS present within the first 3 months of life with cholestasis; however, those with severe extrahepatic manifestations (usually caused by associated congenital heart disease) may present at birth or even be identified by prenatal US. Although many forms of congenital heart disease have been associated with ALGS (e.g., tetralogy of Fallot and transposition of the great arteries), the most common is peripheral pulmonary stenosis. The characteristic facial features are frequently difficult to appreciate in the neonatal period but include a prominent forehead and pointed chin, giving the face a triangular appearance, deep-set eyes with hypertelorism, and a saddle nose.

When cholestatic jaundice occurs in the first 6 weeks of life, care must be taken to discriminate ALGS from alternate etiologies of neonatal cholestasis, particularly BA and other treatable etiologies for which timely initiation of treatment may change outcome. Initial evaluation should include serum biochemistries, abdominal US, and echocardiogram when a heart murmur is identified. In ALGS, the conjugated hyperbilirubinemia is associated with elevated serum aminotransferases and GGT, reflective of the biliary involvement. If there is clinical concern for ALGS, a spinal radiograph should be obtained to evaluate for hemivertebra or butterfly vertebra, and an ophthalmologic evaluation for posterior embryotoxin is recommended.

Although a liver biopsy is not required for the diagnosis of ALGS when other stereotypical syndromic features are present, one should be performed when the diagnosis is in question. The histopathology in ALGS is characterized by bile ductular paucity; however, this is not pathognomonic. In the preterm infant the number of bile ducts is normally diminished, and hence care must be taken to not incorrectly make the diagnosis of pathologic paucity (Kamath, Piccoli, 2014) (Fig. 77.4). In term infants and older children, the normal bile duct to portal tract ratio ranges from

0.9 to 1.8; ratios less than 0.9 are suggestive of paucity. Given the normal developmental progression of the biliary system in infancy and the importance of excluding BA, infants with cholestatic jaundice and elevated GGT usually require liver biopsy and hepatobiliary scintigraphy and may require an intraoperative cholangiogram to verify patency of the extrahepatic biliary system.

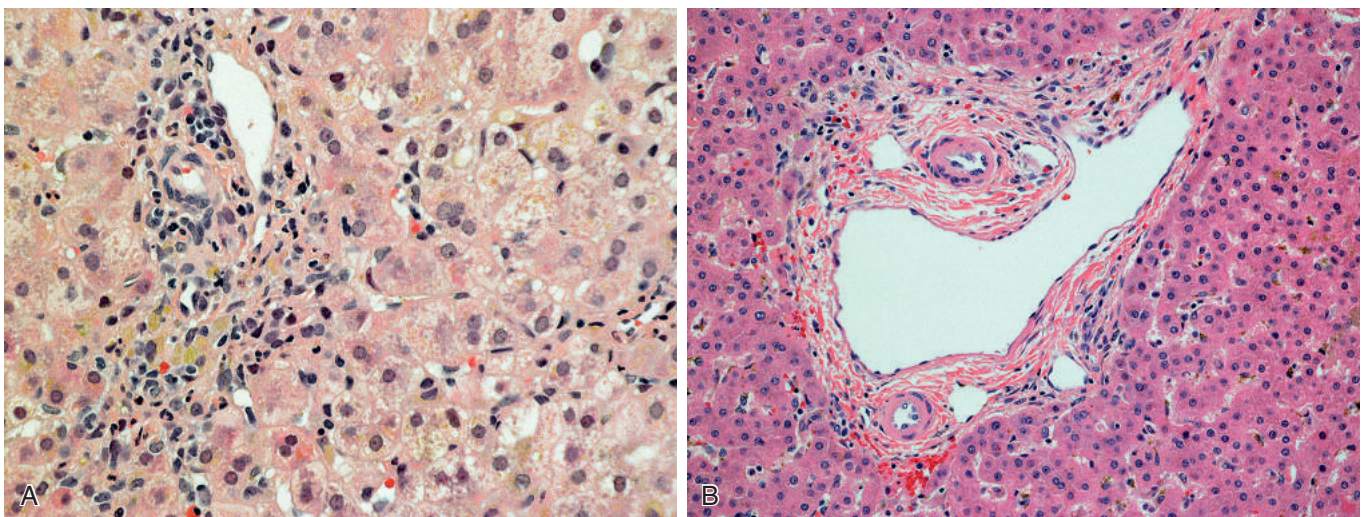
Metabolic bone disease is especially striking in ALGS. Elevated ALP commonly reflects abnormal bone metabolism in addition to the biliary disease. Serum bile salt levels can be extremely elevated, even in the absence of jaundice, leading to intractable and refractory pruritus. Hypercholesterolemia and hypertriglyceridemia can lead to the development of xanthomas, most prominent on extensor surfaces and areas of minor trauma including the diaper area and plantar surfaces of feet, abdomen, and neck.

Treatment of ALGS is directed at improving or maintaining adequate nutrition, treating the complications if there is cholestasis, and supporting the cardiovascular health. The introduction of this section has details regarding nutritional management of cholestatic infants.

α -1 Antitrypsin Deficiency

α -1 Antitrypsin (A1AT) deficiency is the most common genetic cause of liver disease and affects approximately 1 in 2000 live births (Perlmutter, 1998; Perlmutter, 2000; Perlmutter, 2007). It is an autosomal dominant disorder that results in a reduction in α -1 antitrypsin, a serine protease produced in the liver whose role is to inhibit other proteases and elastases that can lead to cellular destruction. The genetic defect results in a single amino acid substitution within the A1AT protein, resulting in abnormal molecular folding, and inability of the protein to be processed beyond the Golgi apparatus. Excessive hepatic accumulation of the abnormal A1AT protein results in hepatocellular injury.

The clinical phenotype of A1AT deficiency includes both liver and pulmonary manifestations. Pulmonary disease generally manifests in adulthood, whereas liver involvement commonly presents in neonates. Liver disease in A1AT is most often



• **Fig. 77.4** Alagille Syndrome Histology. (A) Hematoxylin and eosin stain of a liver biopsy from a 3-month-old boy demonstrating loss of bile ducts in a portal triad. This bile duct paucity is accompanied by hepatocellular cholestasis. The hepatocytes also demonstrate swelling. (B) At 10 months of age, another boy was transplanted for Alagille syndrome. On hematoxylin and eosin stain, his liver demonstrated paucity of bile ducts in the portal triads. Mild hepatocellular cholestasis is also present in this biopsy.

characterized by cholestatic jaundice, but hepatosplenomegaly and ascites can also be seen in the most advanced cases.

A1AT deficiency is diagnosed by serologic testing and liver histology. The most specific serum test is A1AT phenotyping (Pi type). Although commonly used as a less costly and more widely available screening test, the serum levels of A1AT can be falsely elevated into the normal range in times of systemic inflammation or infection and so should not be used in the diagnosis of A1AT. A1AT variants are named according to their electrophoretic migration pattern (Pierce, Eradio, 1979). The normal protein is designated M, and the S and Z variants are the most common, leading to a reduction in serum A1AT. The homozygous PiZZ is named because it has the slowest gel migration and causes the most severe disease phenotype. Generally liver disease manifests only in PiZZ, PiSZ, or, rarely, PiMZ variants (Ranes, Stoller, 2005).

The classic, but not pathognomonic, histologic finding in A1AT deficiency is periodic acid-Schiff (PAS)-positive diastase-resistant eosinophilic globules within the hepatocytes, representing the accumulated abnormal protein trapped within the endoplasmic reticulum. Liver histology may also demonstrate bile duct destruction, proliferation, and, potentially, bile duct paucity, making it important to exclude BA and ALGS.

Although recombinant A1AT has been used for the treatment of the pulmonary manifestations, management of the associated liver disease is primarily supportive, as there are no specific or targeted therapies currently available. As in all disorders resulting in cholestasis, fat malabsorption is common with A1AT deficiency. Cholestatic infants usually benefit from MCT-rich formula to aid the fat absorption and supplementation with fat-soluble vitamins as needed. Additionally, ursodeoxycholic acid may be utilized to improve bile flow; however, no study has demonstrated clear benefit. Historically, breastfeeding was thought to be of some benefit; however, in a study comparing formula-fed with breastfed infants, no benefit was found (Udall et al., 1985; Labrune et al., 1989). To prevent acceleration of pulmonary manifestations, including early emphysema, avoidance of smoking and environmental pollution is critical.

For infants and children with end-stage liver disease, liver transplantation is indicated. As most A1AT is manufactured in the liver, the recipient assumes the donor's Pi phenotype and, posttransplant, experiences normal serum levels of the functional protein, decreased risk of pulmonary disease, and no chance of recurrent disease in the transplanted organ.

Cystic Fibrosis Liver Disease

While cystic fibrosis (CF) is common, affecting approximately 1 in 2500 births in North America, CF-related liver disease is uncommon in the neonatal period. It is estimated that less than 2% of infants with CF present with cholestasis (Narkowicz, Waasdorp Hurtado, 2014). Given the low incidence of CF-related liver disease in neonates, testing for CF beyond state-mandated newborn screens should be reserved for those infants in whom alternate causes of cholestasis have been excluded or in infants with other typical features of CF, including meconium ileus or inadequate weight gain despite theoretically adequate caloric intake.

Disorders of Bile Acid Synthesis

There are several steps in the synthesis of bile acids that may be disrupted, leading to an accumulation of hepatotoxic bile acid intermediates. Bile acid synthesis disorders are rare, with a prevalence

of 1 in 50,000 in the general population. In the neonatal period, phenotypes of bile acid synthetic disorders include acute hepatitis, acute liver failure, persistent cholestasis, and progressive chronic hepatitis.

Cholic acid and chenodeoxycholic acid are the primary bile acids in humans, and disruption at any step in their synthesis results in the accumulation of toxic intermediate metabolites. Liver injury associated with disorders of bile acid synthesis occurs secondary to direct hepatocellular injury from accumulation of toxic intermediates or secondary to the accumulation of cholesterol, drugs, and other toxins within the liver from abnormal bile excretion.

The most common clinical presentation of disorders of bile acid synthesis include neonatal jaundice, failure to thrive, hepatosplenomegaly, metabolic bone disease, and bleeding early in childhood. Some disorders are associated with progressive neurologic disease, manifesting with seizures, developmental delay, deafness, blindness, and neuromuscular weakness.

Laboratory testing in infants with bile acid synthetic disorders demonstrates normal or low serum bile acid levels, elevated serum aminotransferases, normal GGT, and complications of fat malabsorption, including fat-soluble vitamin deficiency. If serum bile acids are found to be low, urinary bile acids should be measured for identification of the particular synthetic defect. Liver biopsies are generally nonspecific and can demonstrate canalicular bile plugging, inflammation without bile duct proliferation, or giant cell transformation (Setchell, Heubi, 2006).

Treatment of inborn errors of bile acid synthesis focuses on supporting normal growth and supplementation of fat-soluble vitamins. Treatment with cholic acid is a preferred therapy for the most common disorders of primary bile acid synthesis because it suppresses the production of the toxic bile acid intermediates. Ursodiol is not indicated as it does not suppress production of abnormal bile acid intermediates.

Progressive Familial Intrahepatic Cholestasis

Progressive familial intrahepatic cholestasis (PFIC) is a group of autosomal recessive disorders characterized by defective bile export leading to cholestasis. This group of disorders is classified based on the genetic mutation, and they are named PFIC 1, PFIC 2, and PFIC 3. Liver disease in PFIC results from accumulation of bile salts within the hepatocytes leading to profound cholestasis, fat-soluble vitamin deficiency, and intractable pruritus.

PFIC 1, also known as Byler disease, is caused by a mutation in the gene *ATP8B1* on chromosome 18q21-22, which encodes for a protein flippase (FIC 1) that facilitates the flipping of aminophospholipids from the outer to inner canalicular membrane. As the gene is also expressed in many extrahepatic tissues, affected individuals may also have short stature, deafness, pancreatitis, and persistent diarrhea.

PFIC 2 results from a defect in the bile canalicular bile salt export pump (BSEP) caused by a mutation in the gene *ABCB11* on chromosome 2q24. BSEP is responsible for transporting bile acids from inside the hepatocyte to the canaliculus. Disruption of BSEP results in accumulation of bile acids within the hepatocyte resulting in severe cholestasis and rapid progression to end-stage liver disease. PFIC 2 presents earlier and is a more rapidly progressive liver disease than PFIC 1. Children with PFIC 2 have an increased risk of developing hepatocellular carcinoma, a risk that persists even after liver transplantation.

PFIC 3 is caused by a mutation in the gene *ABCB4* on chromosome 7q21, which encodes for multidrug resistance-associated

protein 3 (MDR3) and mediates flopping of aminophospholipids from the inner to outer canalicular lipid bilayer. Rather than a deficiency in bile acid export, patients with PFIC 3 have a deficiency in phospholipid export. The resultant bile lacks phospholipids, making the micelles unstable and toxic to bile ducts, leading to a progressive intrahepatic cholangiopathy. In contrast to PFIC 1 and 2, only a third of children with PFIC 3 present with cholestasis during infancy, with most presenting in later childhood and adolescence. When infants with PFIC 3 do present with liver disease, they commonly have cholesterol gallstones complicating their intrahepatic cholestasis.

Infants with PFIC generally have markedly elevated serum bile acid levels with only mildly elevated serum bilirubin. The characteristic biochemical markers of PFIC 1 and 2 are a normal or low GGT, normal serum cholesterol, and only mild transaminitis; however, specific diagnosis requires genetic testing. PFIC 3 presents with an elevated GGT in the absence of extrahepatic biliary obstruction. The intrahepatic cholestasis that is commonly seen in PFIC often progresses to end-stage liver disease.

Treatment for PFIC initially focuses on the nutritional management of cholestasis because of insufficient absorption of fat and fat-soluble vitamins. Additionally, aggressive treatment of debilitating pruritus with ursodiol, antihistamines, cholestyramine, rifampin, and opioid antagonists is often required. In refractory cases, treatment may include partial biliary diversion, interruption of the enterohepatic circulation by surgical ileal exclusion, and liver transplantation (Englert et al., 2007).

Congenital Hepatic Fibrosis

Congenital hepatic fibrosis (CHF) is a hereditary malformation of the bile ducts resulting from failure of remodeling during embryogenesis. It is an autosomal recessive disorder that may present in isolation but is more often seen as a feature of several syndromes, including Ivemark, Berdet–Biedl, Caroli, and Joubert syndromes, autosomal recessive polycystic kidney disease (ARPKD), and disorder of glycosylation type 1b.

Caroli syndrome is characterized by CHF and ductal ectasia with cystic dilation of intrahepatic ducts. Infants with CHF most commonly present with portal hypertension and splenomegaly, although some present with cholestasis caused by cholangitis. Characteristically, CHF is associated with normal transaminases and intact synthetic function despite advancing portal hypertension. When complicated with cholangitis, transient elevation of GGT and conjugated bilirubin are typically seen. Diagnosis of Caroli syndrome may be made by imaging with abdominal US with Doppler or magnetic resonance imaging (MRI) of the liver with magnetic resonance cholangiopancreatography. When associated with ARPKD, CHF can be diagnosed clinically, but if in isolation histologic diagnosis is necessary.

Treatment of CHF is aimed at treating complications of portal hypertension and preventing and treating cholangitis. Portosystemic shunting is commonly required to decompress advanced portal hypertension. In Caroli syndrome, focal cystic dilation may be amenable to liver lobectomy, but, if diffuse, liver transplantation is required.

Infections

Congenital or perinatal infections and sepsis are common causes of neonatal liver cholestasis, hepatitis, and sometimes liver failure. A careful history and physical examination may suggest infection

as an etiology of neonatal liver disease. For ill-appearing infants with cholestasis, a rapid evaluation for bacterial infection (such as sepsis or urinary tract infection) is recommended. Judicial selection of antimicrobials must be considered, as several are known to exacerbate cholestasis by displacing bilirubin from albumin (e.g., ceftriaxone) or cause direct hepatotoxicity (e.g., sulfamethoxazole/trimethoprim and fluconazole) (Murray and Horslen, 2014). In addition to common bacterial infections, TORCH infections (toxoplasmosis, rubella, cytomegalovirus, herpes, and syphilis) as well as infections with hepatitis B, parvovirus B19, adenovirus, or echoviruses can result in neonatal cholestasis and hepatitis. For more detail regarding diagnosis and specific treatments for congenital or neonatal infections, please refer to Section IX.

Parenteral Nutrition–Associated Liver Disease

Parenteral nutrition–associated liver disease (PNALD) is an important and common cause of hepatitis, cholestasis, and liver-related morbidity in the neonatal period. It is estimated that between 33% and 85% of premature infants who receive parenteral nutrition for more than 7 days develop PNALD (Duro et al., 2011; Koseesirikul et al., 2012). The primary phenotypic manifestation of PNALD in infants is cholestasis. When total parenteral nutrition (TPN) is utilized for a short duration, generally less than 2 weeks, the transient liver inflammation generally completely resolves. However, prolonged use increases the risk for irreversible and severe liver disease, which may ultimately result in liver failure (Nanji, Anderson, 1985; Beath et al., 1995). PNALD often presents as hepatic steatosis with elevated serum aminotransferases, cholelithiasis, and cholestasis. Several risk factors have been identified that contribute to the development of PNALD and include prematurity, low birth weight, lack of enteral feeding, sepsis, short gut syndrome, and necrotizing enterocolitis (Btaiche, Khalidi, 2002; Rangel et al., 2012). The diagnosis of PNALD in an infant receiving TPN is suggested by the presence of a serum conjugated bilirubin level less than 2 mg/dL and ALT greater than two times the upper limit of normal.

Management of PNALD includes early initiation and continuation of enteral feeding, lipid minimization to less than 1 g/kg per day, and prevention of infection. Ursodiol at a dose of 20 to 30 mg/kg per day in divided doses may be utilized to improve or stimulate bile flow (Kowdley, 2000; Chen, et al., 2004; Al-Hathlol et al., 2006). Additionally, the use of omega-6 fatty acid or fish oil–based, rather than soy-based, lipid formulations have been shown to be effective at resolving cholestasis (Murray and Horslen, 2014). Recognition and early intervention are required to prevent irreversible liver damage, which can progress to end-stage liver disease.

Metabolic Liver Disease

Inborn errors of metabolism often present with elevated serum aminotransferases, hepatomegaly, or metabolic derangements. There are several clinical phenotypes of metabolic liver disease, including acute liver failure, encephalopathy, cholestasis, and isolated hepatomegaly. Tables 77.2–77.3 summarize the clinical phenotypes of neonatal metabolic liver disease and etiologies of acute neonatal liver failure.

Disorders of Carbohydrate Metabolism

Galactosemia

Galactosemia results from an inability to metabolize galactose secondary to a deficiency in one of the following: galactokinase,

**TABLE
77.2****Common Clinical Presentations of Metabolic Liver Diseases in the Neonatal Period**

| Clinical Presentation | Metabolic Disorder | Laboratory Investigation |
|-----------------------|---|--|
| Acute liver failure | Galactosemia Tyrosinemia Hereditary fructose intolerance | Erythrocyte galactose-1-phosphate uridyl transferase activity, DNA mutational analysis Urine—succinylacetone Screen—urine reducing substances Genetic testing Enzyme activity analysis |
| | Mitochondrial defects Fatty acid oxidation defects Gestational alloimmune liver disease | Serum lactate, pyruvate Fibroblast enzymatic assay, acyl carnitine profile, genetic testing Ferritin, liver biopsy with C5b-9 staining |
| Encephalopathy | Fatty acid oxidation defects Organic acidemias Urea cycle defects | As above (ferritin, liver biopsy with C5b-9 staining) Urine organic acids Serum ammonia level, serum amino acid profile |
| | | |
| Cholestasis | Peroxisomal disorders | Specialized screening for urine metabolites such as very long chain fatty acids, pibecolic acid, phytanic acid, pristanic acid |
| | Lysosomal storage disorders | Liver biopsy, leukocyte glucocerebrosidase activity, genetic mutational analysis |
| Hepatomegaly | Glycogen storage diseases Lysosomal storage disorders | Liver or fibroblast enzymatic assay, genetic testing See above (liver biopsy, leukocyte glucocerebrosidase activity and genetic mutational analysis) |
| | | |

**TABLE
77.3****Causes of Neonatal Acute Liver Failure**

| | |
|--------------------------|--|
| Infections | Herpes simplex virus Human herpesvirus 6 Cytomegalovirus Adenovirus Influenza Hepatitis B Bacterial sepsis Enterovirus Parvovirus B19 Malaria |
| Metabolic/Genetic | Galactosemia Tyrosinemia type 1 Hereditary fructose intolerance Fructose 1,6-bisphosphatase deficiency Fatty acid oxidation defects Mitochondrial defects |
| Immune-Mediated | Gestational alloimmune liver disease Autoimmune hemolytic anemia with giant cell hepatitis Hemophagocytic lymphohistiocytosis |
| Vascular | Ischemia Heart failure |
| Toxic | Drugs Toxins |
| Neoplastic | Leukemia |

galactose-1-phosphate uridyl transferase (Gal-1-PUT), or uridine diphosphate galactose-4-epimerase. Gal-1-PUT deficiency is the most common cause of galactosemia and results in the inability to metabolize galactose into glucose-1-phosphate. It is an autosomal recessive disorder with an incidence of 1 per 60,000 live births (Squires, Heubi, 2014). Abnormal galactose metabolism results in accumulation of toxic metabolites in the liver, brain, kidney, and eye lens.

Classically, galactosemia presents within the first few weeks of life after infants ingest breast milk or milk-based formulas that contain lactose. Presenting symptoms may include failure to thrive, jaundice, vomiting, and diarrhea. Occasionally, infants may present acutely, with *Escherichia coli* sepsis with severe acidosis, jaundice, and coagulopathy. Additional clinical findings may include hepatomegaly, ascites, bleeding, hypotonia, edema, and bulging fontanelle. Although affected infants will spill reducing sugar in their urine (positive urine reducing substances) while still ingesting galactose, the gold standard of diagnosis is demonstration of a complete absence of Gal-1-PUT activity via a quantitative red blood cell (RBC) assay. Many newborn screens may identify variants of the disease, resulting in varying activity of Gal-1-PUT. It should be noted that, because the assay is of RBC enzyme activity, analysis post-RBC transfusion will give unreliable results.

Treatment of galactosemia centers on the immediate stabilization of the critically ill infant as well as urgent restriction of galactose from the diet. Treatment is generally supportive and involves intravenous fluids with glucose, vitamin K, antibiotics, and initiation of a soy-based (nongalactose-containing) formula. As infants graduate to solid food, continued avoidance of lactose-containing foods is recommended. Despite treatment, many children will have some degree of developmental delay.

Hereditary Fructose Intolerance

Hereditary fructose intolerance (HFI) is an autosomal recessive disorder characterized by a deficiency in fructose-1-phosphate aldolase (aldolase B), which is important in both glycolysis and gluconeogenesis, and plays a critical role in the metabolism of fructose. The incidence is approximately 1 per 20,000 live births (Squires, Heubi,

2014). Approximately 75% of dietary fructose is metabolized by the liver, with the remainder metabolized by the kidneys and small bowel. The deficiency in aldolase B leads to a toxic accumulation of fructose-1-phosphate and traps phosphate in an unusable form, thus depleting adenosine triphosphate (ATP) stores and, in turn, inhibiting normal gluconeogenesis and glycogenolysis.

Most infants with HFI are healthy until ingestion of fructose or sucrose (disaccharide of glucose and fructose). Upon ingestion of fructose they begin accumulating the toxic metabolites, developing metabolic derangements that lead to vomiting, hepatomegaly, and failure to thrive. Diagnosis may be suspected after a careful dietary history is obtained, and laboratory evidence of acute liver failure, hypoglycemia, and proximal renal tubular acidosis is found. Urine can be tested for the presence of reducing substances, as fructose in the urine will give a positive test, but this is not specific. Definitive diagnosis requires confirmation with genetic testing for mutations in the *ALDOB* gene, located at 9q22.3, or enzyme analysis from liver tissue.

Treatment of HFI requires prompt removal of dietary fructose and sucrose. Complete elimination is seldom achievable, and there are no established thresholds of required restriction. Additionally, as the severity of the enzyme deficiency is heterogeneous, some patients may develop chronic symptoms despite treatment (Stanbury, 1983). Fortunately, with nearly complete dietary restriction of fructose and sucrose, most children with HFI display normal growth and development.

Glycogen Storage Diseases

Glycogen is a glucose polymer that is primarily stored in the liver and muscle and is required for glucose homeostasis during fasting. There are 12 recognized glycogen storage diseases (GSD), but only types I, III, and IV primarily manifest as neonatal liver disease.

GSD type I, also known as von Gierke disease, named after its discoverer, Edgar von Gierke, is a rare autosomal recessive disease with an incidence of approximately 1 per 100,000 live births (Squires, Heubi, 2014). There are several defects that lead to the clinical manifestation of GSD type I; however, the end result is absence or decreased activity of glucose-6-phosphatase. Infants with GSD type I generally present with hepatomegaly and fasting hypoglycemia, often first noted when children begin to sleep more than 4 hours. Lactic acidosis, hyperuricemia, hyperlipidemia, hyperphosphatemia, neutropenia (specific to GSD type Ib), and bleeding secondary to platelet dysfunction are other clinical manifestations experienced by those affected with GSD type I.

Diagnosis is confirmed by DNA mutational analysis of the potentially affected genes. GSD Ia accounts for 80% of cases and results from mutation of *G6PC*, the gene for glucose-6-phosphatase, located on chromosome 17q21, and GSD Ib represents less than 20% of cases and results from mutations of the gene *G6PT1*, the G6P transporter. Prenatal diagnosis is possible via chorionic villus sampling.

Treatment in infancy consists of maintaining euglycemia with frequent feedings and continuous enteral feeds overnight with a high-glucose, low-fat formula. In older infants and children, a carbohydrate-balanced diet and frequent feedings that include the addition of cornstarch are recommended. Cornstarch provides a long-lasting source of glucose because it is slowly degraded by α -amylase and hence negates the need for hourly glucose intake. With adequate metabolic and glycemic control, children generally grow and develop normally.

GSD type III (Cori or Forbes disease) is an autosomal recessive disease that results from deficiency of the glycogen debranching

enzyme, amylo-1,6-glucosidase, and occurs in 1 in 100,000 live births. The presentation in infancy is similar to that of GSD type I, although the hypoglycemia is not usually as rapid and severe, and failure to thrive may be more prominent. The skeletal muscle (hypotonia) and cardiac manifestations (cardiomyopathy) occur later in childhood. Diagnosis is made by directly measuring the activity of amylo-1,6-glucosidase in the liver or, more recently, doing mutational analysis on the associated gene, *AGL*. Treatment mirrors that of GSD type I, with efforts to maintain euglycemia.

GSD type IV (Andersen disease) is an autosomal recessive disorder caused by a mutation in the *GBE1* gene, resulting in deficiency of glycogen branching enzyme; it affects roughly 800,000 individuals worldwide and accounts for 3% of the cases of GSD. GSD type IV may present prenatally with hydrops or postnatally with hypotonia, heart failure (secondary to cardiomyopathy), contractures, and muscle atrophy. Diagnosis is based upon the absence of branching enzyme activity in skin fibroblasts or detection of mutations in the *GBE1* gene. Treatment consists of liver transplantation when progression to end-stage liver disease has occurred (usually in the third decade of life); however, care must be taken in patient selection as extrahepatic manifestations may be life-limiting.

Disorders of Amino Acid Metabolism

Tyrosinemia Type 1

Tyrosinemia is an autosomal recessive disorder with an incidence of 1 in 100,000 live births. It results from deficiency of fumarylacetoacetate hydrolase, the enzyme responsible for the final step of tyrosine degradation (Squires, Heubi, 2014). Tyrosinemia generally presents acutely in the neonatal period and should be included in the differential of fulminant neonatal liver failure. Failure to thrive, vomiting, ascites, coagulopathy, hypoglycemia, and hyperbilirubinemia may be the initial presenting signs and symptoms. In older infants and children, a more chronic presentation typified by growth failure, Fanconi syndrome, and neurologic manifestations may develop. Children with tyrosinemia type 1 carry a long-term risk of developing hepatocellular carcinoma. Diagnosis is made by measuring elevated urinary succinylacetone.

Treatment in the neonatal period consists of correcting metabolic derangements, treating sepsis, and correcting coagulopathy, followed by the restriction of dietary tyrosine using specific formulas. Treatment with diet alone, however, results in less than 40% survival at 1 year of age (Holme, Lindstedt, 1998; McKiernan, 2006; Masurel-Paulet et al., 2008). Treatment with NTBC (2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione, nitisinone) has been demonstrated to improve survival to greater than 85% at 1 year of age and is now the standard of care in the treatment of tyrosinemia (Mohan et al., 1999). NTBC works by reversibly inhibiting 4-hydroxyphenylpyruvate dioxygenase, hence preventing the formation of maleylacetoacetic acid and fumarylacetoacetic acid, the precursors to the hepatotoxic compound succinylacetone.

Maple Syrup Urine Disease

Maple syrup urine disease (MSUD), like other rare disorders of amino acid metabolism, is autosomal recessive and screened for on routine newborn screening tests throughout the United States. Caused by the deficiency in the branched chain α -ketoacid dehydrogenase complex, the disease results in accumulation of the branch chain amino acids and their toxic ketoacids. Most infants present with vomiting, acidosis, and evolving neurocognitive dysfunction or intermittent altered mental status. The disorder is

named from the particularly sweet odor, reminiscent of maple syrup, which emanates from the urine of affected infants. Laboratory analysis may reveal mildly elevated transaminases, hypoglycemia, and hyperammonemia. Diagnosis is made by the identification of excessive branched chain amino acids (valine, leucine, and isoleucine) in the serum. Treatment is dietary, with restriction of branched chain amino acids by limiting protein intake, supplementation with specialized formula, and care not to induce catabolism. The clinical course and natural history of MSUD and other disorders of amino acid metabolism are characterized by intermittent episodes of metabolic crises, including ketosis, acidosis, and hyperammonemia, which may be treated with intravenous glucose administration, arginine, and, rarely, dialysis. Liver transplantation may be considered for those patients in whom severe metabolic crises are threatening to cause evolving developmental delays.

Disorders of Organic Acid Metabolism

The autosomal recessive methylmalonic acid, isovaleric acid, and 3-hydroxy-3-methylglutaryl (HMG)-coenzyme A (CoA) lyase deficiencies are rare and make up disorders of organic acid metabolism. Most are screened for on routine newborn screening in the United States but are confirmed with the identification of a particular pattern of organic acids in the urine. Collectively, they present with metabolic crises, similar to MSUD. Treatment is largely by avoidance of catabolism and by tailored diets.

Fatty Acid Oxidation Defects

Fatty acid oxidation (FAO) is responsible for the production of the cellular energy that is required for normal cardiac and skeletal muscle function and glucose homeostasis. FAO largely occurs within mitochondria. The end result of FAO is the production of ketones, which serve as a critical energy source when glucose stores are depleted. Defects in FAO include: medium-chain acyl-CoA dehydrogenase deficiency (MCADD), long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD), HMG-CoA synthase deficiency, and carnitineacylcarnitine translocase (CACT) deficiency.

MCADD is an autosomal recessive disorder that affects the metabolism of medium-chain fatty acids and results in fasting hypoglycemia and accumulation of toxic acyl-CoA. The clinical presentation is generally recurrent lethargy, emesis, coma, and even death secondary to life-threatening hypoglycemia. Laboratory evaluation reveals hypoglycemia, hepatitis, hyperammonemia, hypoketosis, and hyperuricemia. Confirmation of diagnosis is made by fibroblast enzymatic assays or genetic testing. Treatment is avoidance of hypoglycemia, but infants are at risk of sudden death due to metabolic derangements, and many develop significant developmental delays.

LCHADD is an error of fatty acid metabolism that affects the mitochondrial trifunctional complex, the primary function of which is to metabolize long-chain fatty acids (found in oils and milk). The classic presentation of a neonate with LCHADD is acute onset of poor feeding in the newborn period, accompanied by lethargy, hypotonia, and hypoketotic hypoglycemia. Older children may go on to develop neuropathy and retinopathy. LCHADD in a fetus predisposes the mother to develop *hemolysis, liver dysfunction, low platelets* (HELLP) syndrome, and acute fatty liver of pregnancy. Diagnosis is suggested by newborn screening and confirmed by measuring LCHADD activity in fibroblasts or muscle or mutational analysis. Treatment is largely dietary, with a low-fat,

high-carbohydrate diet and supplementation with MCTs. Morbidity is high, despite dietary intervention.

HMG-CoA synthase deficiency results in the failure to form ketones in times of stress, resulting in hypoketotic hypoglycemia. Infants may present with hepatomegaly, encephalopathy, or coma in the setting of acute illness or fasting. Generally, serum aminotransferases are normal, as is serum lactate. Diagnosis is suggested by the absence of urinary ketones with normal acyl carnitine profile and confirmed by molecular testing. Treatment is largely avoidance of fasting.

CACT is necessary to transport long-chain fatty acids from the cytosol into the mitochondrion, which supports the production of ATP. Most patients with deficiency in CACT present in the neonatal period with hypoketotic hypoglycemia, hepatic dysfunction, cardiomyopathy, and seizures. Laboratory investigation often reveals hypoketosis, hypoglycemia, hyperammonemia, elevated creatinine kinase, elevated serum aminotransferases, low carnitine levels, and abnormal acyl carnitine profile. Diagnosis is confirmed by genetic testing. Treatment is dietary and involves a low-fat diet with supplementation of MCTs and carnitine. Morbidity and mortality are high in the presence of cardiomyopathy.

Urea Cycle Defects

One of the primary functions of the liver is ureagenesis, to detoxify ammonia, which is the end product of amino acid metabolism. The accumulation of ammonia and glutamine lead to hepatocellular and central nervous system dysfunction. The most common disorders of ureagenesis include ornithine transcarboxylase deficiency, carbamoyl phosphate synthase, and citrullinemia. These disorders generally present in the neonatal period with hyperammonemia and, potentially, cerebral edema, in the first few days of life. The clinical presentation may be confused with sepsis. Diagnosis is suggested by the presence of hyperammonemia with low serum blood urea nitrogen and typical patterns of abnormal elevations in serum amino acids. Diagnosis is confirmed by molecular genetic testing. Treatment should be directed by a metabolic specialist and largely focuses on maintaining serum ammonia within normal range, preventing catabolism, and utilizing a protein-restricted formula with one-half of the protein content as essential amino acids. Liver transplantation may be considered in patients with severe recurrent episodes of metabolic crises with hyperammonemia.

Mitochondrial Hepatopathies

Mitochondrial hepatopathies secondary to respiratory chain complex deficiencies may present in the neonatal period as acute liver failure. Additionally, mitochondrial disorders should be suspected in neonates who present in the first week of life with hypoglycemia, hypotonia, seizures, and evolving hepatic synthetic dysfunction. Diagnosis is suggested by elevated lactate and specifically with lactate-pyruvate ratios greater than 30. With evolving mitochondrial DNA mutational analysis some disorders may be identified; however, few targeted therapies are available. Liver transplantation may be considered if additional comorbidities do not suggest poor post-transplant survival.

Lysosomal Storage Disorders

Lysosomal storage disorders generally present with hepatosplenomegaly as well as an array of systemic symptoms. The most common

lysosomal storage disorders are Gaucher disease, Niemann–Pick disease type C (NPD-C), and lysosomal acid lipase deficiency.

Gaucher disease is an autosomal recessive genetic disease that results from the deficiency of a lysosomal enzyme, glucocerebrosidase, leading to an accumulation of glycolipids within lysosomes. The incidence is approximately 1 per 75,000 live births but is more prevalent in infants of Ashkenazi Jewish descent (Squires, Heubi, 2014). Clinical suspicion should be raised in infants who present with hepatosplenomegaly and normal to mildly elevated liver enzymes. Diagnosis is supported by liver biopsy, which demonstrates macrophages filled with lipid, also known as Gaucher cells; however, the diagnosis is confirmed by reduced glucocerebrosidase activity in leukocytes or by mutational analysis. Prenatal diagnosis is available by chorionic villus sampling or amniocentesis. Targeted treatment with enzyme replacement therapy with recombinant glucocerebrosidase is recommended for symptomatic children.

NPD-C is autosomal recessive and caused by mutations in *NPC1* and *NPC2*. These mutations result in impaired processing and transport of low-density lipoprotein (LDL) cholesterol, which clinically manifests as hepatosplenomegaly and progressive neurocognitive degeneration with dystonia, seizures, dysphagia, and ataxia. Laboratory findings characteristic of NPD-C include decreased high-density lipoprotein, hypertriglyceridemia, and increased LDL. Diagnosis is confirmed by fibroblast cell culture and genetic testing. Treatment is largely supportive, as bone marrow transplantation, liver transplantation, and lipid-lowering medications have not been shown to confer survival benefit.

Lysosomal acid lipase deficiency results in two distinct phenotypes: Wolman disease and cholesteryl ester storage disease (CESD). Both occur secondary to abnormal lysosomal accumulation of cholesteryl esters, triglycerides, and other lipids. Phenotype severity depends on the extent of reduction in lysosomal acid lipase activity. Wolman disease is the most severe manifestation of lysosomal acid lipase deficiency. Infants typically present in the first few weeks of life with failure to thrive, steatorrhea, hepatosplenomegaly, and jaundice. Death generally occurs within the first year of life. Diagnosis is suggested by calcifications of adrenal glands, cholestasis, elevated transaminases, liver synthetic dysfunction, and a normal plasma lipid profile. Liver biopsies have a characteristic orange-yellow appearance secondary to lipid and fat accumulation. CESD generally presents in older children or even in adulthood. Treatment is largely supportive, although trials of recombinant lysosomal acid lipase are available.

Gestational Alloimmune Liver Disease

Historically, gestational alloimmune liver disease (GALD) was referred to as neonatal hemochromatosis, a term used to describe the clinical phenotype of severe neonatal liver disease resulting from an inborn error of iron metabolism, causing hepatic and extrahepatic siderosis. Recently, advances in understanding of the pathogenesis debunk earlier suppositions that GALD results from primary iron overload. Evidence now suggests that iron overload is the phenotype that results from GALD (Bonilla et al., 2012; Whittington, 2012; Zoller, Kinsley, 2012). While nearly all nonhematopoietic iron overload is due to GALD, there are a few rare associations other than GALD. These include trisomy 21, mitochondrial DNA depletion syndrome, and bile acid synthetic defects.

The pathogenesis of GALD stems from complement-mediated hepatocyte injury and alloimmunity activated by maternal immunoglobulin G (IgG) (the only immunoglobulin that freely crosses the placenta). Once sensitization has occurred, maternal antifetal hepatocyte IgG binds to fetal liver antigen. Subsequently,

complement-mediated destruction of fetal hepatocytes results in neonatal liver mass that is 10%–25% of normal. The unaffected fetal liver normally produces hepcidin to regulate placental iron delivery (Bonilla, 2012), but in GALD the drastically reduced amount of hepcidin fails to appropriately regulate iron influx, resulting in iron overload.

GALD should always be suspected in infants who manifest prenatal or neonatal liver disease, especially in liver failure. Infants with GALD are typically sick at birth. In the fetal period, oligohydramnios or intrauterine growth restriction are common. Affected newborns generally present in the neonatal period with acute liver failure with hypoglycemia and coagulopathy or, more rarely, with cirrhosis. Cholestasis generally evolves over time but is not present at birth. Laboratory findings may include normal to slightly elevated ALT, elevated ferritin (n/mL) levels (generally >800 but commonly exceeding 15,000), alpha fetoprotein (ng/mL) greater than 80,000 but typically greater than 300,000, and iron saturation greater than 90%. Imaging may demonstrate extrahepatic siderosis effecting the pancreas, myocardium, thyroid, and salivary glands.

Diagnosis is suggested by liver biopsy demonstrating reduced hepatocyte volume and the presence of C5b-9 complex on immunohistochemistry (Pan et al., 2010). Biopsies may also show hepatocyte collapse, fibrosis, and siderosis (Fig. 77.5). However, demonstration of extrahepatic siderosis is necessary to confirm the diagnosis. This may be accomplished by tissue biopsy or MRI. Generally, it may be preferable to obtain mucosal tissue (submucosal glandular biopsy). This may be followed by MRI if the biopsy is negative and diagnosis is still suspected. Unfortunately, GALD is often diagnosed at autopsy where extrahepatic siderosis is observed.

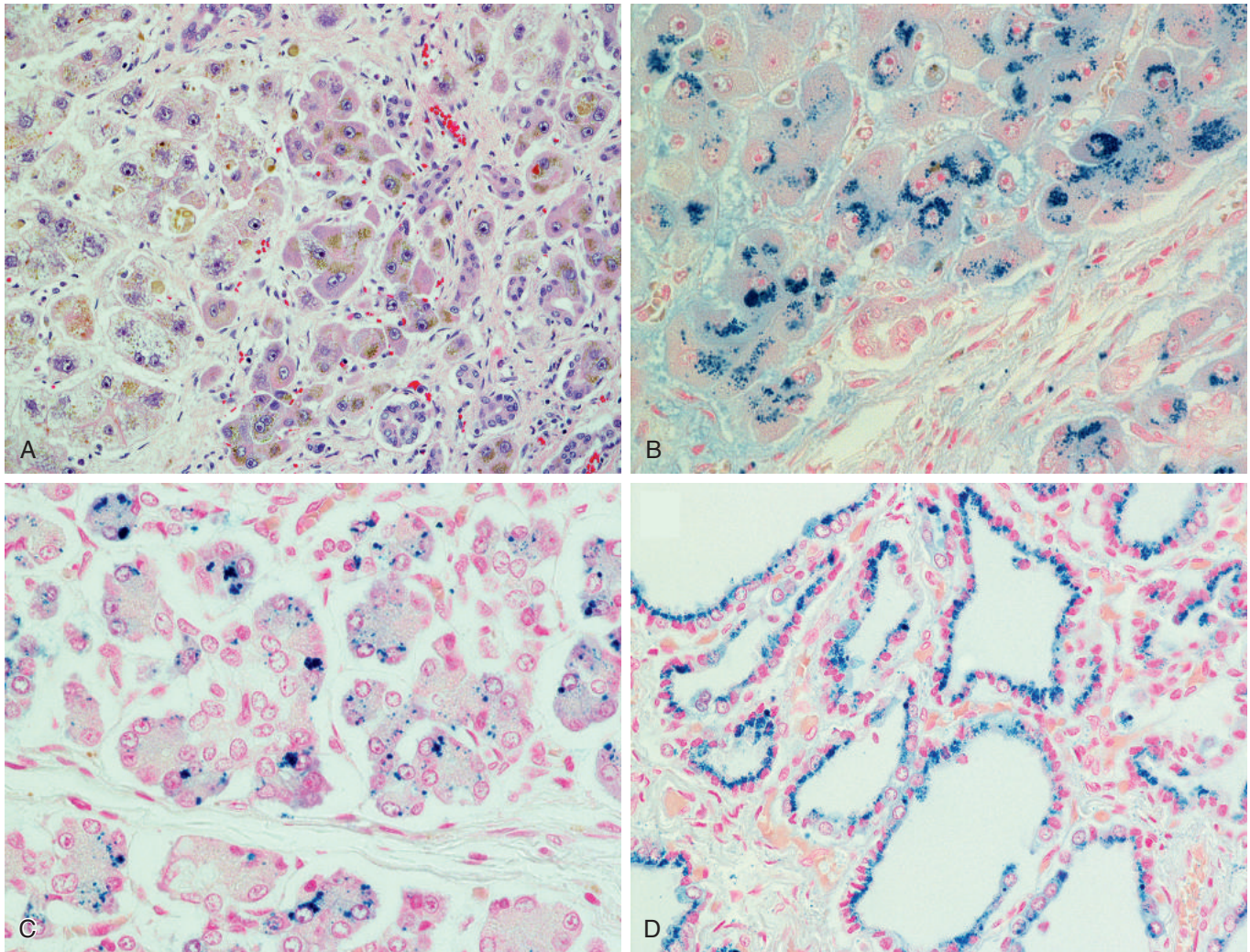
Treatment attempts to block existing antibody action by administration of high-dose intravenous immunoglobulin (IVIG) (1 g/kg) as well as to remove existing antibody with double-volume exchange transfusion. This regimen has been shown to improve survival to between 75% and 80% without liver transplantation (Rand et al., 2009). Before this treatment regimen, outcomes were very poor with older medical therapies such as iron chelation and antioxidants, which have no role in current treatment recommendations. GALD remains a common indication for liver transplantation in the neonatal period; however, transplantation in these patients is complicated by small infant size and multiorgan failure.

Because of the rapid progression and high mortality associated with GALD, if it is suspected, an infant should receive a dose of IVIG immediately, even before the diagnostic work-up is complete. Side effects of IVIG are minimal, and IVIG has minimal impact on the natural history of alternate causes of neonatal liver failure. If diagnosis is confirmed, then the double-volume exchange transfusion should be employed followed by a second dose of IVIG.

The probability of GALD diagnosis in each subsequent infant born to a mother whose previous child was born with GALD is greater than 90% (Whittington, Kelly, 2008). However, GALD is thought to be congenital and familial but not hereditary (Whittington, 2007). Evidence for prevention of GALD is evolving. Given the high risk of subsequent infants developing GALD following an index birth of an affected infant, all mothers should be treated during pregnancy with recurring infusions of IVIG at 14 weeks gestational age, 16 weeks, and weekly from 18 weeks until delivery (Whittington, Hibbard, 2004).

Vascular Malformations

Vascular malformations of the liver generally present in the neonatal period if they are associated with cutaneous malformations or result



• **Fig. 77.5** Gestational Alloimmune Liver Disease Histology. (A) Hematoxylin and eosin stain of a liver demonstrating pericellular fibrosis. Many hepatocytes contain pigment, some of which is hemosiderin, and some of which is bile. (B) The corresponding Gomori iron stain shows iron deposition within hepatocytes. (C) Iron stain demonstrating iron deposition in the pancreas acinar epithelial cells. (D) Iron stain demonstrating iron deposition in thyroid follicular cells.

in significant systemic shunting of blood. Types of shunts may include hepatic artery to hepatic vein, hepatic artery to portal vein, or from portal vein to systemic circulation (portosystemic shunts).

Arteriovenous Malformations

Arteriovenous malformations that shunt large volumes of blood may present in the neonatal period with anemia, heart failure, portal hypertension, or hepatomegaly depending on the vascular involvement. Treatment includes embolization, surgical ligation, or resection. Liver transplantation is reserved for those lesions that are not amenable to more limited surgical intervention and that result in life-threatening complications.

Congenital Portosystemic Shunts

Congenital portosystemic shunts allow for intestinal venous blood to bypass the liver and shunt directly into the systemic venous system. They may be either intrahepatic or extrahepatic in origin (Fig. 77.6–Fig. 77.7). Extrahepatic portosystemic shunts are known

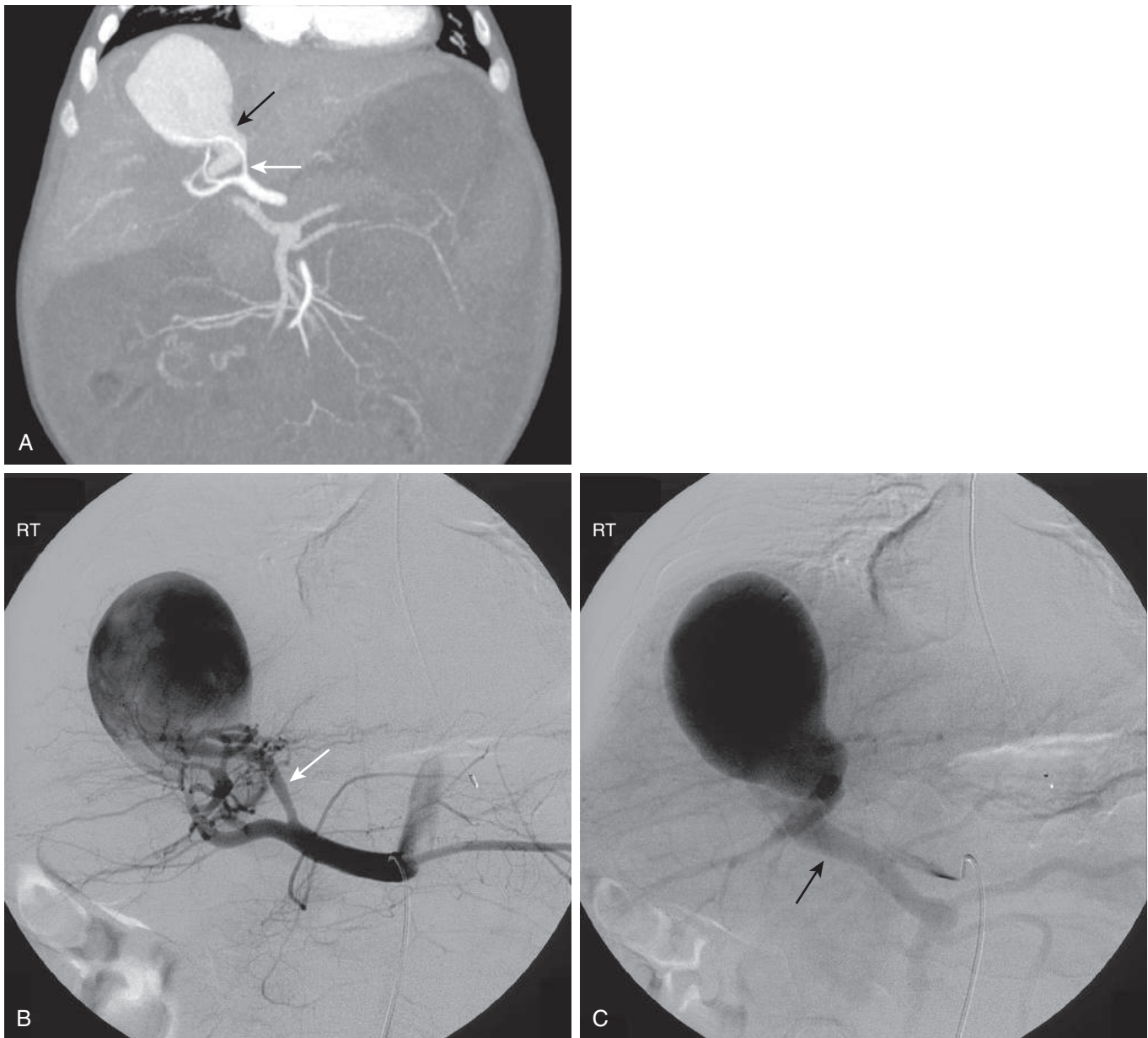
as “Abernathy malformations” (Fig. 77.8). Persistence of fetal circulation with a patent ductus venosus may allow for intrahepatic shunting from the left portal vein to a hepatic vein.

Portosystemic shunts are most commonly identified incidentally on abdominal imaging in individuals with portal hypertension or other vascular malformations. If diverting significant portal blood flow past the liver, they may lead to elevated serum ammonia. Portal hypertension is not usually seen with portosystemic shunts.

Treatment of congenital portosystemic shunts varies and depends on the severity of clinical sequelae. Several may close spontaneously; however, those that are contributing to encephalopathy and subsequent developmental delay or cardiopulmonary compromise should be considered for closure. Techniques may include embolization, surgical ligation, or, rarely, liver transplantation (Ohnishi, 2005; Gillespie et al., 2006).

Hereditary Hemorrhagic Telangiectasia

Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant disease characterized by mucosal and cutaneous



• **Fig. 77.6** Congenital Intrahepatic Arteriportal Fistula. (A) Coronal maximum intensity projection image from an arterial phase computed tomography scan of the abdomen demonstrates a fistulous connection between the left hepatic artery (*white arrow*) and left portal vein (*black arrow*) with a large associated aneurysm. Digital subtraction angiogram images of the celiac axis in arterial (B) and delayed (C) phases redemonstrate the arteriportal fistula supplied by the left hepatic artery (*white arrow*) with shunting into the portal venous system (*black arrow*). (Images courtesy of Dr. Eric Monroe, Interventional Radiology, Seattle Children's Hospital, Seattle, Washington.)

telangiectasias and visceral vascular malformations. The visceral organs most commonly affected include the lungs, liver, intestine, and brain. The prevalence is estimated to be approximately 1 per 10,000 live births. Diagnosis of HHT is based on fulfilling greater than three of four clinical criteria and genetic testing. The clinical criteria include spontaneous, recurrent epistaxis, multiple cutaneous and oral cavity telangiectasias, the presence of visceral vascular lesions, and a first-degree relative with HHT.

Most infants with HHT will present with high-output heart failure and potentially portal hypertension secondary to arteriportal

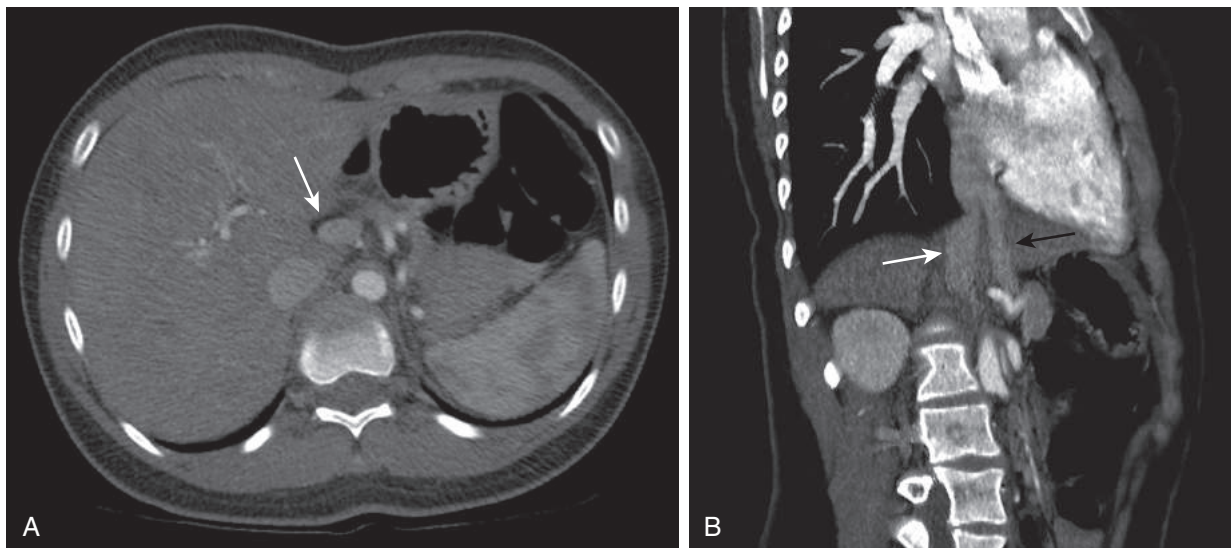
shunting. An ischemic cholangiopathy may result if hepatic arterial blood shunts away from bile ducts.

As with all liver-associated vascular lesions, diagnosis is accomplished by Doppler-enhanced US of the abdomen. In the case of HHT, US most commonly demonstrates a dilated hepatic artery with elevated hepatic artery flow and intrahepatic vascularity. On computed tomography (CT), the presence of multiple telangiectasia leads to heterogeneous enhancement of the liver. Liver biopsy carries high risk of bleeding.

Liver transplantation may be considered in the setting of heart failure, biliary ischemia, or severe portal hypertension.



• **Fig. 77.7** Congenital Intrahepatic Portosystemic Shunt. (A) Sagittal image from a venous phase computed tomography scan of the abdomen demonstrates an anomalous portosystemic shunt between the anterior division of the right portal vein (*white arrow*) and middle hepatic vein (*black arrow*). (B) Right anterior oblique digital subtraction angiogram image following trans-shunt catheterization of the portal vein redemonstrates the broad portosystemic shunt between portal vein (*white arrow*) and middle hepatic vein (*black arrow*). (Images courtesy of Dr. Eric Monroe, Interventional Radiology, Seattle Children's Hospital, Seattle, Washington.)



• **Fig. 77.8** Abernathy Malformation. (A) Axial image from a late arterial phase computed tomography (CT) scan demonstrates a craniocaudal course of the portal vein (*white arrow*) and absent portal vein within the hepatic hilum. (B) Multiplanar reformat image from the CT confirms an abnormal course of the portal vein (*black arrow*) draining to the right atrium, parallel to the inferior vena cava (*white arrow*). (Images courtesy of Dr. Eric Monroe, Interventional Radiology, Seattle Children's Hospital, Seattle, Washington.)

Infantile Hepatic Hemangiomas

Infantile hepatic hemangiomas (IHHs) are the most common benign tumor of infancy, affecting as many as 1%–2% of newborns and upwards of 10% of infants by 1 year of age (Boon et al.,

1996). Most infants present with associated cutaneous or subcutaneous lesions; however, many present with isolated vascular lesions of the liver, brain, and lungs. IHH should be suspected in infants with five or more cutaneous hemangiomas, and this physical finding should prompt investigation for visceral involvement.

IHH is the most common pediatric tumor of the liver and may be characterized as focal, multifocal, or diffuse. Most infants with IHH present before 6 months of age and experience initial progression of the size of lesions from birth to 12 months, followed by a period of regression and involution by about 2 to 10 years of age.

Focal IHH is usually incidental and may be initially seen on prenatal US. They are not associated with cutaneous findings and are typically asymptomatic; larger lesions may lead to high-output heart failure. Diagnosis is by US imaging, identifying a hyperechoic or hypoechoic lesion with heterogeneous echotexture with vascular flow on Doppler interrogation. On MRI, lesions are noted to be hypointense on T1-weighted images and hyperintense on T2-weighted scans with centripetal enhancement with gadolinium contrast.

Multifocal and diffuse IHH are associated with multiple cutaneous hemangiomas. Multifocal IHH is typically not apparent at birth but presents within the first few weeks of life when the hemangiomas become more prominent. Symptomatic infants present with hepatomegaly, heart failure, anemia, and occasionally thrombocytopenia secondary to consumption, hemorrhage, or thrombosis within the hemangioma. Rarely, hemangiomas may stimulate inactivation of thyroxine, resulting in hypothyroidism. Occasionally, rupture of hemangiomas may result in intraperitoneal hemorrhage. US and MRI imaging are similar to that of focal IHH but with more numerous lesions. Treatment is reserved for symptomatic infants, as asymptomatic infants may be observed and followed with serial imaging. Medical therapy employs propranolol at a dose of 1 to 3 mg/kg per day, continued until the proliferation phase is completed around 9 to 12 months of age. Second-line treatments for propranolol failures include steroids, vincristine, and alfa 2a interferon (Leaute-Labreze et al., 2008; Mazereeuw-Hautier et al., 2010). Embolization or surgical resection may be utilized if medical therapy fails or in the setting of significant organ dysfunction. Liver transplantation has been performed in critical clinical circumstances without other options (Christison-Lagay et al., 2007; Dickie et al., 2009). Rarely, multifocal IHH may be associated with hepatic angiosarcoma.

Liver Masses

Hepatoblastoma

While many solid and cystic tumors of the liver may present in the neonatal period, the most common malignant tumor is hepatoblastoma. Risk factors for the development of hepatoblastoma include prematurity, low birth weight, familial adenomatous polyposis, trisomy 18, GSD Ia, Beckwith–Wiedemann syndrome, and Li–Faumeni syndrome. Hepatoblastoma most commonly presents as an abdominal mass associated with weight loss, abdominal pain, and decreased oral intake. Diagnosis is made by typical radiologic findings, including a well-defined but heterogeneous mass that may have areas of necrosis, hemorrhage, or calcification. MRI is the preferred mode of imaging. Biopsy to differentiate epithelial subtype (fetal, embryonal, macrotrabecular, and small cell undifferentiated) is required. Treatment consists primarily of

resection, augmented by chemotherapy; when not resectable, liver transplantation may be considered.

Congenital Hepatic Cysts

Congenital hepatic cysts are one of the most common benign liver masses of infancy. With modern imaging techniques, simple hepatic cysts are frequently diagnosed prenatally by US. There is a wide spectrum of pathologies resulting in simple hepatic cysts. Simple cysts are generally thought to develop from aberrant bile ducts and are not generally associated with cystic lesions of other organs (Rogers et al., 2007). They are characterized by a layer of cuboidal or columnar epithelium surrounding a collection of serous fluid. Most simple hepatic cysts are incidental, asymptomatic, do not require intervention, and may shrink or resolve over time. It is recommended that periodic, serial USs be utilized to survey for interval growth or resolution. Indications for surgical intervention include interval growth, large size, which may cause compression of nearby structures or cause risk for rupture, or those that become symptomatic (Rogers, 2007).

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78

Developmental Hematology

SANDRA E. JUUL AND ROBERT D. CHRISTENSEN

KEY POINTS

- Pluripotential stem cells sustain hematopoietic function throughout a person's lifetime; the fate of developing cells is influenced by the microenvironment.
- The site of erythropoiesis changes over development, progressing from the yolk sac to the aortogonadomesonephron, to the liver, and then to the bone marrow. Erythropoietin (Epo) is the principal factor regulating secondary erythropoiesis.
- Hemoglobin tetramers change over development. Oxygen affinity decreases as hemoglobins switch from embryonic to adult forms.
- The optimal hematocrit trigger for neonatal transfusion remains unknown because the benefits and risks of transfusion at any given hematocrit remain unproven.
- The need for transfusions in the neonatal intensive care unit can be reduced by delayed cord clamping or cord milking, reducing phlebotomy-related losses, and using erythropoiesis-stimulating agents (Epo or darbepoetin)
- Most platelet transfusions given in the neonatal intensive care unit are prophylactic, meaning that they are transfusions in nonbleeding neonates with low platelet counts. The platelet count at which the benefits of platelet transfusion outweigh the risks is uncertain. The "platelet mass" (platelet count times mean platelet volume) may provide a better transfusion trigger than the platelet count alone. In general, 10–20 mL of single donor platelets per kilogram should raise the platelet count by more than 100,000/ μ L. Use of volume-reduced or pooled platelets should be avoided because processing results in platelet activation and decreased function.

Introduction to Embryonic Hematopoiesis

Hematopoiesis is the process by which self-renewing multipotential stem cells give rise to all of the differentiated blood cells (Fig. 78.1). This process involves the coordinated expression of growth factors, some of which act on primitive progenitors that can give rise to multiple cell lineages and others that support clonal maturation of lineage-committed multipotential hematopoietic stem cells (HSCs). Hematopoiesis begins in the embryo, with the first lymphoid progenitors emerging within the embryo and yolk sac before stem cell detection at embryonic day 7.5 (Lin and Yoder *et al.*, 2014). By day 10 HSCs are present in the aortogonadomesonephron and activity then shifts to the liver and finally to the bone marrow (Song *et al.*, 2016). Each cell lineage undergoes

developmental changes that are unique and specific (Yoder, 2014). Details of these systems and the resulting clinical effect of these changes are reviewed in the following chapters.

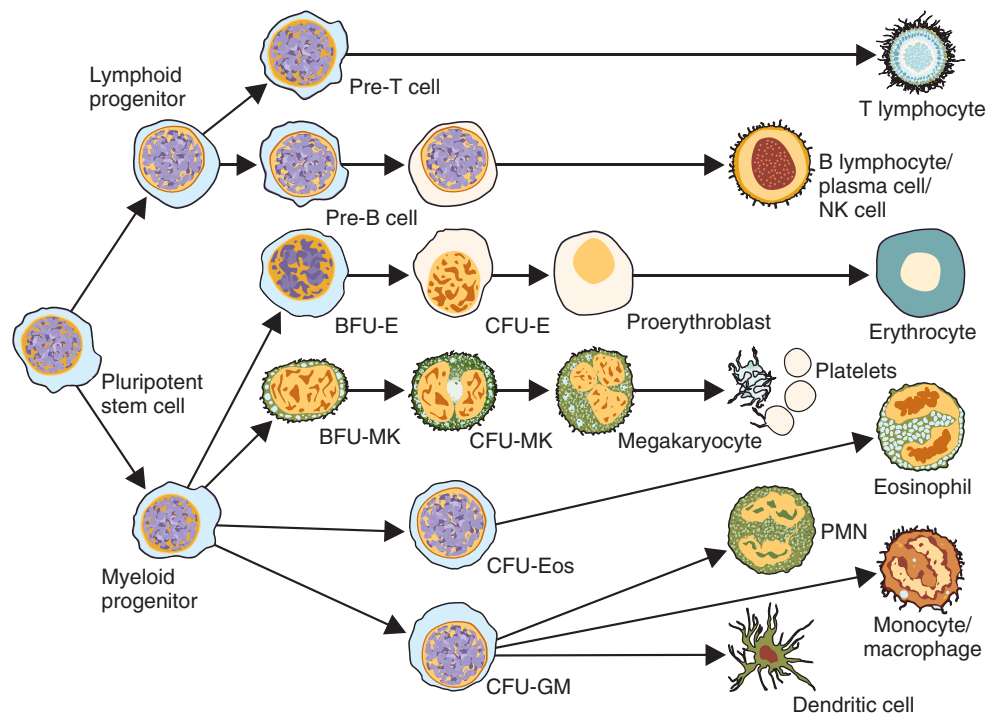
Stem Cell Biology

Pluripotential stem cells sustain marrow function throughout a person's lifetime. A unique characteristic of these cells is that their direct offspring include at least one identical daughter cell, thus perpetuating the population. In contrast, progenitor cells are more differentiated and give rise only to cells more differentiated than themselves. The fate of any particular developing cell is determined in large part by its microenvironment.

The developmental changes in the number, function, and location of HSCs are of interest to transplantation biologists and gene therapists. The proliferative capacity of HSCs differs with the anatomic source of the cells and with the age at which the cells are harvested. The sensitivity of these cells to recombinant cytokines also changes with age. Improving the understanding of the ontogeny of these cells may be helpful in optimizing their clinical use.

Embryonic and fetal HSCs are capable of repopulating adult organisms (Easterbrook *et al.*, 2016). In contrast, transplanted adult stem cells have a lower capacity for self-renewal, sometimes resulting in late graft failure. This might be because stem cells harvested from adults continue to express the adult differentiation program, even if transplanted into a neonatal environment, indicating an irreversible change in gene expression (Dziedosz *et al.*, 2016). Other explanations for the decrease in proliferative potential may have to do with DNA damage over time and loss of telomere repeats with each stem cell division, limiting the replicative potential (Warren and Rossi, 2009).

Ongoing research focuses on optimizing stem cell harvesting techniques. Cell-surface markers, which are dependent on cell maturity and gestational age, are often used to identify and separate HSCs with use of monoclonal antibodies and fluorescence-activated cell sorting analysis. For example, CD34, a cell-surface sialomucin, is an antigen commonly used to select HSCs and early erythropoietic progenitor cells. Combining CD34 positivity with the absence of lineage-specific markers allows selection of a population highly enriched for cells desired for transplant. Research is also focused on optimizing stem cell harvest sites. Both bone marrow and umbilical cord blood are rich in stem cells and have long been used as sources of progenitor cells. The collection of stem cells



• **Fig. 78.1** Overview of Hematopoiesis. Hematopoietic lineages are outlined in this simplified overview of hematopoiesis. *BFU-E*, Burst-forming unit–erythroid; *BFU-MK*, burst-forming unit–megakaryocyte; *CFU-E*, colony-forming unit–erythroid; *CFU-Eos*, colony-forming unit–eosinophil; *CFU-MK*, colony-forming unit–megakaryocyte; *CFU-GM*, colony-forming unit–granulocyte–macrophage; *NK*, natural killer; *PMN*, polymorphonuclear leukocyte. (Courtesy of Alexander R. Vermillion.)

from peripheral blood by stimulated apheresis, with ex vivo expansion of select populations, is now also an option (Brugger et al., 2000).

Umbilical cord blood (UCB) can be harvested as a source of HSCs and used for transplant in patients with marrow failure, malignancy, or immunodeficiency (Alfraih et al., 2016; Kekre and Autin, 2016). Hematopoietic progenitor cells in UCB tend to have a high proliferation index compared with cells harvested from adult marrow (Schoemans et al., 2006). Because of the immaturity of the UCB cells, HLA matching is less stringent for UCB, allowing more efficient donor unit identification compared with a bone marrow registry (Barker et al., 2002). This feature also allows improved matching ability for patients in minority ethnic populations since bone marrow registry matches are frequently in short supply. Promising strategies to increase cells available for transplant include combining multiple units of UCB (Barker et al., 2005) and ex vivo expansion of UCB units.

The first successful use of UCB for HSC transplant purposes was in 1989, between HLA-identical siblings, for severe aplastic anemia from Fanconi anemia (Gluckman et al., 1989). Since then, thousands of UCB transplants have been performed to treat malignancies such as acute lymphoblastic leukemia, acute myeloid leukemia, and chronic myeloid leukemia; bone marrow disorders such as Fanconi anemia; immunodeficiencies such as severe combined immune deficiency; metabolic disorders; and hemoglobinopathies (Kekre and Antin, 2016).

UCB can be stored in public or private commercial banks. Donation of harvested cells to public UCB banks allows storage in a central repository available to all individuals in need of

transplant. Private banking makes the stored UCB available at a cost for future use by individuals or a family. However, there is a low likelihood of any one individual needing an autologous UCB transplant (Roura et al., 2015).

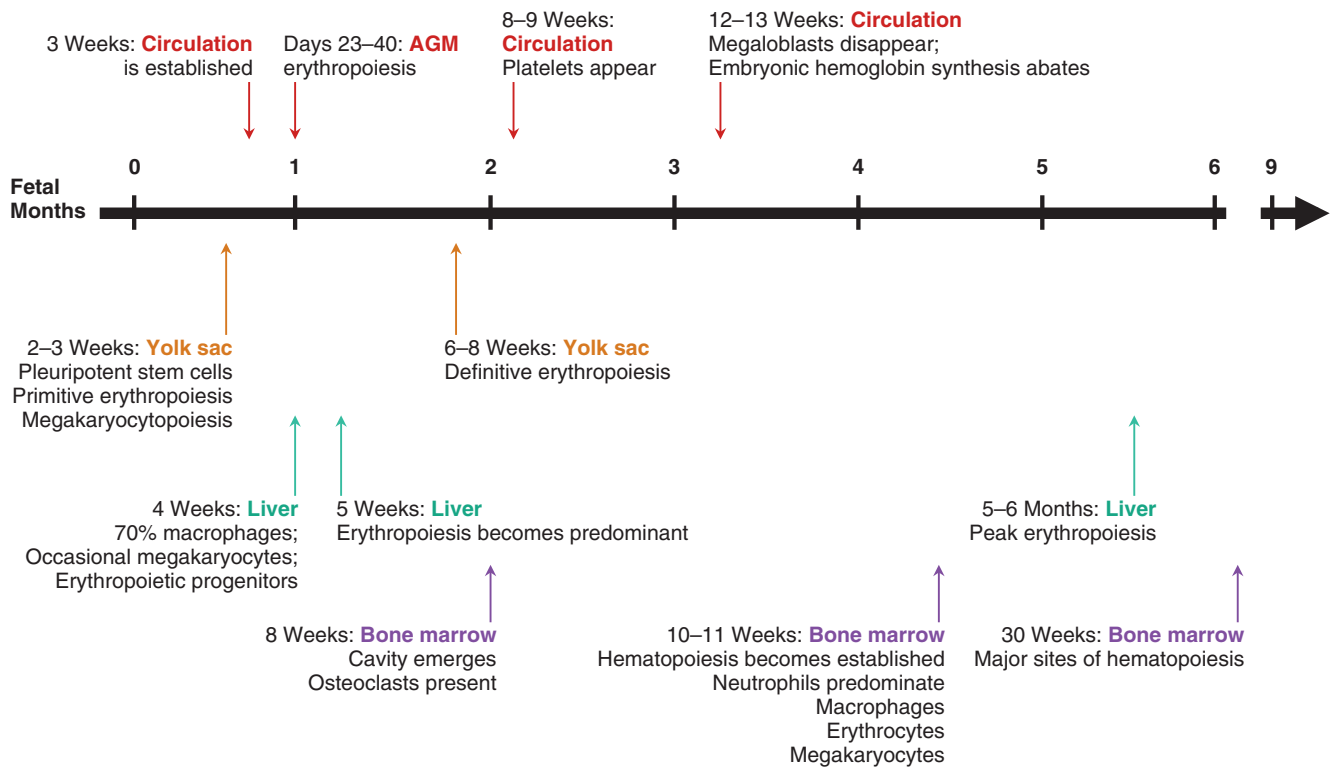
Delaying cord clamping by 60 seconds increases the blood volume of the neonate and increases the endowment of iron. In theory, this practice might result in insufficient UCB remaining for banking. The Canadian Task Force on Preventive Health Care suggests that delayed cord clamping has significant benefits for the neonate and that sufficient blood typically remains in the umbilical cord and placenta after delayed clamping for UCB banking. The task force suggests that both techniques can generally be accomplished (Armson et al., 2015).

Developmental Aspects of Erythropoiesis

Erythropoiesis is the process of perpetual production of red blood cells (RBCs). Serial adaptations occur throughout development so as to meet the changing oxygen demands of the embryo, fetus, and neonate. The type of cells produced, the locations in which they are produced, and even the microenvironments within these locations change as development proceeds (Fig. 78.2). The molecular mechanisms involved in instituting, regulating, and maintaining these adaptations are complex.

Primitive and Definitive Erythropoiesis

During development, two types of RBCs are formed: primitive and definitive erythrocytes. The liver is the primary organ of



• **Fig. 78.2** Changing Sites of Hematopoiesis During Human Gestation. Fetal gestation is shown in months along the *central horizontal arrow*. The timing of significant events during hematopoiesis is shown, beginning with primitive erythropoiesis and megakaryocytopoiesis in the yolk sac and ending with definitive hematopoiesis in the bone marrow during late gestation. AGM, Aortogonadomesonephron.

hematopoiesis during fetal life, but primitive RBCs are first formed in the yolk sac (Yoshimoto and Yoder, 2009). Large primitive RBCs are produced in blood islands of the yolk sac days after implantation of the embryo. These cells enter the newly formed vasculature of the embryo, where they continue to divide and differentiate for several days. This process is only minimally responsive to Epo (Palis, 2014). Primitive erythroblasts are large ($>20\ \mu\text{m}$), CD34 negative, and contain predominantly embryonic hemoglobin. Hemoglobin synthesis continues until cell replication ceases (Boussios and Bertles, 1988). In mice a transition to definitive erythropoiesis occurs at embryonic day 13.5 (full gestation is 21 days). Definitive erythropoiesis is characterized by smaller ($<20\ \mu\text{m}$) CD34-positive erythroblasts, which produce fetal and adult hemoglobins, extruding their nuclei when mature. Unlike primitive erythropoiesis, this process is dependent on Janus kinase signal transduction and Epo stimulation (Neubauer et al., 1998). Primitive erythroblasts normally undergo apoptosis, becoming extinct during fetal life, whereas definitive erythroblasts are able to self-renew (Dieterlen-Lievre, 1997).

Switch of the Primary Site of Erythropoiesis

Humans have four main sites of embryonic and fetal erythropoiesis—yolk sac, aorta (ventral aspect), liver, and marrow. In rodents the spleen is also an important site of hematopoiesis, but there is no evidence for this in healthy humans (Calhoun et al., 1996). Studies using an in vitro embryonic stem cell differentiation system showed that endothelial cells, primitive hematopoietic cells, and definitive blood-cell colonies arise from a common fetal, liver,

kinase-1 (Flk-1)-expressing progenitor (Lancrin et al., 2009). Between embryonic day 8 and embryonic day 11.5, runt-related transcription factor 1 is required for the formation of HSCs and their progenitors. Primitive progenitor cells first develop in the yolk sac, followed by the rise of definitive progenitors, also in the yolk sac (Palis, 2008). Another source of the early definitive progenitors is the ventral aspect of the embryonic aorta (Tavian and Peault, 2005; Palis, 2008). Once circulation is established, progenitors from all lines are detected in the blood, then in the liver, and finally in the marrow (Tavian et al., 2001).

Yolk Sac

The yolk sac is an extraembryonic structure that can be subdivided into the primary and secondary yolk sac. The primary yolk sac is transient and has no known hematopoietic function. In humans it forms by proliferation and differentiation of primitive endodermal cells 7 to 8 days after conception. These endodermal cells give rise to mesodermal precursors (intermediate cells). The primary yolk sac then collapses into small vesicles, and the secondary yolk sac is formed from its remnants at 12 to 15 days after conception. By 16 to 19 days, primitive erythropoiesis is found in the human yolk sac (Kelemen and Janossa, 1980; Kennedy et al., 1997). The secondary yolk sac is an active site of protein synthesis, nutrient transport, and hematopoiesis (Enders and King, 1993). Primitive hematopoietic cells, adherent to surrounding endothelial cells, are first observed at day 16 in the mesodermal layer. These hematopoietic–endothelial cell masses have been described as *blood islands*. As maturation proceeds, these blood islands migrate toward each other, merging to form a network of capillaries. Small clusters of undifferentiated

cells, the hemangioblasts, and clusters of primitive erythroblasts are observed in the small vessels present at this developmental stage (Enders and King, 1993). As differentiation proceeds, endothelial and hematopoietic cell lineages emerge. These cell types share common molecular markers and responsiveness to a cohort of growth factors, and, depending on the microenvironment, can be derived from a common stem cell in culture (Eichmann et al., 1997; Lux et al., 2008; Lancrin et al., 2009).

After the sixth week after conception, definitive erythroblasts are found in the yolk sac. A decline in yolk sac hematopoiesis is observed after the eighth week (Enders and King, 1993). Yolk sac–derived hematopoietic cells have more restricted potential *in vivo*, as only RBCs and macrophages are present in the yolk sac (Enzan, 1986), while progenitor cells in the liver develop into the full spectrum of hematopoietic cells. However, when yolk sac–derived stem cells are cultured *in vitro* or are transplanted, they are multipotent, illustrating the importance of the microenvironment in the development of committed cell lineages.

Aortogonadomesonephron

Another site of early erythropoietic activity in the developing human embryo is the ventral aspect of the aorta, in the periumbilical region (Tavian and Peault, 2005). At around the 23rd postconceptional day in humans, the multipotent hematopoietic progenitor cells in this region are more numerous than in the yolk sac or the liver. By day 40 of gestation, hematopoiesis in this site is concluded.

Liver

A short time after the onset of blood circulation (week 4 to week 5 of gestation), erythropoiesis begins in the liver (Kelemen and Janossa, 1980). As in the yolk sac, primitive erythroblasts initially predominate. However, over the next 4 weeks, definitive erythrocytes become the predominant RBC form. During this time the liver mass increases 40-fold, with hematopoietic cells constituting 60% of the liver by week 11 to week 12 (Thomas and Yoffey, 1964). Meanwhile, other hematopoietic cell types are also produced in the liver. Early in this process (5 weeks), macrophages predominate, with approximately one granulocyte to every nine macrophages (Slayton et al., 1998b). In contrast to the yolk sac, during the period of peak hepatic hematopoiesis (week 6 to week 18), production of all hematopoietic cell lines (erythrocytes, macrophages, megakaryocytes, granulocytes, and lymphocytes) occurs. Between 18 and 21 weeks of gestation, hematopoiesis in the liver diminishes, but the liver continues as an erythropoietic organ until term.

Bone Marrow

As hepatic hematopoiesis diminishes, the bone marrow becomes the primary site of erythropoiesis and remains so throughout postnatal life. The process of erythropoiesis in marrow begins at about 8 weeks, again with primitive erythrocytosis (Kelemen and Janossa, 1980). Over the next few gestational weeks, a switch to definitive erythropoiesis occurs, and by 14 weeks, only definitive erythroblasts are present. As with the liver, production of all hematopoietic cells occurs in bone marrow. Erythropoietic cells constitute a maximum of 35% of total bone marrow cells at week 12 of gestation, falling to between 20% and 30% thereafter (Charbord et al., 1996).

Factors Influencing the Sites of Erythropoiesis

The microenvironment at each site of hematopoiesis influences the type and timing of hematopoietic development. The microen-

vironment includes hematopoietic growth factors and cytokines, as well as the extracellular matrix in which the cells proliferate.

Growth factors are thought to act mainly as permissive and/or selective signals, allowing already committed cell types to proliferate and differentiate. Growth factors important for definitive erythropoiesis include Epo, stem cell factor, interleukin (IL)-3, thrombopoietin (TPO), and possibly insulin and insulin-like growth factor I, both of which act as nonessential survival factors for CD34⁺ cells (Ratajczak et al., 1998). These growth factors work in concert to promote definitive erythropoiesis. However, Epo is the primary growth factor: in the absence of Epo signaling, as in the case for both Epo-null and Epo receptor-null mutations, definitive erythropoiesis does not occur. Null mutations of either Epo or its receptor are lethal at 13.5 days of gestation in the mouse, because of severe anemia.

Extramedullary Hematopoiesis

Extramedullary hematopoiesis can occur after the bone marrow has been established as the primary site of erythropoiesis. Diseases inducing this occurrence include hemolytic conditions, congenital rubella, cytomegalovirus infection, and parvovirus B19 infection. Extramedullary hematopoiesis can occur in the liver, spleen, adrenal glands, pancreas, thyroid, endocardium, testes, uterus, skin, or brain (Yamamoto et al., 2016). When the skin is involved in a neonate, the classic “blueberry muffin” rash is seen, typical of congenital rubella or cytomegalovirus infection.

Ontogeny of Erythrocytes

The earliest precursor cells specific to the erythroid lineage are the burst-forming unit–erythroid (BFU-E) cells. BFU-E cells have low numbers of Epo receptors (Sawada et al., 1990) and respond to Epo, as well as granulocyte–macrophage colony-stimulating factor (GM-CSF), and IL-3. As these cells mature into erythroid colony forming unit–erythroid and proerythroblasts, they become highly dependent on Epo, which is reflected by the high density of Epo receptors on the cell membrane (up to 1000 per cell) (Broudy et al., 1991). Mature erythroblasts have fewer Epo receptors and are less sensitive to Epo stimulation, and reticulocytes and erythrocytes have no Epo receptors and are unresponsive to Epo. The principal functions of mature erythrocyte metabolism are to maintain adequate adenosine triphosphate (ATP) stores, to produce reducing substances to act as antioxidants, and to produce 2,3-diphosphoglycerate (2,3-DPG), which modifies the oxygen affinity of hemoglobin.

Important developmental changes occur in hematocrit, reticulocyte count, and RBC morphology, membrane content, deformability, life span, and metabolism. Over the course of gestation, there is an expected rise in hematocrit, from (36 ± 3)% at 18 to 20 weeks of gestation (fetal samples) to (61 ± 7)% expected at term birth. To maintain this increase in hematocrit and blood volume (up to 7 mL/day during the last trimester), the production of approximately 50 × 10⁹ erythrocytes per day is required. During this same period of fetal development, erythrocyte size (the mean cell volume) decreases from 134 ± 9 femtoliter (fL) to 119 ± 9 fL (Christensen et al., 2008). At term the mean cell volume is larger than that of normal healthy adults and drops postnatally, reaching a nadir at 4 to 6 months. It then increases to reach adult values (88 ± 8 fL) by approximately 1 year.

Reticulocytes are near mature erythrocytes released from the bone marrow into the circulation. Although the nucleus has been

extruded, they retain cytoplasmic organelles such as ribosomes, mitochondria, and Golgi bodies for approximately 24 hours. These newly released cells can be differentiated from mature RBCs by manual methods that involve staining with new methylene blue or brilliant cresyl blue, which stain the nucleic acid within the cells. They can also be enumerated by electronic means that involve flow-cytometric gating of cells that are larger and contain more nucleic acid than mature RBCs. The reticulocyte count can be used to assess the level of erythrocyte production, because high values indicate active erythropoiesis, while low numbers indicate low levels of erythropoiesis. At birth, reticulocyte counts in preterm infants tend to be higher than in term infants (400,000/ μ L–550,000/ μ L vs 200,000/ μ L–400,000/ μ L) (Henry and Christensen, 2015a). Absolute reticulocyte counts, reticulocyte percentage of total RBCs, and corrected reticulocyte counts can be obtained. In general, when neonates are being evaluated, the absolute reticulocyte count is the most helpful (Christensen et al., 2016b).

RBC morphology is quite heterogeneous in preterm and term infants as compared with adults. Irregularly shaped cells such as poikilocytes, acanthocytes, schizocytes, and burr cells are common in the blood smears of neonates. This reflects developmental changes in cell membrane deformability and flexibility. The neonatal RBC has a life span of approximately 70 to 80 days as compared with 120 days for the adult RBC.

Developmental Changes in the Regulation of Erythropoiesis

The principal growth factor that regulates erythropoiesis is Epo. This 30.4-kDa glycoprotein contains 165 amino acids and is extensively glycosylated, with 40% carbohydrate content. Epo maintains RBC production during fetal, neonatal, and adult life by inhibiting apoptosis of erythroid progenitors and by stimulating their proliferation and differentiation into normoblasts (Jelkmann, 1992). Since very little Epo crosses the placenta, the Epo concentrations measured in the fetus reflect fetal synthesis (Widness et al., 1991; Eichhorn et al., 1993). Epo production begins early in fetal life, and Epo has been identified in extraembryonic coelomic fluid and amniotic fluid (Campbell et al., 1992). The primary site of Epo production during fetal life is the liver, with transition to the kidney postnatally (Fahnenstich and Dame, 1996). This transition is mediated in part by expression of *GATA4* (Dame et al., 2004). Production of Epo is stimulated by hypoxia via hypoxia-inducible factor (HIF) 1 and 2 pathways (Dioum et al., 2009; Fisher et al., 2009; Lam et al., 2009; Semenza, 2009; Webb et al., 2009). HIF is a DNA-binding complex composed of two subunits: HIF-1 β , which is not oxygen responsive and is constitutively expressed, and either HIF-1 α or HIF-2 α , which are highly oxygen sensitive. The HIF complex is, in turn, regulated by prolyl hydroxylase domain enzymes 1–3 (Weidemann and Johnson, 2009). Elevated Epo concentrations (up to 8000 mU/mL) have been reported in pathologic states such as fetal hypoxia, anemia, and placental insufficiency and in infants of diabetic mothers (Teramo and Widness, 2009).

During fetal development, circulating Epo concentrations range from 4 mU/mL at 16 weeks of gestation to 40 mU/mL at term (Forestier et al., 1991; Fahnenstich et al., 1996). An unhealthy intrauterine environment can result in increased Epo production, reflecting fetal hypoxemia (Halmesmaki et al., 1990; Ruth et al., 1990). In healthy term infants, serum Epo concentrations decrease after birth to reach a nadir between the fourth and sixth week of age (Ruth et al., 1990). By 10 to 12 weeks, they reach adult

concentrations (approximately 15 mU/mL) (Kling et al., 1996). These changes in Epo concentrations are consistent with the changes in hemoglobin and hematocrit seen following term birth (physiologic anemia). In premature infants the anemia is more severe and persists longer, leading to anemia of prematurity. Epo concentrations in these infants are inappropriately low, forming the rationale for recombinant human Epo therapy.

Ontogeny, Organization, and Structure of Hemoglobins

Hemoglobin is a tetrameric molecule comprising two pairs of polypeptide subunits. As development proceeds, various hemoglobins are constructed by a combination of two α -like globins (ζ or α) with two β -like globins (ϵ , γ , δ , or β) to form hemoglobin tetramers. These tetramers include the embryonic hemoglobins, hemoglobin Gower 1 ($\zeta_2\epsilon_2$), hemoglobin Gower 2 ($\alpha_2\epsilon_2$), and hemoglobin Portland 1 ($\zeta_2\gamma_2$), fetal hemoglobin (hemoglobin F) ($\alpha_2\gamma_2$), and the adult hemoglobins A ($\alpha_2\beta_2$) and A₂ ($\alpha_2\delta_2$). Their expression and proportion depend on gestational age but can be modified by external mechanisms. The basic function of the various hemoglobins is similar, but their oxygen affinity differs. As the hemoglobins switch from embryonic to fetal to adult forms, oxygen affinity decreases. Thus the switch from embryonic to fetal to adult hemoglobin synthesis is a major mechanism by which the developing fetus adapts from the relatively hypoxic intrauterine environment to the relatively oxygen-rich extrauterine environment (Dzierzak and Phillipsen, 2013).

Changes in Hemoglobin Synthesis With Development

The genes within the α -globin and β -globin families are expressed according to a strict ontogenetic schedule, and the quantitative expression of the genes from each of these families is strictly balanced and coordinated (Bard, 2000). Hemoglobin synthesis begins around 14 days after conception, with synthesis of ζ -globin and ϵ -globin chains. These are replaced by the synthesis of α -globin and γ -globin chains by the fifth to seventh week of gestation (hemoglobin Gower 2, hemoglobin Portland 1, and hemoglobin F become predominant) (Gale et al., 1979). By 12 weeks of gestation, hemoglobin F ($\alpha_2\gamma_2$) accounts for almost all of the hemoglobin produced (Cividalli et al., 1974). After the 20th week of gestation, no ϵ -globin chains are produced, but the production of the ζ -globin chains can persist through the last trimester in pathologic conditions such as homozygous α -thalassemia. Expression of the γ -globin gene peaks during midgestation and declines rapidly during the last month of fetal gestation. β -Globin synthesis, required for hemoglobin A, starts at the sixth week of gestation, increasing as γ -globin synthesis declines, a transition that continues to the sixth month of life (Kazazian and Woodhead, 1973). Thus hemoglobin A synthesis quantitatively increases first after the 30th week of gestation. At the end of the last trimester a rapid switch from the synthesis of fetal hemoglobin to adult hemoglobin occurs, falling from 85% at 34 weeks of gestation to 60%–80% at birth (Peri et al., 1998). The synthesis of δ -globin chains, required for hemoglobin A₂ ($\alpha_2\delta_2$), begins at the 34th to 35th week of gestation. After birth, a rapid increase in hemoglobin A and hemoglobin A₂ synthesis occurs.

Red Blood Cell Transfusion

RBCs can be transfused to anemic patients to simultaneously increase the recipient's blood volume and RBC content, thereby increasing oxygen-carrying capacity. The technical ability to store blood for future transfusion was developed in the early 1900s

(Rous and Turner, 1916). In the early years, RBCs were kept viable in a citrate and glucose solution, but current solutions make it possible to store cells for up to 42 days in solutions such as citrate–phosphate–dextrose, citrate–phosphate–dextrose–adenine, and various additive solutions, which may contain additional dextrose, mannitol, and adenine (Nemkov et al., 2016). The hematocrit of “packed RBCs” typically ranges from 55% to 80%. During storage RBCs undergo metabolic and structural changes. 2,3-DGP, antioxidant, and ATP contents decrease, glycolysis decreases, osmotic fragility increases, and deformability decreases (Matthews et al., 2015). ATP-dependent membrane pumps become dysfunctional, and extracellular potassium content increases at a rate of 1 milliequivalent per day, which can be dangerous when large volumes are transfused quickly. Oxidative damage occurs in lipids and proteins during storage and irradiation (Remy et al., 2015). Proinflammatory compounds accumulate during storage of blood, particularly if it is not leukoreduced. After an RBC transfusion, the mean potential life span of the RBC is 85 days, with a mean half-life of 43 ± 11 days (Strauss et al., 2004).

The risks of RBC transfusion may be due to the storage process, the transfusion itself, and the association with oxidative damage. Because of the storage process and increasing age of the stored blood, RBC transfusion exposes the recipient to high levels of potassium, glucose, hydrogen, and lactic acid; the clinical significance depends on the age of the blood and the volume and speed of transfusion (Remy et al., 2015). Although rare, transfusion-transmitted bacterial infections can occur because of bacterial contamination of stored blood (Niu et al., 2006). Other risks of RBC transfusion include viral infections, transfusion-related acute lung injury, and graft-versus-host reaction (Lieberman et al., 2014). Multiple RBC transfusions in the absence of significant phlebotomy-related loss may also put the patient at risk of iron overload and oxidative injury (Park and Kim, 2015). There is 200 mg of iron in a 420-mL unit of whole blood, which is then processed into 250-mL units of packed RBCs (Porter, 2001), so a unit of blood with a hematocrit of 60% has an iron content of 0.7 mg/mL, which becomes available as the cells turn over. There is increased unbound iron in stored blood, which may increase the amount of reactive oxygen species (Hirano et al., 2001). In retrospective and observational studies, increased numbers of RBC transfusions have been associated with retinopathy of prematurity (Dani et al., 2001), bronchopulmonary dysplasia, necrotizing enterocolitis, and diuretic use (Valieva et al., 2009).

Optimal hematocrit values and indications for transfusions in infants in the neonatal intensive care unit (NICU) remain controversial. In the past, infants were transfused if the hematocrit fell below 40%. Because of the risk of transfusions and lack of evidence for benefit, more restrictive transfusion guidelines have been proposed (dos Santos et al., 2011). Most studies comparing restrictive with liberal transfusion practices in premature infants have not demonstrated a substantial and lasting benefit to maintaining higher hemoglobin levels (Bell et al., 2005; Kirpalani et al., 2006). In one study, neurodevelopmental outcome at school age was worse with liberal NICU transfusion practice (Nopoulos et al., 2011). Currently, there is no reliable marker to identify the need for transfusions in neonates, although the following have been studied: clinical indicators (Wardle and Weindling, 2001), lactic acid concentration (Wardle and Weindling, 2001; Takahashi et al., 2009), echocardiographic findings (Alkalay et al., 2003), and near infrared spectroscopic monitoring of cerebral and visceral oxygenation (Sandal et al., 2014).

Delayed clamping of the umbilical cord at birth, for 60 to 90 seconds, is a safe and effective means of increasing the baseline hematocrit of preterm neonates (Brocato et al., 2016). Mounting evidence indicates that cord milking or stripping, a more rapid means of accomplishing a placental/cord blood transfusion at birth, is equally safe and effective (Christensen et al., 2014; Katheria et al., 2015). Current practices for transfusion avoidance in the NICU include either delayed cord clamping or cord milking, plus efforts to reduce phlebotomy-related losses. The latter includes drawing all the blood needed for laboratory tests at birth using otherwise discarded fetal blood in the umbilical cord and thereby not drawing blood from small preterm infants on admission to the NICU (Henry et al., 2015b). An additional means of avoiding transfusions is to use erythropoiesis-stimulating agents, Epo, or long-acting darbepoetin. These can keep hemoglobin levels higher than otherwise, reduce transfusion requirements, and perhaps lead to better neurodevelopmental outcomes (Ohls et al., 2013, 2014, 2015, 2016).

Bilirubin Metabolism

The primary source of bilirubin in the fetus and neonate is metabolism of heme derived from hemoglobin in circulating erythrocytes. The rate-limiting step in heme breakdown is the formation of biliverdin, a process controlled by heme oxygenase (Rodgers and Stevenson, 1990). After heme breakdown the iron is recycled, carbon monoxide is liberated and exhaled, and biliverdin is reduced to bilirubin IX α by biliverdin reductase. In utero, unconjugated bilirubin is processed by the mother after placental transfer. Postnatally, exhaled carbon monoxide can be quantified to assess the heme breakdown rate (Christensen et al., 2016a). Unconjugated bilirubin is lipophilic and tightly bound to circulating albumin. The conjugation of bilirubin results in a relatively polar, water-soluble molecule, bilirubin diglucuronide, which can be excreted. This process occurs in the liver and is dependent on ligandin, a transfer protein, and the enzyme uridine diphosphoglucuronyl transferase. The conjugating ability of the fetus and newborn is impaired relative to that of older individuals because of reduced transferase activity and low levels of uridine diphosphoglucuronic acid (Dennerly et al., 2001).

Developmental Aspects of Megakaryocytopoiesis

Platelets are small (7.5 fL) anucleated fragments of megakaryocytes that circulate as relatively smooth disks when unactivated. The normal circulating life span of a platelet is 10 days. Platelets provide hemostasis when a breach of the vascular endothelial lining occurs and they activate and adhere to the exposed subendothelium. Activated platelets generate mediators, including the potent vasoconstrictor thromboxane A₂ and adenosine diphosphate, both of which further contribute to hemostatic plug formation (Gremmel et al., 2016).

Sites of Megakaryocyte Production

Megakaryocytopoiesis is the process by which megakaryocytes, and ultimately platelets, develop. As with erythropoiesis, the sites of megakaryocytopoiesis change during embryonic and fetal development. In mouse development, megakaryocytes are found in the early yolk sac (McGrath and Palis, 2005). These cells, when

cultured in the presence of stem cell factor, IL-3, IL-6, Epo, TPO, and granulocyte colony-stimulating factor (G-CSF) produce not only BFU-E cells but also megakaryocyte colonies. Megakaryocyte progenitors share a common progenitor with primitive hematopoietic cells (McGrath and Palis, 2005). In humans, electron micrograph studies have shown megakaryocytes present in the liver and circulatory system as early as 8 weeks after conception (Hesseldahl and Falck-Larsen, 1971).

Megakaryocyte Precursors

Megakaryocytopoiesis begins with the pluripotent HSCs, which give rise to myeloid progenitor cells (colony-forming unit–spleen cells), then burst-forming unit–megakaryocyte cells, followed by colony-forming unit–megakaryocyte cells (see Fig. 78.1). Further maturation brings these small mononuclear cells that are largely indistinguishable from monocytes to large polyploid cells, which are easily recognized on the basis of their phenotype. The process of megakaryocyte differentiation has been separated into four stages. Stage I cells, or megakaryoblasts, are the smallest and most immature. As cells mature through stage II (promegakaryocytes), stage III (granular megakaryocytes), and stage IV (mature megakaryocytes), the nucleus becomes multilobed, the cytoplasm becomes increasingly eosinophilic by Wright–Giemsa staining, and cellular size increases from 6–24 μm up to 50 μm . The presence of granules increases steadily until in the mature cells they become organized into “platelet fields.” Unlike in other cell lines, as the nucleus of megakaryocytes matures, it undergoes endomitosis or endoreduplication, a process by which cell ploidy is increased in the absence of cell division. Megakaryocytes from adults typically have a modal ploidy of 16N, while comparable samples from preterm or term infants have a significantly lower ploidy of less than 8N (Slayton et al., 2005). Megakaryocytes from newborns are also typically smaller than those in adults, although they manifest features of mature megakaryocytes (Fuchs, et al., 2012). Adult-size megakaryocytes appear by 2 years of age. Typically, smaller cells with lower ploidy produce fewer platelets than do larger cells with higher ploidy. Despite this, the platelet counts of fetuses are only slightly lower than those of adults (Wiedmeier et al., 2009).

Control of Megakaryocytopoiesis

Platelets serve the primary function of hemostasis but also participate in antimicrobial defense and tissue repair (Grozovsky et al., 2015). Multiple cytokines participate in the process of megakaryocytopoiesis; however, TPO is the principal one. Stem cell factor, IL-3, and IL-6 increase ploidy and the size of megakaryocytes. IL-11 also stimulates the proliferation of megakaryocyte progenitors and induces megakaryocyte maturation, while still other growth factors such as Epo, stem cell factor, GM-CSF, IL-1, basic fibroblast growth factor, platelet-derived growth factor, and interferon- γ have a less clearly defined role. Some cytokines inhibit thrombopoiesis, including transforming growth factor β and platelet factor 4 (Gewirtz et al., 1995). The magnitude of the influence of these growth factors changes with development.

Thrombopoietin

The presence of a growth factor regulating platelet formation was hypothesized in the 1950s but was not realized until 1994 when the protein was isolated by Kaushansky (2015). TPO is composed of 332 amino acids and contains two domains. The amino terminal is the active domain (153 amino acids) and bears marked homology

to Epo. TPO is produced primarily by the liver, although other tissues express small amounts. It acts as a potent stimulator of all stages of megakaryocyte development by binding to its specific cell-surface receptor, c-myeloproliferative leukemia (MPL). In TPO- and c-MPL-knockout models, platelet production is 10%–15% of that of controls, confirming that TPO is the primary regulator of platelet production but also indicating that alternative pathways exist for megakaryocytopoiesis. TPO is bound to the surface of platelets by its receptor, thereby reducing megakaryocyte exposure to the hormone (Kaushansky, 2015). Serum TPO concentrations tend to be lower in preterm infants than in older infants and children, and the TPO response to thrombocytopenia is less robust as gestational age decreases. This is counterbalanced by an increased sensitivity of megakaryocyte precursors to TPO (Liu and Sola, 2011).

Developmental Changes in Platelet Count

Fetal platelet counts increase with gestation. At 15 weeks the average platelet count is 187,000/ μL , increasing to 274,000/ μL at term. Overall, preterm infants have slightly lower platelet counts than do adults with a broader range of normal (100,000/ μL –450,000/ μL) (Wiedmeier et al., 2009; Henry and Christensen, 2015a).

Platelet Transfusions

Clinical practice regarding platelet transfusion is inconsistent since no evidence-based guidelines are available (Cremer et al., 2016). In a 2005 Web-based survey of neonatologists in the United States and Canada, wide variations in practice were noted, with platelet transfusions frequently administered to nonbleeding neonates with platelet counts greater than 50,000/ μL . This practice was particularly common during indomethacin treatment, before or after procedures or operations, or after diagnosis of intraventricular hemorrhages (Josephson et al., 2009). This is concerning because several studies show a correlation between the number of platelet transfusions received by hospitalized neonates and mortality rate (Garcia et al., 2001; Baer et al., 2007). In a study of 1600 thrombocytopenic NICU patients, those who received platelet transfusions had higher mortality rates: 2% for those with no transfusions, 11% for those with 1 or 2 transfusions, 35% for those with more than 10 transfusions, and 50% with 20 or more transfusions (Baer et al., 2007). This was partially due to the underlying illness that required the infant to be transfused. However, since transfusion practices differ so widely, some of the increased mortality could statistically be ascribed to harmful effects of multiple platelet transfusions. In an attempt to create a reasonable, yet safe approach to platelet transfusions, two guidelines were prospectively compared: one based on platelet count and the other based on platelet mass (platelet count times mean platelet volume). Fewer patients were transfused when platelet mass was used as a transfusion trigger (Gerday et al., 2009). Roberts et al. (2008) recommended that prophylactic transfusions of platelets be avoided after the first week of life unless the platelet count falls below 30,000/ μL . During the first week of life, they recommend a transfusion trigger of 50,000/ μL for preterm infants. For patients with platelet counts greater than 50,000/ μL , transfusions should be reserved for those with active serious bleeding. Another controversy concerns the preparation of platelets: single donor, multiple donor, single unit, or volume-reduced multiple unit preparations. In general, 10- to 20-mL single donor platelets per kilogram should raise the platelet count by more than 100,000/ μL . In the absence of consumption, some of the donor

platelets should remain in the recipient's circulation for 1 week. Use of volume-reduced or pooled platelets should be avoided because processing results in platelet activation and decreased function.

Developmental Aspects of Granulocytopoiesis

Early hematopoiesis is characterized almost exclusively by erythropoiesis, although a small number of macrophages are produced in the yolk sac. After circulation begins in the fourth to fifth week of gestation, macrophages appear in the liver, brain, and lungs. During the fifth week, hematopoiesis begins in the liver, and the first hematopoietic cells to appear are macrophages (Kelemen and Janossa, 1980). Whether Kupffer cells originate in the yolk sac and migrate to the liver or arise de novo in the liver is unknown. The marrow space begins to develop around the eighth week after conception, and as occurs in the liver, the first hematopoietic cells to appear in the bones are phagocytes (Kelemen and Janossa, 1980; Slayton et al., 1998b). These phagocytic osteoclasts seem to core out the marrow space. When hematopoiesis is established in the marrow at 10 to 11 weeks after conception, primarily neutrophils are produced, in contrast to the liver, where primarily macrophages are present (Slayton et al., 1998a, 1998b).

The thymus appears around 8 weeks after conception. T-cell progenitors are thought to migrate from the fetal liver to the thymus at 8 to 9 weeks after conception (Haynes et al., 1989), and by the 10th week, lymphoid cells constitute 95% of this organ, with granulocyte precursors and macrophages making up the remainder. B-cell precursors first appear in the omentum and the fetal liver at 8 weeks after conception. B-cell production in the omentum occurs transiently from 8 to 12 weeks (Solvason and Kearney, 1992), while production continues in the fetal liver. Regulatory T cells are found in the thymus and secondary lymphatic organs in the early second trimester and are proposed to be involved in self-reactivity and immune tolerance (Cupedo et al., 2005; Izcue and Powrie, 2005).

The spleen is an important secondary lymphatic organ in humans. The human spleen does not have intrinsic granulocytopoiesis and erythropoiesis activities or produce hematopoietic growth factors (Calhoun et al., 1996), and lymphocytes appear to migrate there through fetal blood. Lymphocytes begin to appear in the spleen around 11 weeks after conception. By the 22nd week, 70% of the cells are lymphocytes.

Overview of Hematopoietic Cytokines

Hematopoietic growth factors can be classified into two groups: those responsible for the regulation of myeloid and erythroid growth and differentiation, called *colony-stimulating factors*, and those concerned with immunity, called *lymphokines*. Once sequenced, lymphokines are assigned IL numbers. There is a great deal of

functional overlap between hematopoietic growth factors (redundancy), and each growth factor has a multiplicity of biologic actions (pleiotropy). Thus more than one cytokine controls cells in any cell lineage, and most factors affect cells in more than one lineage (Vaidya and Kale, 2015).

Epo, GM-CSF, and G-CSF belong to a family of hematopoietic cytokines that share a tertiary structure and function by binding to specific cell-surface receptors. Specific ligand binding results in allosteric changes in the receptor molecules, which, depending on the type of receptor, results either in protein kinase activation, as with macrophage colony-stimulating factor (Bourette and Rohrschneider, 2000), or in a cascade of intracellular signaling via the Janus kinase 2 mechanism, as is characterized by Epo (Kuhrt and Wojchowski, 2015).

Many of the hematopoietic cytokines were discovered on the basis of their growth-promoting effects on hematopoietic cells or their specific immune functions. It was initially assumed that their effects were specific to the hematopoietic system. This view was incomplete. Functional receptors are expressed by other nonhematopoietic cells, with clear nonhematopoietic functions as reviewed by Li et al. (2015) and Juul and Pet (2015). For example, both glia and neurons produce many of the cytokines once thought to be restricted to the hematopoietic system. Furthermore, they express receptors for these peptides, indicating the capability of both paracrine and autocrine interaction. Epo and G-CSF are both available in recombinant form and are approved by the US Food and Drug Administration for clinical use.

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79

Neonatal Bleeding and Thrombotic Disorders

MATTHEW A. SAXONHOUSE

KEY POINTS

- Acquired or inherited coagulation disorders should be considered in any neonate that suffers significant hemorrhage.
- Treatment for specific coagulation disorders should be provided in consultation with pediatric hematology and based on the most current guidelines.
- Thromboembolism (TE) is a significant problem affecting both term and preterm neonates.
- Most neonates that experience a significant TE event have acquired risk factors and/or a prothrombotic disorder.
- Proper imaging is essential for accurately identifying neonatal TE events.
- The use of central venous/arterial catheters significantly increases a neonate's risk for thrombosis.
- Recommendations for treatment for neonatal TE events are based on expert opinion and data from case studies/series.
- Care for neonates with coagulation disorders or significant TE events should occur in a tertiary referral center that has appropriate subspecialty support.

Neonatal bleeding and thrombotic disorders may present a diagnostic challenge to the physician. Excess and/or deficiencies of certain coagulation/anticoagulation proteins coupled with multiple acquired and/or prothrombotic risk factors and/or thrombocytopenia can result in a hemorrhagic or thromboembolic (TE) emergency. The timely diagnosis of a congenital hemorrhagic disorder can potentially avoid significant long-term sequelae, while the lack of randomized clinical trials addressing the management of neonatal thromboses can leave a neonatologist guessing on what the optimum treatment strategy should be. In this chapter, we first review the neonatal hemostatic system. Neonatal hemorrhagic disorders are presented with a discussion of current treatment options. Congenital and acquired risk factors and common sites for neonatal thromboses are reviewed. Finally, suggested evaluations for neonates with TE emergencies as well as the latest treatment options are presented.

The Neonatal Hemostatic System

Blood vessel injury caused by damaged endothelium leads to bleeding. If this bleeding is not curtailed, pathologic hemorrhage

will occur. Hemostasis refers to the process in which bleeding is controlled at the site of damaged endothelium (Roberts et al., 2006). The process of hemostasis involves the interaction between endothelium, subendothelium, platelets, circulating cells, and plasma proteins. Blood vessel injury leads to an immediate, local response of both plasma and cellular components. Thrombosis is confined to the area of injury, leading to eventual tissue repair. A model of hemostasis combining the vascular, platelet, and plasma phases is presented in Video 79.1. The end result is a localized, firm thrombosis, leading to cessation of bleeding. The thrombosis then serves as a scaffold for tissue repair, leading to complete restoration of the endothelial lining (Young, 2015). Fibrinolysis, as demonstrated in Video 79.2, must occur for blood vessel repair and return of blood flow.

Hemostatic processes are regulated by natural anticoagulants (Video 79.3), whose job is to contain these processes at the site of injury and to prevent these reactions from becoming systemic and pathologic (Young, 2015). Deficiencies in natural anticoagulation proteins may lead to the formation of pathologic thromboses during the neonatal period, and these disorders are described later. The complex interaction of the hemostatic, fibrinolytic, and anticoagulant components of the hemostatic system results in a well-balanced machine that allows for hemostasis to occur at the site of injury and for fibrinolysis to follow, facilitating a localized tissue repair process.

Developmental Hemostasis

The neonatal hemostatic system differs significantly from that of older children and adults (Will, 2015). The concentrations of the procoagulant, anticoagulant, and fibrinolytic proteins, compared with adults, are shown in Table 79.1. Essentially all of the procoagulant proteins, except for fibrinogen, factors V and VIII, and von Willebrand factor (vWF), are lower. These lower levels are balanced by lower levels of anticoagulant proteins, except for alpha2-macroglobulin, which is the only anticoagulant protein that is elevated during the neonatal period. All of the fibrinolytic proteins are reduced. These differences place a neonate in a “relative” prothrombotic state, but other factors balance the system and prevent a term or “well” premature neonate from experiencing spontaneous thrombosis. However, many acquired (Table 79.2) and/or prothrombotic risk factors may disrupt this balance, shifting the neonate into a prothrombotic state.

TABLE 79.1 Neonatal Coagulation/Anticoagulation/Fibrinolytic Protein Levels Compared With Adult Levels

| | Protein Levels Elevated Compared With Adult Values | Protein Levels Decreased Compared With Adult Values |
|---------------|---|--|
| Procoagulant | Fibrinogen Factor V ^a Factor VIII vWF | ^b Factors II, VII, IX, X, XI, XII, XIII Prekallikrein High-molecular-weight kininogen |
| Anticoagulant | Alpha2-macroglobulin | Antithrombin III Heparin cofactor II Protein C Protein S |
| Fibrinolytic | Tissue plasminogen activator Plasminogen activator inhibitor | Plasminogen α_2 -antiplasmin |

^aFactor V levels are low on day of life 1 but reach adult values within days after birth (Will, 2015).

^bLevels are 50% of adult values (Will, 2015).

vWF, von Willebrand factor.

Adapted from Manco-Johnson M. Controversies in neonatal thrombotic disorders. In: Ohls RY ed. *Hematology, Immunology and Infectious Disease: Neonatology Questions and Controversies*. Philadelphia, PA: Saunders Elsevier; 2008, 59; with permission.

TABLE 79.2 Acquired Risk Factors for the Development of Neonatal Thromboses

| Maternal Risk Factors | Delivery Risk Factors | Neonatal Risk Factors |
|---------------------------------|--------------------------------|--|
| Infertility | Emergent cesarean section | Central venous/arterial catheters ^a |
| Oligohydramnios | Fetal heart rate abnormalities | Congenital heart disease |
| Prothrombotic disorder | Instrumentation | Sepsis |
| Preeclampsia | Meconium-stained fluid | Meningitis |
| Diabetes | | Birth asphyxia |
| Intrauterine growth restriction | | Respiratory distress syndrome |
| Chorioamnionitis | | Dehydration |
| Prolonged rupture of membranes | | Congenital nephritic/nephrotic syndrome |
| Autoimmune disorders | | Necrotizing enterocolitis |
| | | Polycythemia |
| | | Pulmonary hypertension |
| | | Surgery |
| | | Extracorporeal membrane oxygenation |
| | | Medications (steroids) |

^aGreatest risk factor for thrombosis.

From Saxonhouse MA, Manco-Johnson MJ. The evaluation and management of neonatal coagulation disorders. *Semin Perinatol*. 2009;33:56; with permission; and data from the following references Nowak-Gottl et al., 1997b, 2003; van Ommen et al., 2001; Manco-Johnson et al., 2002; Golomb et al., 2004; Kosch et al., 2004; Chalmers, 2005; Lee et al., 2005; Wu et al., 2005; Alloglu et al., 2006; Kenet and Nowak-Gottl, 2006; Beardsley, 2007; Boffa and Lachassinne, 2007; Lau et al., 2007; Raju et al., 2007; Wasay et al., 2008.

TABLE 79.3 Laboratory Diagnostic Criterion for the Bleeding Newborn^a

| Disorder | PT | APTT | Platelets | Fibrinogen |
|------------------------------------|--------|-----------------------|-----------|------------|
| Hemophilia A | Normal | ↑↑↑ | Normal | Normal |
| Hemophilia B | Normal | ↑↑↑ | Normal | Normal |
| Hemophilia C | Normal | ↑↑ | Normal | Normal |
| Factor XIII deficiency | Normal | Normal | Normal | Normal |
| Factor II, V, and X deficiency | ↑↑ | ↑↑ | Normal | Normal |
| Hemorrhagic disease of the newborn | ↑↑↑ | Normal/↑ ^b | Normal | Normal |
| DIC ^c | ↑↑↑ | ↑↑↑ | Low | Low |
| Liver disease ^c | ↑↑↑ | ↑↑↑ | Low | Low |
| vWD | Normal | Normal/↑ | Normal | Normal |
| Hypofibrinogenemia | ↑↑↑ | ↑↑↑ | Normal | Low |
| Dysfibrinogenemia | ↑↑↑ | ↑↑↑ | Normal | Normal/Low |

^aA complete blood count and coagulation screening test should be performed for any bleeding infant.

^bAPTT values may be prolonged but not as severe as the elevated PT value.

^cTo differentiate between DIC and liver disease, a factor VIII value should be obtained. Factor VIII values will be normal in infants with liver disease but low in infants with DIC.

APTT, Activated partial thromboplastin time(s); DIC, disseminated intravascular coagulation; PT, prothrombin time(s); vWD, von Willebrand disease.

References (Campbell and Bolton-Maggs, 2015; Young, 2015; Jaffray et al., 2016).

The exact reasons for the differences in the fetal and neonatal hemostatic system, compared with adults, are unclear but supported by recent theories. The lower levels of antithrombin III (ATIII) are balanced by elevated concentrations of alpha2-macroglobulin, allowing for unrestricted angiogenesis during intense growth while maintaining an effective anticoagulant pathway (Monagle et al., 2006; Monagle and Massicotte, 2011). Lower levels of vitamin K during fetal growth may reduce the synthesis of osteocalcin, preventing premature fetal cartilage maturation (Booth, 1997).

Despite having a balanced hemostatic system at birth, neonatal prothrombin time (PT) and activated partial thromboplastin time (APTT) are elevated when compared with adults. Elevated tissue factor (TF) in neonates compensates for the lower levels of tissue factor pathway inhibitor (TFPI) and ATIII. Some aspects of decreased platelet aggregation in neonates are compensated for with higher levels of vWF and factor VIII (FVIII). Therefore despite the lower levels of coagulation proteins, neonates are able to form effective thromboses.

The neonatal hemostatic system undergoes numerous changes over the first 6 months of life, and many of the lower protein levels reach adult values during this time period.

Bleeding Disorders in the Neonate

When a neonatologist is faced with either an actively bleeding infant or one that has suffered a significant hemorrhage, acquired or inherited coagulation defects must be considered, especially if the neonate has a normal platelet count. Laboratory diagnostic criteria for the bleeding newborn are shown in Table 79.3. Inherited

TABLE 79.4 Acquired and Inherited Bleeding Disorders in Neonates

| Inherited | Classification | Symptoms |
|--|--|--|
| Hemophilia A (FVIII) Hemophilia B (FIX) | Severe (<1%) Moderate (1–5%) Mild (>40%) | Bleeding after circumcision and/or blood draws, ICH, extracranial hemorrhage, excessive bruising, muscle hematomas, bleeding after surgery |
| Fibrinogen deficiency | Decreased levels (heterozygote): hypofibrinogenemia Absent levels (homozygote): afibrinogenemia Dysfunctional Dysfibrinogenemia | Prolonged bleeding from umbilical stump, bleeding after circumcision, ICH, or mucocutaneous bleeding |
| Factor II (prothrombin) Factor V | Commonly associated with congenital anomalies, particularly cardiac defects | Mucocutaneous bleeding, ICH, prolonged bleeding from umbilical stump, bleeding after procedures |
| Factor VII Factor X Factor XI | Levels do not correlate well with bleeding phenotype | |
| Factor XIII | Levels do not correlate well with bleeding phenotype | Umbilical cord stump bleeding, ICH, bleeding after procedures |
| Acquired | Classification | Symptoms |
| DIC Liver disease | | Prolonged bleeding after venipuncture/heel sticks, jaundice, pulmonary hemorrhage |
| Vitamin K deficiency | Early <24 hours Classical 1–7 days Late ≥2 weeks | Cephalohematoma, umbilical stump bleeding, ICH GI bleeding, umbilical stump bleeding, mucocutaneous, circumcision, ICH ICH, mucocutaneous, GI bleeding |

DIC, Disseminated intravascular coagulation; *FVIII*, factor VIII; *FIX*, factor IX; *GI*, gastrointestinal; *ICH*, intracranial hemorrhage.
From Saxenhouse MA, Manco-Johnson MJ. The evaluation and management of neonatal coagulation disorders, *Semin Perinatol*. 2009;33:56; with permission.
References (Campbell and Bolton-Maggs, 2015; Young, 2015; Jaffray et al., 2016).

and acquired coagulation defects and the most common symptoms associated with their presentation in the neonate are shown in [Table 79.4](#). It is important to obtain a detailed family and delivery history for any neonate experiencing significant bleeding, as certain clues may be presented, allowing the clinician to perform a focused approach.

Laboratory Investigation

The first approach (initial screen) to any neonate with a suspected bleeding disorder should be a complete blood count (CBC) and coagulation screen (PT, APTT, and fibrinogen). More specific testing should then be performed to make the correct diagnosis. It is important for the neonatologist to remember that the method of sample collection may affect sample results: for example, heel-stick samples may result in platelet clumping, which will produce a falsely low platelet count and should never be used for coagulation screening. Elevated hematocrit levels greater than 55% may result in prolonged diluted coagulation times. In addition, the collection of blood samples through heparinized arterial or venous lines will prolong the APTT, unless the specimen has the heparin absorbed and/or adequate blood is removed from the line before the sample collection in order to clear heparin from the line. When interpreting values from coagulation screening and more detailed testing, values should be interpreted using age-adjusted normal ranges based on gestational age and days of life; these are published elsewhere ([Andrew et al., 1987, 1988b, 1990](#)).

Hemophilia

The most common congenital bleeding disorders are hemophilia A (FVIII deficiency) and hemophilia B (factor IX deficiency), both being inherited as sex-linked recessive. The incidence of hemophilia A is 1 per 5000 males and for hemophilia B is 1 per 20,000 males ([Soucie et al., 1998](#)). Approximately one-third of cases will occur in the absence of a positive family history ([Chalmers, 2004b](#)). Heterozygous females may have mild hemophilia as a result of nonrandom X-chromosome inactivation ([Young, 2015](#)). The severity of hemophilia is determined by the type of mutation and the part of the protein that is affected ([Young, 2015](#)). With the absence of either FVIII or factor IX, there is reduced thrombin formation on the surface of activated platelets, resulting in a thrombosis with poor structural integrity that is more susceptible to fibrinolysis, and as a result, bleeding occurs. The lower the factor level, the greater the potential for more severe early onset bleeding. Approximately 70% of patients with hemophilia are diagnosed during the first month of life, with the mean age of patients with hemophilia having their first bleed by 28.5 days ([Conway and Hilgartner, 1994; Chalmers, 2004b](#)). The most common presentation of hemophilia in the neonate is excessive bleeding, either after circumcision or surgery. Further classifications of hemophilia A and B, based on factor activity level, and the types of bleeding that neonates may present with are shown in [Table 79.4](#).

Most symptomatic infants will have significant prolongation of their APTT; however, if the diagnosis is suspected, specific

TABLE 79.5 Treatment for Congenital/Acquired Bleeding Disorders

| Factor | Replacement Therapy |
|--------------------------------|---|
| Hemophilia A | Factor VIII concentrate |
| Hemophilia B | Factor IX concentrate |
| Fibrinogen | Fibrinogen concentrate FFP or cryoprecipitate if concentrate unavailable |
| Factor II | Prothrombin complex concentrate (if available) FFP if concentrate unavailable |
| Factor V | FFP |
| Factor VII | Recombinant factor VIIa or prothrombin complex concentrate (if available) |
| Factor X | Prothrombin complex concentrate (if available) FFP if concentrate unavailable |
| Factor XI | FFP |
| Factor XIII | Plasma derived and recombinant (only for A subunit deletions) FXIII concentrate Cryoprecipitate if concentrate unavailable |
| Early vitamin K deficiency | Parenteral vitamin K |
| Classical vitamin K deficiency | Prothrombin concentrates for active bleeding |
| Late vitamin K deficiency | |
| ^a DIC | FFP and cryoprecipitate, platelets |
| ^a Liver disease | Cryoprecipitate, FFP, prothrombin complex concentrates, platelets, vitamin K, recombinant factor VIIa |

^aTherapies for bleeding patients, not abnormal laboratory testing.

DIC, Disseminated intravascular coagulation; FFP, fresh frozen plasma; FXIII, factor XIII.

From Saxtonhouse MA, Manco-Johnson MJ. The evaluation and management of neonatal coagulation disorders. *Semin Perinatol*. 2009;33:56; with permission.

References (Campbell and Bolton-Maggs, 2015; Young, 2015; Jaffray et al., 2016).

factor levels should always be obtained. If there is a strong family history for hemophilia, factor levels may be screened from cord blood samples, but severity of disease should always be determined from samples obtained after the infant is born.

Because FVIII levels are increased during the neonatal period, the diagnosis of mild hemophilia A may be difficult, and confirmatory testing should be done at 6 to 12 months of age. In addition, lower levels of factor IX at birth may also make the diagnosis of mild hemophilia B difficult, and testing should also be repeated at 6 to 12 months of life. Treatment is replacement of the specific factor and should be done in consultation with pediatric hematology, as there are a number of factor concentrates that are commercially available for both FVIII and factor IX deficiency (Table 79.5) (Young, 2015). Many neonates do not require treatment during the neonatal period, as they may not manifest any bleeding symptoms. Adjuvant therapies for hemophilia (used only in conjunction with pediatric hematology), such as desmopressin, may increase factors to hemostatic levels, which can be useful for management of minor bleeding (Young, 2015). Fresh frozen plasma (FFP) should only be used in the instance of acute hemorrhage when confirmatory

testing is not yet available (Chalmers, 2004a; Kulkarni and Lusher, 2001).

von Willebrand Disease

The primary plasma protein required for platelet adhesion, vWF, also has a role in platelet aggregation and serves as the carrier for FVIII (Young, 2015). Absence, reduction, or abnormal function of vWF results in defects in platelet adhesion and aggregation, increasing one's risk for bleeding. The effectiveness of vWF as an adhesive protein relies on multimerization of the protein, resulting in very large molecules comprising what are known as high-molecular-weight multimers (Young, 2015). Neonates have higher plasma concentrations of vWF and an increased proportion of high-molecular-weight vWF multimers. As a result, presentation of von Willebrand disease (vWD) during the neonatal period is rare (Weinstein et al., 1989).

The spectrum of vWD consists of quantitative (types 1 and 3) or qualitative defects (types 2A, 2B, 2M, and 2N). Type 1 is due to reduced amounts of vWF resulting from either decreased production/secretion of vWF or increased clearance of circulating vWF (Young, 2015). Type 3 is caused by complete absence of vWF and is usually the result of severe gene mutations and has the highest potential for presentation during the neonatal period. Type 2A is caused by defects in multimerization that result in an absence of large and medium-sized multimers, affecting platelet adhesion (case reports in neonates). Type 2B is due to a mutation that leads to increased binding of the high-molecular-weight multimers to platelets, removing them from the available pool in the plasma (case reports in neonates). Type 2M is due to a mutation resulting in the inability of vWF to bind to platelets. Type 2N affects vWFs' ability to bind to FVIII and results in a reduction of circulating levels of FVIII (Donner et al., 1987; Sadler et al., 2006; Branchford and Di Paola, 2012; Ng et al., 2015). Acquired vWD syndrome is extremely rare and has been associated with Wilms tumor, congenital heart disease, and systemic lupus erythematosus (Young, 2015).

Suspicion for vWD in a neonate requires specialized testing. Coagulation testing may demonstrate an isolated prolonged APTT and prolongation of epinephrine and ADP closure times as measured by the PFA-100. Further testing would evaluate the levels of vWF (vWF antigen assay), platelet-binding function (ristocetin cofactor assay), and FVIII-binding function (FVIII activity) (Branchford and Di Paola, 2012; Ng et al., 2015). Assistance by a pediatric hematologist should occur if a diagnosis is suspected (Young, 2015).

The management of type 3 vWD should consist of factor replacement using an intermediate purity FVIII concentrate containing the high-molecular-weight multimers of vWF (Sadler et al., 2006; Ng et al., 2015). Desmopressin should be reserved for those patients with type 1 vWD but should only occur after consultation with a pediatric hematologist (Young, 2015).

Other Rare Inherited Coagulation Disorders

There are other rare factor deficiencies, inherited as autosomal recessive, in 1 in 500,000 to 1 in 2,000,000 live births, representing 3%–5% of all coagulation disorders (Acharya et al., 2004; Palla et al., 2015). A complete listing of the disorders and their potential symptoms are displayed in Table 79.4. Severe deficiencies of fibrinogen, factor VII, factor X, and factor XIII are the most likely (of the rare coagulation disorders) to present during the neonatal period. The majority of these deficiencies will present with an

abnormality in the coagulation screen (Table 79.3). Further testing for the specific abnormality will then confirm the diagnosis (Jaffray et al., 2016). The clinician must remember that newborn levels of many of these factors are lower to start with, and therefore diagnosis may be difficult and must be confirmed by 6 to 12 months of life. Treatment for the various deficiencies is shown in Table 79.5.

Factor XIII is composed of two subunits and cross-links with fibrin to stabilize clots. Low levels of factor XIII do not prolong the PT or APTT. Therefore any neonate with concerns for a coagulation disorder that has a normal platelet count, normal fibrinogen levels, and normal PT and APTT values should be screened for factor XIII deficiency via a quantitative assay. Treatment is shown in Table 79.5.

Acquired Coagulation Disorders

Vitamin K Deficiency

Vitamin K is found in leafy green vegetables as vitamin K₁ (phylloquinone) and is synthesized as vitamin K₂ in intestinal bacteria (Saxonhouse and Manco-Johnson, 2009). It is an essential cofactor for the γ -carboxylation process of factors II, VII, IX, and X and protein C and protein S (Jaffray et al., 2016). Insufficient bacterial colonization of the colon at birth, inadequate dietary intake in solely breastfed infants, and poor transfer across the placenta places neonates at risk for vitamin K deficiency bleeding (VKDB). The different forms and their clinical presentation are shown in Table 79.4. Treatment is shown in Table 79.5.

Early VKDB is due to maternal ingestion of oral anticoagulants, anticonvulsants, and antituberculosis agents. These agents cross the placenta and interfere with vitamin K metabolism. Classical VKDB occurs because of a physiologic deficiency in vitamin K at birth combined with a sole breast milk diet or inadequate feeding. Late VKDB presents in an infant that is either solely breastfed who receives an inadequate dose of vitamin K (none or one oral dose) or has an associated disease process that interferes with the absorption or supply of vitamin K such as diarrhea, cystic fibrosis, α_1 -antitrypsin deficiency, biliary atresia, hepatitis, and celiac disease. In the absence of vitamin K prophylaxis, the incidence of late VKDB is 4 to 10 per 100,000 births (Sutor, 1995). When intramuscular (IM) vitamin K prophylaxis is provided, the incidence of late VKDB decreases to 0.24 to 3.2 per 100,000 live births (Schulte et al., 2014).

If VKDB is suspected, coagulation screening should be performed and will usually demonstrate isolated prolongation of the PT, followed by prolongation of the APTT. The prolongation of the PT is usually out of proportion to the elevation of the APTT. Fibrinogen concentration and platelet counts will be normal. In addition, decreased concentrations of factors II, VII, IX, and X will occur. An alternative is to obtain an undercarboxylated or abnormal coagulation factor II measurement (Clarke and Shearer, 2007). This factor is released into the bloodstream in the very early stages of vitamin K deficiency, and it can be detected well before changes in the PT become manifest. The adoption of this single test in clinical practice may improve the early diagnosis of VKDB, resulting in decreased incidences of intracranial hemorrhage (ICH).

When presented with a patient with suspected VKDB, parenteral treatment (intravenous, IM, or subcutaneous injection) with vitamin K should immediately occur. Improvement of the PT and APTT 2 to 6 hours after the administration of parenteral vitamin K will confirm the diagnosis. However, if a patient suspected of having

VKDB presents with severe hemorrhage, additional therapy with prothrombin complex concentrates (contain all the vitamin K-dependent factors; not available at every institution) aimed at immediate correction of factor deficiencies should occur (Young, 2015).

Common practice in the United States is to provide all infants 1.0 mg (0.3 mg/kg for infants <1000 g and 0.5 mg for infants >1000 g but <32 weeks' gestation) of IM vitamin K on the first day of life (Saxonhouse and Manco-Johnson, 2009). This single dose has been found to prevent both classical and late VKDB, even in infants with cholestatic jaundice (American Academy of Pediatrics Committee on Fetus and Newborn, 2003; McNinch et al., 2007). The safety of IM vitamin K has been questioned because of the reported association between IM vitamin K administration in newborns and an increased incidence of childhood cancer (Golding et al., 1990, 1992). Further studies have concluded no association between IM vitamin K and childhood leukemia or other cancers (Ross and Davies, 2000). Further research has suggested a prenatal origin of childhood leukemia, further weakening the plausibility of a causal relationship between IM vitamin K and cancer (Wiemels et al., 1999).

Proper oral administration of vitamin K has been shown to have an efficacy similar to that of IM vitamin K in the prevention of early and classical VKDB (von Kries and Hanawa, 1993; Puckett and Offringa, 2000). However, cases of late VKDB began to increase and surveillance data from four countries revealed oral prophylaxis failures of 1.2 to 1.8 per 100,000 live births (Cornelissen et al., 1997), with higher rates of 2 to 4 per 100,000 cases when incomplete oral dosing was observed. More recent data have demonstrated that weekly or daily oral dosing of vitamin K protects almost all babies from VKDB, and late VKDB remains confined to breastfed infants who received no vitamin K or just one oral dose (Hansen et al., 2003; van Hasselt et al., 2008).

A more recent study evaluated the association of VKDB in breastfed infants with unrecognized biliary atresia and oral vs IM dosing of vitamin K (Witt et al., 2016). Of 91 breastfed infants diagnosed with biliary atresia, 25 received a 2-mg IM dose after birth compared with 55 and 11 infants receiving 25- μ g and 150- μ g oral daily dosing, respectively; 4% of the infants that received IM dosing experienced vitamin K deficiency bleeding compared with 82% (both groups) of the infants that received only oral dosing. More importantly, 0% of the infants who received IM dosing experienced ICH compared with 40% and 27%, respectively, in the oral dosing groups (Witt et al., 2016).

Disseminated Intravascular Coagulation

A complex process involving the activation and dysregulation of the coagulation and inflammatory systems, disseminated intravascular coagulation (DIC), results in massive thrombin generation with widespread fibrin deposition and consumption of coagulation proteins and platelets, ultimately leading to multiorgan damage (Chalmers, 2004b). DIC always occurs as a secondary event, and many perinatal and neonatal problems are associated with it. These include birth asphyxia, respiratory distress syndrome, meconium aspiration syndrome, infection, necrotizing enterocolitis, hypothermia, severe placental insufficiency, homozygous protein C/S deficiency, and thrombosis (Chalmers, 2004b).

The diagnosis of DIC is made in an ill neonate with supporting laboratory parameters (Table 79.3). The most important aspect of treatment for DIC is to treat the underlying disorder. However, once DIC is established, it can be difficult to control (Chalmers, 2004b). The focus of acute management in the neonate is to

support adequate hemostasis to reduce the risk of spontaneous hemorrhage. This is usually achieved with platelet transfusions, FFP, or cryoprecipitate (Williams et al., 2002). Another option is to inhibit the activation of the coagulation system using heparin. However, trials in neonates have not been conclusive, and the risk of bleeding may be increased (Gobel et al., 1980; Albisetti et al., 2005).

Liver Disease

Acute liver disease is rare in the neonatal population. Illnesses that can lead to acute liver failure include congenital heart disease with low cardiac output, birth asphyxia, extrahepatic biliary atresia, inborn errors of metabolism, hemophagocytic syndrome, and viral hepatitis. Mechanisms contributing to hemostatic abnormalities include decreased synthesis of coagulation factors, activation of the coagulation and fibrinolytic systems, poor clearance of activated hemostatic components, loss of coagulation proteins into ascitic fluid, thrombocytopenia, platelet dysfunction, and vitamin K deficiency (Albisetti et al., 2005). The diagnosis of acute liver disease in the neonate should include elevated liver enzymes, direct hyperbilirubinemia, prolonged PT and APTT, thrombocytopenia, elevated ammonia concentrations, decreased fibrinogen levels, and decreased concentrations of factors VII and V (Table 79.3). A normal FVIII concentration, reflecting extrahepatic synthesis, can help distinguish primary liver disease from DIC (Albisetti et al., 2005). Treatment, in addition to treating the underlying cause of liver disease (if able), includes prothrombin complex concentrates (if available; Young, 2015), cryoprecipitate (if fibrinogen is low), FFP, recombinant factor VIIa (for bleeding not responsive to platelets and plasma products), platelets, and vitamin K.

Neonatal Thrombosis

Epidemiology

Neonatal TE is becoming an increasingly recognized condition. The exact incidence of neonatal TE varies with the types of thromboses reported and how centers screened for them. Four registries have reported data on the epidemiology of neonatal TE: the German registry reported an incidence of 5.1 events per 100,000 live births (Nowak-Gottl et al., 1997b); the Canadian registry reported 2.4 clinically apparent events (excluding stroke) per 1000 neonatal intensive care unit (NICU) admissions (Schmidt and Andrew, 1995); the Dutch registry reported 0.07 events per 10,000 children for venous thrombosis (van Ommen et al., 2001); and the most recent reports from the Italian registry reported an incidence of 3.4 to 6.5 per 10,000 live births and 5.8 to 6.6 per 1000 NICU admissions for noncerebral systemic venous and arterial thrombotic events (Saracco et al., 2016). All four registries observed that thromboses occurred in both term and preterm infants, with the Italian registry reporting a higher incidence among preterm infants. The German, Canadian, and Dutch registries reported an equal incidence among males and females other than renal vein thrombosis, which affected more male neonates (Schmidt and Andrew, 1995; Nowak-Gottl et al., 1997b; van Ommen et al., 2001), while the Italian registry reported a higher incidence for all types of thromboses among male neonates (Saracco et al., 2016). These registries also demonstrated that the majority of venous thromboses in neonates are associated with central venous catheters (CVCs) (Schmidt and Zipursky, 1984; Nowak-Gottl et al., 1997b; Saracco et al., 2016; van Ommen et al., 2001). The recurrence

rate for neonatal TE remains low and ranges from 3.3% to 7% (Kurnik et al., 2003).

Risk Factors for Neonatal Thromboembolism

Hypercoagulability, disturbances in blood flow, and endothelial damage/disruption (Virchow triad) all pertain in some degree to neonates admitted to the NICU (Young, 2015). Therefore it is understandable as to why neonates have the highest rate of TE events among pediatric patients. As demonstrated in Table 79.1, neonates, particularly premature neonates, are deficient in the anticoagulant proteins, especially protein C and protein S. Prothrombotic disorders may also be present, shifting the neonate into a hypercoagulable state. Hypovolemic, septic, or cardiogenic shock may affect an ill neonate, resulting in hypotension and thus disrupting blood flow to vital organs. Polycythemia or certain cardiac lesions may also result in sluggish blood flow and/or hyperviscosity, increasing the risk for thrombosis. However, the presence of CVC and arterial catheters is by far the greatest acquired risk for the development of TE in neonates (Schmidt and Andrew, 1995; Nowak-Gottl et al., 1997b; van Ommen et al., 2001; Greenway et al., 2004; Beardsley, 2007). Insertion, infusion of hyperosmolar substances, and length of time that a catheter remains in place may disrupt the endothelium, increasing the risk for thrombosis. The effects of different types of catheter materials, catheter design, and lumen number have been evaluated, and these reviews have only demonstrated that fewer thrombotic complications were associated with end-hole umbilical arterial catheters (UACs) (Barrington, 2000a, 2000b, 2000c, 2000d). Risk factors that have been implicated in neonatal TE are shown in Table 79.2.

Prothrombotic Disorders: Pathophysiology and Their Role in Neonatal Thromboembolism

A genetic mutation resulting in the complete absence or severe deficiency of an inhibitor of hemostasis, the production of an inhibitor of hemostasis that has inadequate function despite normal levels, or an overproduction of a procoagulant protein or cofactor greatly increases a neonate's risk for TE. The more severe the mutation, the greater the risk for the development of severe thrombosis. Homozygous prothrombotic disorders, such as severe protein C, protein S, or ATIII deficiency, usually present in newborns with severe clinical manifestations, mainly purpura fulminans (Monagle et al., 2004). The debate that continues is how the inheritance of a heterozygous prothrombotic mutation increases a neonate's risk for symptomatic TE. Despite having a heterozygote prothrombotic mutation, a neonate may never experience a symptomatic TE event, thus demonstrating that the inheritance of a prothrombotic trait does not guarantee a symptomatic TE event. Other acquired risk factors (Table 79.2) are usually present. Prothrombotic disorders that have been associated with neonatal TE are shown in Box 79.1.

Neonatal registries have demonstrated that greater than 80% of neonates with symptomatic thromboses have acquired risk factors present (Table 79.2) (Schmidt and Andrew, 1995; Nowak-Gottl et al., 1997b; van Ommen et al., 2001; Saracco et al., 2016). Therefore it appears that the combination of acquired factors and the presence of a prothrombotic mutation may represent the perfect storm for neonatal TE. Registry data and other case studies have demonstrated that the majority of symptomatic neonatal TE events stem either from the association of multiple prothrombotic defects or the combination of prothrombotic defects and environmental or clinical conditions (Schmidt and Andrew, 1995; Nowak-Gottl

• BOX 79.1 Prothrombotic Disorders Implicated in the Development of Neonatal Thromboembolism

Factor V Leiden mutation (most common)
 Factor II *G20210A* gene mutation (1%–2% of Caucasians)
 Increased apolipoprotein(a)
 Methylene tetrahydrofolate reductase gene mutation (*MTHFR C677T*) genotype
 Hyperhomocysteinemia
 Protein C deficiency
 Protein S deficiency
 Antithrombin III deficiency
 Heparin cofactor II deficiency
 Dysfibrinogenemia
PAI-1 4g/5G gene mutation
 Increased levels of factors VIII, IX, XI, or fibrinogen
 Antiphospholipid antibodies (including anticardiolipin antibodies, lupus anticoagulant)
 Chromosome 2q
 Chromosome 2q13 deletion

References (Alioglu et al., 2006; Boffa and Lachassinne, 2007; Bucciarelli et al., 1999; Dahlback et al., 1996; Kenet et al., 2010; Nowak-Gottl et al., 1999b, 2003; Rosendaal, 2005; Saxonhouse and Burchfield, 2009).

Adapted from Saxonhouse MA. Thrombosis in the neonatal intensive care unit. Clin Perinatol. 2015;42:651–673.

et al., 1997a, 1997b, 2001; van Ommen et al., 2001; Khan et al., 1991). It is therefore currently recommended that pediatric patients, including neonates, with thrombosis (regardless of other acquired risk factors) be tested for prothrombotic traits (Box 79.1) (Manco-Johnson et al., 2002). A more detailed laboratory approach based on type of thrombosis and acquired risk factors is presented later.

The most common prothrombotic disorder is the factor V Leiden mutation, which reduces the inactivation of activated factor V by its regulator, activated protein C (Zoller et al., 2014). The presence of the prothrombin 20210 mutation has the potential to increase circulating concentrations of prothrombin by 15%–30% (Poort et al., 1996; Junker et al., 1999). The exact role regarding mutations in the methylenetetrahydrofolate reductase (*MTHFR*) enzyme and risk for neonatal TE remains unclear (Couto et al., 2004). The mutation results in a defect in the remethylation of homocysteine into methionine, with the end result being hyperhomocysteinemia. Increased levels of homocysteine have been speculated as an increased risk for premature vascular disease and arterial thrombosis (Eldibany and Caprini, 2007; Khan et al., 1991). However, most studies have associated an increased risk for thrombosis because of elevated homocysteine levels (kidney damage) and not with the genetic mutation. Thus the *MTHFR* gene mutation and the risk of neonatal arterial thrombosis remain controversial (Rook et al., 2005; Brenner, 2006).

The activated protein C system, consisting of protein C plus protein S and factor V as cofactors, cleaves and inactivates factors V and VIII. Mutations that affect the quantity or quality of these proteins may contribute to an increase in neonatal venous thrombosis. ATIII, when bound to heparin sulfate, inhibits thrombin and activated factors IX, X, and XI. Mutations may result in deficiencies in ATIII production, increasing thrombosis risk (Schneppenheim and Greiner, 2006). Elevations in lipoprotein(a) (reduces fibrinolytic capacity) have been implicated as a significant risk factor for both venous and arterial thrombosis in the German

and Dutch registries; however, generalizing these findings to all populations remains unclear (Nowak-Gottl et al., 1999b, 1999c).

Lupus anticoagulant, anticardiolipin antibody, anti- β_2 -glycoprotein-I antibodies, and other maternal antiphospholipid antibodies may play a significant role in neonatal TE, especially neonatal stroke. These antibodies either lead to thromboses in the placenta or are transported across the placenta and may serve as a nidus for thrombosis formation (Chalmers, 2005; Manco-Johnson, 2008; Nelson, 2008). A recent review of 16 infants with a history of maternal antiphospholipid antibodies and perinatal thrombosis found that 13/16 had arterial thromboses, with 8/16 presenting as strokes. Therefore mothers of and/or infants with significant arterial or venous TE events should be screened for the presence of antiphospholipid antibodies (Boffa and Lachassinne, 2007).

Locations of Neonatal Thromboses, Imaging Modalities to Diagnose Them, and Management Guidelines for Specific Thromboses

Neonatal TE events may occur in a variety of locations (Table 79.6). The majority of symptomatic events are usually identified by proper imaging modalities (Table 79.6). However, difficult intravenous access, desire to limit radiation exposure, lower glomerular filtration rates, and inaccessibility to perform specific testing at the bedside limit neonatal use of many of the gold standard techniques used in adults and children (Rajagopal et al., 2016). Therefore Doppler ultrasonography is the most widely and safely used modality. The small diameter of certain vessels, low pulse pressure, and the presence of a CVC at the site of thrombus may limit interpretation of the results (Rajagopal et al., 2016).

Arterial Thromboses

Perinatal Arterial Ischemic Stroke

The majority of neonatal arterial TE events are represented by perinatal arterial ischemic stroke (PAIS). Perinatal stroke refers to an insult, ischemic or hemorrhagic, occurring from 20 weeks' gestational age to 28 days' postnatally. The subcategories of perinatal stroke and their presenting symptoms are shown in Table 79.7 (Greenway et al., 2004; Raju et al., 2007; Gacio et al., 2015). Arterial stroke in neonates is referred to as PAIS for the remainder of the chapter (cerebral sinovenous thromboses are presented later). PAIS refers to a condition with acute encephalopathy, seizures, or neurologic deficits presenting in the term or preterm infant before the 29th postnatal day with brain imaging confirming a parenchymal infarct in the appropriate arterial territory (Fig. 79.1) (Fernandez-Lopez et al., 2014; Gacio et al., 2015). PAIS is responsible for 22%–70% of congenital hemiplegic cerebral palsy in neonates (Lynch and Nelson, 2001; Lynch et al., 2001; Chalmers, 2005) as well as other neurologic comorbidities, including seizure disorders, delayed language development, and behavioral disorders (Nelson, 2008).

The incidence of PAIS ranges from 20 to 63.4 per 100,000 live births and depends on whether or not events diagnosed after the first month of life were included (Schulzke et al., 2005; Laugesaar et al., 2007). Most abnormalities occur in the left hemisphere within the distribution of the middle cerebral artery, with anterior and posterior cerebral artery lesions being less common (Chalmers, 2005; Hunt and Inder, 2006). The origin of the left carotid artery from the aorta allows a more direct vascular route to the brain as a corridor for cardiac emboli. Rarely, multifocal

TABLE 79.6**Locations of Neonatal Thromboses and the Best Imaging Modalities to Diagnose Them**

| Vessel | Type of Thromboses (Vessels Potentially Involved) | Imaging Modality |
|-----------------|--|-------------------------------------|
| Arterial | Perinatal arterial ischemic stroke (Left middle cerebral artery, anterior cerebral artery, posterior cerebral artery) | Diffusion-weighted MRI/MRA |
| | Iatrogenic (Abdominal aorta, radial artery, renal artery, mesenteric artery, popliteal artery) | Doppler ultrasound |
| | Spontaneous (Iliac artery, left pulmonary artery, aortic arch, descending aorta, renal artery) | |
| Venous | Iatrogenic/spontaneous vessel occlusion (SVC, IVC, hepatic vein, subclavian vein, abdominal veins, peripheral veins) | |
| | Renal vein | |
| | Portal vein | |
| | Cerebral sinovenous (Superior sagittal sinus, transverse sinuses of the superficial venous system, straight sinus of the deep system) | Diffusion-weighted MRI w/venography |
| | Congenital heart disease related (Right/left atria, right/left ventricle, SVC, IVC) | Echocardiography |

IVC, Inferior vena cava; MRA, magnetic resonance angiogram; MRI, magnetic resonance imaging; SVC, superior vena cava.

Adapted from Saxonhouse MA. Management of neonatal thrombosis. *Clin Perinatol.* 2012;39:192–193; with permission.

References (Golomb et al., 2004; Chalmers, 2005; Wasay et al., 2008; Sharathkumar et al., 2009; Elhassan et al., 2010; Nagel et al., 2010; Tridapalli et al., 2010; Monagle et al., 2012).

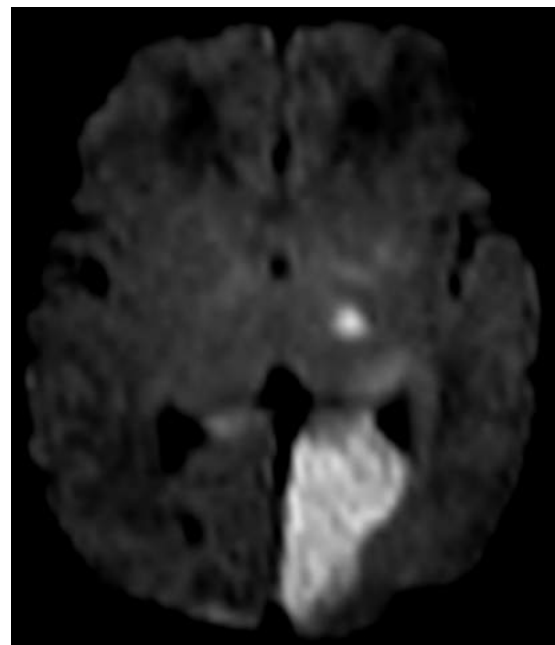
TABLE 79.7**Subcategories of Perinatal Stroke and Their Presenting Symptoms**

| Subcategory | Description | Symptoms |
|---|--|--|
| Neonatal arterial ischemic stroke | Occurs after birth but before 29th day of life | Seizures (focal), apnea, chewing or bicycling movements, |
| Perinatal arterial ischemic stroke | Difficult to establish whether stroke occurred before, during, or after childbirth but occurs from 20 weeks' gestational age until birth | persistent feeding difficulties |
| Presumed perinatal ischemic stroke | Subtle initial symptoms with later sequelae observed | |
| Neonatal cerebral sinovenous thrombosis | | Seizures, apnea, lethargy, irritability, poor feeding, jitteriness, thrombocytopenia, anemia, changes in muscle tone |

References (deVeber et al., 2001; Golomb et al., 2004; Chalmers, 2005; Hunt and Inder, 2006; Raju et al., 2007; Kersbergen et al., 2011; Moharir et al., 2011; van der Aa et al., 2014; Gacio et al., 2015).

cerebral infarctions can occur, and these tend to be embolic in origin (Fig. 79.2). Many potential risk factors have been implicated in the etiology of PAIS (Table 79.2).

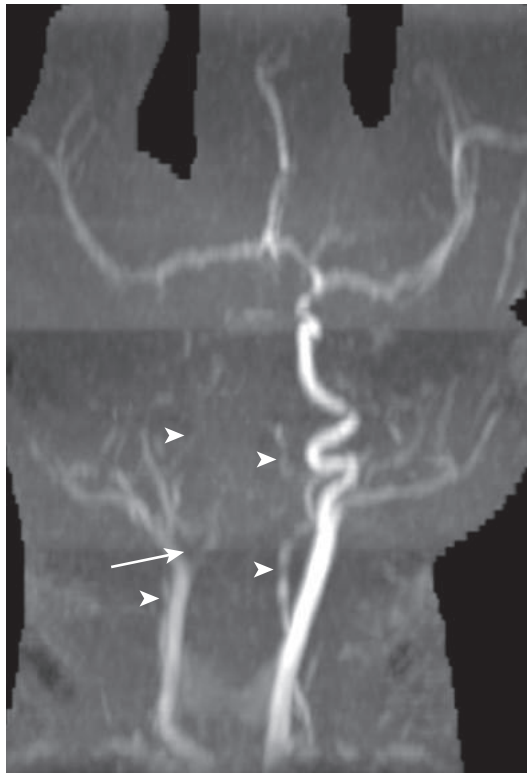
Placental pathology is a major risk factor for PAIS. Maternal/fetal conditions that may result in decreased placental reserve, thromboinflammatory processes leading to placental blood vessel



• **Fig. 79.1** Axial diffusion-weighted magnetic resonance imaging demonstrating areas of high signal in bilateral frontal and parietal lobes, left occipital lobe, left thalamus, and left pons.

thrombosis, and sudden catastrophic events may result in emboli that break off from the placental circulation and enter the fetal circulation, passing through a patent foramen ovale with a predilection to enter the left middle cerebral artery (Elbers et al., 2011).

Prothrombotic disorders (Box 79.1) have a role in PAIS (Kenet et al., 2010). In a study of 91 full-term neonates with PAIS, prothrombotic risk factors, most commonly lipoprotein(a), were identified in 68% of these neonates compared with 25% in controls (Gunther et al., 2000). A more recent study of 24 neonates with



• **Fig. 79.2** Three-dimensional (3D) maximum intensity projection image from a 3D time-of-flight magnetic resonance angiography demonstrates occlusion of the right internal carotid artery at its origin (*arrow*). The vertebral arteries are diminutive (*arrowheads*), and the basilar artery is not visualized.

PAIS identified prothrombotic disorders (protein C deficiency most common) in 28.6% of neonates (Mercuri et al., 2001). There has also been speculation that the presence of a prothrombotic disorder in a neonate with PAIS may indicate a worse neurologic outcome (Mercuri et al., 2001).

There are no randomized clinical trials addressing the management of neonates with PAIS. Current guidelines from the American College of Chest Physicians recommend anticoagulation for neonates with PAIS if there is an ongoing cardioembolic source or if there is evidence of recurrent PAIS. Otherwise, supportive care is recommended (Monagle et al., 2012; van der Aa et al., 2014). Treatment, if necessary, ultimately depends on the acute clinical situation and the specific hemorrhagic risks for that neonate.

Magnetic resonance imaging (MRI) with diffusion-weighted imaging (DW MRI) is the most sensitive technique for the early detection of PAIS (Venkataraman et al., 2004). This technique allows for the detection of cerebral edema, which is an early sign of cerebral ischemic damage (Lovblad et al., 2001; Mader et al., 2002). Angiography allows for the detection of thromboses if there is a history of instrumentation/difficult delivery. Although cranial ultrasound (CUS) is the least-invasive method for diagnosing PAIS, it is also the least sensitive. One study found that 75% of cases of neonatal PAIS were missed when CUS was used (Estan and Hope, 1997). Therefore PAIS should never be excluded in any neonate based on negative computed tomography or CUS results.

Iatrogenic/Spontaneous Arterial Thromboses

Used for continuous monitoring of arterial blood pressure and blood gases, UACs and peripheral arterial lines (PALs) are frequently



• **Fig. 79.3** Arterial Thrombosis With Skin Necrosis.



• **Fig. 79.4** Aortic Thrombus Diagnosed by Magnetic Resonance Imaging.

used in ill neonates admitted to the NICU. Approximately 10%–64% of newborns admitted to the NICU require placement of a UAC (Saxonhouse, 2015). Femoral arterial catheters are used less frequently but are more commonly used in neonates with congenital heart disease or those requiring extensive surgery. Despite their important role, neonatologists must remember that complications from arterial catheters occur and range from line dysfunction/infection to limb/organ-threatening ischemia, as demonstrated in Figs. 79.3–79.4.

The incidence of arterial thromboses from UACs based on autopsy reports has ranged from 9% to 28% (Richardson et al., 2002), whereas the incidence of PAL-related thromboses is unknown. Potential short-term and long-term complications of UACs include mesenteric ischemia, hypertension, renal dysfunction/failure, limb loss, and congestive heart failure (Seibert et al., 1991; Greenberg et al., 1998; Nouri et al., 2007). High UAC positioning (T6–9) has been found to have fewer clinical complications (Barrington, 2000d), while low-dose continuous heparin infusion at 1 u/mL prolongs catheter patency but does not reduce the risk of thrombosis (Barrington, 2000c). Clinical signs of PAL-related



• **Fig. 79.5** Arterial Thrombosis of Digital Artery.

thrombosis include limb ischemia, pale or cold extremities distal to the cannulation site, weak or absent pulse, and decreased or immeasurable blood pressure (Fig. 79.5) (Veldman et al., 2008). Catheter material, duration of placement, and solutions infused influence the risk for both UAC-related and PAL-related thrombosis.

Suspicion or confirmation of an arterial thrombosis should warrant immediate removal of the arterial catheter. Vascular spasm may occur, and removal of the catheter may simply resolve symptoms. If symptoms of spasm persist, further actions are needed. An approach to the neonate with vascular spasm is shown in Fig. 79.6. If thrombosis is suspected, the most sensitive method to diagnose an arterial catheter-related thrombosis is contrast angiography (Greenway et al., 2004), but because of its risk in neonates, it is generally reserved for older children and adults. As a result, Doppler ultrasound is mainly employed.

Management options for neonates with a peripheral arterial catheter-related TE event are to remove the catheter with only supportive care, anticoagulation following catheter removal if there are nonlimb-threatening symptoms, or thrombolysis if there are limb-threatening symptoms (Monagle et al., 2012).

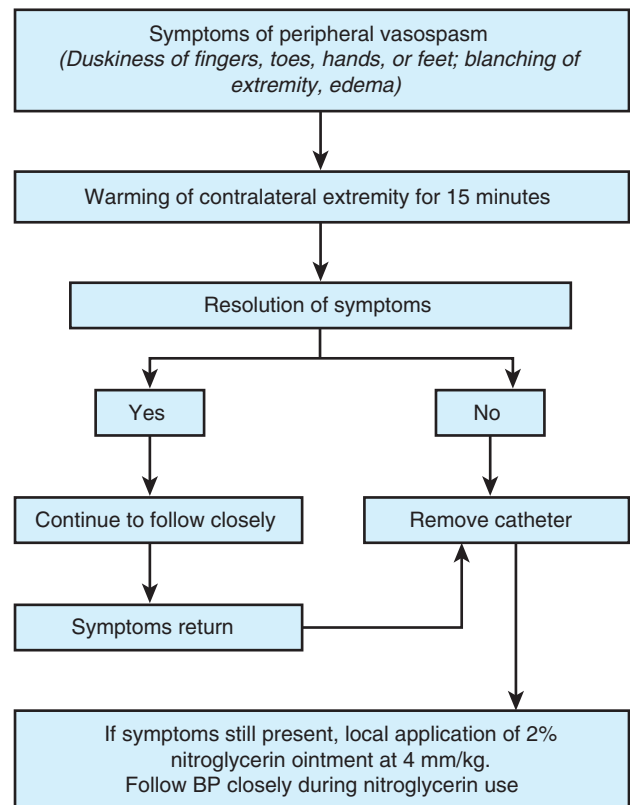
Management options for femoral arterial catheter-related thromboses are shown in Table 79.8. Dosing guidelines for thrombolysis and/or anticoagulation related to clinically significant iatrogenic or idiopathic aortic thromboses are shown in Tables 79.9–79.11.

Venous Thrombosis

The biggest risk for the development of venous thromboses in neonates admitted to the NICU is the presence of a CVC. Additional risk factors (Table 79.2) combined with the presence of a CVC further increase this risk.

Catheter-Related Thrombosis (Umbilical Venous Catheters and Peripherally Inserted Central Venous Catheters)

Premature and sick newborns greatly benefit from CVCs. Infusion of life-saving medications and enhanced nutrition lead to better outcomes, but risks for thrombosis, infection, malfunction, and death exist. Neonatologists and others caring for neonates admitted to the NICU must weigh the benefit versus the risk every day that



• **Fig. 79.6** Management of Peripheral Vasospasm. Current recommendations for the evaluation and management for neonates with peripheral vasospasm caused by complications from peripheral arterial lines and umbilical arterial catheters. Nitroglycerin dosing is provided. BP, Blood pressure.

a CVC remains in place, and the catheter should be removed as soon as necessary. Damage to blood vessel walls during insertion, disrupted blood flow, infusion of substances that damage endothelium, and thrombogenic catheter materials are the main reasons that thromboses develop (Bhat and Monagle, 2012; Sellitto and Messina, 2012).

Neonates, especially very low birth weight infants (<1500 g), routinely have umbilical venous catheters (UVCs) placed during the first few days of life as peripheral access may be difficult to secure. Despite their clinical importance, UVCs represent the most common cause for thrombosis in premature infants (Rajagopal et al., 2016). The exact incidence of UVC-related thrombosis varies, but recent reports have ranged from 21% to 71% (dependent on how aggressive centers screened for asymptomatic thrombosis) and include portal vein, renal vein, and inferior vena cava (IVC) thrombosis (Bhat and Monagle, 2012). Autopsy studies have estimated that 20%–65% of infants who die with a UVC in situ have microscopic evidence of TE (Schmidt and Zipursky, 1984; Kulkarni and Lusher, 2001; Tanke et al., 1994). Intracardiac thrombosis from UVC placement ranges from 1.8% to 5.3% (Bhat and Monagle, 2012).

Clinical signs and symptoms suggestive of a UVC-related thrombosis include persistent infection, persistent thrombocytopenia, line dysfunction, and bilateral lower limb edema (Fig. 79.7) (Monagle et al., 2012). The Centers for Disease Control and Prevention recommend that use of UVCs be limited to 14 days (O'Grady et al., 2002). A randomized trial comparing long-term UVC use (up to 28 days) with short-term (7 to 10 days) use

TABLE 79.8 Management of Femoral Artery Thrombosis

| Treatment | Severity of Thrombosis | Description |
|---|---|---|
| IV UFH (see Table 79.9) | Acute femoral artery thrombosis but nonlimb threatening | Treatment should continue until clot resolution but should not be for more than 5–7 days. May convert to LMWH (Table 79.10) after 48–72 hours of treatment and continue until clot resolution |
| IV UFH followed by rt-TPA if clot does not improve or symptoms worsen (see Tables 79.9 and 79.11) | Limb-threatening or organ-threatening femoral artery thrombosis | Begin IV UFH (see Table 79.9), but if symptoms do not improve within 24–48 hours, may begin rt-TPA with UFH (see Table 79.11). If rt-TPA contraindicated, may need surgery |

LMWH, Low-molecular-weight heparin; UFH, unfractionated heparin; rt-TPA, recombinant tissue type plasminogen activator.
Reference (Monagle et al., 2012).

TABLE 79.9 Clinical Indications and Recommended Dosing Guidelines for Unfractionated Heparin Therapy in Neonates

| Clinical Indication | Current Recommended Dosing | Appropriate Monitoring (Applied to All Dosing Regimens) |
|---|--|---|
| Symptomatic thrombus but nonlimb/life threatening | <p><28 weeks' GA Maintenance dose: 15 U/kg per h ^aBolus dose: 25 units/kg IV over 10 minutes</p> <p>28–37 weeks' GA Maintenance dose: 15 U/kg per h ^aBolus dose: 50 units/kg over 10 minutes</p> <p>>37 weeks' GA Maintenance dose: 28 U/kg per h ^aBolus dose: 100 units/kg over 10 minutes</p> | <p>Maintain anti-factor Xa level of 0.35–0.7 units/mL (APTT of 60–85 seconds) Check antifactor Xa level 6 hours after initiating therapy. If provide loading dose, check levels 6 hours after loading dose. If need to make changes in dosing, check levels 6 hours after each change in infusion rate Antifactor Xa levels should be checked daily during treatment. Complete blood count, platelet count, and coagulation screening (including APTT, PT, and fibrinogen) should be performed before starting UFH therapy. Platelet count should be repeated daily for 2–3 days once therapeutic levels are achieved and at least twice weekly thereafter.</p> |

^aBolus dosing should be performed only if there is a significant risk or evidence of thrombus progression (Bhatt et al., 2015).
Treatment with UFH should be limited to 10–14 days.
APTT, Activated partial thromboplastin time; GA, gestational age; IV, intravenous; PT, prothrombin time; UFH, unfractionated heparin.
Adapted from Armstrong-Wells JL, Manco-Johnson MJ. Neonatal thrombosis. In: de Alarcon P, Werner EJ, Christensen RD eds, *Neonatal Hematology*. New York, NY: Cambridge University Press; 2013; 282 with permission.
Data from references (Manco-Johnson, 2006; Monagle et al., 2012; Armstrong-Wells, 2013).

TABLE 79.10 Clinical Indications and Recommended Dosing Guidelines for LMWH Therapy in Neonates

| Clinical Situation | Traditional Dosing | Other Recommended Dosing (Armstrong-Wells, 2013) | Prophylactic Dosing | Appropriate Monitoring |
|--|-----------------------------------|--|----------------------------|---|
| Symptomatic thrombus but nonlimb threatening | Any GA 1.5 mg/kg SQ every 12 h | <p><28 weeks' gestation 1.25 mg/kg SQ every 12 h</p> <p>28–37 weeks' gestation 1.5 mg/kg SQ every 12 h</p> <p>>37 weeks' gestation 1.625 mg/kg SQ every 12 h</p> | 0.75–1 mg/kg SQ every 12 h | <p>Goal of antiFXa levels of 0.5–1.0 U/mL Check level 4 hours after 2nd dose and then every few days or weekly (Molinari et al., 2014). Dosing adjustments based on anti-FXA levels are published elsewhere (Monagle et al., 2012). If infant with high hemorrhagic profile, use dosing regimen of 1 mg/kg SQ every 12 h (Monagle et al., 2012)</p> |

GA, Gestational age; FX, factor X; LMWH, low-molecular-weight heparin; SQ, subcutaneous.
Adapted from Armstrong-Wells JL, Manco-Johnson MJ. Neonatal thrombosis. In: de Alarcon P, Werner EJ, Christensen RD (eds), *Neonatal Hematology*. New York: Cambridge University Press; 2013; 282; with permission.
Data from references (Streif et al., 2003; Michaels et al., 2004; Obaid et al., 2004; Manco-Johnson, 2006; Malowany et al., 2007, 2008; Monagle et al., 2012).

TABLE 79.11**Clinical Indications and Recommended Dosing Guidelines for rt-TPA Therapy in Neonates**

| Clinical Situation | Gestational Age | Recommended Dosing | Appropriate Monitoring |
|--------------------------------|-----------------|---|--|
| Limb/life-threatening thrombus | <28 weeks | 0.03–0.06 mg/kg per h Infuse UFH at 10 U/kg per h | Dose escalation up to 0.24 mg/kg per h can be considered but has to be done slowly with continuing monitoring of the patient (see Table 79.17). Supplementation with plasminogen (FFP at 10 mL/kg) before commencing therapy is recommended to ensure adequate thrombolysis (Monagle et al., 2012). |
| | >28 weeks | May use same as for <28 weeks or may use 0.1–0.5 mg/kg per h for 6–12 hours with repeat daily dosing for up to 3 days Infuse UFH at 10 U/kg per h during rt-TPA infusion | |

FFP, Fresh frozen plasma; rt-TPA, recombinant tissue type plasminogen activator; UFH, unfractionated heparin.

–If no clot dissolution occurs 12–24 hours after starting infusion and/or D-dimers are not increasing, may give an additional 10 mL of FFP to provide additional plasminogen to increase efficacy of rt-TPA.

–Discontinue infusion when clot dissolved (maximum of 96 hours).

–Maintain fibrinogen levels greater than 100 mg/dL (provide cryoprecipitate if less than 100 mg/dL) and platelet counts greater than $50 \times 10^9/\mu\text{L}$.

Adapted from Armstrong-Wells JL, Manco-Johnson MJ. Neonatal thrombosis. In: de Alarcon P, Werner EJ, Christensen RD eds, *Neonatal Hematology*. New York, NY: Cambridge University Press; 2013; 282; with permission.

Data from references (Wang et al., 2003; Manco-Johnson, 2006).



• **Fig. 79.7** Femoral Deep Vein Thrombus. Note the swollen leg, with venous congestion pattern on the skin.

followed by peripheral central venous line placement demonstrated that 4% of neonates in the short-term group compared with 7% of neonates in the long-term group developed significant thromboses (detected by echocardiogram). However, all of the thrombi were at the site of the UVC tip, and none of the neonates required treatment (Butler-O'Hara et al., 2006).

To enhance nutrition and to administer long-term parenteral medications, peripherally inserted central venous catheters (PICVs) or surgically placed CVCs are routinely placed in preterm and ill neonates and tend to remain in place for weeks until treatment is finished or an infant reaches full enteral feedings. Clinical signs of PICV and surgically placed CVC-related thrombosis include unilateral limb swelling/pain/dyscoloration, superior vena cava syndrome, chylothorax, chylopericardium, intracardiac thrombosis, unresolving sepsis, thrombocytopenia, and cardiac failure (Andrew et al., 2001; Saxonhouse and Burchfield, 2009; Rajagopal et al., 2016).

The gold standard for diagnosing venous TE is MR venogram; however, this modality is difficult to perform in neonates (Greenway et al., 2004). Ultrasound is therefore the most widely and safely used modality. False-negative results often occur when evaluating

for thromboses in the upper central venous system because of obstruction of the distal subclavian veins by the clavicles. In addition, compression of veins in a central location by the probe is not feasible because of the neonate's rib cage (Albisetti et al., 2005).

Long-term complications of venous TE include chronic venous obstruction with prominent cutaneous collateral circulation, chylothorax, portal hypertension, and embolism. More children are now presenting with postthrombotic syndrome who had a previous history of a neonatal venous TE (Barnes et al., 2002).

There are two options regarding the management for CVC-related thromboses. The first is to initiate anticoagulation followed by the removal of the catheter after 3 to 5 days of anticoagulation. Anticoagulation (dosing provided in Tables 79.9–79.10) should continue until there is resolution of the thrombus, and treatment may last from 2 weeks to 3 months, depending on the size, location, and symptoms of the thrombus (Monagle et al., 2012). The second option is to provide supportive care only with immediate removal of the catheter. Radiologic monitoring should continue for clot extension. If clot extension does occur, then anticoagulation should begin (Monagle et al., 2012).

Intracardiac Thromboses and Thromboses in Infants With Complex Congenital Heart Disease

The placement of a CVC into the right atrium may lead to damage of the endocardium, inducing either pericardial tamponade and/or the development of intracardiac thrombi. The development of intracardiac vegetations secondary to CVC infections may expose an infant to prolonged infection and dissemination of septic emboli. Thrombus formation in the right atrium and superior vena cava has been associated with a high endocarditis risk, sepsis persistence, pulmonary artery obstruction, ventricular dysfunction, acute hemodynamic compromise, and death (Schmidt and Andrew, 1995; Torres-Valdivieso et al., 2003; Hermansen and Hermansen, 2005; Ulloa-Ricard et al., 2015).

Thrombosis is a common complication reported in neonates undergoing repair for complex congenital heart disease (Chollette et al., 2007). Less is known about neonates undergoing initial palliative procedures for single ventricle physiology. A retrospective review of neonates who underwent palliative repair found that in patients who died and had autopsies available, 33% of deaths were attributable to thrombosis (Fenton et al., 2003). A more recent

review of 22 neonates who underwent palliative repair between 1 and 11 days of life found evidence of thrombi in 5 infants (23%) (Chollette et al., 2007).

A recent case-control study performed at a high-level referral NICU demonstrated an incidence of intracardiac thrombosis of 22.5 cases per 1000 admissions (Ulloa-Ricardez et al., 2015). Many of these infants were critically ill and had complex congenital heart disease. Major risk factors from this study included prematurity, maternal history of gestational diabetes/diabetes mellitus, surgical cut-down technique to place a CVC, and *Staphylococcus epidermidis* infection. In addition, almost one-quarter of these infants died despite receiving treatment for the thrombosis (Ulloa-Ricardez et al., 2015).

Echocardiography is the preferred modality for diagnosing either right atrial thrombus formation, intracardiac vegetations, or thrombus formation in infants with single ventricle physiology. Signs suggestive of an atrial thrombus include new-onset murmur, persistent sepsis, persistent thrombocytopenia, and cardiac failure. The surgical approach to remove the thrombus in neonates is not feasible, especially the premature infant. There have been case reports using recombinant tissue type plasminogen activator (rt-TPA) for thrombolysis of catheter-related atrial thrombus in the neonate (Rimensberger et al., 2001; Torres-Valdivieso et al., 2003; Wang et al., 2003; Tardin et al., 2007). rt-TPA has also been used successfully in the management of premature infants with infective endocarditis (Marks et al., 2002).¹⁶

Renal Vein Thrombosis

The majority of cases of neonatal renal vein thrombosis (RVT) present in preterm infants usually during the first month of life (Bokenkamp et al., 2000; Lau et al., 2007). RVT is the most common spontaneous venous TE in neonates, with an incidence of about 0.5 per 1000 NICU admissions (Schmidt and Andrew, 1995). A review of 271 neonatal cases demonstrated that 70% of cases were unilateral, with 64% of these involving the left kidney, and males were more frequently affected (Messinger et al., 2006; Lau et al., 2007). These findings were supported by a more recent review of 10 cases (Bidadi et al., 2016). The cardinal signs suggestive of RVT are macroscopic hematuria, a palpable abdominal mass, and thrombocytopenia, with the most recent study demonstrating this triad in 50% of patients (Bidadi et al., 2016). Other symptoms include oliguria, proteinuria, acute renal failure, and hypertension. Risk factors (shown in Table 79.2) are frequently found in RVT cases. The most recent study demonstrated risk factors in 90% of infants with RVT, with 50% having at least three risk factors: prematurity and perinatal asphyxia are the most common (Bidadi et al., 2016). Prothrombotic risk factors have been found in varying numbers of neonatal cases of RVT, with studies finding at least one prothrombotic risk factor in 17%–67% of cases (Kosch et al., 2004; Marks et al., 2005; Lau et al., 2007; Bidadi et al., 2016). Because of the potential association of prothrombotic disorders and RVT, an evaluation for an inherited thrombophilia is warranted.

The diagnosis of RVT is usually made via Doppler ultrasound. Radiographic criteria for RVT include presence of echogenic clot, venous distention secondary to the presence of a thrombus, or absence of flow (Bidadi et al., 2016).

The treatment for RVT is controversial, as there are no large randomized clinical trials addressing the issue. However, complications of RVT are serious and include adrenal hemorrhage, extension of the clot into the IVC, renal failure, hypertension, and death (Lau et al., 2007). One study demonstrated the development of hypertension in 19% and 22% of neonates with unilateral and

**TABLE
79.12**

Management of Renal Vein Thrombosis

| | Unilateral RVT | Bilateral RVT |
|--|--|--|
| Absence of renal impairment or extension into the inferior vena cava | Supportive care with monitoring of the RVT for extension If extension occurs, anticoagulation for 6 weeks to 3 months | Supportive care with monitoring of the RVT for extension If extension occurs, anticoagulation for 6 weeks to 3 months |
| Extension into the inferior vena cava | Anticoagulation ^a | Anticoagulation ^a |
| Renal failure | N/A | Initial thrombolytic therapy with rt-TPA ^a , followed by anticoagulation |

^aFor dosing options, please see Tables 79.9–79.11.

rt-TPA, Recombinant tissue type plasminogen activator; RVT, renal vein thrombosis.

Adapted from Saxonhouse MA. Management of neonatal thrombosis. *Clin Perinatol*. 2012;39:195; with permission.

Data from references (Kosch et al., 2004; Marks et al., 2005; Messinger et al., 2006; Lau et al., 2007; Monagle et al., 2012).

bilateral RVT, respectively (Lau et al., 2007). The most recent treatment recommendations based on observational and case series and expert opinion are shown in Table 79.12 (Monagle et al., 2012). The most recent analysis demonstrated that anticoagulation and fibrinolysis appeared to prevent worsening of renal failure in neonates with bilateral RVT. However, renal atrophy did not seem to change whether supportive care or anticoagulation/fibrinolysis was used, suggesting that many of these events may be in utero and chronic in nature (Bidadi et al., 2016).

Portal Vein Thrombosis

The two main risk factors for the development of portal vein thrombosis (PVT) in neonates are sepsis/omphalitis and UVC use (Williams and Chan, 2011). Most cases tend to be clinically silent, making the diagnosis difficult. Incidences vary (because of aggressiveness of imaging in asymptomatic patients) between 1% and 43% (Kim et al., 2001; Williams and Chan, 2011). Ultrasound is the preferred modality for diagnosis, and findings of cavernous transformation of the portal vein with subsequent splenomegaly and reversal of portal flow are used to document its severity (Williams and Chan, 2011). Spontaneous resolution of asymptomatic PVT is relatively common, but the detection of PVT, even in asymptomatic patients, warrants close observation in order to follow for signs of portal hypertension. This complication may manifest itself up to 10 years after the neonatal period (Williams and Chan, 2011). Management options, based on limited clinical data, are provided in Table 79.13 (Williams and Chan, 2011).

Cerebral Sinovenous Thrombosis

Cerebral sinovenous thrombosis (CSVT) represents a subcategory of perinatal stroke (O'Brien, 2015). Symptoms are similar to PAIS and include seizures, apnea, lethargy, irritability, poor feeding, jitteriness, or changes in muscle tone (deVeber et al., 2001; Wasay et al., 2008). These may be accompanied by anemia and/or thrombocytopenia. A recent review demonstrated that 90% of children who presented with CSVT had predisposing risk factors

(Table 79.2 and Box 79.1), with 16% of these patients having multiple risk factors (Wasay et al., 2008; Kenet et al., 2010). The most common risk factors were infections (40%), perinatal complications (25%), and prothrombotic traits (13%) (Wasay et al., 2008). The presentation of any of the above symptoms in a neonate with any of these risk factors should alert the clinician to screen for CSVT.

The superficial and lateral sinuses are the most frequently involved vessels (Chalmers, 2005), and up to 30% of cases have reported

venous infarction with subsequent hemorrhage (Wu et al., 2003). Impaired or absent venous drainage in one of the cerebral sinuses leads to increased venous pressure, vasogenic edema, and secondary infarction. Intraventricular hemorrhages (especially in term neonates) and hemorrhages within the caudate nucleus and thalamus are associated with thrombosis of the deep cerebral venous sinuses. Therefore the presence of an intraventricular hemorrhage or thalamic hemorrhage in a term or late preterm infant warrants exclusion of CSVT (Wu et al., 2003). Diagnosis of CSVT is best made through diffusion MRI with venography (Manco-Johnson, 2006; Monagle et al., 2012; O'Brien, 2015; Rajagopal et al., 2016).

Neonatal CSVT mortality rates have ranged from 2% to 24% (Moharir et al., 2011). Long-term follow-up of neonates diagnosed with CSVT has demonstrated neurologic deficits, consisting of cerebral palsy, epilepsy, and cognitive impairments in 10%–80% of infants (Berfelo et al., 2010; Kersbergen et al., 2011; Moharir et al., 2011).

The role of anticoagulation for CSVT is controversial, and recommendations for management are not based on clinical trials (Jordan et al., 2010; Moharir et al., 2010; Monagle et al., 2012). Current treatment recommendations are shown in Fig. 79.8. Surgery is reserved for those with hydrocephalus or large intracerebral hematomas with mass effect.

Prothrombotic Laboratory Evaluation for Clinically Symptomatic Neonatal Thromboses

The question remains whether every neonate diagnosed with a clinically significant thrombosis should have a full evaluation for a prothrombotic disorder (Box 79.1). Does the presence of acquired risk factors (Table 79.2) provide enough risk to avoid searching for a possible prothrombotic trait? Does a critically ill neonate with a CVC-related venous thrombosis warrant a full prothrombotic evaluation (Greenway et al., 2004; Gursel et al., 2007; Turebylu et al., 2007)? Definitive answers to these questions remain unknown, but based on the current evidence from data registries, case series, case reports, and expert opinion, a stepwise investigation for a prothrombotic disorder should be performed based on the type and severity of the thrombosis and the number of acquired risk

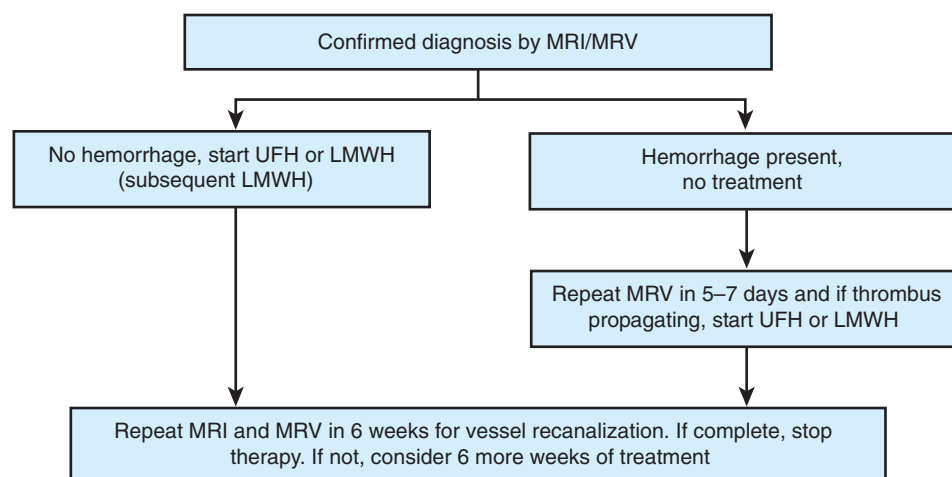
TABLE 79.13 Management Options for Neonates With Portal Venous Thrombosis

| Treatment Plan | Description of PVT | Recommended Ultrasound (US) Follow-Up |
|------------------------------|---|---|
| Observation | No extension observed and infant clinically stable | 7–10 days |
| Anticoagulation ^a | Extension into the IVC, RA, and/or RV but no end-organ compromise | 10 days If thrombus resolved, may stop therapy. If still present, treat for 6 weeks to 3 months, depending on US follow-up |
| Thrombolysis ^a | End-organ compromise with extension of the thrombosis into the IVC, RA, and/or RV | Daily May stop thrombolysis when symptoms improve but would transition to anticoagulation |

^aFor dosing options, please see Tables 79.9–79.11.

IVC, Inferior vena cava; RA, right atrium; RV, right ventricle; US, ultrasound.

Adapted from Williams S, Chan AK. Neonatal portal vein thrombosis: diagnosis and management. *Semin Fetal Neonatal Med.* 2011;16:337; with permission.



• **Fig. 79.8** Management of Neonatal Cerebral Sinovenous Thrombosis. The current recommendations for appropriate evaluation, management, and follow-up of neonates diagnosed with cerebral sinovenous thrombosis. If either unfractionated heparin (UFH) or low-molecular-weight heparin is provided, dosing guidelines are provided in Tables 79.9 and 79.10. LMWH, Low-molecular-weight heparin; MRI, magnetic resonance imaging; MRV, magnetic resonance venography; UFH, unfractionated heparin.

TABLE 79.14 Evaluation for Prothrombotic Disorder: Presence of Acquired Risk Factors (see also Table 79.2)

| Laboratory Testing | Collection Tube |
|--|-------------------------|
| Antiphospholipid antibody panel, anticardiolipin and lupus anticoagulant ^a (IgG, IgM) | Citratd plasma |
| Protein C activity ^b | Citratd plasma (1.8 mL) |
| Protein S activity ^b | |
| Lipoprotein(a) (in Caucasian neonates) ^b | |
| Plasminogen level ^b (if considering thrombolytic therapy) | |
| ATIII (activity assay) ^b | EDTA (1 mL) |
| Factor V Leiden ^c | |
| Prothrombin G ^c | |

^aMay be performed from maternal serum during first few months of life.

^bProtein-based assays are affected by the acute thrombosis and must be repeated at 3–6 months of life, before a definitive diagnosis may be made. Therefore, recommend that complete evaluation (excluding DNA-based assays) be performed at 3–6 months of life (Nowak-Gottl et al., 2003; Manco-Johnson, 2006). If anticoagulation is being administered, then these assays should be obtained 14–30 days after discontinuing the anticoagulant. Lipoprotein(a) levels may need to be repeated at 8–12 months of life.

^cDNA-based assays.

ATIII, Antithrombin III; EDTA, ethylenediaminetetraacetic acid; IgG, immunoglobulin G; IgM, immunoglobulin M.

Adapted from Saxonhouse MA, Manco-Johnson MJ. The evaluation and management of neonatal coagulation disorders. *Semin Perinatol.* 2009;33:59; with permission.

• BOX 79.2 Key Points Regarding the Laboratory Evaluation for a Prothrombotic Disorder

- Because of many of the pro/anticoagulation protein levels being lower than adult values, the diagnosis of a coagulation disorder may be difficult in the immediate neonatal period.
- Certain protein-based assays may aid in treatment during the neonatal period (antithrombin III and plasminogen assays) and may be performed during the neonatal period.
- DNA-based assays are accurate and may be obtained at any time.
- Lipoprotein(a) concentrations increase during the first year of life and should be repeated at 8–12 months of life if values obtained at 3–6 months are low, especially in Caucasian individuals.
- The different evaluations listed below are based on the presence of acquired risk factors, type of thrombosis, severity of thrombosis, and treatment regimen.
- Baseline complete blood count, prothrombin time, activated partial thromboplastin time, and fibrinogen levels should be obtained shortly after the acute event.
- Antiphospholipid antibody panels should be obtained from the mother and may warrant coordination with obstetrics.
- Placental pathology, especially in cases of perinatal arterial ischemic stroke, should be requested.

Adapted from Saxonhouse MA. Thrombosis in the neonatal intensive care unit. *Clin Perinatol.* 2015;42:651–673.

factors. Timing of the evaluation is important, with certain aspects being performed immediately following an acute event and other aspects being performed at 3 to 6 months of life. Key points and a comprehensive laboratory evaluation based on acquired risk factors are listed in Table 79.14, Table 79.15, and Box 79.2. The clinician

TABLE 79.15 Evaluation for Prothrombotic Disorder: No Acquired Risk Factors Present (see also Table 79.2)

| Laboratory Testing | Collection Tube |
|--|-----------------|
| Antiphospholipid antibody panel, anticardiolipin and lupus anticoagulant (IgG, IgM) ^a | Citratd plasma |
| Protein C activity ^b | Citratd plasma |
| Protein S activity ^b | |
| Antithrombin (activity assay) ^b | |
| Factor V Leiden ^c | EDTA |
| Prothrombin G ^c | |
| MTHFR ^c | |
| PAI-1 4G/5G mutation ^c | Citratd plasma |
| Homocysteine ^b | |
| Lipoprotein(a) ^b | Citratd plasma |
| FVIII activity ^b | |
| FXII activity ^b | |
| Plasminogen activity ^b | |
| Heparin cofactor II ^b | |

^aMay be performed from maternal serum during first few months of life.

^bProtein-based assays are affected by the acute TE event and must be repeated at 3–6 months of life, before a definitive diagnosis may be made. Therefore, recommend that complete evaluation (excluding DNA-based assays) be performed at 3–6 months of life (Manco-Johnson, 2006; Nowak-Gottl et al., 2003). If anticoagulation is being administered, then these assays should be obtained 14–30 days after discontinuing the anticoagulant. Lipoprotein(a) levels may need to be repeated at 8–12 months of life.

^cDNA-based assays.

EDTA, Ethylenediaminetetraacetic acid; FVIII, factor VIII; FXII, factor XII; IgG, immunoglobulin G; IgM, immunoglobulin M; MTHFR, methylenetetrahydrofolate reductase; PAI-1, plasminogen activator inhibitor-1.

Adapted from Saxonhouse MA, Manco-Johnson MJ. The evaluation and management of neonatal coagulation disorders. *Semin Perinatol.* 2009;33:59; with permission.

may choose to add or delete tests based on the presentation, family history, or timeframe of the event.

The large amount of blood that traditionally has been required to perform this evaluation has always been a criticism: therefore the evaluation should take place at an experienced tertiary care center that has the ability to perform these tests or has a reliable referral center. This approach dramatically reduces the amount of blood required, as it is specifically designed for neonates. Although anticoagulation treatment is not usually started based on a prothrombotic evaluation, the identification of a disorder may help prevent future thromboses during childhood and adolescence. Anticoagulation before certain medical or surgical procedures may help to avoid the development of a significant thromboses and truly be beneficial to the future health of the patient.

Management of Thrombosis

The use of anticoagulant/fibrinolytic therapy in the NICU carries the significant risk for bleeding, especially ICH. Many clinicians are hesitant to start therapy, even when the benefits outweigh the risks. Specific neonatal formulations for antithrombotic agents are limited, making accurate and reproducible weight-adjusted dosing difficult (Manco-Johnson, 2006; Monagle et al., 2012).

The majority of recommendations and dosing regimens for anticoagulant/fibrinolytic therapy in neonates are based on case series, cohort studies, and expert opinion. Goals for fibrinolysis are restoration of blood flow preserving organ function and

TABLE 79.16 Contraindications for Anticoagulation/Thrombolysis

| | Absolute | Relative |
|---------------------------|---|---|
| Medical Conditions | <ol style="list-style-type: none"> 1. CNS surgery or ischemia (including birth asphyxia) within 10 days 2. Active bleeding 3. Invasive procedures within 3 days 4. Seizures within 48 hours | <ol style="list-style-type: none"> 1. Platelet count $<50 \times 10^4/\mu\text{L}$ ($100 \times 10^4/\mu\text{L}$ for ill neonates) 2. Fibrinogen concentration $<100 \text{ mg/dL}$ 3. INR >2 4. Severe coagulation deficiency 5. Hypertension |

CNS, Central nervous system; INR, international normalized ratio.

Adapted from Manco-Johnson M. Controversies in neonatal thrombotic disorders. In: Ohls RY ed., *Hematology, Immunology and Infectious Disease: Neonatology Questions and Controversies*. Philadelphia, PA: Saunders Elsevier; 2008;68; with permission.

Data from references (Greenway et al., 2004; Manco-Johnson, 2006; Thornburg and Pipe, 2006; Beardsley, 2007; Monagle et al., 2012).

preventing catastrophic long-term complications, including death. Goals for anticoagulation are prevention of recurrent thromboses and serious sequelae from embolism (Bhatt et al., 2015). Before initiating any therapy, consideration for serious complications must be noted, and the treatment's benefits must outweigh its risks, especially in the premature infant. Family discussions highlighting the risks and goals for treatment must be documented before initiating any therapy. Treatment should occur at a tertiary care center that has proper laboratory, blood bank, pharmacy, pediatric radiological subspecialty, pediatric hematological subspecialty, and pediatric surgical support (Monagle et al., 2012). The clinician should refer to the most recent American College of Chest Physicians (CHEST) guidelines (Monagle et al., 2012) or may use the service 1-800-NO CLOTS to receive up-to-date management guidance. Before initiating any treatment, absolute and relative contraindications to antithrombotic therapy should be reviewed (Table 79.16).

Unfractionated Heparin

The use of unfractionated heparin (UFH) should be limited to clinically symptomatic thromboses that are not life or limb threatening. Heparin inhibits numerous coagulation factors and platelet function, produces a release of TFPI from endothelial cells, and enhances fibrinolysis (Schulman, 2013). UFH achieves its main anticoagulant effect by binding to antithrombin, catalyzing its ability to inactivate thrombin and factor Xa (Pratt and Church, 1991). The dosing of UFH in neonates is affected by many factors, including lower AT levels, increased binding of UFH to plasma proteins, and faster clearance in neonates compared with adults (McDonald et al., 1981; Andrew et al., 1988a, 1992; Monagle et al., 2006). These differences explain why neonates tend to require a higher infusion rate to reach therapeutic levels than do adults (Newall et al., 2009a; Ignjatovic et al., 2010). Current dosing guidelines for neonates with and without loading doses are provided in Table 79.9. Dosing regimens for UFH tend to vary, based on the source referenced and age of the infant. Larger prospective studies are needed to better define UFH dosing in neonates and whether or not a loading dose is necessary.

The most recent review of UFH dosing in neonates *does not recommend* a loading dose (Bhatt et al., 2015). Increased bleeding risk with use of a loading dose and low risk for recurrent thrombosis with no evidence suggesting that recurrent thromboses are due to

subtherapeutic antifactor Xa levels during the first 24 hours of anticoagulation are why loading doses were not recommended (Bhatt et al., 2015). Loading doses should be reserved for neonates with significant risk or evidence for thrombus progression.

Proper laboratory monitoring of UFH therapy is crucial to reducing bleeding complications (Table 79.9). The two currently used tests are the anti-Xa assay and the APTT. The anti-Xa assay measures UFH's ability to catalyze AT's inhibition of factor Xa (Bhatt et al., 2015). An anti-Xa value of 0.35–0.7 U/mL is considered therapeutic (Basu et al., 1972; Levine et al., 1994). There are two types of anti-Xa assays: one with exogenous AT added and another without exogenous AT (Newall et al., 2009b). Clinicians should be aware which assay their laboratory is using before monitoring is performed (Bhatt et al., 2015). The APTT allows for quick and easy measurement of the effect of UFH (Bhatt et al., 2015), using an APTT value of 60–85 seconds serves as an indicator of therapeutic anticoagulation in neonates (Monagle et al., 2012). Neonatal limitations of the APTT include a higher baseline value and a smaller prolongation with UFH, thus underestimating or overestimating in vivo heparin activity. Based on these limitations, one possible approach to measuring therapeutic anticoagulation using UFH in neonates is to use a combination of the anti-Xa assay and APTT (dependent on whether centers can perform both tests) (Bhatt et al., 2015). After initiating UFH therapy, an anti-Xa level and an APTT should be measured 6 hours later (Monagle et al., 2012). If both are therapeutic, daily CBC and anti-Xa and APTT levels should be monitored. This specific regimen is difficult to perform in neonates (especially premature) as significant phlebotomy losses occur, so a more reasonable approach is to perform both tests 6 hours after initiating therapy and then monitoring anti-Xa levels with periodic APTT values (Table 79.9). If the APTT is elevated and out of proportion to the anti-Xa level, a possible coagulopathy may exist (Bhatt et al., 2015). If the APTT is low compared with the anti-Xa level, an elevated FVIII level may exist (Kurekci et al., 2003; Goldenberg et al., 2004). Finally, a persistently low anti-Xa level suggests antithrombin deficiency, and a level should be obtained (Bhatt et al., 2015). Supplementation may need to occur, but one must remember that dosing is based on lower antithrombin levels (Manco-Johnson, 2006, 2008; Monagle et al., 2012). UFH therapy should be limited for short-term use, and attempts to convert to lower molecular weight heparin (LMWH) should be done if longer therapy is required. Therapy is usually limited to 2 to 14 days, but data to support this recommendation are lacking (Monagle et al., 2012).

Bleeding is the most significant complication of UFH therapy in neonates. The largest study evaluating 38 neonates being treated for CSVT had a bleeding risk of 8% (Moharir et al., 2010). More recently, a risk of 11% was observed with UFH therapy in infants less than 6 months old (Schechter et al., 2012). Because of the risks for intraventricular hemorrhage in preterm and term infants, CUS should be performed before and during treatment. Treatment of hemorrhage usually only requires cessation of the infusion because of UFH's short half-life. If the infusion is stopped but bleeding continues, then protamine should be given at a dose of 1 mg per 100 units of heparin received if the last heparin dose was administered less than 30 minutes previously and 0.25 mg protamine per 100 units of heparin if the last heparin dose was administered as long as 120 minutes before (Saxonhouse and Manco-Johnson, 2009). Other complications such as heparin-induced thrombocytopenia, although common in adults and children, rarely occurs in neonates, and only a few cases have been

reported (Spadone et al., 1992; Martchenko and Boshkov, 2005). The most recent systematic review in neonates demonstrated an incidence of 0% among 335 neonates treated with UFH (Avila et al., 2013). A drop in the platelet count by 50% or persistent platelet counts of less than 70–100,000/mm³ occurring 5 to 10 days after the first exposure to heparin should alert the clinician for this possible diagnosis. Lepirudin can be considered in neonates with heparin-induced thrombocytopenia, but its use has not been adequately studied in neonates (Thornburg and Pipe, 2006). Prolonged therapy with UFH (>14 days) should be avoided to prevent potential loss of calcium from bones (Bhatt et al., 2015).

Low-Molecular-Weight Heparin

Low-molecular-weight heparin (LMWH), specifically enoxaparin, has become the neonatal anticoagulant of choice because of a reduced risk of hemorrhage, subcutaneous (SQ) dosing, and reduced monitoring requirements (Streif et al., 2003; Michaels et al., 2004; Thornburg and Pipe, 2006). LMWH specifically acts against factor Xa, and its administration can be achieved either through SQ injection or the use of an indwelling SQ catheter (Insuflo; Unomedical, Birkerød, Denmark) (Manco-Johnson, 2006; Streif et al., 2003). The use of the SQ catheter reduces the number of needle sticks from 14 per week to 1 per week. However, case reports and reviews of SQ catheter usage in neonates have reported that greater than 50% of neonates experience minor adverse events, including induration, leakage, and bruising (Malowany et al., 2007). Other case reports have reported major adverse events caused by SQ catheters in three infants and one case of an infected hematoma (Streif et al., 2003; Malowany et al., 2007, 2008; van Elteren et al., 2011a, 2011b).

Recommended dosing and monitoring for infants less than 2 months of age are provided in Table 79.10. Antifactor Xa levels should be followed 4 hours after the second dose, with a goal level of 0.5–1.0 U/mL (Malowany et al., 2008). Once therapeutic, levels should be followed 1 to 2 times per week (obtained 4 hours after a specified dose). Guidelines for adjusting LMWH therapy are presented elsewhere (Monagle et al., 2012).

Maintaining therapeutic levels in neonates may be a challenge. Lack of pediatric formulations and rapid growth rates in premature infants are the main reasons (Manco-Johnson, 2006; McNinch et al., 2007). A recent review evaluating enoxaparin use in 240 neonates found that the mean maintenance dose of enoxaparin ranged from 1.48 to 2.27 mg/kg every 12 hours for all infants but was higher for preterm neonates at 1.9 to 2.27 mg/kg every 12 h (Malowany et al., 2007, 2008). These findings have influenced current recommended dosing (Table 79.10) (Malowany et al., 2008).

LMWH therapy has been effective in the NICU with centers reporting either partial or complete resolution of TE events in 59%–100% of neonates treated (Malowany et al., 2007, 2008).

Recombinant Tissue Type Plasminogen Activator

Fibrinolytic agents, specifically recombinant tissue type plasminogen activator (rt-TPA), convert plasminogen to plasmin, which cleaves fibrinogen to fibrin and fibrin degradation products (see Video 79.2). rt-TPA use in neonates should *only* be considered for limb-threatening or organ-threatening thromboses and acute atrial thrombi (Wang et al., 2003; Manco-Johnson, 2008; Monagle et al., 2012). Treatment in neonates is affected by low baseline plasminogen levels.

The safety and efficacy of rt-TPA treatment in neonates have been reported in case series and cohort studies demonstrating complete or partial clot lysis in 84%–94% of cases (Hartmann et al., 2001). Five of these cases used low-dose systemic rt-TPA treatment, demonstrating effectiveness in all neonates treated, with one preterm infant requiring discontinuation of therapy because of the development of a subdural hematoma (Wang et al., 2003). Based on limited data, recommended dosing is provided in Table 79.11, with very careful laboratory and radiologic monitoring required, and these recommendations are provided in Table 79.17. rt-TPA treatment does not inhibit clot propagation or directly affect hypercoagulability; therefore simultaneous infusion of UFH at 5–10 U/kg per hour is recommended (Wang et al., 2003; Manco-Johnson, 2006). Once the clot is adequately lysed, rt-TPA

TABLE 79.17 Monitoring Recommendations for Thrombolytic Therapy in Neonates

| Testing | When Performed | Levels Desired (if Applicable)* |
|-----------------------------------|---|---|
| Imaging of thrombosis | Before initiation of treatment Every 12–24 hours during treatment | |
| Fibrinogen level | Before initiation of treatment 4–6 hours after starting treatment Every 12–24 hours | Minimum of 100 mg/dL ¹⁰ Supplement with cryoprecipitate |
| Platelet count | Before initiation of treatment 4–6 hours after starting treatment Every 12–24 hours | Minimum of 50–100 × 10 ⁴ /μL ¹⁰ , dependent upon bleeding risk |
| Cranial imaging | Before initiation of treatment Daily during treatment | |
| Coagulation testing | Before initiation of treatment 4–6 hours after starting treatment Every 12–24 hours | |
| Plasminogen | Before initiation of treatment 4–6 hours after starting treatment Every 12–24 hours | Adequate to achieve thrombolysis Supplementation with plasminogen (FFP) before commencing therapy is recommended to ensure adequate thrombolysis ^{11,108} |
| Line associated or mucosal oozing | At all clinical assessments | Topical thrombin prn |

FFP, Fresh frozen plasma.

Adapted from Saxonhouse MA. Management of neonatal thrombosis. *Clin Perinatol.* 2012;39:191–208; with permission.

Data from references (Nowak-Gottl et al., 1999a; Wang et al., 2003; Thornburg and Pipe, 2006).

therapy should be discontinued, with anticoagulants, preferably LMWH, started.

Surgery

The use of microsurgical techniques and combined microsurgical and thrombolytic regimens has the potential to rapidly restore blood flow, avoiding tissue loss, without major bleeding complications, especially in patients with peripheral arterial occlusion (Coombs et al., 2006). One center's experience of 11 patients with arterial vascular access–associated thrombosis, secondary to PAL complications, described 5 patients that required arteriotomy, embolectomy, and subsequent microvascular reconstruction (Coombs et al., 2006). When faced with a significant arterial thrombosis, especially one from a PAL, surgery may be entertained but should only take place at an experienced institution.

New Anticoagulants

Of the new anticoagulant agents in adults, there is little experience in children, especially neonates. Recombinant hirudin (lepirudin) is a direct thrombin inhibitor and may be used for neonatal cases of heparin-induced thrombocytopenia (Thornburg and Pipe, 2006). The direct oral thrombin inhibitor (melagatran) has potential in the NICU but remains to be tested. Other oral antifactor Xa agents are currently being tested in the NICU and may have a major role in long-term neonatal anticoagulation. New catheter materials that are in the clinical and preclinical stage of development, such as those coated with antithrombin–heparin covalent complex, may prove to be useful in the future (Greenway et al., 2004).

Conclusion

Hemorrhagic and TE emergencies may affect neonates admitted to the NICU. Neonatologists and others caring for newborns should routinely refer to updated guidelines on what are the best treatments. The lack of randomized clinical trials for the management of TE emergencies forces neonatologists to base their medical decisions from registry and expert guidelines. The ultimate goal is to treat effectively without causing additional harm. This may be difficult when treatments, for both hemorrhage and TE, have significant risks. As our knowledge improves of the premature infant, so will our understanding of the neonatal hemostatic system. The importance of randomized clinical trials and improved center data registries

investigating treatment options for neonatal hemorrhage and TE thrombosis cannot be ignored. As we begin to answer the mysteries of the neonatal hemostatic system, it is only hoped that we uncover what are the best preventive and safest treatment strategies.

Suggested Readings

- Bhatt MD, Paes BA, Chan AK. How to use unfractionated heparin to treat neonatal thrombosis in clinical practice. *Blood Coagul Fibrinolysis*. 2015;27(6):605-614.
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Complete references used in this text can be found online at www.expertconsult.com

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Neonatal Platelet Disorders

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KEY POINTS

- Mild to moderate, early-onset thrombocytopenia in well-appearing small for gestational age neonates is usually caused by placental insufficiency and resolves spontaneously within 10–14 days.
- Neonates with platelet counts less than $50 \times 10^9/L$ in the first day of life should be screened for neonatal alloimmune thrombocytopenia and should be treated with random donor platelets (\pm intravenous immunoglobulin) as the first line of therapy, unless human platelet antigen (HPA)-1b1b and HPA-5a5a platelets are immediately available for use.
- The only published randomized controlled trial of neonatal platelet transfusions was limited to infants less than 1500 g during the first week of life and showed no differences in the frequency or severity of intraventricular hemorrhages between neonates transfused for platelet counts less than $60 \times 10^9/L$ versus less than $150 \times 10^9/L$.
- The risk of bleeding in thrombocytopenic neonates is multifactorial, but gestational age less than 28 weeks, postnatal age less than 10 days, and diagnosis of necrotizing enterocolitis are stronger predictors of bleeding than the platelet count.
- The most severe congenital platelet function defects, which can present with bleeding in neonatal life, are Bernard–Soulier syndrome and Glanzmann thrombasthenia.

Fetal and Neonatal Platelet Production

A mounting body of evidence arising over the last decade has clearly demonstrated that there are substantial morphologic and biologic differences between fetal/neonatal and adult megakaryocytes and platelets. Developmental stage-specific differences are ontogenetically important, because they allow the fetus to maintain stable platelet counts while the blood volume is rapidly expanding, in a time period characterized by exceptionally rapid growth. The complex process of platelet production can be represented as consisting of four main steps: (1) the production of thrombopoietic factors (mainly thrombopoietin [TPO]), (2) the proliferation of megakaryocyte progenitors, (3) the differentiation and maturation of megakaryocytes through a unique process of endomitosis, and finally, (4) the production and release of platelets into the circulation.

Studies culturing megakaryocyte progenitors derived from term and preterm umbilical cord blood, fetal blood (18–22 weeks' gestation), or fetal bone marrow have documented that fetal/neonatal megakaryocyte progenitors proliferate at a significantly higher rate compared with their adult counterparts. Additionally, fetal and

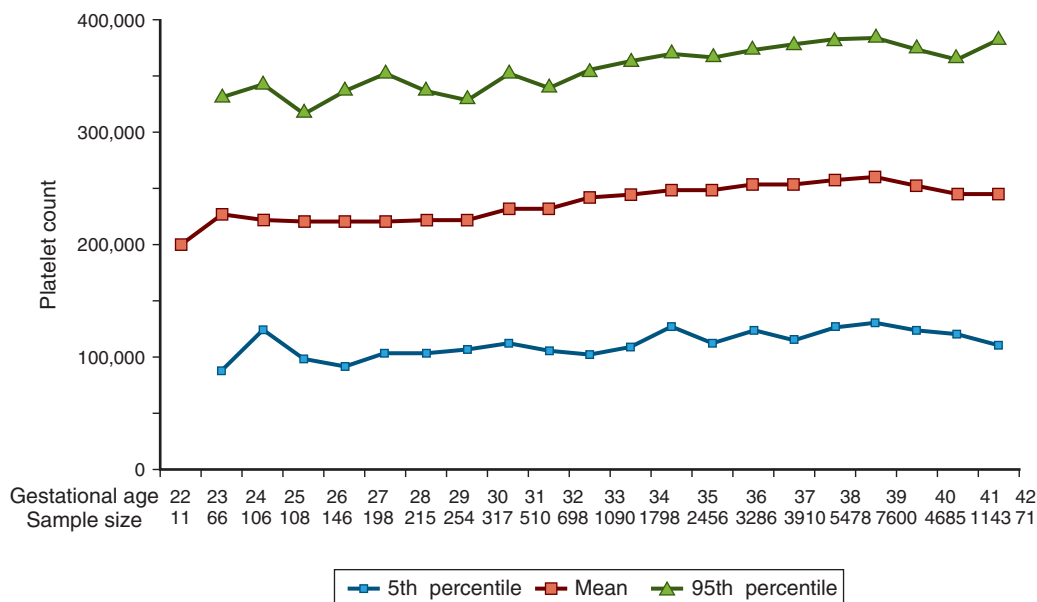
neonatal megakaryocytes are substantially smaller and have lower ploidy levels than adult megakaryocytes. Contrary to adult megakaryocytes, however, which mature as their ploidy level increases, neonatal megakaryocytes are fully mature and capable of platelet production despite their small size and low ploidy. This dissociation between proliferation, polyploidization, and cytoplasmic maturation is a hallmark feature of neonatal megakaryopoiesis. The net result of this process is the production of large numbers of low-ploidy but highly mature megakaryocytes, with which fetuses and neonates populate their rapidly expanding bone marrow space and blood volume, while maintaining normal platelet counts. As developmental processes are easily disturbed, however, sick neonates, and particularly very low birth weight infants (VLBW) (birth weight <1500 g), are at high risk of thrombocytopenia.

Platelet Counts During Development and Reference Ranges

Recently, Wiedmeier and collaborators published the largest study on neonatal platelet counts conducted to date, which included approximately 47,000 infants delivered between 22 and 42 weeks' gestation (Wiedmeier et al., 2009). This study showed that platelet counts at birth increased with advancing gestational age (Fig. 80.1), by approximately $2 \times 10^9/L$ for each week of gestation. Importantly, while the mean platelet count was greater than $200 \times 10^9/L$ even in the most preterm infants, the fifth percentile was $104 \times 10^9/L$ for those less than 32 weeks' gestation and $123 \times 10^9/L$ for late-preterm and term neonates (see Fig. 80.1) (Wiedmeier et al., 2009). These findings suggested that different definitions of thrombocytopenia and thrombocytosis should be applied to preterm infants. In that regard, however, it is important to emphasize that the reference ranges in that study were generated by eliminating the top 5% and the bottom 5% of all available values, rather than excluding values based on diagnoses. Thus these should be considered “epidemiologic reference ranges” rather than “normal ranges.” Nevertheless, these data suggest that platelet counts between 100 and $150 \times 10^9/L$ might be more frequent among otherwise healthy extremely preterm infants than among full-term neonates or older children/adults.

Platelet Function and Primary Hemostasis

Multiple studies evaluating platelet adhesion, aggregation, and activation have shown that neonatal platelets are hyporesponsive in vitro to most agonists, compared with adult platelets (Israels et al., 1997; Rajasekhar et al., 1997), and this hyporeactivity is



• **Fig. 80.1** First recorded platelet counts, obtained in the first 3 days after birth, in neonates born at 22 to 42 weeks' gestation. Mean values are indicated by the red line, and the 5th and 95th percentiles are shown in the blue and green lines, respectively. (Adapted from Wiedmeier SE, Henry E, Sola-Visner MC, Christensen RD. Platelet reference ranges for neonates, defined using data from over 47,000 patients in a multihospital healthcare system. *J Perinatol.* 2009;29:130–136.)

more pronounced in preterm infants (Sitaru et al., 2005; Ucar et al., 2005). Platelet aggregation studies demonstrated that platelets from neonatal (full-term) cord blood were less responsive than adult platelets to agonists such as adenosine diphosphate (ADP), epinephrine, collagen, thrombin, and thromboxane analogues (Israels et al., 2003). Similar results were obtained in flow cytometric platelet activation studies, which showed decreased expression of surface activation markers in neonatal platelets stimulated with thrombin, ADP, and epinephrine (Israels et al., 2003; Sitaru et al., 2005). Different mechanisms account for the hyporeactivity of neonatal platelets to various agents: (1) the hyporesponsiveness to epinephrine is due to fewer α_2 -adrenergic receptors, the binding sites for epinephrine (Corby and O'Barr, 1981); (2) the reduced response to collagen probably results from impaired calcium mobilization (Gelman et al., 1996); (3) the decreased response to thromboxane results from differences in signaling downstream from the receptor in neonatal platelets (Israels et al., 1997); and (4) the decreased responsiveness to thrombin is related to reduced expression of Protease-Activated Receptor 1 and 4 (PAR 1 and PAR 4) in neonatal platelets (Schlagenhauf et al., 2010).

Surprisingly, while the hypofunctional platelet phenotype in vitro would predict a bleeding tendency, healthy full-term neonates have normal to enhanced primary hemostasis, compared with older children or adults. Bleeding times (BTs) in healthy term neonates are shorter than BTs in adults (Andrew et al., 1990a). Similarly, studies using the platelet function analyzer (PFA-100; an in vitro test of primary hemostasis that measures the time it takes to occlude a small aperture, or closure time [CT]) found that cord blood samples from term neonates exhibited shorter CTs than samples from older children or adults (Israels et al., 2001; Boudewijns et al., 2003). The results of these studies suggest that there is an enhanced platelet/vessel wall interaction in full-term neonates, probably related to their higher hematocrits, higher mean corpuscular volumes, and higher concentrations of von Willebrand factors (vWFs; particularly its ultralong polymers) (Andrew et al., 1990b),

all factors that—combined effectively—counteract the neonatal platelet hyporeactivity. Taken together, the available evidence strongly suggests that the in vitro platelet hyporeactivity of healthy full-term infants is an integral part of a carefully balanced and well-functioning neonatal hemostatic system, rather than a developmental deficiency.

These compensatory mechanisms might be less well developed in preterm infants, whose platelets are also more hyporeactive than those of full-term infants, leading to longer BTs (Del Vecchio et al., 2008) and therefore a less balanced and probably more vulnerable hemostatic system. Specifically, BTs performed on the first day of life were longer in preterm compared with term infants, with neonates less than 33 weeks' gestation exhibiting the longest BTs (Del Vecchio et al., 2008). Saxonhouse et al. (2010) found that PFA-100 CTs from nonthrombocytopenic neonates were inversely correlated to gestational age in both cord blood and neonatal peripheral blood samples obtained on the first day of life. Importantly, however, while these BTs and CTs were longer in preterm compared with term neonates, they were still near or within the normal range for adults, suggesting that healthy preterm neonates also have adequate primary hemostasis. Data regarding how disease processes perturb this delicate system, particularly in the preterm neonate, are lacking.

In vitro studies using flow cytometry or the cone and platelet analyzer showed that the neonatal platelet function improves significantly and nearly normalizes by 10–14 days, even in preterm infants (Sitaru et al., 2005; Ucar et al., 2005; Bednarek et al., 2009). Consistent with this, Del Vecchio et al. (2008) found that, by day 10 of life, all infants had shorter BTs than at birth, and early gestational age-related differences had disappeared. Moreover, little or no further shortening occurred between days 10 and 30. While no causal association has been demonstrated, this period overlaps with the period of highest risk of bleeding among preterm neonatal intensive care unit (NICU) patients: namely, during the first 10 days of life (Stanworth et al., 2009b).

Thrombocytosis in the Neonate

Depending on the pathogenesis, thrombocytosis can be classified as primary (essential) or secondary (reactive). Primary thrombocytosis is a myeloproliferative disorder, caused by monoclonal or polyclonal abnormalities of hematopoietic cells or by abnormalities in TPO biology, leading to uncontrolled platelet production. Patients with primary thrombocytosis might be at risk for bleeding or thrombotic complications, sometimes requiring therapy. However, primary thrombocytosis is extremely rare in children, with an estimated incidence of 1 in 10 million (Dame and Sutor, 2005).

Secondary or reactive thrombocytosis, in contrast, has its highest incidence in neonates and infants. Based on severity, thrombocytosis has been traditionally classified as mild (platelet counts between 500 and $700 \times 10^9/L$), moderate (700 – $900 \times 10^9/L$), severe (900 – $1000 \times 10^9/L$), and extreme thrombocytosis (platelet counts $> 1000 \times 10^9/L$). In a recent study of platelet counts in 47,000 neonates over the first 90 days of life, the 95th percentile upper reference range was as high as $750 \times 10^9/L$, suggesting that mild thrombocytosis might not be pathologic in this population.

The most common causes of reactive thrombocytosis in neonates and children are infections, tissue damage (surgeries, trauma, burns), and anemia (frequently iron deficiency). Reactive thrombocytosis has also been described in association with medications (i.e., corticosteroids), maternal exposure to methadone or psychopharmaceutical drugs, and metabolic diseases, myopathies, or neurofibromatosis. In childhood, reactive thrombocytosis usually does not lead to thromboembolic or hemorrhagic complications, and for that reason therapy with anticoagulants or platelet function inhibitors (i.e., aspirin) is usually not indicated in asymptomatic children, even if the platelet count is greater than $1000 \times 10^9/L$. Individually tailored thrombosis prophylaxis should be considered only in neonates with additional thrombotic risk factors, such as maternal antiphospholipid syndrome or cardiac malformation. In a recent retrospective study of 26 episodes of extreme thrombocytosis (platelet counts $\geq 1000 \times 10^9/L$) in neonates and young infants, 12 patients had had a recent infection, 8 patients had recent surgery (median of 9 and 13 days before the diagnosis of thrombocytosis, respectively), 4 patients were growing premature infants with anemia, 1 patient had methadone withdrawal syndrome, and 1 patient had congenital adrenal hyperplasia. In 20 of the 26 episodes, for which data were available, a reduction in platelet count to less than $1000 \times 10^9/L$ was noted after 7 days (range 1 to 30). No patient had hemorrhagic or thrombotic complications, and only 1 patient was treated with low-dose aspirin because of concomitant complex congenital heart disease generating concerns about potential thrombosis (Wiedmeier et al., 2010).

Thrombocytopenia in the Neonate

Thrombocytopenia in neonates (as in adults) has traditionally been defined as a platelet count of less than $150 \times 10^9/L$ and has been classified as mild (100 – $150 \times 10^9/L$), moderate (50 – $99 \times 10^9/L$), and severe ($<50 \times 10^9/L$). However, consistent with the data by (Wiedmeier et al., 2009), platelet counts in the 100 to $149 \times 10^9/L$ range are somewhat more common among neonates than adults. The incidence of thrombocytopenia in neonates varies significantly, depending on the population studied. Based on the traditional definitions, large studies in unselected populations established an overall incidence of neonatal thrombocytopenia of 0.7% – 0.9% (Dreyfus et al., 1997; Uhrynowska et al., 1997). However, when focusing on neonates admitted to the NICU, the

incidence of thrombocytopenia is much higher, ranging from 18% to 35% (Mehta et al., 1980; Castle et al., 1986; Oren et al., 1994). The incidence of thrombocytopenia is also inversely correlated to the gestational age so that the most immature neonates are the most frequently affected: platelet counts less than $150 \times 10^9/L$ were found at least once during the hospital stay in 70% of infants with a birth weight of less than 1000 g (Christensen et al., 2006).

Approach to the Thrombocytopenic Neonate—Evaluation and Classification

When evaluating a thrombocytopenic neonate, the first step to narrow the differential diagnosis is to classify the thrombocytopenia as either *early onset* (within the first 72 hours of life) or *late onset* (after 72 hours of life) and to determine whether the infant is clinically ill or well. Importantly, infection/sepsis should always be considered near the top of the differential diagnosis (regardless of the time of presentation and the infant's appearance), as any delay in diagnosis and treatment can have life-threatening consequences.

Early-Onset Thrombocytopenia (Fig. 80.2, Box 80.1)

The most frequent cause of early-onset thrombocytopenia in a well-appearing neonate is placental insufficiency, seen in infants born to mothers with pregnancy-induced hypertension/preeclampsia

• BOX 80.1 Classification of Neonatal Thrombocytopenia by Time of Presentation

Early-Onset Thrombocytopenia

Chronic fetal hypoxia (hypertension/preeclampsia, diabetes)—IUGR/SGA
 Immune-mediated thrombocytopenia (NAIT, autoimmune thrombocytopenia)
 Early-onset sepsis (GBS, *Escherichia coli*)
 Congenital viral/parasitic infection (HIV, CMV, toxoplasma, enterovirus)
 Birth asphyxia
 Renal vein thrombosis
 Polycythemia
 Chromosomal anomalies (trisomy 21, 18, 13)
 Congenital thrombocytopenia (TAR, ATRUS syndrome, Wiskott–Aldrich syndrome)
 Congenital leukemia

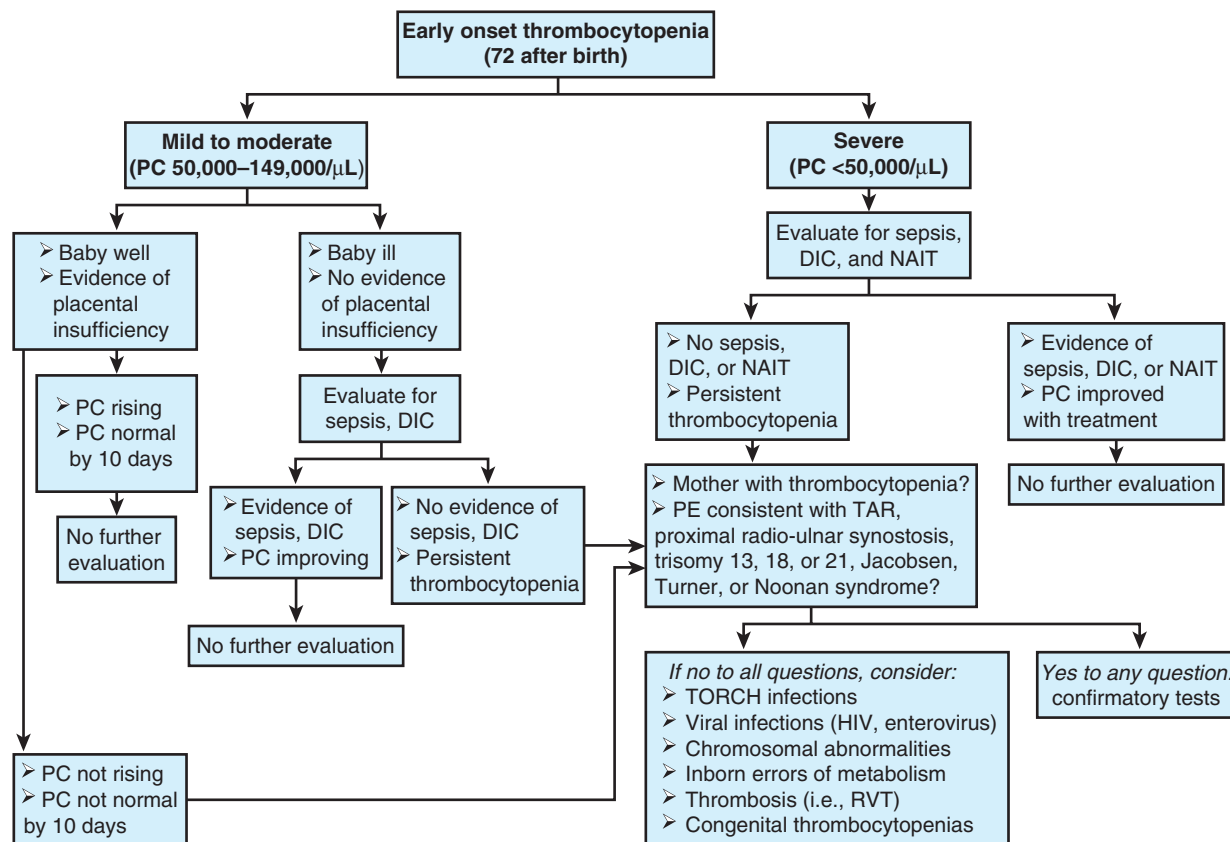
Late-Onset Thrombocytopenia

Late-onset infection/sepsis (bacterial, fungal)
 NEC
 Viral infection (HSV, acquired CMV, enterovirus, adenovirus)
 Thrombosis (catheter related)
 Drug-induced thrombocytopenia (antibiotics, heparin)
 Fanconi anemia

Can Present Both Early and Late

Sepsis/DIC
 Inborn errors of metabolism (Gaucher disease, methylmalonic acidemia)
 Kasabach–Merritt syndrome

ATRUS, Amegakaryocytic thrombocytopenia with proximal radio-ulnar synostosis; CMV, cytomegalovirus; DIC, disseminated intravascular coagulation; GBS, group B streptococcus; HIV, human immunodeficiency virus; HSV, herpes simplex virus; IUGR, intrauterine growth restriction; NAIT, neonatal alloimmune thrombocytopenia; NEC, necrotizing enterocolitis; SGA, small for gestational age; TAR, thrombocytopenia-absent radius syndrome.



• **Fig. 80.2** Guidelines for the evaluation of neonates with early-onset thrombocytopenia (≤ 72 hours of life). DIC, Disseminated intravascular coagulation; HIV, human immunodeficiency virus; NAIT, neonatal alloimmune thrombocytopenia; PC, platelet count; PE, physical examination; RVT, renal vein thrombosis; TAR, thrombocytopenia-absent radius syndrome; TORCH, Toxoplasmosis, Other, Rubella, Cytomegalovirus, Herpes simplex virus infection complex. (Adapted from Deschmann E, Saxonhouse M, Sola-Visner M. Chapter 47: Thrombocytopenia. In: Eichenwald EC, Hansen AR, Martin CR, Stark AR, eds. *Cloherly and Stark's Manual of Neonatal Care*. 8th ed. Philadelphia, PA: Wolters Kluwer; 2016:630–640.)

and in those with intrauterine growth restriction (Murray and Roberts, 1996; Murray et al., 1998). This thrombocytopenia is always mild to moderate, presents immediately or shortly after birth, and resolves within 7 to 10 days. In a recent large cohort study, approximately one-third of small for gestational age (SGA) infants had thrombocytopenia ($<150 \times 10^9/L$) during the first week of life, compared with only 10% of non-SGA-matched infants. This type of thrombocytopenia was associated with low mortality (2%), as long as there was no identified cause for the SGA other than placental insufficiency (i.e., genetic syndrome or congenital infection) (Christensen et al., 2015). If an SGA otherwise nondysmorphic infant with mild to moderate thrombocytopenia remains clinically stable and the platelet count normalizes within 10 days, no further evaluation is necessary. However, if the thrombocytopenia becomes severe and/or persists for greater than 10 days, further investigation is indicated.

Severe early-onset thrombocytopenia in an otherwise healthy infant should trigger suspicion for an immune-mediated thrombocytopenia, either autoimmune (if the mother is also thrombocytopenic) or alloimmune (if the mother has a normal platelet count). These varieties of thrombocytopenia are discussed in detail below. Early-onset thrombocytopenia of any severity in an *ill-appearing* term or preterm neonate should prompt evaluation for sepsis, congenital viral or parasitic infections, or disseminated

intravascular coagulation (DIC). DIC is most frequently associated with sepsis but can also be secondary to birth asphyxia (Suzuki and Morishita, 1998).

In addition to these considerations, the affected neonate should be carefully examined for any radial and thumb abnormalities (suggestive of thrombocytopenia-absent radii [TAR] syndrome, amegakaryocytic thrombocytopenia with radio-ulnar synostosis, or Fanconi anemia). The inability to rotate the forearm on physical examination, in the presence of severe early-onset thrombocytopenia, suggests the rare diagnosis of congenital amegakaryocytic thrombocytopenia with proximal radio-ulnar synostosis (ATRUS). Dysmorphic features on physical examination should warrant investigation for other genetic disorders associated with early-onset thrombocytopenia: most commonly, trisomy 21, trisomy 18, trisomy 13, Turner syndrome, Noonan syndrome, and Jacobsen syndrome.

The presence of hepatomegaly and/or splenomegaly is suggestive of viral infection, although it can also be seen in hemophagocytic syndrome and liver failure from different etiologies (i.e., hemochromatosis). Other diagnoses, such as renal vein thrombosis, Kasabach–Merritt syndrome, and inborn errors of metabolism (mainly propionic acidemia and methylmalonic acidemia) should be considered and evaluated for based on specific clinical indications (i.e., hematuria in renal vein thrombosis, presence of a vascular tumor in Kasabach–Merritt syndrome).

Late-Onset Thrombocytopenia (Fig. 80.3, Box 80.1)

The most common causes of thrombocytopenia of any severity presenting after 72 hours of life are sepsis (bacterial or fungal) and necrotizing enterocolitis (NEC). Affected infants are usually ill appearing and have other signs suggestive of sepsis and/or NEC. However, it is important to keep in mind that *thrombocytopenia can be the first presenting sign of these processes and can precede clinical deterioration*. Appropriate treatment (i.e., antibiotics, supportive respiratory and cardiovascular care, bowel rest in case of NEC, and surgery in case of surgical NEC) usually improves the platelet count in 1 to 2 weeks, although in some infants the thrombocytopenia persists for several weeks. The reasons underlying this prolonged thrombocytopenia are unclear.

If bacterial/fungal sepsis and NEC are ruled out, viral infections such as herpes simplex virus (HSV), cytomegalovirus (CMV), or enterovirus should be considered. These viral infections are frequently accompanied by abnormal liver enzymes. If the infant has or has recently had a central venous or arterial catheter, thromboses should be part of the differential diagnosis, although they only cause thrombocytopenia if the thrombus is enlarging or is infected. Finally, drug-induced thrombocytopenia is rare in neonates but should be considered if the infant is clinically well, other potential etiologies have been ruled out, and he/she is receiving heparin, antibiotics (penicillins, cephalosporins, metronidazole, vancomycin, or rifampin), indomethacin, famotidine, cimetidine, phenobarbital, or phenytoin, among others (Aster and Bougie, 2007; Aster et al., 2009). Other less common causes of late-onset thrombocytopenia include inborn errors of metabolism and Fanconi anemia (rare).

Novel tools to evaluate platelet production, which aid in the evaluation of thrombocytopenia of unclear etiology, have been recently developed and are likely to become widely available to clinicians in the near future. Among those, the immature platelet

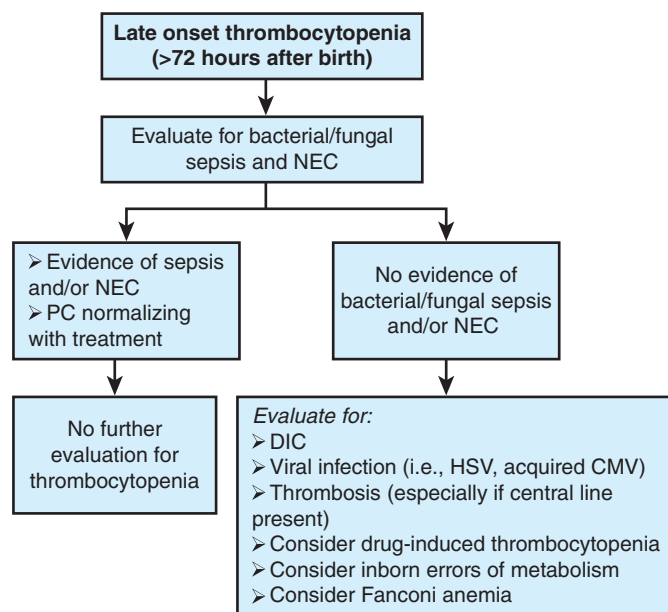
fraction (IPF) measures the percentage of newly released platelets (platelet age <24 hours). The IPF can be measured in a standard hematologic cell counter (Sysmex XE-2100 Automated Hematology System, Sysmex Corporation, Kobe, Japan) as part of the complete cell count and can help differentiate thrombocytopenias associated with decreased platelet production from those with increased platelet destruction, in a manner similar to the use of reticulocyte counts to evaluate anemia (Abe et al., 2006). Thus an elevated IPF would suggest platelet consumption (as in neonatal alloimmune thrombocytopenia [NAIT] or DIC), while a decreased IPF would be consistent with a hyporegenerative thrombocytopenia, as in bone marrow suppression or failure. Reference intervals for immature platelet fraction percentage (IPF%) and absolute immature platelet fraction (A-IPF) have been evaluated by Ko et al. (2013) in a large number of healthy adult individuals and in full-term umbilical cord blood samples (Table 80.1), as well as in nonthrombocytopenic NICU patients by Cremer et al. (2010) (IPF% = $4.1 \pm 1.8\%$; A-IPF = $9.5 \pm 3.8 \times 10^9/L$). Notably, reference ranges for IPF in neonates are higher compared with adults and children (Strauss et al., 2011; Ko et al., 2013). Recent studies have shown the usefulness of the IPF to evaluate mechanisms of thrombocytopenia and to predict platelet recovery in neonates (Cremer et al., 2009, 2010). In patients with NEC and severe thrombocytopenia, a low absolute

TABLE 80.1 Reference Intervals for Platelet Counts and Immature Platelet Fractions in Healthy Adults and Term Neonates (Umbilical Cord Blood)

| | HEALTHY INDIVIDUALS | | | Umbilical Cord Blood |
|---|---------------------|-------------------|--------------------|----------------------|
| | Total (n = 2152) | Men (n = 1252) | Women (n = 900) | (n = 133) |
| PLATELET COUNTS ($\times 10^9/L$) | | | | |
| Reference interval | 162–347 | 161–338 | 164–360 | 191–392 |
| Lower limit (95% CI) | 160–164 | 158–164 | 160–169 | 168–208 |
| Upper limit (95% CI) | 340–353 | 326–344 | 351–372 | 364–447 |
| IPF% | | | | |
| Reference interval | 0.5–3.3 | 0.5–3.1 | 0.5–3.4 | 0.7–3.8 |
| Lower limit (95% CI) | 0.5–0.5 | 0.5–0.6 | 0.5–0.5 | 0.7–0.9 |
| Upper limit (95% CI) | 3.2–3.4 | 3.0–3.3 | 3.3–3.5 | 3.0–3.8 |
| A-IPF ($\times 10^9/L$) | | | | |
| Reference interval | 1.25–7.02 | 1.30–6.80 | 1.21–7.15 | 1.94–9.69 |
| Lower limit (95% CI) | 1.19–1.30 | 1.20–1.41 | 1.10–1.27 | 1.66–2.58 |
| Upper limit (95% CI) | 6.75–7.24 | 6.49–7.16 | 6.9–7.48 | 7.96–10.57 |

A-IPF, Absolute immature platelet fraction; CI, confidence interval; IPF%, immature platelet fraction percentage.

From Ko YJ, Kim H, Hur M, et al. Establishment of reference interval for immature platelet fraction. *Int J Lab Hematol*. 2013;35:528–533.



•Fig. 80.3 Guidelines for the evaluation of neonates with late-onset thrombocytopenia (>72 hours of life). CMV, Cytomegalovirus; DIC, disseminated intravascular coagulation; HSV, herpes simplex virus; PC, platelet count; NEC, necrotizing enterocolitis. (Adapted from Deschmann E, Saxonhouse M, Sola-Visner M. Chapter 47: Thrombocytopenia. In: Eichenwald EC, Hansen AR, Martin CR, Stark AR, eds. *Cloherly and Stark's Manual of Neonatal Care*. 8th ed. Philadelphia, PA: Wolters Kluwer; 2016:630–640.)

IPF has been associated with a poor prognosis and high mortality. The IPF is also particularly helpful to guide the diagnostic evaluation of infants with thrombocytopenia of unclear etiology and can help differentiate rare congenital thrombocytopenias (usually low) from NAIT (usually elevated).

Immune Thrombocytopenia

Immune thrombocytopenia occurs because of the passive transfer of antibodies from the maternal to the fetal circulation. There are two distinct types of immune-mediated thrombocytopenia: (1) NAIT and (2) autoimmune thrombocytopenia. In NAIT, the antibody is produced in the mother against a specific human platelet antigen (HPA) present in the fetus but absent in the mother. The antigen is inherited from the father of the fetus.

The antigens responsible for NAIT are results of single nucleotide polymorphisms in the gene encoding any of the main glycoproteins located on the platelet surface, particularly glycoprotein (GP)IIb/IIIa. The first platelet antigen was identified by van Loghem et al. (1959) and was designated Zw-a (later PLA1). The initial nomenclature for these antigens came from the name of the patients, leading to confusion in the field. In 1990, a simplified system for HPA nomenclature was described, in which each antigen was given an HPA number (von dem Borne and Decary, 1990). Antigens were numbered chronologically, according to the date of their initial report. The biallelic antigens were given an alphabetic designation of “a” or “b” in the order of their frequency (higher frequency for “a”). Thus the Zw-a/PLA1 antigen was renamed HPA-1, with its two serologic forms designated as HPA-1a for the common form and HPA-1b for the less common form (the latter corresponding to PLA2). Sixteen HPA antigens have been identified so far. The frequency of each antigen varies within ethnic groups: in whites, antibodies to HPA-1a are the major cause of NAIT, followed by HPA-5a and, less frequently, HPA-9b, HPA-3a and HPA-3b, and HPA-15. Antibodies to HPA-4b are the predominant cause of NAIT in the Japanese population (Mueller-Eckhardt et al., 1994).

The anti-HPA antibody produced in the maternal serum crosses the placenta and reaches the fetal circulation, leading to platelet destruction, apoptosis of early megakaryocyte progenitors (therefore decreased platelet production) (Liu et al., 2015), and thrombocytopenia. In autoimmune thrombocytopenia, the antibody is directed against an antigen on the mother's own platelets (autoantibody) as well as on the infant's platelets. The maternal autoantibody also crosses the placenta (both passively and actively transported), resulting in destruction of fetal platelets and thrombocytopenia.

Neonatal Alloimmune Thrombocytopenia

NAIT is the most common underlying cause of early-onset, severe thrombocytopenia, with an incidence among live born neonates of 0.5–1.5 per 1000 births (Bertrand et al., 2011). The true incidence of the disease is likely higher, however, since the milder cases might go undetected and the most severe cases lead to intrauterine death. Intrauterine death or intracranial hemorrhage (ICH) may occur as early as at 14 to 16 weeks' gestation, resulting in a relatively high incidence of intrauterine ICH (>10%) (Bertrand et al., 2011). The overall incidence of ICH (prenatal and postnatal) is particularly high in this population, affecting up to 20% of infants with NAIT and potentially leading to lifelong consequences. ICH may occur during the first pregnancy and has a recurrence risk close to 100% in subsequent pregnancies in the absence of prenatal treatment (Bussel et al., 1997).

NAIT should be considered in any neonate who presents with severe thrombocytopenia at birth or shortly thereafter, particularly in the absence of other risk factors, clinical signs, or abnormalities in the physical examination. In a study of more than 200 neonates with thrombocytopenia, using a *platelet count of less than $50 \times 10^9/L$* in the first day of life as a screening indicator identified 90% of the patients with NAIT (Bussel et al., 2005). Based on this observation, *it is currently recommended that all neonates with platelet counts in this range in the first day of life be screened for NAIT*. In addition, the combination of severe neonatal thrombocytopenia with a parenchymal (rather than intraventricular) ICH is highly suggestive of NAIT.

Laboratory Investigation

When NAIT is suspected, blood should be collected from the mother and father and submitted for testing. The initial antigen screening should include HPA-1, HPA-3, and HPA-5. This evaluation should identify approximately 90% of cases of NAIT. However, if the diagnosis is strongly suspected and the initial evaluation is negative, further testing should be undertaken for HPA-9 and HPA-15 (and HPA-4 if the parents are of Asian descent) (Bussel and Sola-Visner, 2009). If positive, these tests will reveal an antibody in the mother's plasma directed against the specific platelet antigen in the father. If blood cannot be collected from the parents in a timely fashion, neonatal serum may be screened for the presence of antiplatelet antibodies. However, a low antibody concentration in the neonate, coupled with binding of the antibodies to the infant's platelets, can lead to false-negative results. It is still unclear if there is any correlation between the affinity of the antibodies and the severity of disease (Bertrand and Kaplan, 2014). Because of the complexity of testing, evaluations should be performed in an experienced reference laboratory that has a large number of typed controls available for antibody detection and the appropriate DNA-based technology to type multiple antigens. In rare cases, antibodies may be hard to detect in samples drawn at the time of delivery; therefore when the clinical diagnosis is most likely NAIT, follow-up serology tests should be performed.

Brain imaging studies (cranial ultrasound) should be performed as soon as NAIT is suspected, *regardless of the presence or absence of neurologic manifestations*, because findings from these studies will dictate the aggressiveness of the treatment regimen for the affected infant and for the mother's future pregnancies. The clinical course of NAIT is short in most cases, often resolving almost entirely within 2 weeks. However, to confirm the diagnosis, it is imperative to follow the platelet count frequently until a normal count is achieved.

Management

The management of NAIT differs, depending on the specific clinical scenario: (1) suspected NAIT (unknown pregnancy); (2) known case of NAIT; and (3) prenatal management of pregnant woman with previous history of NAIT.

Management of the Neonate With Suspected Neonatal Alloimmune Thrombocytopenia (Pregnancy Unknown to the Mother)

Based on recent data demonstrating that a large proportion of infants with NAIT respond to random donor platelet transfusions, this is now considered the first line of therapy for infants in whom NAIT is suspected (Kiefel et al., 2006). If the patient is clinically stable and does not have an ICH, platelets are usually given when the platelet count is less than $30 \times 10^9/L$, although this is arbitrary. In the case of a preterm infant, or a clinically unstable infant (i.e.,

TABLE 80.2 Proposed Platelet Transfusion Recommendation

| Platelet Count ($\times 10^3/\mu\text{L}$) | Recommendations |
|---|--|
| <30 | <i>Transfuse all</i> |
| 30–49 | <i>Transfuse if:</i> <ul style="list-style-type: none"> • BW <1500 g and ≤ 7 days old • Clinically unstable • Recent diagnosis of NEC • Concurrent coagulopathy • Recent major hemorrhage (i.e., grade 3 or 4 IVH) • Prior to surgical procedure • Postoperative period (72 hours) |
| 50–100 | <i>Transfuse if:</i> <ul style="list-style-type: none"> • Active bleeding • NAIT with intracranial hemorrhage • Before and after neurosurgical procedures |

BW, Birth weight; IVH, intraventricular hemorrhage; NAIT, neonatal alloimmune thrombocytopenia; NEC, necrotizing enterocolitis.

From Deschmann E, Saxonhouse M, Sola-Visner M. Chapter 47: Thrombocytopenia. In: Eichenwald EC, Hansen AR, Martin CR, Stark AR, eds. *Cloherly and Stark's Manual of Neonatal Care*. 8th ed. Philadelphia, PA: Wolters Kluwer; 2016:630–640.

respiratory distress, infection), a platelet transfusion is usually given when the platelet count falls below $50 \times 10^9/\text{L}$ during the first week of life (Table 80.2). In addition to platelets, if the diagnosis of NAIT is confirmed or strongly suspected, intravenous immune globulin (IVIG) (1 g/kg per day for up to 2 consecutive days) may be administered to increase the patient's own platelets and potentially to protect the transfused platelets (Mueller-Eckhardt et al., 1989). Because in NAIT the platelet count usually falls after birth, IVIG may be given when the platelet count is between 30 and $50 \times 10^9/\text{L}$ in a stable neonate, to try to prevent a further drop.

If the patient has evidence of an ICH, the goal is to maintain a platelet count greater than $100 \times 10^9/\text{L}$. However, this may be challenging in the setting of NAIT. In all of these scenarios, it is important to keep in mind that some infants with NAIT fail to respond to random donor platelets and IVIG. For that reason, the blood bank should be immediately alerted about any infant with suspected NAIT, and arrangements should be made to secure a source of antigen-negative platelets (either from HPA-1b1b and HPA-5a5a donors, which should be compatible in >90% of cases or from the mother) as soon as possible, so they are available if there is no response to the initial therapies. If maternal platelets are used, they need to be concentrated to decrease the amount of antiplatelet antibodies (present in the mother's plasma) infused into the infant. Platelets can also be washed to eliminate the plasma, although this induces more damage to the platelets than concentrating them (Bussel and Sola-Visner, 2009). Of note, in some European countries, HPA-1b1b and HPA-5a5a platelets are maintained in the blood bank inventory and are immediately available for use. In those cases, these are preferable to random donor platelets and/or IVIG and should be the first line of therapy.

Management of the Neonate With Known Neonatal Alloimmune Thrombocytopenia

When a neonate is born to a mother who had a previous pregnancy affected by confirmed NAIT, genotypically matched platelets (e.g., HPA-1b1b platelets) should be available in the blood bank at the

time of delivery and should be the first-line treatment if the infant is thrombocytopenic.

Management of Pregnant Women With Previous History of Neonatal Alloimmune Thrombocytopenia

Mothers who delivered an infant with NAIT should be followed in high-risk obstetric clinics during all future pregnancies as the reoccurrence rate is high, reaching 100% if the father is homozygous dominant for the causative HPA. However, in all cases fetal genetic testing should be performed. Recently, noninvasive methods have become available through cell-free DNA testing from the mother's plasma. The intensity of prenatal treatment will be based on the severity of the thrombocytopenia and the presence or absence of ICH in the previously affected fetus. This is particularly important to assess the risk of developing an ICH in the current pregnancy and to minimize this risk. Current recommendations involve maternal treatment with IVIG (0.5–2 g/kg per week) \pm steroids (0.5–1 mg/kg per day prednisone), starting at 12 or at 20 to 26 weeks' gestation, depending on whether the previously affected fetus suffered an ICH and if so at what time during pregnancy (Bussel and Sola-Visner, 2009). Most recent studies showed that the combination of IVIG and steroids is the most efficient treatment (Bertrand et al., 2011). Regarding mode of delivery, elective cesarean section is recommended in most countries, regardless of ICH status in the previous and current fetus, to avoid ICH (Bertrand and Kaplan, 2014).

Autoimmune Thrombocytopenia

The diagnosis of neonatal autoimmune thrombocytopenia should be considered in any neonate who has early-onset thrombocytopenia and a maternal history of either immune thrombocytopenic purpura (ITP) or an autoimmune disease (with or without thrombocytopenia). A retrospective study of obstetric patients who had ITP (including a high number of mothers who had thrombocytopenia during their pregnancies) demonstrated a relatively high incidence of affected babies: 25% of neonates had thrombocytopenia at birth; the thrombocytopenia was severe in 9%, and 15% received treatment for it (Webert et al., 2003). Other large studies confirmed an incidence of severe neonatal thrombocytopenia in this population ranging from 8.9% to 14.7%, with ICH occurring in 0%–1.5% of affected neonates (Kaplan et al., 1990; Samuels et al., 1990; Burrows and Kelton, 1993). Based on these data, we recommend that all neonates born to mothers who have autoimmune diseases undergo a screening platelet count at or shortly after birth. If the platelet count is normal, no further evaluation is necessary. If the neonate has mild thrombocytopenia, however, the platelet count should be repeated in 2 to 3 days, since this type of thrombocytopenia usually reaches the nadir between days 2 and 5 after birth. If the platelet count is less than $30 \times 10^9/\text{L}$, IVIG (1 g/kg, repeated if necessary) is the first line of therapy. Random donor platelets, in addition to IVIG, should be provided if the neonate has evidence of active bleeding, although some authors give them in addition to IVIG when the platelet count is less than $30 \times 10^9/\text{L}$ and provide IVIG alone for platelet counts between 30 and $50 \times 10^9/\text{L}$. Cranial imaging (cranial ultrasound) should be obtained in all infants with platelet counts less than $50 \times 10^9/\text{L}$, to evaluate for ICH. Importantly, neonatal thrombocytopenia secondary to maternal ITP may last for weeks to months (unlike NAIT, which usually resolves within 2 weeks) and requires long-term monitoring and sometimes a second dose of IVIG at 4 to 6 weeks of life. Recently, it has been reported that the transfer of antiplatelet

antibodies (immunoglobulin A type) from mothers with ITP through breastfeeding can be associated with persistent neonatal thrombocytopenia (Hauschner et al., 2015).

Maternal Management

Even if the mother has severe ITP, it appears that fetal hemorrhage in utero is very rare, compared with the small but definite risk of such hemorrhage in alloimmune thrombocytopenia. Consequently, treatment of ITP during pregnancy and delivery is mostly based on the risk of maternal hemorrhage (Provan et al., 2010). A small prospective randomized trial of low-dose betamethasone (1.5 mg/day orally) failed to prevent thrombocytopenia in newborns (Christiaens et al., 1990). IVIG given prenatally to the mother with ITP has also not been clearly shown to affect the fetal platelet count.

There is in general little correlation between fetal platelet counts and maternal platelet counts, platelet antibody levels, or history of maternal splenectomy. Attempts to measure the fetal platelet count before delivery are not recommended, because of the risk associated with such attempts. The only reliable predictive measure of neonatal thrombocytopenia in a mother with ITP has been found to be a history of neonatal thrombocytopenia in previous pregnancy (Hachisuga et al., 2014). In regard to the mode of delivery, there is no evidence that cesarean section is safer for the fetus with thrombocytopenia than uncomplicated vaginal delivery. Given this fact, combined with the difficulty predicting severe thrombocytopenia in neonates and the very low risk of serious hemorrhage, the 2010 International Consensus Report on the Investigation and Management of Primary Immune Thrombocytopenia concluded that the mode of delivery in ITP patients should be determined by purely obstetric indications (Provan et al., 2010). However, interventions that increase the risk of bleeding in the fetus should be avoided, such as vacuum extraction or forceps delivery.

Congenital Thrombocytopenias

Congenital thrombocytopenias are rare conditions that most often present at birth and are therefore classified as early-onset thrombocytopenias. This is a heterogeneous group of diseases, with variable clinical manifestations. Often, but not always, patients with congenital thrombocytopenia have dysmorphic features and associated abnormalities as part of a genetic syndrome. If a neonate presents with radial abnormalities, the differential diagnosis includes TAR syndrome, amegakaryocytic thrombocytopenia with radio-ulnar

synostosis, and Fanconi anemia. Although thrombocytopenia associated with Fanconi anemia almost always presents later (during childhood), few isolated neonatal cases have been reported (Gershanik et al., 1972). In these patients, thumb abnormalities are frequently found, and chromosomal fragility testing is nearly always diagnostic. If the infant has radial abnormalities with normal-appearing thumbs, TAR syndrome should be considered (Hedberg and Lipton, 1988). The platelet count is usually less than $50 \times 10^9/L$, and the white cell count is elevated in greater than 90% of TAR syndrome patients, sometimes exceeding $100 \times 10^9/L$ and mimicking congenital leukemia. Infants that survive the first year of life generally do well, since the platelet count then spontaneously improves to low-normal levels that are maintained through life (Geddis, 2006). The inability to rotate the forearm on physical examination, in the presence of severe early-onset thrombocytopenia, suggests the rare diagnosis of congenital ATRUS. Radiologic examination of the upper extremities in these infants confirms the proximal synostosis of the radial and ulnar bones (Sola et al., 2004). Most (but not all cases) of ATRUS are associated with *Hox-A11* mutations (Thompson and Nguyen, 2000; Horvat-Switzer and Thompson, 2006) and require bone marrow transplantation. Other genetic disorders associated with early-onset thrombocytopenia include trisomy 21, trisomy 18, trisomy 13, Turner syndrome, Noonan syndrome, and Jacobsen syndrome. Cases of Noonan syndrome presenting with mild dysmorphic features and very severe neonatal thrombocytopenia (mimicking congenital amegakaryocytic thrombocytopenia [CAMT]) have been recently described, so genetic testing should be performed in children who present with a CAMT-like picture and no mutations in the *C-Mpl* gene (Christensen et al., 2013).

In nonsyndromic cases of congenital thrombocytopenia, there is usually a history of chronic thrombocytopenia in family members. These conditions belong to a heterogeneous group of diseases, and, because of diverse clinical manifestations and laboratory findings, the diagnosis is challenging. Often the size of the platelets helps in the differential diagnosis (Table 80.3). Myosin heavy chain-9 (MYH-9)-related disorders (May-Hegglin anomaly, Fechner syndrome, Sebastian syndrome) are inherited in autosomal dominant fashion and present with macro thrombocytopenia (Pauzner et al., 2009). Other congenital thrombocytopenias presenting with large platelets include Bernard-Soulier syndrome (with autosomal recessive inheritance) and X-linked macrothrombocytopenia. Wiskott-Aldrich syndrome, in contrast, should be suspected in male neonates with severe thrombocytopenia and small platelets

TABLE 80.3 Classification of Congenital Thrombocytopenias by Platelet Size

| Small Platelets | Normal-Sized Platelets | Large Platelets |
|---|---|--|
| Wiskott-Aldrich syndrome (X-linked microthrombocytopenia) | CAMT | MYH-9-associated thrombocytopenia (May-Hegglin anomaly, Sebastian syndrome, Fechtner syndrome, Epstein syndrome) |
| | TAR syndrome | Bernard-Soulier syndrome |
| | Thrombocytopenia associated with trisomies (13, 18, 21) | X-linked macrothrombocytopenia |
| | | Paris-Trousseau syndrome |
| | | Gray platelet syndrome |

CAMT, Congenital amegakaryocytic thrombocytopenia; MYH-9, myosin heavy chain-9; TAR, thrombocytopenia-absent radius.

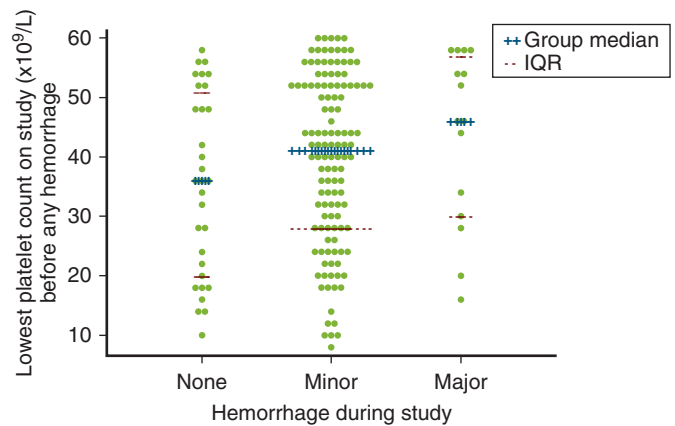
(≤ 7 femtoliter), particularly if they develop eczema. The diagnosis of CAMT is challenging as it presents with normal-sized platelets and may not be diagnosed in the newborn period (when it is usually confused with alloimmune thrombocytopenia) because of the lack of other clinical features. A summary of congenital thrombocytopenias and their associated features is provided in Table 80.4 (Smock and Perkins, 2014). These conditions are often paired with platelet dysfunction as well.

The outcomes for patients with congenital thrombocytopenia are variable and depend on the specific disorders. Patients with CAMT often develop bone marrow failure and pancytopenia and require bone marrow transplant. The thrombocytopenia in TAR patients is severe at birth but improves over time, often reaching platelet counts greater than $100 \times 10^9/L$. The hematologic outcome of MYH-9-related disorders is good, although patients frequently develop nephritis, hearing loss, and cataracts later in life.

Management of Neonatal Thrombocytopenia

Platelet Transfusions in the Neonatal Intensive Care Unit

Platelet transfusion is the primary modality of treatment for neonatal thrombocytopenia, but there is lack of consensus regarding platelet transfusion thresholds. Recent studies have shown that there is great variability in neonatal transfusion practices in the United States and worldwide (Kahn et al., 2003; Josephson et al., 2009; Stanworth et al., 2009b; Cremer et al., 2011). To a large extent, this is attributable to the absence of scientific evidence in the field, the high incidence of bleeding in this population, and the known platelet hyporeactivity of neonates. Generally, neonates are transfused at higher platelet counts than older children and adults, and thus platelet transfusions are a frequent intervention in the NICU. Despite the high frequency of transfusions, only one randomized trial has compared different platelet transfusion thresholds in neonates. This trial was limited to VLBW infants in the first week of life and excluded patients with severe thrombocytopenia (platelet counts $< 50 \times 10^9/L$) (Andrew et al., 1993). The investigators found no differences in the incidence or severity of intraventricular hemorrhages (IVHs) between a group of neonates transfused for any platelet count less than $150 \times 10^9/L$ and a group transfused only for counts less than $60 \times 10^9/L$. Based on these findings, the investigators concluded that transfusing VLBW infants with platelet counts greater than $60 \times 10^9/L$ does not reduce the risk of IVH. In the contemporary prospective multicenter observational Platelet for Neonatal Transfusion-study 1 (PlaNeT-1), platelet transfusions were administered at a median platelet count of $27 \times 10^9/L$. In a secondary analysis, the temporal association between platelet transfusions and minor bleeding was assessed. This analysis showed that neonates had 21% fewer bleeding events during the 12 hours following a platelet transfusion, compared with the 12 hours before the transfusion (Muthukumar et al., 2012). However, these findings should be interpreted with caution, partly because of the study design (observational study being prone to confounders and lacking a control group) and because these results were part of a secondary analysis. A more recent study by von Lindern et al. (2011) compared bleeding outcomes in an NICU that used liberal transfusion thresholds with an NICU that used restrictive transfusion thresholds. The study found no significant differences in bleeding outcomes between these units.



• **Fig. 80.4** Lowest platelet counts in neonates with and without major or minor hemorrhage. Hatched lines indicate group medians; dashed lines represent interquartile ranges (IQRs). The lowest counts before hemorrhage are shown for neonates with minor or major hemorrhage, and the lowest counts during the thrombocytopenic episode are presented for neonates with no bleeding. (Adapted from Stanworth SJ, Clarke P, Watts T, et al. Prospective, observational study of outcomes in neonates with severe thrombocytopenia. *Pediatrics*. 2009;124:e826–e834.)

The relationship between degree of thrombocytopenia and bleeding risk has been assessed in a number of neonatal studies (Andrew et al., 1987; Stanworth et al., 2009b). The PlaNeT-1 study found that 9% of thrombocytopenic neonates experienced clinically significant bleeding (most commonly intracranial): 87% of these hemorrhages occurred during the first 2 weeks of life, and 87% were in neonates less than 28 weeks' gestation. Importantly, nadir platelet counts were similar in infants who experienced clinically significant bleeding compared with those with no or only minor bleeding (Fig. 80.4). A secondary analysis looking at all bleeding events (minor as well as clinically significant) found that a lower nadir platelet count was associated with only a slightly increased number of bleeding events. In that study, the strongest predictors of bleeding were gestational age less than 28 weeks, postnatal age less than 10 days, and a diagnosis of NEC (Muthukumar et al., 2012), suggesting that factors other than the platelet count are the most important determinants of bleeding risk. Consistently, a recent study by Sparger et al. (2016) found that the risk of IVH was higher in VLBW infants with thrombocytopenia in the first week of life, compared with nonthrombocytopenic neonates, but there was no correlation between degree of thrombocytopenia and IVH risk. Furthermore, platelet transfusions (which in this study were mostly given for platelet counts between 50 and $100 \times 10^9/L$) did not reduce the risk of IVH, further supporting the lack of efficacy of platelet transfusions given at these platelet counts to prevent IVH. Based on this limited evidence, coming mostly from observational studies, we currently propose administering platelet transfusions to neonates according to the criteria shown in Table 80.2.

There is more consensus in regard to the platelet product that should be transfused. Most experts agree that neonates should receive 10–15 mL/kg of a standard platelet suspension, either a platelet concentrate (“random-donor platelets”) or apheresis platelets. Each random-donor platelet unit has approximately 50 mL of volume and contains approximately 10×10^9 platelets per 10 mL (Strauss, 2000). There is no need to pool more than one random-donor unit for a neonatal transfusion, a practice that only increases donor exposures and induces platelet activation, without any benefit. Two additional important considerations in neonatology are the

**TABLE
80.4****Genetic and Clinical Characteristics of Congenital Thrombocytopenias**

| Disease | Mode of Inheritance | Degree of Thrombocytopenia | Platelet Size | Other Clinical/Pathologic Features | Genetic Defect |
|--|---------------------|---|---|---|---|
| Congenital amegakaryocytic thrombocytopenia | AR | Severe—type 1 Mild to moderate—type 2 | Normal | Absence of megakaryocytes in marrow Develop bone marrow failure/pancytopenia, markedly elevated TPO levels | Mutations in <i>MPL</i> gene |
| TAR | AR | Severe at birth but usually less severe with age | Normal | Congenital malformations including bilateral absent radii, skeletal and cardiac abnormalities, often have cow's milk intolerance, elevated TPO levels. Decreased to absent megakaryocytes in marrow at birth, low to normal numbers later in life | Unknown. Mutation in exon junction complex subunit <i>RBM8A</i> gene. Micro deletion of chromosome 1q21.1 (not causative) |
| Bernard–Soulier syndrome | AR | Mild | Large | Abnormal platelet function. Decreased GPIb complex on platelets. Marrow has normal to slightly increased normal megakaryocytes. | Mutations in <i>GP1BA</i> , <i>GP1BB</i> , or <i>GP9</i> genes |
| <i>MYH9</i> -related disorders May–Hegglin anomaly Sebastian syndrome Fechtner syndrome Epstein syndrome | AD | Mild–moderate Mild–moderate Mild–moderate Mild–moderate Mild–moderate | Large Large Large Large Large | All will have Dohle-like bodies in myeloid cells. Nephritis, hearing loss, and cataracts Nephritis and hearing loss | Mutation in <i>MYH9</i> gene on 22q12.3-13.1, variable penetrance |
| Familial platelet disorder with predisposition to acute myelogenous leukemia | AD | Moderate | Normal | Predisposition to develop AML or MDS Abnormal platelet aggregation/prolonged bleeding times Abnormal platelet function Decreased megakaryocytes in marrow | Heterozygous <i>RUNX1</i> mutation on 8q22 |
| Mediterranean macrothrombocytopenia | AD | Mild | Large | Seen in patients from Italy and Balkan peninsula. May represent carrier state for Bernard–Soulier syndrome | Mutation in <i>GP1A</i> gene on chromosome 17 |
| Paris–Trousseau syndrome | AD | Moderate to severe at birth but improve with age to near normal | Large | Congenital abnormalities including psychomotor retardation, trigonocephaly, facial dysmorphism, and cardiac abnormalities. Dysmegakaryopoiesis with many micromegakaryocytes and abnormal α -granules | Mutation in <i>FLI1</i> gene on 11q23.3 |
| Gray platelet syndrome | AR | Mild to moderate | Large | Develop myelofibrosis and splenomegaly. Platelets lack α -granules and appear agranular and gray. Variable bleeding | Unknown. Mutations in <i>NBEAL2</i> gene in AR disease |
| X-linked microthrombocytopenia (Wiskott–Aldrich syndrome) | X | Variable—usually mild to moderate | Small | Normal to increased megakaryocytes. Splenic destruction thought to cause small platelet size and thrombocytopenia. Patients may have eczema and T-cell lymphopenia. Increased incidence of autoimmune disorders and lymphoma | Mutation in <i>WAS</i> gene at Xp11.4-p11.21 |
| X-linked thrombocytopenia with dyserythropoiesis (<i>GATA1</i>) | X | Moderate to severe | Large to normal | Usually have bleeding and bruising with abnormal platelet function. Anemia. Hypercellular marrow with dysplastic erythroid and megakaryocytic precursors | Mutation in <i>GATA1</i> gene at Xp11.23 |

AD, Autosomal dominant; AML, acute myelogenous leukemia; AR, autosomal recessive; GPIb, glycoprotein Ib; MDS, myelodysplastic syndrome; TAR, thrombocytopenia-absent radius; TPO, thrombopoietin; X, X-linked.

From Smock KJ, Perkins SL. Thrombocytopenia: an update. *Int J Lab Hematol*. 2014;36:269–278.

prevention of transfusion-transmitted CMV infections and graft-versus-host disease (GVHD). Most blood banks provide either CMV-negative or leuko-reduced products to neonates, both of which significantly reduce (but do not completely eliminate) the risk of transfusion-transmitted CMV. Transfusion of CMV-negative and leuko-reduced blood products completely prevented transmission of CMV to VLBW infants in a recent study (Josephson et al., 2014). GVHD is prevented by irradiating cellular blood products before transfusion. Of note, most neonatal cases of GVHD have been reported in neonates with underlying immunodeficiencies, receiving intrauterine or large-volume transfusions (i.e., double exchange transfusions), or receiving blood products from a first-degree relative. Thus these are all absolute indications for irradiating blood products (Strauss, 2000).

When making platelet transfusion decisions, it is important for neonatologists to be aware of the risks associated with these transfusions. In the case of platelet suspensions, the risk of bacterial contamination is higher than the combined risk of all viral infections for which platelets are routinely tested. This is because platelet suspensions are stored in the blood bank at room temperature for up to 5 days, which increases the risk of bacterial growth. In addition, platelet transfusions can induce transfusion-associated lung injury (TRALI), a process characterized by the onset of hypoxemia and bilateral pulmonary infiltrates within 6 hours of a transfusion (Goldman et al., 2005). Given that neonates have frequent episodes of respiratory decompensation due to variable causes, TRALI is likely to be under-recognized in the NICU. Several publications have also shown a strong association between the number of platelet transfusions and the mortality rate among NICU patients (Del Vecchio et al., 2001; Garcia et al., 2001; Baer et al., 2007; Stanworth et al., 2009a). It is unclear from these studies whether this association simply reflects sicker patients receiving more platelets or whether platelet transfusions adversely affect outcomes. Nevertheless, while we await data from well-designed randomized controlled studies (the ongoing PlaNeT-2 trial is comparing 25 vs $50 \times 10^9/L$ as platelet transfusion thresholds for neonates) (Curley et al., 2014), neonatologists should undertake platelet transfusions thoughtfully, carefully balancing the risks and benefits in each individual patient.

Alternative Tests to Guide Platelet Transfusions

While, currently, platelet transfusions are administered on the basis of platelet counts or platelet mass (platelet count \times platelet volume), the evidence strongly suggests that factors other than the degree of thrombocytopenia determine the bleeding risk. Thus larger studies are needed to better characterize the platelet function and the hemostatic profile of preterm infants and their changes over time and in response to illness. Along these lines, it will also be important to develop and validate tests that can be used in preterm infants and that incorporate both platelet count and function to evaluate hemostasis, rather than individual platelet factors alone.

The PFA-100 is an *in vitro* test of primary hemostasis that provides a quantitative measurement of platelet adhesion, activation, and aggregation in whole blood (Kundu et al., 1995). As PFA-100 CTs represent global measurements of primary hemostasis, they are particularly attractive in neonates, since many factors contribute to their finely balanced hemostatic system. A recent pilot study measuring PFA-100 CTs in thrombocytopenic neonates of various gestational and postnatal ages showed that closure time-adenosine diphosphate (CT-ADP) was a better marker for evaluating neonatal platelet function than closure time-epinephrine (CT-Epi) and identified a threshold effect of platelet counts on CT-ADP: all

infants with platelet counts greater than $90 \times 10^9/L$ had normal CT-ADP, and most infants with platelet counts less than $90 \times 10^9/L$ had normal or minimally prolonged CT-ADP, but a few exhibited significant prolongations (Deschmann et al., 2014). It is unknown whether a prolonged CT-ADP is a better predictor of bleeding risk than the platelet count alone.

Nontransfusal Therapies

While severe thrombocytopenia resolves within 14 days in 80% of affected neonates, in approximately 10% it persists for greater than 30 days (Murray et al., 2002), resulting in multiple platelet transfusions (>20) (Dohner et al., 2009). In 2008, two TPO mimetics, romiplostim and eltrombopag, were approved by the US Food and Drug Administration (FDA) for the treatment of adults with chronic immune thrombocytopenic purpura, and, more recently, eltrombopag was approved for use in other varieties of chronic thrombocytopenia (i.e., chronic hepatitis C, aplastic anemia). In 2015 (Grainger et al., 2015), eltrombopag was approved by the FDA for use in children with symptomatic chronic immune thrombocytopenia. Recent publications have reported the use of eltrombopag in adult and pediatric patients with various hematologic disorders, including inherited thrombocytopenia associated with *MYH-9* mutations (Pecci et al., 2010; Favier et al., 2013), Wiskott–Aldrich syndrome (Gerrits et al., 2015), and aplastic anemia (Olness et al., 2012; Desmond et al., 2014). Thus it is plausible that eltrombopag would also be considered as a therapeutic alternative in neonates, infants, and children in the first year of life with different varieties of thrombocytopenia. Both agents, romiplostim and eltrombopag, begin to raise platelet counts 4 to 6 days after the initiation of the treatment and reach peak platelet counts at 10 to 14 days. Because of these pharmacodynamic characteristics, they would be only appropriate for selected infants with thrombocytopenia severe enough to warrant treatment and expected to last longer than 10 to 14 days. Difficulties in predicting the duration of thrombocytopenia in neonates have hampered the use of any TPO mimetics in neonates and infants less than 12 months old, which so far has been limited to sporadic reports (Christensen et al., 2013). It is likely that only a very small subset of thrombocytopenic neonates would be appropriate candidates for treatment with these medications.

Platelet Function Disorders

Etiology/Pathophysiology

Platelets play a major role in hemostasis, both by supporting the cellular structure for the primary platelet plug and also by providing a phospholipid surface on which the plasma elements involved in coagulation can bind. Thus a decrease in the platelet count and/or poor platelet function can result in bleeding symptoms. Platelet function disorders can be broadly categorized as congenital or acquired.

Most platelet function defects seen in the NICU are acquired and can be due to medications, medical conditions, or medical interventions. The list of medications that can affect platelet function is extensive, but the most common medications resulting in platelet dysfunction include aspirin and other nonsteroidal antiinflammatory drugs (indomethacin and ibuprofen), prostacyclin, certain anti-convulsants (valproic acid in particular), and some antibiotics (beta-lactams). Among the medical disorders associated with platelet dysfunction, the most common and best described is uremia.

Although the exact mechanism by which the platelets are affected is not clear, a prevailing theory is that the accumulation of certain substances associated with uremia disrupts the platelet phospholipid surface. Finally, certain medical procedures are associated with platelet dysfunction, with the most common being the use of extracorporeal circuits (extracorporeal membrane oxygenation, cardiopulmonary bypass, and hemodialysis). Therapeutic hypothermia, currently standard of care for infants with hypoxic–ischemic encephalopathy, also is associated with transient platelet dysfunction (Christensen et al., 2012).

Congenital platelet function disorders occur as a result of defects or deficiencies in any of a multitude of components (functional, structural, and regulatory proteins) required for normal platelet function. The most severe platelet function defects, which can present in neonatal life, result from deficiency or absence in the GPs located on the platelet surface: Bernard–Soulier syndrome, caused by deficiency of GPIb (the vWF receptor), and Glanzmann thrombasthenia, caused by a deficiency of GPIIb/IIIa, the fibrinogen receptor (Nurden and Nurden, 2008). Both have been reported to present with bleeding in neonatal life, although they most frequently present later in childhood.

Most of the other inherited platelet function defects are mild and very rarely present in the newborn period. Secretory platelet disorders have defective platelet granules and cause mild to moderate bleeding. These include δ -storage pool defects (including the common ADP secretion defect and the less common absence of dense bodies associated with Hermansky–Pudlak syndrome) and α -granule defects (gray platelet syndrome). Over the last decade, the availability of better genetic tools has led to the identification of the genetic causes of many of these platelet functional defects (i.e., mutations in *NBEAL2* cause gray platelet syndrome) and of novel mutations associated with platelet dysfunction and bleeding. A thorough review of these disorders is outside of the scope of this chapter, particularly because the degree of bleeding associated with most of these conditions rarely manifests in the neonatal period.

Clinical Presentation

Patients with platelet function disorders present with bleeding signs similar to thrombocytopenia, including mucocutaneous bleeding (nose, mouth, gastrointestinal tract, genitourinary tract) and bruising. The extent, location, and nature of the bruises are generally related to birth trauma and invasive procedures. Bernard–Soulier syndrome patients present with mild thrombocytopenia, giant platelets on the blood smear (macrothrombocytopenia), and mucosal-type bleeding caused by platelet dysfunction. Patients with Bernard–Soulier syndrome have been reported to present as neonates with gastrointestinal bleeding, bleeding after circumcision, and bleeding after cardiac catheterization in a patient with DiGeorge syndrome (Lopez et al., 1998). Glanzmann thrombasthenia is a rare autosomal recessive platelet function disorder, caused by a deficiency or abnormality of GPIIb/IIIa expression on the platelet surface. In neonates, the most common manifestations of Glanzmann thrombasthenia are generalized purpura or bleeding after circumcision, although more serious hemorrhages have also been described (George et al., 1990).

Diagnosis

The diagnosis of platelet function disorders in neonates is problematic, because many of the traditional tests require a large amount of blood (platelet aggregation studies) or lack reference values for

neonates. The traditional assay of platelet function is platelet aggregometry. This assay uses a set concentration of platelet-rich plasma and assesses platelet aggregation via light transmission after addition of a variety of platelet agonists (ADP, epinephrine, ristocetin, arachidonic acid, collagen, and thrombin-related activation peptide). Neonates have reduced platelet aggregation compared with older children and adults (based on cord blood values), and therefore the interpretation of this test in neonates is difficult. The major limitation of platelet aggregometry in neonates has been the large amount of blood required (20–30 mL, depending on the platelet count and how many agonists will be tested). Recently, whole blood aggregometry assays have been developed and are becoming increasingly available. These assays require significantly less blood than traditional platelet aggregometry, thus making them accessible to neonates. However, the lack of neonatal reference ranges has hampered the wider use of aggregometry in this age group.

There are two other tests that can screen for platelet function disorders; however, both have significant limitations. The original screening test is the BT, a test that is difficult to perform, particularly in neonates, and is rarely offered clinically. The PFA-100 is a widely used laboratory assay available in most coagulation laboratories and is a useful screening assay to evaluate for disorders of primary hemostasis. Normal ranges for the PFA-100 have been established in cord blood (Israels, 2009) and more recently in neonatal blood (Saxonhouse et al., 2010). The blood volume required for this test is much less than for aggregometry, and thus it can be used in neonates. Patients with severe platelet function defects, such as Bernard–Soulier syndrome and Glanzmann thrombasthenia, typically have significantly abnormal results. The PFA-100 is also often abnormal in the milder disorders such as the common ADP secretion defects; however, its sensitivity for these disorders is not sufficient to allow such defects to be ruled out if the results are normal. The PFA-100 is also abnormal in patients on aspirin and clopidogrel and ticlopidine (the collagen/ADP cartridge). Neonates on indomethacin, ibuprofen, or certain antibiotics can also have a prolonged PFA-100. The effects of other medications known to affect platelet function are not clear. Thus the PFA-100 is a useful screen for the platelet function defects. However, it cannot be performed when the patient is on certain medications, and it cannot rule out milder platelet function defects.

In general, it is reasonable to start with a complete blood count and a review of the peripheral blood smear. The combination of mild thrombocytopenia and large platelets (macrothrombocytopenia) is suggestive of Bernard–Soulier syndrome, which can be confirmed using flow cytometry to evaluate for the presence of GPIb on the platelet surface. Similarly, GPIIb/IIIa surface expression can be assessed to evaluate for Glanzmann thrombasthenia. This evaluation can be done in a small blood volume and is accurate in neonatal life. Some platelet function defects also lead to easily identifiable ultrastructural changes in the platelets that can be visualized by electron microscopy. In particular, a deficiency or absence of dense bodies (δ -storage pool deficiency) or α -granules (gray platelet syndrome) can be demonstrated by electron microscopy.

More recently, a high-throughput sequencing platform targeting 63 genes relevant for bleeding and platelet disorders was generated, which allows the efficient molecular diagnosis of bleeding and platelet disorders, avoiding the need for multiple sequential tests to narrow the diagnostic possibilities. This DNA-based diagnostic platform will probably become the new approach to patients with suspected inherited platelet function disorders (Simeoni et al., 2016).

Treatment

The management of congenital platelet function defects relies on several medications and platelet transfusions in dire situations when the medications or local measures are ineffective. In acquired conditions, reversal of the condition that led to the platelet dysfunction will reverse the platelet defect, but this is not always possible. In such situations, the approach to management of bleeding is mostly based on platelet transfusions.

Several medications have nonspecific mechanisms whereby they can enhance hemostasis when platelet function is abnormal: these include desmopressin, antifibrinolytic agents, and recombinant activated factor VIIa (rFVIIa). Desmopressin has been demonstrated to improve platelet function in many congenital disorders, in uremia, and during cardiopulmonary bypass. However, desmopressin is generally not used in children less than 2 years of age, because it can lead to vasodilatation, resulting in reductions in blood pressure sufficient to lead to clinical signs, but mostly because it is an analogue of antidiuretic hormone and can induce hyponatremic seizures. Neonates are at a particularly high risk for this complication; thus desmopressin is not recommended in this age group.

rFVIIa was developed for the management of bleeding in patients with hemophilia and inhibitors; however, it has been shown to also be effective for managing severe bleeding in patients with severe platelet function defects. It is licensed in Europe for the management of bleeding in patients with Glanzmann thrombasthenia who are refractory to platelet transfusions. The major risk of rFVIIa is the risk of thrombosis. Thus it is suggested that rFVIIa be used only for patients with severe bleeding in whom standard therapeutic measures have failed. The use of this agent in neonates has been reported, and it appears that neonates are at increased risk for thrombosis from this agent compared with older children (Young et al., 2009). There is no consensus, however, since another study evaluating the risk of thrombotic events associated with rFVIIa use in neonates showed a prevalence of 7.5% in bleeding and/or coagulopathic neonates, although this prevalence was similar in those neonates who received fresh frozen plasma (Puetz et al., 2009). Antifibrinolytic agents might also be helpful, particularly later in life, for epistaxis and menorrhagia.

For severe bleeding that has not responded to the measures just described, a platelet transfusion should be given in order to provide normally functioning platelets. Although most platelet function defects are mild enough that this will never be required, for the

more severe disorders such as Bernard–Soulier syndrome and Glanzmann thrombasthenia, a platelet transfusion may be lifesaving. The risks associated with a platelet transfusion are no different from those for other patients, with one important exception: patients with Bernard–Soulier syndrome and Glanzmann thrombasthenia are at risk for alloimmunization, resulting in the formation of antibodies to GPIb and GPIIb/IIIa, respectively. Once these antibodies develop, future platelet transfusions are likely to be ineffective. Thus it is imperative to withhold platelet transfusions for these patients except in life-threatening hemorrhage, because it may be possible to use this therapy only once in a patient's lifetime. Finally, local measures are extremely important in the management of bleeding.

Suggested Readings

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Neonatal Erythrocyte Disorders

ROBERT D. CHRISTENSEN

KEY POINTS

- Anemia and polycythemia are defined during the neonatal period in accordance with reference intervals appropriate for gestational and postnatal age. Anemia corresponds to a hemoglobin or hematocrit value below the fifth percentile lower reference interval, and polycythemia corresponds to a value above the 95th percentile (sometimes the 97.5th percentile value is used) upper reference interval.
- Anemia in a fetus or neonate can be categorized kinetically as the result of erythropoietic failure, hemorrhage, or hemolysis.
- Polycythemia in a fetus or neonate can be categorized kinetically as the result of increased erythropoiesis in utero (accompanying fetal hypoxia), intravascular fluid contraction, or hypertransfusion.
- Adopting guidelines for preventing and treating neonatal anemia can bring consistency, foster quality improvement research, and reduce costs.

Anemia corresponds to a pathologically low hematocrit (or blood hemoglobin concentration or circulating erythrocyte count), and polycythemia corresponds to a pathologically high value ([Henry and Christensen, 2015a](#)). By convention, anemia is diagnosed in a neonate when the hematocrit, hemoglobin level, or red blood cell (RBC) count falls below the fifth percentile lower reference interval for gestational and postnatal age. In like manner, polycythemia is diagnosed in a neonate when these measures are above the 95th percentile (or 97.5th percentile) upper reference interval. To review this topic, this chapter has been divided into four parts: (1) the reference intervals with which neonatal anemia and neonatal polycythemia are diagnosed, (2) neonatal anemia, (3) neonatal polycythemia, and (4) treatment considerations.

Reference Intervals

Maxwell M. Wintrobe is sometimes referred to as the father of clinical hematology ([Christensen et al., 2016b](#)). Many of his discoveries and inventions in the 1930s and 1940s remain central to modern hematology practice. They include the hematocrit and the RBC indices (mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC]). When asked about these, Wintrobe explained his motivation: “I discovered that there were no

reliable normal blood values. What was called ‘normal’ was based on only a few counts that had been made in the nineteenth century. So I proceeded to collect normal blood values. Others elsewhere, also mindful of this deficiency, were beginning to do the same.”

The need for accurate normal ranges exists in modern neonatal hematology. Unlike the case in adult hematology, “reference intervals” are used in neonatology in place of the “normal ranges.” The difference is that reference intervals are constructed from selected clinically performed laboratory tests, not from tests performed on healthy volunteers. To approximate a normal range, the laboratory test results included in a neonatal reference interval data set include results only from patients with minimal disease or with disease not known to be related to the test under consideration ([Henry and Christensen, 2015a](#)). For instance, reference intervals for the hematocrit exclude data from neonates with clinical issues known to affect the hematocrit, such as recipients of RBC transfusions, those with a diagnosis of hemolytic disease, or those who have a diagnosis of anemia or polycythemia.

Reference intervals for hematocrit and hemoglobin level of neonates on the day of birth are shown in [Fig. 81.1](#). Reference intervals for hematocrit and hemoglobin level during the first 28 days after birth are shown in [Fig. 81.2](#). The fall in hemoglobin level and hematocrit during the first few days after birth occurs too rapidly to be explained by reduced RBC production alone and suggests an element of transient, mild, but active hemolysis. The theory of a physiologic hemolysis during the first few days after birth is supported by elevated exhaled carbon monoxide (CO) concentrations during the first week after birth ([Tidmarsh et al., 2014](#)). CO is released when heme is metabolized to bilirubin, and thus the higher exhaled CO levels initially suggest more rapid heme degradation during the first few days ([Christensen et al., 2015b, 2016c](#)). The mechanism responsible for this mild, transient, physiologic hemolysis in neonates and its purposes in nature are under investigation ([Song et al., 2015](#)).

Erythrocyte indices can help categorize anemias. The average size of erythrocytes is reflected by the MCV (measured in femtoliters), and the average amount of hemoglobin per erythrocyte is reflected by the MCH (measured in pictograms). [Fig. 81.3](#) shows the reference intervals for the MCV and MCH on the day of birth according to gestational age ([Christensen et al., 2008](#)). Unlike the MCV and MCH, the concentration of hemoglobin in RBCs (the MCHC) does not change during gestation.

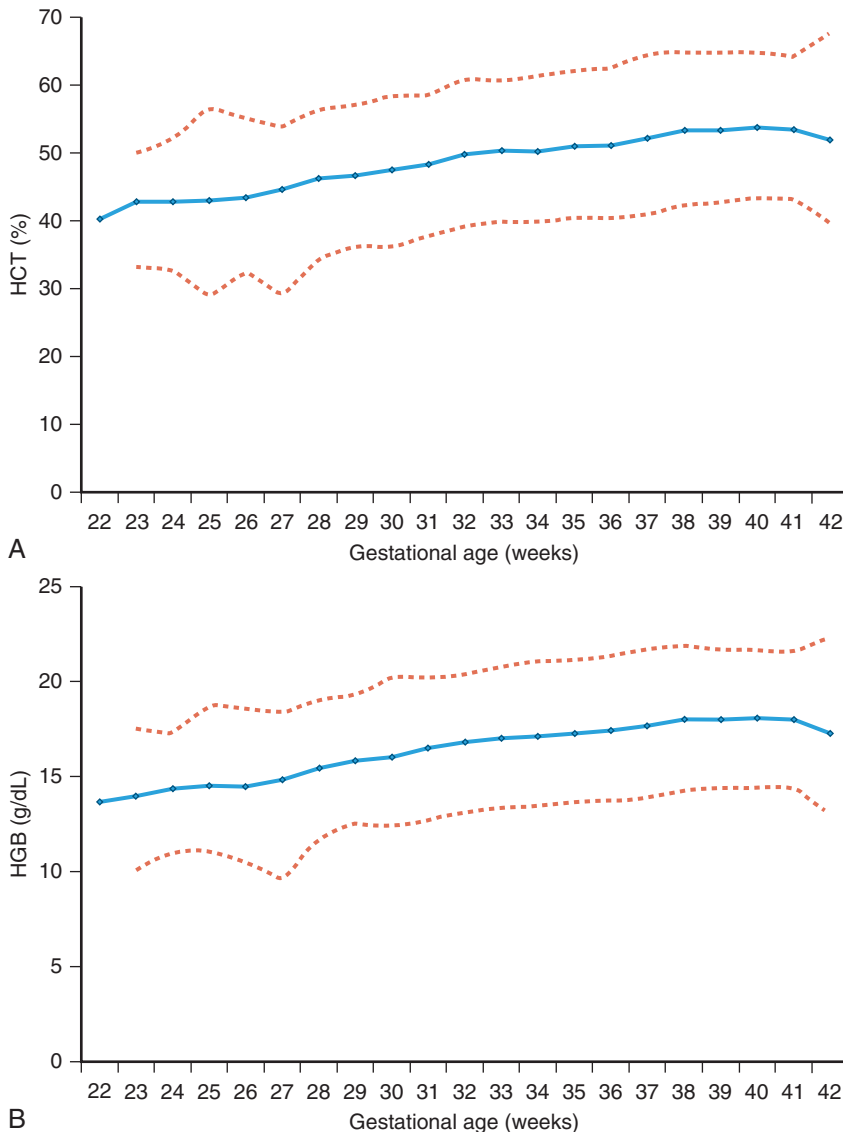
RBC morphology of neonates is characterized by anisocytosis, variation in erythrocyte size (when compared with blood films of adults), and by poikilocytosis, variation in erythrocyte shape. Anisocytosis can be quantified by the RBC distribution width (RDW; %), such that the higher the RDW, the more anisocytosis is present. Reference intervals for RDW during the neonatal period are shown in Fig. 81.4. The poikilocytosis of neonates includes the presence of a few target cells and stomatocytes and schistocytes (Christensen et al., 2014e, 2015d).

Reticulocyte counts and reticulocyte parameters can also be helpful in categorizing anemias (Christensen et al., 2016a). Neonatal reference ranges for the absolute reticulocyte count, the immature reticulocyte fraction, and the reticulocyte hemoglobin content are shown in Fig. 81.5.

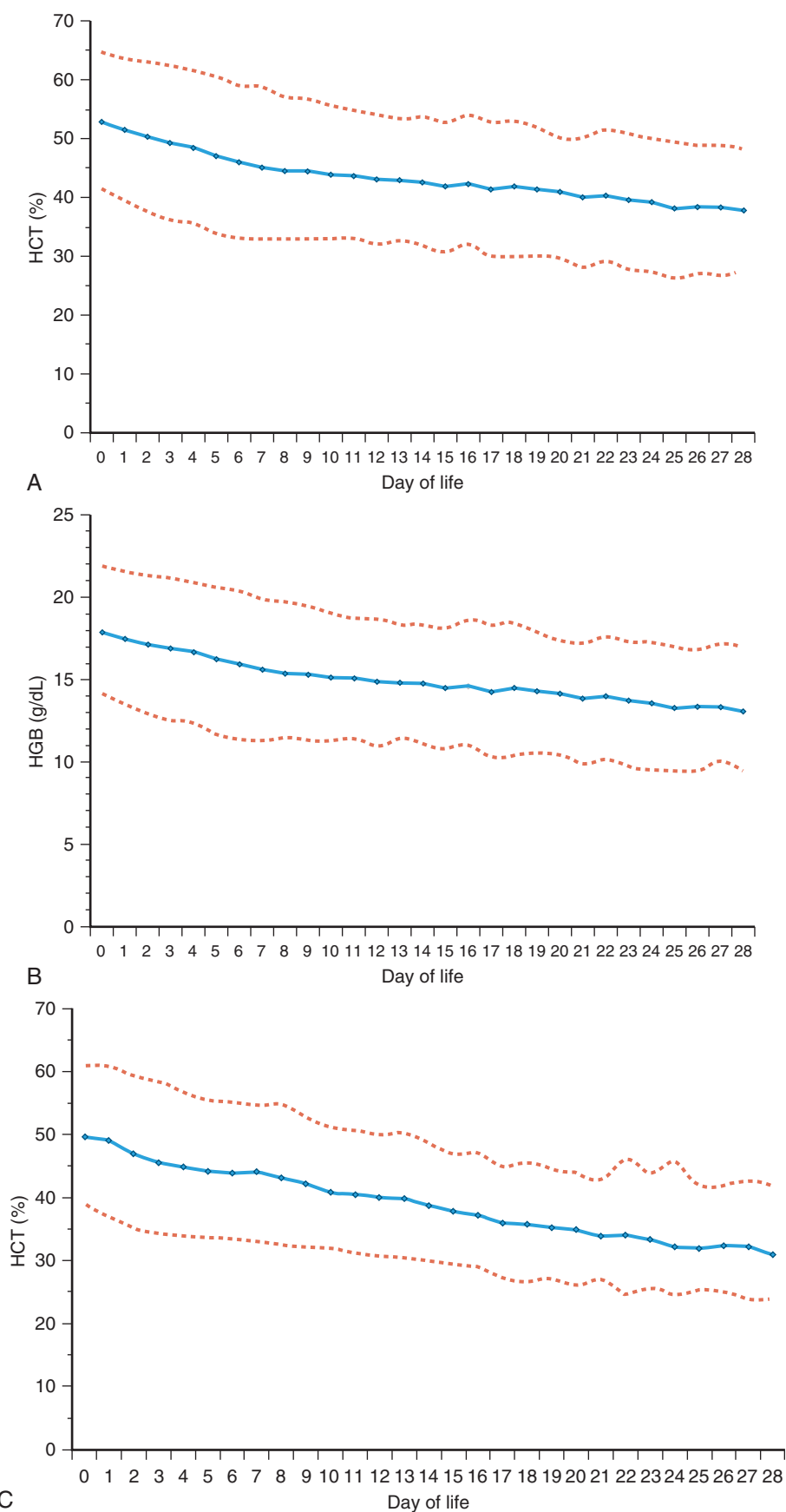
Nucleated RBCs are seen regularly on blood smears during the first day of life but are uncommonly seen in the circulation after day 3, unless intermittent or chronic hypoxemia is present (Christensen et al., 2011b). Reference intervals for the number of nucleated RBCs per microliter and reference intervals for the number of nucleated RBCs per 100 leukocytes according to gestational age are shown in Fig. 81.6.

Marrow cellularity in the fetus is relatively high. Erythroid precursors account for 30%–65% of nucleated marrow cells at birth, and myeloid cells account for 45%–75% (Gairdner, 1952). The myeloid-to-erythroid ratio at birth is approximately 1.5:1. Marrow cellularity decreases after birth, attaining a density that is normal for adults by 1 to 3 months. Initially, this decrease in cellularity results from a rapid decline in RBC production. At 1 week of age, erythroid elements account for only 8%–12% of nucleated cells, and the myeloid-to-erythroid ratio exceeds 6:1. The normal adult proportion of myeloid to erythroid precursors is not established until the third month. Both the percentage and the absolute number of lymphocytes increase during the first 2 months, such that by 3 months of age they constitute nearly 50% of marrow nucleated cells. Differential counts of bone marrow aspirates from preterm infants are similar to those of term infants (Christensen, 2000).

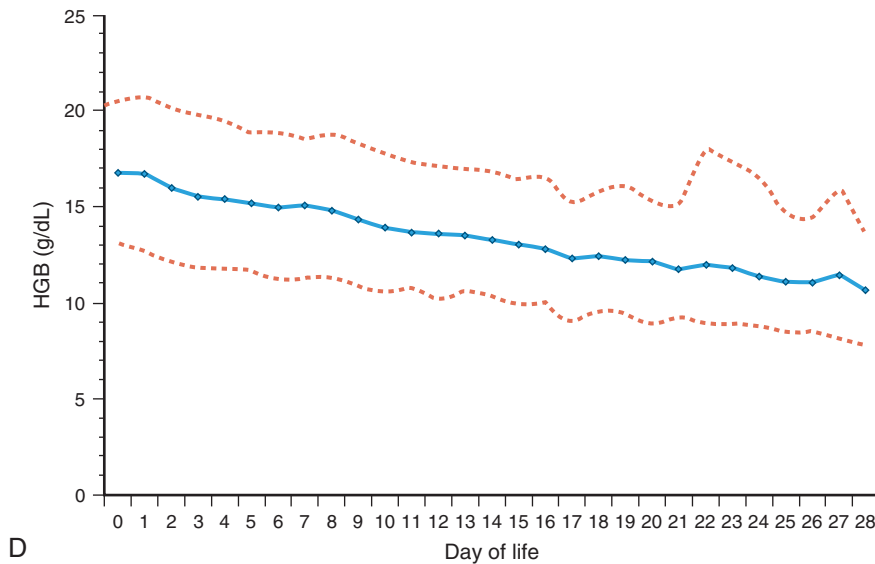
In newborns the hemoglobin concentration and hematocrit of capillary blood are 5%–10% higher than those of venous blood (Oettinger and Mills, 1949). The difference between capillary and venous values is greatest at birth and disappears by about 3 months of age. The discrepancy is greatest in preterm infants and



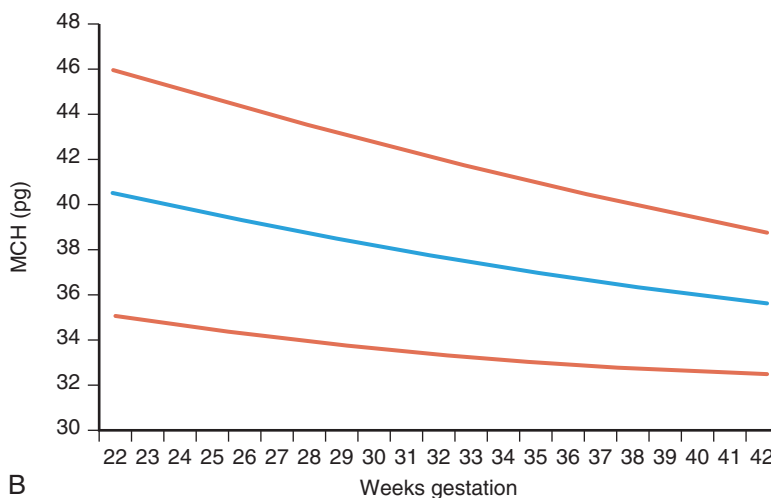
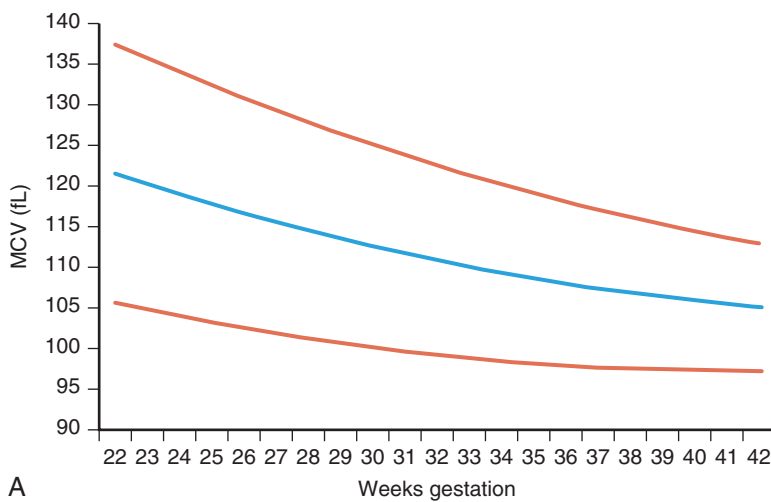
• **Fig. 81.1** Reference intervals for hematocrit (A) and blood hemoglobin concentration (B) on the day of birth. Values are given as a function of gestational age. Dashed lines indicate 5th and 95th percentile reference interval. Solid line indicates mean. *HCT*, Hematocrit; *HGB*, hemoglobin. (From Jopling J, Henry E, Wiedmeier SE, Christensen RD. Reference ranges for hematocrit and blood hemoglobin concentration during the neonatal period: data from a multihospital health care system. *Pediatrics*. 2009;123:e333–e337.)



• **Fig. 81.2** Reference intervals for hematocrit (HCT) and blood hemoglobin (HGB) concentration during the first month after birth: (A) HCT from late preterm and term neonates; (B) HGB concentration from late preterm and term neonates; (C) HCT from preterm neonates.

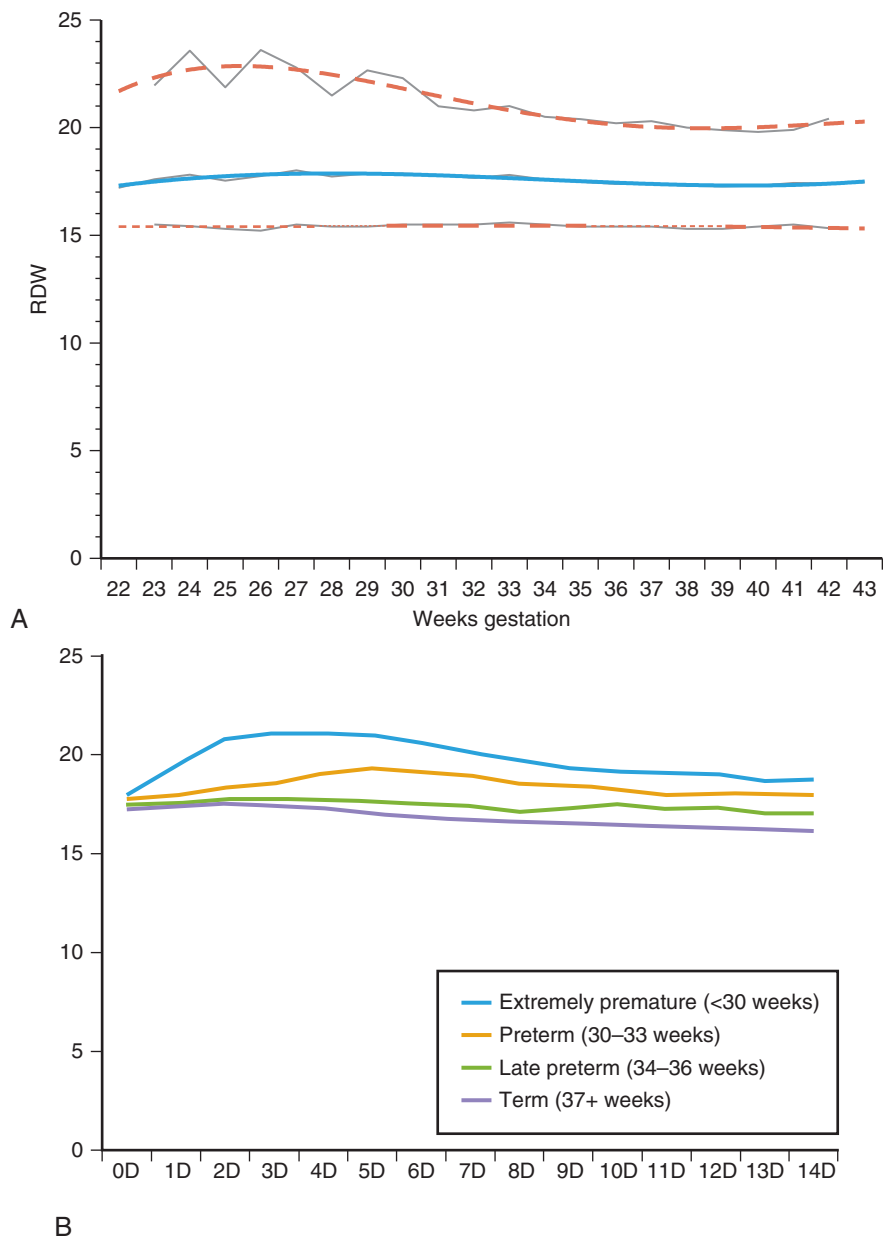


• **Fig. 81.2, cont'd (D)** HGB concentration from preterm infants. Dashed lines indicate 5th and 95th percentile reference interval. Solid line indicates mean. (From Jopling J, Henry E, Wiedmeier SE, Christensen RD. Reference ranges for hematocrit and blood hemoglobin concentration during the neonatal period: data from a multihospital health care system. *Pediatrics*. 2009;123:e333–e337.)



• **Fig. 81.3** Reference intervals for mean corpuscular volume (A) and mean corpuscular hemoglobin (B) on the day of birth according to gestational age. The *lower line* shows the fifth percentile lower reference interval limit, the *middle line* shows the median values, and the *upper line* shows the 95th percentile upper reference interval limit. *MCH*, Mean corpuscular hemoglobin; *MCV*, mean corpuscular volume. (From Christensen RD, Jopling J, Henry E, Wiedmeier SE. The erythrocyte indices of neonates, defined using data from over 12,000 patients in a multihospital health care system. *J Perinatol*. 2008;28:24–28.)

• **Fig. 81.4** Reference intervals for red blood cell distribution width (RDW) of neonates: (A) RDW on the day of birth, shown by weeks of gestation; (B) RDW over the first 14 days after birth, divided into four gestational-age groups. *D*, Day(s). (From Christensen RD, Yaish HM, Henry E, Bennett ST. Red blood cell distribution width: reference intervals for neonates. *J Matern Fetal Neonatal Med.* 2015;28:883–888)



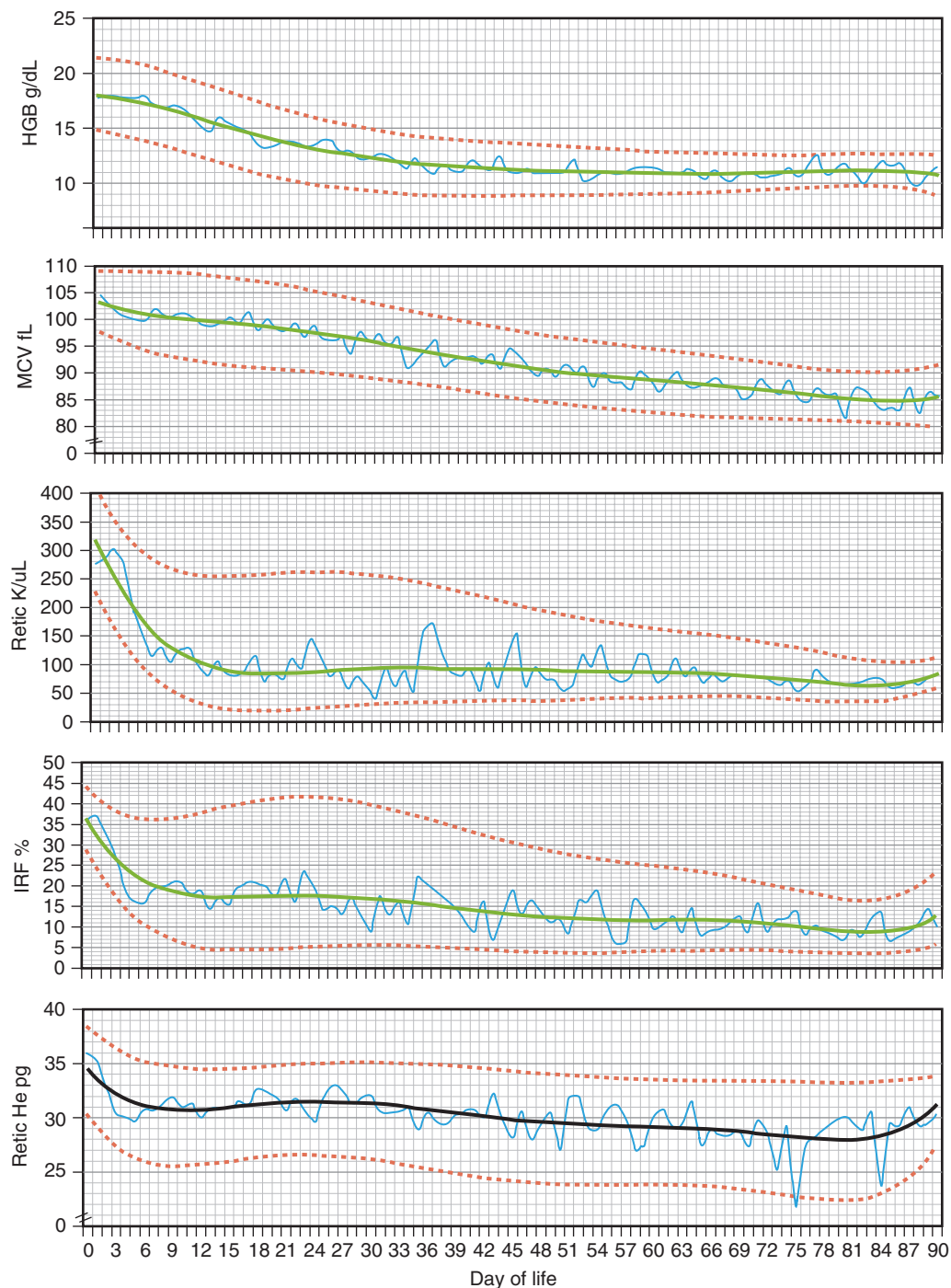
in those with hypotension, hypovolemia, and acidosis (Linderkamp, 1977).

Measuring the circulating RBC volume in a fetus or neonate is challenging. Mock et al. (2001) used a nonradioactive method based on in vivo dilution of biotinylated RBCs enumerated by flow cytometry to estimate the correlation between hematocrit and circulating RBC volume in infants weighing less than 1300 g. They found that venous hematocrit values correlated highly with the circulating erythrocyte volume ($r = 0.907$; $P < .0001$) (Strauss et al., 2003).

Neonates have a shorter RBC survival than do children and adults. The life span of RBCs from term infants is estimated to be 60 to 80 days with use of the ^{51}Cr method (Pearson and Vertrees, 1961) and 45 to 70 days with use of methods involving ^{59}Fe (Pearson, 1967). Fetal studies using ^{14}C cyanate-labeled RBCs in sheep revealed an average RBC life span of 64 ± 6 days (Brace et al., 2000). Brace et al. found that the mean RBC life span

increased linearly from 35 to 107 days as the fetal age increased from 97 days (midgestation) to 136 days (term). In a similar fashion, neonatal RBCs transfused into adults have a short survival (Pearson, 1967), indicating that factors intrinsic to the newborn RBC are responsible. This conclusion has gained further support by the demonstration that adult RBCs survive normally in newborn recipients (Brace et al., 2000). The life span frequency function is not parametrically distributed, in that most cells are destroyed before the mean survival is reached. Shortened RBC survival corresponds with erythropoietic rates at birth that are three to five times greater than those of normal adults.

At term the placenta and umbilical cord contain 75 to 125 mL of blood (30 to 40 mL/kg), or approximately one-fourth to one-third of the fetal blood volume. Umbilical arteries typically constrict shortly after birth, but the umbilical vein can remain dilated. Early studies suggested that newborns held below the level of the placenta can receive half of the placental blood volume (30 to 50 mL, or



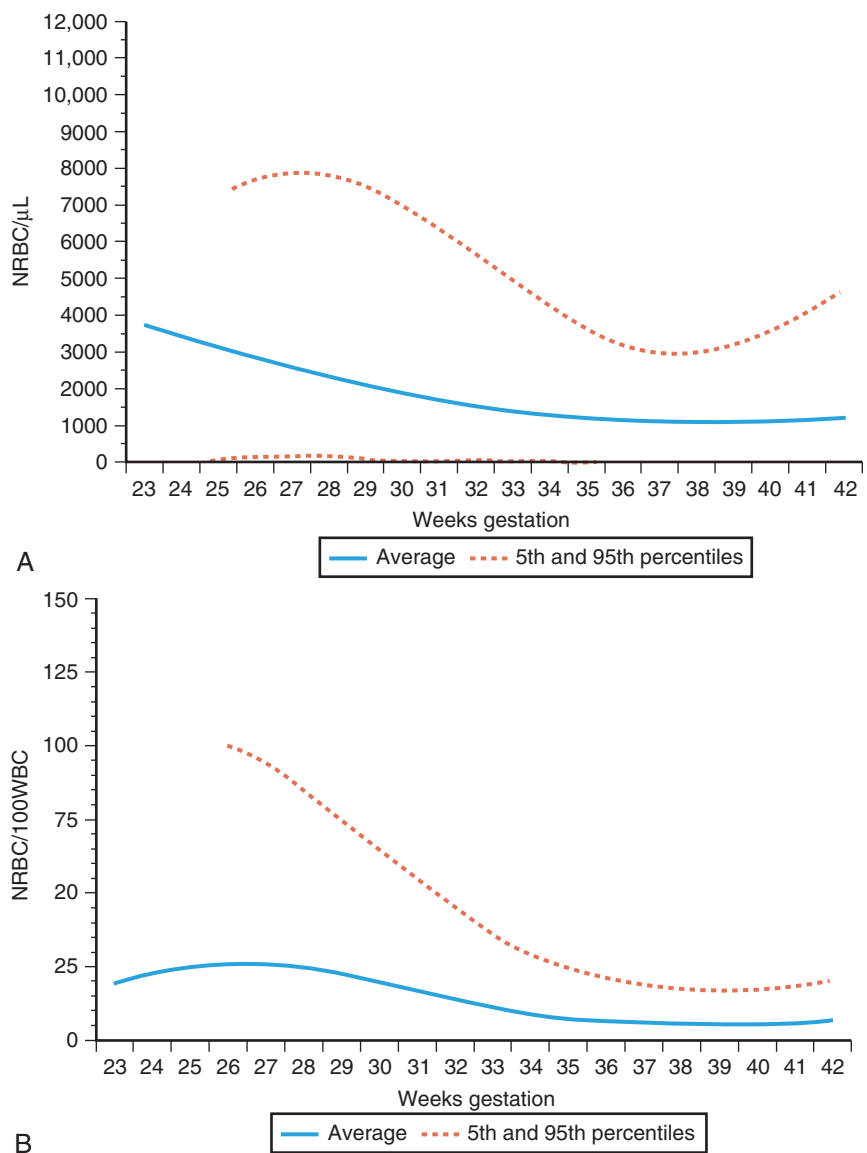
• **Fig. 81.5** Reference intervals for absolute reticulocyte count, immature reticulocyte fraction, and reticulocyte hemoglobin content during the first 90 days after birth. Dashed lines indicate 5th and 95th percentile reference interval. Solid line indicates mean. *HGB*, Hemoglobin; *IRF*, immature reticulocyte fraction; *Retic K*, absolute reticulocyte count; *Retic He*, reticulocyte hemoglobin; *MCV*, mean corpuscular volume. (From Christensen RD, Henry E, Bennett ST, Yaish HM. Reference intervals for reticulocyte parameters of infants during their first 90 days after birth. *J Perinatol*. 2016;36:61–66.)

10 to 15 mL/kg) in 1 minute. Some studies showed that infants held above the placenta can lose 20 to 30 mL of blood back into the placenta per minute (Linderkamp, 1978; Linderkamp et al., 1992), but a more recent study from Argentina refutes this, reporting no effect of position (neonate held at the level of the introitus compared with the mother's abdomen or chest) on the volume of

placental transfusion (Vain et al., 2014). The blood volume of infants with early cord clamping averages 72 mL/kg, whereas the blood volume of infants with cord clamping delayed for 1 minute averages 93 mL/kg.

Linderkamp et al. compared postnatal alterations in blood viscosity, hematocrit, plasma viscosity, RBC aggregation, and

• **Fig. 81.6** Reference intervals for blood concentrations of nucleated red blood cells (NRBCs) on the day of birth according to gestational age: (A) data expressed as number of NRBCs per microliter of blood; (B) data expressed as number of NRBCs per 100 white blood cells. The *lower line* represents the fifth percentile limit, the *upper line* represents the and 95th percentile limit, and the *middle line* represents the median value. WBC, White blood cells. (From Christensen RD, Henry E, Andres RL, Bennett ST. Neonatal reference ranges for blood concentrations of nucleated red blood cells. *Neonatology*. 2010;99:289–294.)



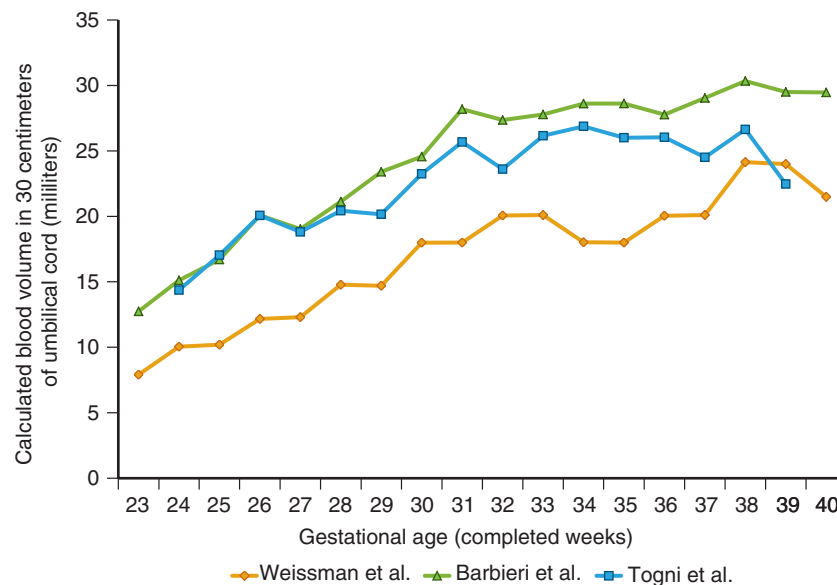
RBC deformability in the first 5 days of postnatal life in term neonates with early (less than 10 seconds) and late (3 minutes) cord clamping (Linderkamp, 1978; Linderkamp et al., 1992). The residual placental blood volume decreased from 52 ± 8 mL/kg neonatal body weight after early cord clamping to 15 ± 4 mL/kg after clamping. The neonatal blood volume was 50% higher in the infants with late cord clamping. Other investigations showed a transfer of about 15 mL/kg body weight by delaying clamping for 60 to 90 seconds (Fig. 81.7) and that this practice reduces intraventricular hemorrhage and late-onset sepsis (Aladangady et al., 2006; Mercer et al., 2006; Strauss et al., 2008). For very low birth weight (VLBW; <1500 g) deliveries, delayed cord clamping or the somewhat more rapid alternative of umbilical cord milking can result in a higher 24-hour hemoglobin concentration and hematocrit and can lower the risk of hypotension, early RBC transfusion, and intraventricular hemorrhage (Rabe et al., 2004, 2009; Carroll and Christensen, 2015). Moreover, drawing all of the needed initial blood studies of VLBW neonates on their admission to the neonatal intensive care unit (NICU) from otherwise discarded blood in the

umbilical cord after placental delivery can result in a higher 24-hour hemoglobin concentration and hematocrit and can lower the risk of initial hypotension (Christensen et al., 2011c, 2014c; Henry et al., 2015).

Neonatal Anemia

Anemia in a neonate can be categorized kinetically as due to either (1) RBC production failure or (2) RBC loss. RBC loss can be the result of bleeding (either occult or obvious blood loss) or hemolysis (Box 81.1). Rarely a neonate has anemia from mixed mechanisms, such as production failure and also bleeding, or disseminated intravascular coagulation (DIC) with both hemolysis and bleeding. However, to facilitate a clear presentation of each element of anemia pathogenesis in neonates, we will discuss the varieties of neonatal anemia using the simple kinetic categorization of production failure, hemorrhage, and hemolysis.

The cause of anemia in a neonate can frequently be suspected on the basis of the history and physical examination findings.



• **Fig. 81.7** In utero volume of blood contained within a 30-cm segment of umbilical cord at various gestational ages. (From Carroll PD, Christensen RD. New and underutilized uses of umbilical cord blood in neonatal care. *Matern Health Neonatol Perinatol*. 2015;1:16.)

• BOX 81.1 Categorization of Anemia in Neonates

Red Blood Cell Production Failure

Anemia of prematurity
Late anemia of Rh hemolytic disease
Neonatal iron deficiency
Diamond–Blackfan anemia and somewhat related conditions

Hemorrhage

Prenatal
Perinatal
Postnatal

Hemolysis

Immune-mediated diseases: Rh, ABO, Kell, Kidd, Duffy, MNS, maternal autoimmune
Nonimmune-mediated acquired diseases: cytomegalovirus infection, toxoplasmosis, syphilis, sepsis, disseminated intravascular coagulation
Erythrocyte cytoskeletal mutations: hereditary spherocytosis, hereditary elliptocytosis, hereditary pyropoikilocytosis
Erythrocyte enzyme abnormalities: glucose 6-phosphate dehydrogenase deficiency, pyruvate kinase deficiency, unstable hemoglobins, alpha and gamma thalassemia

Particular importance is given to family history (anemia, cholelithiasis, jaundice, splenomegaly), maternal medical history (especially infections), and obstetric history (previous pregnancies, length of gestation, difficulty of delivery). The age at which anemia becomes manifest is also of diagnostic importance. Significant anemia at birth is generally the result of blood loss or alloimmune hemolysis. If anemia first occurs after 24 hours, internal hemorrhages and other causes of hemolysis are likely. Anemia that first appears 1 week or more after birth can be caused by RBC enzyme deficiencies, abnormalities in RBC cytoskeletal proteins, hypoplastic RBC disorders, phlebotomy losses, the physiologic anemia of infancy, or anemia of prematurity.

Newborns with anemia from *chronic* intrauterine blood loss or from intrauterine hemolysis generally appear pale at birth but without other signs of distress. However, *acute* blood loss typically produces hypovolemic shock. Neonates with hemolytic anemia are typically jaundiced, while those with anemia caused by blood loss (fetal–maternal or twin–twin transfusion) are generally not jaundiced. In addition, hemolytic anemia is often accompanied by hepatosplenomegaly, and cases of anemia resulting from congenital infection can have other stigmata of infection such as thrombocytopenia and sometimes cutaneous hemorrhages.

Anemia Caused by Failure of Erythrocyte Production

A hyporegenerative erythropoietic state in a neonate can be transient or long lived. The transient varieties typically relate to hypoproduction of erythropoietin (Epo) or to deficiencies of micronutrients such as iron. The long-lived varieties are typically a result of genetic mutations. We will begin this section on RBC production failure with the transient varieties. These include the common “anemia of prematurity,” the “late” anemia following fetal hemolytic disease, and iron deficiency. Then we will review the long-lived congenital hyporegenerative anemias seen in neonates: Diamond–Blackfan anemia (DBA) and somewhat similar disorders.

Anemia of Prematurity

The abrupt transition from the relatively hypoxic uterus to the relatively oxygen-rich extrauterine environment triggers responses that have profound effects on erythropoiesis. During the first 2 months of life, an infant has both the highest (at birth) and the lowest (physiologic nadir) blood hemoglobin concentrations of its entire (healthy) life. Epo levels at birth are usually well above the normal adult reference range but fall rapidly in the first few days after birth, as oxygen delivery to tissues markedly increases (Kling, 2013). By 24 hours after (healthy) birth, the blood Epo

concentration falls below the normal adult range and remains low throughout the first month. The decrease in Epo level is followed by a decline in erythropoiesis, a fall in the reticulocyte count, and a subsequent lowering of the hemoglobin level/hematocrit.

In healthy term neonates the combination of initial physiologic hemolysis, a shortened cell survival, decreased RBC production, and somatic growth with expansion of the blood volume is responsible for a progressive fall of the blood hemoglobin level to a mean of approximately 11 g/dL at 2 months of age. The lower reference interval for infants of this age is approximately 9 g/dL. This nadir is called *physiologic anemia*, because it is not associated with distress and is not prevented with nutritional supplements. Stabilization of the blood hemoglobin concentration is heralded by an increase in the reticulocyte count at 4 to 8 weeks. Thereafter, the hemoglobin concentration rises to a mean level of 12.5 g/dL, at which it remains throughout infancy and early childhood (Kling, 2013).

In contrast to the situation in healthy term infants, those delivered before about 32 completed weeks of gestation typically develop a transient and unique anemia known as *anemia of prematurity*. During the first week or first 2 weeks after birth, while the neonate is in an intensive care unit, anemia secondary to phlebotomy commonly complicates anemia of prematurity. However, after the period of phlebotomy loss has passed and the neonate becomes basically an NICU “grower,” anemia of prematurity becomes manifest as a normocytic, normochromic, hyporegenerative condition, with serum Epo concentrations significantly below those found in adults with similar degrees of anemia (Brown et al., 1984). In its pure state this anemia is not responsive to the administration of iron, folate, or vitamin E, because it is not due to deficiency of those nutrients. Obviously iron deficiency can complicate anemia of prematurity, and both conditions can coexist.

Some preterm infants who develop anemia of prematurity are asymptomatic, whereas others have clear signs of anemia that are alleviated by erythrocyte transfusion. These signs include tachycardia, rapid tiring with nipple feedings, poor weight gain, increased requirements for supplemental oxygen, episodes of apnea and bradycardia, and elevated serum lactate concentrations.

The reason preterm infants do not significantly increase serum Epo concentration during this anemia is not known. Indeed, it is unclear whether production of Epo does in fact increase, but the serum concentration does not rise. Certainly neonates with anemia of prematurity have erythroid progenitors that are sensitive to recombinant Epo in vitro (Shannon et al., 1987; Rhondeau et al., 1988), and their circulating concentrations of other erythropoietic growth factors appear to be adequate (Ohls et al., 1990).

The molecular and cellular mechanisms responsible for anemia of prematurity remain undefined. Potential explanations include the transition from fetal to adult hemoglobin, shortened erythrocyte survival, and hemodilution associated with a rapidly increasing body mass. It is unknown whether preterm infants rely on Epo produced by the liver (the source of Epo in utero) or that produced by the kidney or a combination of the two.

The “Late” Anemia of Rhesus Hemolytic Disease

Neonates with rhesus (Rh) hemolytic disease typically have an early anemia due to hemolysis and sometimes then develop a late anemia due to erythrocyte production failure. This late anemia is usually severest about 1 month after birth, generally days to weeks after the neonate has been discharged home from the NICU (al-Alaiyan and al Omran, 1999). The incidence of late anemia is highest among the neonates with Rh hemolytic disease who received

intrauterine transfusions. This fact led to the speculation that infusing adult hemoglobin into a fetus in utero leads to a quick downregulation of fetal Epo production and that several weeks are required before this is repaired.

The late anemia is characterized by low serum Epo levels and low reticulocyte counts, but the erythroid progenitors are responsive to Epo in vitro (Koenig et al., 1989; Nicaise et al., 2002; Zuppa et al., 2012). Infants with this variety of anemia are sometimes transfused once, or sometimes two or three times, until the hyporegenerative condition eventually resolves. As an alternative to RBC transfusions, dosing with recombinant Epo or with darbepoetin can be attempted (Ohls et al., 1992; Nicaise et al., 2002; Zuppa et al., 2012). We find this approach far less expensive and more acceptable to parents. However, it is effective only when it is begun before the appearance of signs of anemia. If the hematocrit has fallen below about 20% and signs of anemia are obvious, transfusion is the only effective treatment. Darbepoetin can be given once per week or every other week for two or three doses, after which the problem typically and permanently remits.

Neonatal Iron Deficiency

Adequate iron availability is required for erythropoiesis and is also critical for normal prenatal and postnatal brain development (Rao and Georgieff, 2009). Iron deficiency retards an infant's long-term neurodevelopment, which can be irreversible despite later iron supplementation that corrects the iron deficiency (Fretham et al., 2011; Sani et al., 2011; Callahan et al., 2013; Tran et al., 2013). Anemia is a very *late* sign of iron deficiency. The steps toward iron-deficiency anemia are sometimes labeled as follows (Table 81.1): (1) *iron deficiency*, where biochemical measurements show that the levels of serum and storage iron are low, but measures of erythropoiesis are normal and anemia does not yet exist; (2) *iron-deficient erythropoiesis*, where biochemical measurements of the levels of iron and iron stores are low, and erythropoiesis is reduced but anemia does not yet exist; and (3) *iron-deficiency anemia*, with biochemical iron deficiency, limitations in erythropoiesis, and the presence of microcytic hypochromic anemia. Once iron-deficient erythropoiesis exists, the iron deficiency state has almost certainly begun to cause iron-deficient neurologic impediments, including reduced cognitive performance (Mukhopadhyay et al., 2011).

Iron deficiency in a neonate can be defined by a pattern of findings, including low serum iron concentration, low percent iron saturation, low serum ferritin concentration, low hepcidin concentration, elevated zinc protoporphyrin-to-heme ratio, and elevated soluble transferrin receptor concentration. We see this pattern with some regularity, perhaps 10% or more of cases, in neonates who are small for gestational age (SGA) or in infants of diabetic mothers. It is very rare to find a newborn, at birth, with iron-limited erythropoiesis or with iron-deficiency anemia. However, neonates who are even moderately iron deficient at birth can progress to having iron-limited erythropoiesis and then iron-deficiency anemia and iron-deficient neurocognitive delay.

Current iron supplementation guidelines from the American Academy of Pediatrics (AAP) for term infants include beginning iron supplements for breastfed infants at the age of 4 months and screening for iron deficiency at 12 months (Baker et al., 2010). For preterm infants (<37 weeks) the recommendation is to start iron supplementation by 1 month (2 mg/kg per day) and extend this through 12 months. Specific guidelines for iron dosing and iron-deficiency screening of neonates who are SGA infants of diabetic mothers (IDAs) are not included in that report.

TABLE 81.1 Diagnosis of Iron Deficiency in the Neonatal Period

| Severity | Serum and Storage Iron and Compensations | Reticulocyte Hemoglobin Content, Absolute Reticulocyte Count, Immature Reticulocyte Fraction | MCV, MCH, Hemoglobin Level, and Hematocrit |
|-------------------------------|--|--|---|
| Biochemical iron deficiency | Low serum iron level, percent saturation, ferritin level, hepcidin level High soluble transferrin receptor level, zinc protoporphyrin-to-heme ratio | Within the reference range for gestational and postnatal age | Within the reference range for gestational and postnatal age |
| Iron-deficient erythropoiesis | Same direction as above but the derangements are more pronounced | Below the fifth percentile lower reference interval for gestational and postnatal age | Within the reference range for gestational and postnatal age |
| Iron-deficiency anemia | Same direction as above but the derangements are more pronounced | Below the fifth percentile lower reference interval for gestational and postnatal age | Below the fifth percentile lower reference interval for gestational and postnatal age |

MCH, Mean corpuscular hemoglobin; MCV, mean corpuscular volume.

If 10% or more of SGA or IDM neonates are indeed iron deficient at birth, then over the next few weeks, with no supplemental iron, their iron stores will likely drop further. Perhaps some of these neonates drop their iron stores to the point of iron-limited erythropoiesis. Such a state could be accompanied by significant lack of iron availability to the developing central nervous system, imposing a risk of subsequent neurocognitive deficiencies. However, if iron-deficient neonates could be recognized and safely and effectively treated with iron before iron limitation impairs their erythropoiesis and their brain development, better long-term neurodevelopment might result.

At this point, larger studies are needed to identify neonates at risk of congenital iron deficiency and those likely to develop iron-limited erythropoiesis in the neonatal period. Such studies will provide the data needed to inform the design of definitive randomized trials where neonates with biochemical iron deficiency at birth are randomized to receive iron supplements either according to current guidelines or according to a personalized medicine approach. The primary outcome of such studies would be neurocognitive outcomes.

Diamond–Blackfan Anemia and Somewhat Related Conditions

DBA is a hypoplasia of RBCs characterized by abnormally reduced levels of erythroid precursors in the marrow (Narla et al., 2011; Vlachos et al., 2014). It is a disorder of ribosome biogenesis that causes erythroid hypoplasia, because erythroid progenitors are highly sensitive to apoptotic cell death (Horos and von Lindern, 2012; Ellis, 2014; Nakhoul et al., 2014; Dalle et al., 2016; Khincha and Savage, 2016). Although many cases are sporadic, familial autosomal dominant DBA has been estimated to account for about half of cases. At least 11 different ribosomal protein gene mutations have been identified in DBA patients. Syndromes such as DBA that are associated with congenital hyporegenerative anemia are reviewed in Table 81.2. Anemia in DBA is lifelong but the onset of signs and symptoms is variable. Many individuals with DBA are severely anemic in the newborn period and have pallor at birth. Fetal growth restriction, skeletal abnormalities, or other congenital anomalies are seen in almost one-third of patients. The diagnosis of DBA is suggested by anemia and reticulocytopenia at birth or

appearing in the first 6 months of life. Certain unusual features of the RBCs (macrocytosis, elevated fetal hemoglobin (HbF) level, increased adenosine deaminase activity) can assist one in coming to the diagnosis. Also, tests for many of the DBA gene mutations are available in commercial gene diagnostic laboratories (Narla et al., 2011; Vlachos et al., 2014).

Many infants with DBA achieve durable remissions from anemia when treated with corticosteroids. Those who do not respond require chronic RBC transfusions and are at risk of transfusion iron overload. Transfusion-dependent DBA can be cured by allogeneic bone marrow transplant from HLA-compatible siblings (Lipton, 2006). The incidence of cancer is probably increased in DBA patients, on the basis of cases reported to the Diamond Blackfan Anemia Registry of North America (Lipton and Ellis, 2009). Although a variety of solid tumors, in particular osteogenic sarcoma, have been reported, the most common malignancy has been acute myeloid leukemia.

Neonatal Anemia Caused by Hemorrhage

Underlying causes of hemorrhage that result in fetal or neonatal anemia are noted in Box 81.2 and in this section are divided into prenatal, perinatal, and postnatal hemorrhages.

Prenatal Hemorrhage

Approximately 1 in 400 pregnancies is associated with fetal–maternal hemorrhage (FMH) of 30 mL or more, and 1 in 2000 pregnancies is associated with FMH of 100 mL or more (Kosasa et al., 1993). FMH of small volumes of blood is very common. Perhaps as many as 75% of pregnancies can be shown to have 0.01 to 0.1 mL of fetal blood in the maternal circulation. Fetal blood cells can gain entry into the mother's circulation during abortion. This has been reported in 2% of spontaneous abortions and in 5% of induced abortions (Giacola, 1997).

The Kleihauer–Betke stain of maternal blood evaluates the acid elution of hemoglobin from RBCs (Huissoud et al., 2009). HbF resists acid elution to a greater degree than adult hemoglobin. Therefore maternal cells appear clear (termed *ghost cells*), whereas erythrocytes of fetal origin appear pink. False-positive results occur when mothers have an increase in the level of HbF (i.e., sickle cell

TABLE 81.2**Syndromes Associated With Congenital Hyporegenerative Anemia**

| Syndrome | Phenotypic Features | Genotypic Features |
|---|---|--|
| Adenosine deaminase deficiency | Autoimmune hemolytic anemia, reduced erythrocyte adenosine deaminase activity | AR, 20q13.11 |
| Congenital dyserythropoietic anemias | Type I (rare): megaloblastoid erythroid hyperplasia and nuclear chromatin bridges between nuclei Type II (most common): hereditary erythroblastic multinuclearity with positive acidified serum test result, increased lysis to anti-i Type III: erythroblastic multinuclearity ("gigantoblasts"), macrocytosis | Type I: 15q15.1-q15.3 Type II: 20q11.2 Type III: 15q21 |
| Diamond–Blackfan syndrome | Steroid-responsive hypoplastic anemia, often macrocytic after 5 months of age | AR; sporadic mutations and AD inheritance described; 19q13.2, 8p23.3-p22 |
| Dyskeratosis congenita | Hypoproliferative anemia usually presenting between 5 and 15 years of age | X-linked recessive, locus on Xq28; some cases with AD inheritance |
| Fanconi syndrome | Steroid-responsive hypoplastic anemia, reticulocytopenia, some macrocytic RBCs, shortened RBC life span. Cells are hypersensitive to DNA cross-linking agents | AR, multiple genes: complementation; group A 16q24.3; group B Xp22.3; group C 9q22.3; group D2 3p25.3; group E 6p22-p21; group F 11p15; group G 9p13 |
| Osler hemorrhagic telangiectasia syndrome | Hemorrhagic anemia | AD, 9q34.1 |
| Osteopetrosis | Hypoplastic anemia from marrow compression; extramedullary erythropoiesis | AR, 16p13, 11q13.4-q13.5; AD, 1p21; lethal, reduced levels of osteoclasts |
| Pearson syndrome | Hypoplastic sideroblastic anemia, marrow cell vacuolization | Pleioplasmatic rearrangement of mitochondrial DNA; X-linked or AR |
| Peutz–Jeghers syndrome | Iron-deficiency anemia from chronic blood loss | AD, 19p13.3 |
| ATR-X and ATR-16 syndromes | ATR-X: hypochromic, microcytic anemia; mild form of hemoglobin H disease ATR-16: more significant hemoglobin H disease and anemia are present. | ATR-16, 16p13.3, deletions of alpha globin locus |

AD, Autosomal dominant; *AR*, autosomal recessive; *ATR-16*, chromosome 16–related alpha thalassemia/mental retardation; *ATR-X*, X-linked alpha thalassemia/mental retardation; *RBC*, red blood cell.

• BOX 81.2 Underlying Causes of Hemorrhage in the Fetus and Neonate**Prenatal**

Twin–twin transfusion
Fetal–maternal hemorrhage
Trauma with bleeding into cord, placenta, amniotic fluid

Perinatal

Placenta previa
Placental abruption
Vasa previa
Velamentous insertion of the umbilical cord
Nuchal cord
Trauma or incision of the cord or placenta during cesarean delivery
Rupture of the umbilical cord at delivery

Postnatal

Subgaleal hemorrhage
Cephalohematoma
Organ trauma after birth
Pulmonary hemorrhage
Intracranial hemorrhage
Iatrogenic blood loss

disease, thalassemia, and hereditary persistence of HbF). Diagnosing FMH can be difficult when the mother has blood group O and the infant has blood group A or B because fetal cells are rapidly cleared from the maternal circulation by maternal anti-A or anti-B antibodies and therefore are not detected by the Kleihauer–Betke stain.

Severe FMH can be suspected before delivery by decreased fetal movements and a fetal sinusoidal heart rate pattern (Lopriore et al., 1995). Giacoia (1997) reviewed these variables to determine if they correlated with the severity of FMH. Fetal movements for a period ranging between 24 hours and 7 days were absent in 17 of 134 cases evaluated. In this group, six infants survived, five were stillborn, and five died in the neonatal period. A sinusoidal heart rate pattern was reported in 21 cases and was associated with decreased fetal movement in 40% of the cases. No significant difference was found between the cases with a hemorrhage of less than 200 mL versus those with a hemorrhage of more than 200 mL. Significant FMH has been described following maternal trauma (Huisoud et al., 2009). In a larger study from Intermountain Healthcare, we found an incidence of FMH of 1 case per 9160 live births, with 67% of affected neonates having an initial hemoglobin level below 7 g/dL (Christensen et al., 2012). Outcomes were poorer in those with the lowest initial hemoglobin levels, with death or major morbidities in 100% of those with an initial

value below 5 g/dL and in all who were born before 35 weeks of gestation.

Neonates delivered after a significant FMH can be pale, tachycardic, and tachypneic, but they generally do not have marked respiratory distress or a requirement for supplemental oxygen. Their hemoglobin concentration can be as low as 4 to 6 g/dL, and a significant metabolic acidosis is often present in association with poor perfusion. Other causes of pallor can be ruled out once the infant is stable. Infants with asphyxia or chronic anemia caused by hemolysis can also have pallor. These diagnoses can be distinguished from acute hemorrhage on differences in clinical signs. With chronic blood loss, shock is usually absent. Asphyxiated infants are pale and floppy and may have poor peripheral circulation. The hemoglobin level will be stable but might fall if DIC and internal bleeding occur. [Table 81.3](#) lists potential ways to improve the outcome after FMH.

Twin–twin transfusion is a complication of monochorionic twin gestations, occurring in 5%–30% of such pregnancies ([Domergues et al., 1995](#); [Dennis and Winkler, 1997](#)). It involves placental anastomoses that permit transfer of blood from one twin to the other. The perinatal mortality rate can be 70% or more. About 70% of monozygous twin pregnancies have monochorionic placentas. Although vascular anastomoses are present in almost all such twin pregnancies, not all develop twin–twin transfusion.

Acute twin–twin transfusion generally results in twins of similar size but with hemoglobin concentrations that differ by more than 5 g/dL. In chronic twin–twin transfusion the donor becomes progressively anemic and growth retarded, whereas the recipient becomes polycythemic, macrosomic, and sometimes hypertensive. Both twins can develop hydrops; the donor twin becomes hydropic from profound anemia, and the recipient twin becomes hydropic from congestive heart failure. The donor often has low amniotic fluid volumes, whereas the recipient has increased amniotic fluid volumes because of significant differences in blood volume, renal blood flow, and urine output.

Chronic twin–twin transfusion can be diagnosed by serial prenatal ultrasonography measuring cardiomegaly, discordant amniotic fluid production, and fetal growth discrepancy of more than 20%. An in utero diagnosis is not dependent on differences in hemoglobin concentration; however, percutaneous umbilical blood sampling can determine whether hemoglobin concentrations differ by more than 5 g/dL. After birth the donor twin may require transfusions and can have neutropenia, hydrops from severe anemia,

growth retardation, congestive heart failure, and hypoglycemia. The recipient twin is often the sicker of the two, with problems including hypertrophic cardiomyopathy, congestive heart failure, polycythemia, hyperviscosity, respiratory difficulties, hypocalcemia, and hypoglycemia. Neurologic evaluation and imaging are imperative because the risk of prenatally acquired cerebral lesions is 20%–30% in both twins. The incidence of neurologic morbidity following the intrauterine death of one of the fetuses averages 20%–25%. Morbidities include multiple cerebral infarctions, hypoperfusion syndromes from hypotension, and periventricular leukomalacia. Long-term neurologic follow-up is indicated for all survivors of twin–twin transfusion.

Prenatal treatment for twin–twin transfusion consists of close monitoring and reduction amniocenteses to decrease uterine stretch and prolong the pregnancy. Selective feticide of the hydropic twin has been advocated by some and has resulted in the survival of the healthier twin in some studies ([De Lia et al., 1995](#)). Studies suggest treatment in utero using laser ablation of bridging vessels provides the best outcomes, resulting in survival rates increased up to around 50%, with approximately 70% of the pregnancies having at least one survivor ([Ville et al., 1995](#); [van Hetern et al., 1998](#)). The survival rate without morbidity in the surviving twin is approximately 50%. [Supski et al. \(2002\)](#) performed a metaanalysis of 140 cases to correlate types of treatment with outcome. They found no differences in outcome between amnioreduction, fetoscopy, septostomy, or close observation.

Perinatal Hemorrhage

Perinatal blood loss in the fetus can occur with various obstetric complications, such as placenta previa, placental abruption, incision or tearing of the placenta during cesarean delivery, and cord evulsion. When a fetus undergoes significant blood loss back into the placenta, the term *fetoplacental hemorrhage* is used. Placental anomalies such as a multilobed placenta and placental chorioangiomas can be a source of perinatal bleeding ([Kramer et al., 1997](#)).

Placental abruption occurs in 3 to 6 per 1000 live births. The risk factors for placental abruption include prolonged rupture of membranes, severe fetal growth restriction, chorioamnionitis, hypertension, maternal diabetes, cigarette smoking, and advanced maternal age. The incidence of abruption increases with lower gestational age. Neonatal mortality rates from abruption range from 0.8 to 2.0 per 1000 births, or 15%–20% of the deliveries in which significant abruption occurs ([Rasmussen et al., 1997](#)).

**TABLE
81.3**

Potential Ways to Improve Outcomes After Fetal–Maternal Hemorrhage

| Area of Improvement | Potential Means of Improvement |
|--|--|
| Heightened suspicion of FMH by the obstetric staff | Consider FMH when mother reports lack of fetal movement. Perform a nonstress test or biophysical profile when mother reports lack of fetal movement. Consider FMH in cases with “nonreassuring” fetal heart rate pattern after mother reports lack of fetal movement |
| Increased availability of KB or flow cytometry testing for FMH. Advances in flow cytometry or other means of identifying and quantifying FMH | Availability of best test for FHM to all hospitals with labor and delivery services. More rapid turnaround/reporting of results |
| Rapid and consistent communication that FMH is being considered by the obstetric staff. Rapid communication of positive test results | Communication between clinical laboratory and obstetric staff, labor and delivery staff, pediatric staff, neonatal resuscitation team, neonatology staff, and blood bank staff |

FMH, Fetal–maternal hemorrhage; KB, Kleihauer–Betke.

Women with a history of a cesarean delivery and increased parity are at increased risk of placenta previa (McMahon et al., 1997), a condition where part or all of the placenta overlies the cervical os. Cigarette smoking is associated with a 2.6-fold to 4.4-fold increased risk of placenta previa (Chelmow et al., 1996). Prenatal diagnosis of vasa previa (anomalous vessels overlying the internal os of the cervix) can be made with transvaginal color Doppler ultrasonography and should be suspected in cases of antepartum or intrapartum hemorrhage. Although uncommon (1 in 3000 deliveries), the perinatal death rate is high, ranging from 33% to 100% when this condition is undetected before delivery (Chen and Konchak, 1998).

Neonates delivered after placental abruption or after placenta previa can be anemic, but they can also have signs of hypoxia and ischemia. Most of the blood lost in an abruption or previa is maternal blood, but the neonate can have some degree of anemia as well. Therefore when perinatal blood loss is recognized or suspected, the neonate's hemoglobin level should be measured at birth and again 12 hours or so later. A Kleihauer–Betke stain can be performed on maternal blood to determine if fetal hemorrhage can be documented. Monitoring bleeding mothers with ultrasonography might detect placental abnormalities.

Cord rupture caused by traction on a shortened or abnormal umbilical cord usually occurs on the fetal side. Cord aneurysms, varices, and cysts can all lead to a weakened cord. Cord infections (funisitis) can also weaken the cord and increase the risk of rupture. Infants born precipitously may be at increased risk of hemorrhage due to a ruptured cord.

Cord hematomas occur infrequently (1 per 5000 to 6000 deliveries) and can be a cause of fetal blood loss and perinatal death. Intrauterine death can occur from compression of the umbilical vessels by a cord hematoma. Cord hematomas can result from trauma from percutaneous umbilical blood sampling. Hematomas of the cord can be diagnosed in utero by ultrasonography (Deans and Jauniaux, 1998).

Subamniotic hematomas can occur when chorionic vessels rupture near the cord insertion. Most subamniotic hematomas are the result of traction on a normal or shortened umbilical cord and are not noted until after delivery.

Velamentous insertion of the umbilical cord occurs when the umbilical cord enters the membranes distant from the placenta. This is present in 0.5%–2.0% of pregnancies (Benirschke, 1994). Blood vessels left unprotected by Wharton jelly are more likely to tear. Rupture of anomalous vessels in the absence of traction or trauma can occur even if the cord itself attaches centrally or paracentrally. Fetal mortality remains very high in this condition, often because detection by routine ultrasonography is rare (Eddleman et al., 1992).

Postnatal Hemorrhage

Loss of fetal blood into the placenta can occur during delivery and can be a cause of low-grade neonatal anemia. It is reported to occur when the neonate is held significantly higher than the placenta after birth before the cord is clamped. However, such positioning does not typically result in fetoplacental hemorrhage; in fact even when the neonate is on the mother's abdomen, the unclamped cord typically transfuses fetal blood “uphill” from the placenta to the neonate.

It is possible for neonates to lose blood by fetoplacental transfusion when born with a tight nuchal cord, which allows blood to be pumped through umbilical arteries toward the placenta, while constricting flow back from the placenta to the baby through the

umbilical vein, which is more easily constricted because of its thin wall. However, in actual practice in the Intermountain Healthcare series, most cases of nuchal cord did not result in neonatal anemia (Henry et al., 2013).

Hemorrhage into the subgaleal space can occur before but much more typically after birth. This type of hemorrhage is seen most commonly with difficult deliveries requiring vacuum or forceps assistance (Uchil and Arulkumaran, 2003; Kilani et al., 2006; Christensen et al., 2011a). Subgaleal hemorrhages are potentially life threatening and must be recognized as early as possible to prevent significant morbidity or mortality. In the Intermountain Healthcare series, all cases were diagnosed within a few hours of birth, and the latest were diagnosed by 6 to 7 hours after birth (Christensen et al., 2011a). Subgaleal hemorrhage occurs when veins bridging the subgaleal space are torn, allowing blood to accumulate in the large potential space between the galea aponeurotica and the periosteum of the skull. The subgaleal space extends from the orbital ridge to the base of the skull and can accommodate a volume equivalent to a neonate's entire blood volume (Chadwick et al., 1996; Teng and Sayre, 1997).

Subgaleal hematomas can form or can extend because of risk factors such as coagulopathy or asphyxia, but vacuum extraction itself is a risk factor for their development. The diagnosis should be strongly considered in the presence of a ballotable fluid collection in dependent regions of the infant's head, coupled with signs of hypovolemia. Treatment requires restoration of blood volume and control of bleeding. Death from exsanguination because of subgaleal hemorrhage has been reported. One suggested way to estimate the volume of blood lost is by following head circumference: approximately 40 mL of blood has been lost for every 1 cm increase in head circumference that occurs (Chadwick et al., 1996). The duration of vacuum application has been reported to be a predictor of scalp injury, followed by the duration of the second stage of labor and paramedian cup placement. Of those with reported subgaleal hemorrhages, 80%–90% had some history of vacuum- or instrument-assisted delivery. In the Intermountain Healthcare series, all recognized cases of subgaleal hemorrhage that were severe enough to receive a RBC transfusion occurred after vacuum or forceps deliveries. Limiting the frequency and duration of vacuum assistance in high-risk infants might decrease the incidence of subgaleal hematomas.

Anemia appearing after the first 24 hours of life in a nonjaundiced infant can be a sign of hemorrhage. Hemorrhages can be visible or occult. Breech deliveries can be associated with occult bleeding into the renal, adrenal, hepatic, or splenic organs or into the retroperitoneal space. Delivery of macrosomic infants can result in hemorrhage. Infants with overwhelming sepsis can bleed into soft tissue and organs on the basis of DIC.

In addition to causing anemia, adrenal hemorrhage can result in circulatory collapse because of the loss of adrenal function. The incidence of adrenal hemorrhage is 1.7 per 1000 births (Felic, 1995). Adrenal hemorrhage can also affect surrounding organs. Intestinal obstruction and kidney dysfunction have been reported (Pinto and Guignard, 1995). The diagnosis can be made with ultrasonography, during which calcifications or cystic masses are noted. Adrenal hemorrhage can be distinguished from renal vein thrombosis (RVT) by ultrasonography, in that RVT generally results in a solid mass. Occasionally, both entities coexist.

The liver of a neonate is prone to iatrogenic rupture, resulting in high morbidity and high mortality (Davies, 1997). Neonates with this problem can appear asymptomatic until hemoperitoneum occurs. This problem can occur in term and preterm infants (Emma

et al., 1992) and has been associated with chest compressions during cardiopulmonary resuscitation. Surgical intervention has been reported to save some infants, but the mortality is high. Splenic rupture can result from birth trauma or as a result of distention caused by extramedullary hematopoiesis. Abdominal distention and discoloration, scrotal swelling, and pallor are clinical signs of splenic rupture; these can also be seen with adrenal or hepatic hemorrhage.

More rare causes of hemorrhage in the newborn period include hemangiomas of the gastrointestinal tract (Nagaya et al., 1998), vascular malformations of the skin, and hemorrhage into soft tumors, such as giant sacrococcygeal teratomas. Occult intra-abdominal hemorrhage can occur with fetal ovarian cysts, which are usually benign and resolve spontaneously. One case of fetal anemia was diagnosed by a spontaneous hemorrhage into a fetal ovarian cyst and was managed by intrauterine blood transfusions (Abolmakarem et al., 2001).

Anemia Caused by Hemolysis

Characteristic features of erythrocyte morphology can be used to tentatively classify the cause of neonatal hemolytic disease. Figs. 81.8–81.12 illustrate major categories of neonatal hemolytic disorders that are generally readily identifiable by careful examination of a blood smear (Christensen et al., 2014e).

Erythrocytes of children and adults normally circulate for 100 to 120 days. Erythrocyte survival is shorter in neonates: 70 to 90 days in term infants and 50 to 80 days in premature infants (Pearson, 1967). Hemolysis is defined as a further shortening of erythrocyte survival (Christensen et al., 2015b). A practical, rapid, and non-invasive approximation of the RBC life span can be made by quantification of exhaled CO, with higher values corresponding to a higher hemolytic rate and hence a shorter RBC survival (Christensen et al., 2015c).

The mechanism responsible for shortening the erythrocyte life span can involve macrophage recognition of abnormal RBC membrane properties (extravascular hemolysis, within the reticuloendothelial system) or disruption of circulating erythrocytes (intravascular hemolysis). Both mechanisms can occur simultaneously.

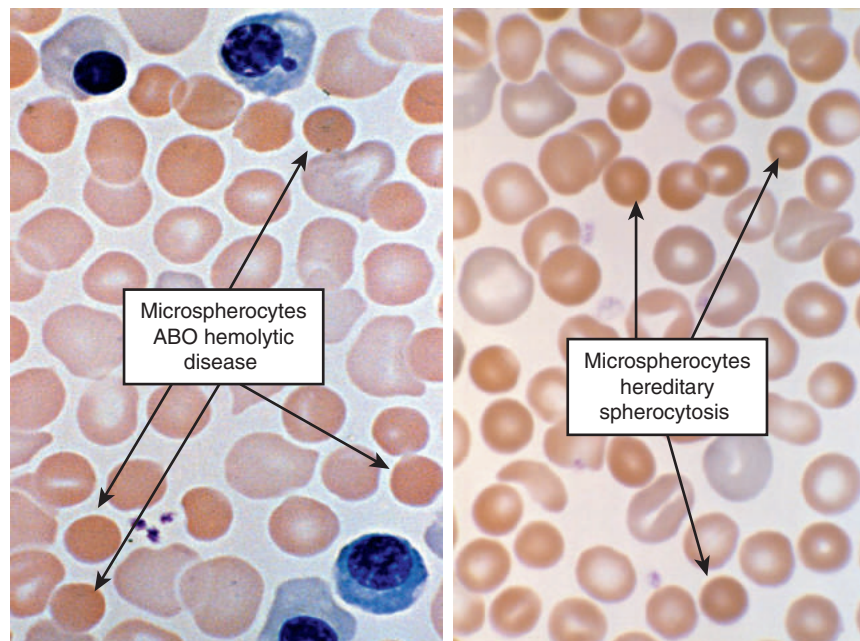
Laboratory tests that support the definition of neonatal hemolysis are shown in Table 81.4. A serum haptoglobin level below the lower detectable limit of the assay and the presence of hemoglobin in the urine in the absence of erythrocytes are both reasonably good indicators of hemolytic disease in neonates. An elevated end-tidal CO concentration is a rapid, practical, and reliable marker of neonatal hemolysis (Christensen and Malleske, 2016).

In older infants and children the usual response to hemolysis is a compensatory increase in erythropoiesis. Because of the increase in erythrocyte production, children with hemolytic disorders sometimes are not anemic or have a hematocrit just at the lower reference interval limit. This occurs when the rate of RBC production increases to exactly match the hemolytic rate. In such cases hyperbilirubinemia and reticulocytosis are seen but anemia is mild. However, neonates are less likely to completely compensate for hemolysis. Perhaps this is because their increased oxygen-carrying capacity, moving from the fetal to the oxygen-rich environment, blunts compensatory erythropoietic activity.

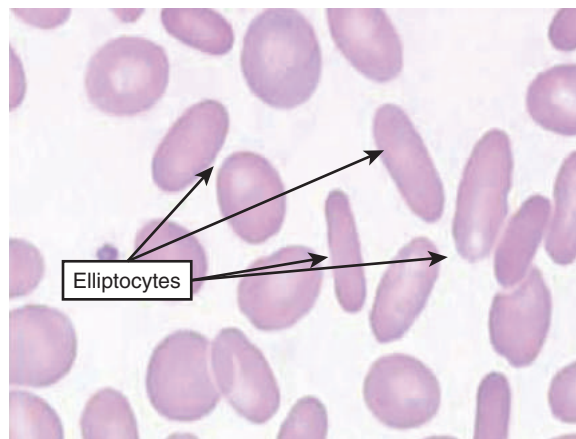
Immune-Mediated Hemolytic Disease of the Neonate

Rhesus

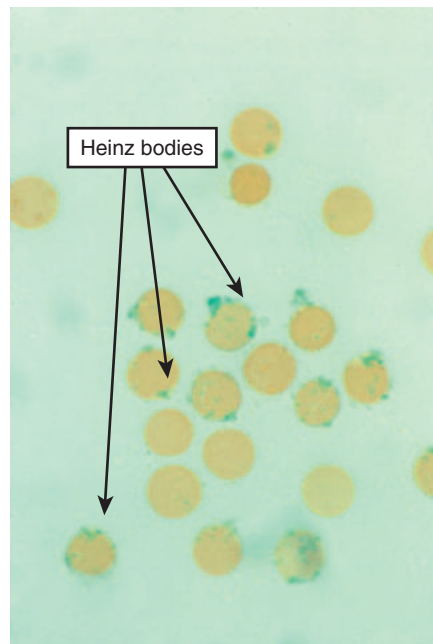
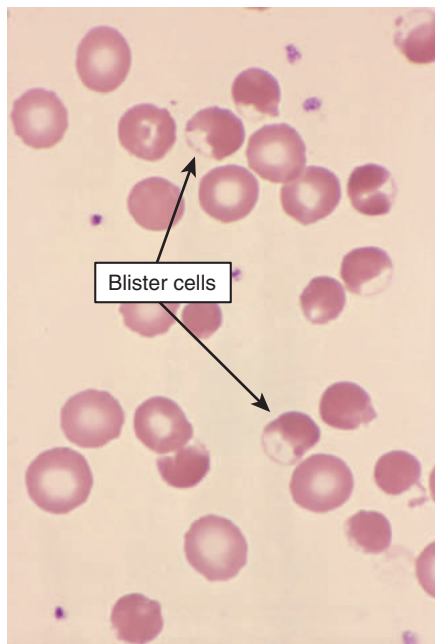
The basis of alloimmune hemolytic anemia is transfer of maternal antibody against fetal RBC antigens inherited from the father and absent in the mother (Huang and Ye, 2010; Christensen and Yaish, 2015, 2016). The severity of the consequent clinical problem ranges from mild neonatal hyperbilirubinemia to severe anemia with hydrops. Since the development of immunoprophylaxis against Rh(D) sensitization, the incidence of alloimmune hemolysis has decreased dramatically, but this remains a global problem in poor



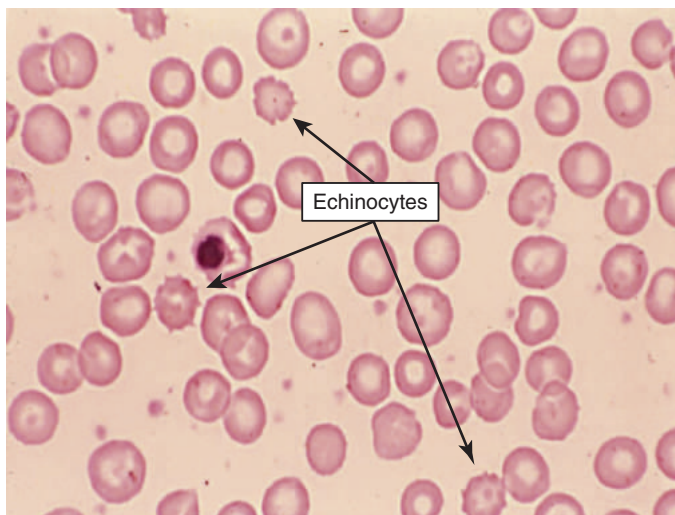
• **Fig. 81.8** Microspheryocytes: neonate with ABO hemolytic disease (left); neonate with hereditary spherocytosis (right).



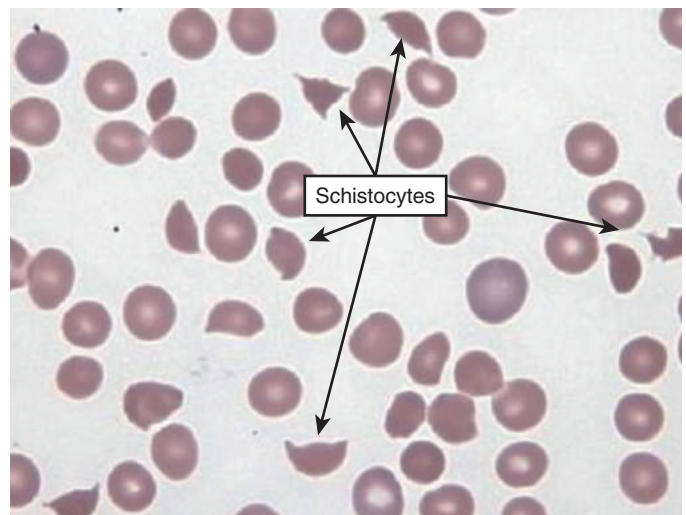
• **Fig. 81.9** Elliptocytes.



• **Fig. 81.10** Bite or blister cells: blister cells from a neonate with glucose 6-phosphate dehydrogenase deficiency (*left*); Heinz bodies from a neonate with glucose 6-phosphate dehydrogenase deficiency (*right*).



• **Fig. 81.11** Echinocytes from a neonate with pyruvate kinase deficiency.



• **Fig. 81.12** Schistocytes from a neonate with disseminated intravascular coagulation associated with birth asphyxia.

TABLE 81.4**Laboratory Tests That Can Be Performed on Jaundiced Neonates to Assess Them for a Hemolytic Condition**

| Test | Reference Intervals | Problems Applying the Test to Preterm Neonates |
|---|---|---|
| Hemoglobinuria | Trace hemoglobin or greater in urine analysis | RBCs in urine are not uncommon in VLBW neonates. When present they render the urinary hemoglobin test invalid for detection of hemolysis. |
| Low or absent serum haptoglobin | A value that is falling or is below the lower limit of detection | Preterm infants without hemolysis might have very low serum haptoglobin concentrations. |
| DAT (direct Coombs test) | Positive DAT or positive indirect antiglobulin test | Accuracy may be less among VLBW neonates. |
| Elevated absolute reticulocyte count | Above upper limit for GA | Preterm infants can normally have elevated reticulocyte counts, according to GA. |
| Elevated immature reticulocyte fraction | Value above 7% at birth or above 3% after 24 h | Not well validated in VLBW neonates |
| Elevated nucleated RBC count | Upper limit 3000/ μ L at birth. Should be <500/ μ L after 3–4 days | Not well validated in VLBW neonates. The numbers of nucleated RBCs are typically higher in younger GA neonates at birth. |
| Abnormal RBC morphology on stained blood film | Microspherocytes, elliptocytes, bite and blister cells, echinocytes, schistocytes | Anisocytosis and poikilocytosis are frequently encountered in healthy preterm neonates. |
| Heinz body preparation | Heinz bodies support diagnosis of hemolysis due to precipitated (unstable) hemoglobin | Less well studied in preterm infants |
| Elevated ETCOc | First week or first 2 weeks the upper limit is 1.7–2 ppm, thereafter the upper limit is 1 ppm | Reference intervals for VLBW neonates are lacking. Cannot perform test if neonate is intubated or receiving nasal cannula O ₂ . Difficult to get accurate reading if respiratory rate is >60 breaths/min |
| Carboxyhemoglobin by cooximetry | Upper limit is 1.5%–2.0% | Reference intervals for VLBW neonates are lacking, and the measurement is not sensitive. |

DAT, Direct antiglobulin test; ETCOc, end-tidal carbon monoxide concentration; GA, gestational age; ppm, parts per million; RBC, red blood cell; VLBW, very low birth weight (<1500 g).

countries (Zipursky and Bhutani, 2015). In the developed world most cases of alloimmune hemolytic disease of the fetus and newborn are now the result of maternal antibodies to ABO, Kell, Duffy, Kidd, and MNS antigens and other Rh antigens, particularly c and E.

The pathogenesis of Rh hemolytic disease of the fetus and newborn was elucidated by Levine et al. (1941). Several Rh antigens are recognized, each of which is detected by specific antibodies (Huang et al., 2010). Rh blood group antigens are distinct erythrocyte membrane-associated proteins. Two of these have separate isoforms (C and c; E and e), which are detected by specific antibodies (anti-C and anti-c; anti-E and anti-e). The most clinically relevant of the membrane Rh proteins to neonatal hemolytic disease is the D antigen. Rh-positive RBCs are those that possess the D antigen. A lowercase *d* is used to denote the absence of the D antigen, or Rh-negative status; it is not a specific antigen in the same way the c and e antigens are.

The Rh proteins are encoded by two genes on chromosome 1, *RHCE* and *RHD*. *RHCE* encodes the C/c and E/e proteins, and *RHD* encodes the D protein. The Rh-negative phenotype results when the *RHD* gene is deleted or nonfunctional on both chromosomes. In most cases the Rh-negative phenotype is also associated with Rh c and Rh e (i.e., Rh cde). The frequency of Rh negativity differs in different racial groups. It is high in whites (15%), lower in blacks (5%), and very low in Asians. The Rh-positive phenotype may result from homozygosity (DD) or heterozygosity (Dd) for the D antigen. In Rh-positive whites,

approximately 44% are homozygous (DD), and 56% are heterozygous (Dd). Knowledge of differences in *RHD* genotype can be helpful because approximately 25% of fetuses of couples with an Rh(D)-negative mother and an Rh(D)-positive father will be Rh(D) negative and thus not susceptible to Rh hemolytic fetal/neonatal hemolysis.

Rh hemolytic disease is rare during the first pregnancy involving an Rh-positive fetus, but the risk increases with each subsequent pregnancy. This is because small volumes of fetal RBCs enter the maternal circulation throughout gestation, although the major fetomaternal bleeding responsible for sensitization occurs during delivery. Once sensitization has occurred, reexposure to Rh(D) RBCs in subsequent pregnancies leads to an anamnestic response, with an increase in the maternal anti-D titer. Therefore significant hemolysis occurring in the first pregnancy indicates previous maternal exposure to Rh-positive RBCs, a consequence of fetal bleeding associated with a previous spontaneous or therapeutic abortion, ectopic pregnancy, or a variety of different prenatal procedures. On occasion the sensitization is a consequence of an earlier transfusion in which Rh-positive RBCs were administered by mistake or in which some other blood component (e.g., platelets) containing Rh(D) RBCs was transfused.

Fetomaternal transfer of erythrocytes (and thereby sensitization) occurs primarily during delivery. The frequency of Rh immune hemolytic disease is much lower if the mother has blood group O (–) and her fetus has blood group A or B (+). The beneficial effect of ABO incompatibility is due to the maternal anti-A and

anti-B antibodies recognizing the corresponding A and B fetal RBCs, leading to their destruction before sensitization to the Rh antigen(s) can occur. Administration of a single intramuscular dose of Rh immune globulin (300 µg) to unsensitized Rh-negative mothers within 72 hours of their delivering an Rh-positive infant led to the virtual elimination of Rh(D) sensitization (Freda et al., 1975). The few treatment failures seen were attributed to fetomaternal bleeding of more than 30 mL at delivery or bleeding that occurred prenatally. This led to the current standard of practice of administering a full dose of Rh immune globulin to all unsensitized Rh-negative women at 28 weeks of gestation, with an additional dose given at birth if the infant is Rh positive. The dose of Rh immune globulin should be increased when there is greater than normal fetomaternal bleeding.

Fetal bilirubin is removed in utero by transfer across the placenta into the maternal circulation. Thus hyperbilirubinemia is not a problem until after delivery. The major threat to the fetus is severe anemia leading to hydrops and intrauterine death.

Mild hemolytic disease is most common, manifested by a positive direct antiglobulin test (DAT) with minimal hemolysis, little or no anemia (cord blood hemoglobin level greater than 14 g/dL), and minimal hyperbilirubinemia (cord blood bilirubin level less than 4 mg/dL). Aside from early phototherapy, these newborns generally require no therapy unless the postnatal rate of the rise in bilirubin level is greater than expected. Moderate hemolytic disease is found in a smaller fraction of affected infants. This is characterized by hemolysis, moderate anemia (cord blood hemoglobin level less than 14 g/dL), and increased cord blood bilirubin levels (greater than 4 mg/dL). The blood may have numerous nucleated RBCs, decreased numbers of platelets, and occasionally a leukemoid reaction with large numbers of immature granulocytes. Infants with Rh disease also may exhibit marked hepatosplenomegaly, a consequence of extramedullary hematopoiesis and sequestration of antibody-coated RBCs. The risk of development of bilirubin encephalopathy is high if these neonates do not receive intensive phototherapy and sometimes exchange transfusion. Severe hemolytic disease is seen in approximately 25% of affected infants, who are either stillborn or hydropic at birth.

Management of seriously affected fetuses is directed at the prevention of severe anemia and death. To accomplish this it first is necessary to identify those fetuses at risk. An increase in the maternal anti-D titer in a previously sensitized Rh-negative woman is a good serologic measure of a fetus in potential jeopardy. A history of neonatal hemolytic disease resulting from anti-D antibodies suggests that the current fetus also may be at risk. In this regard it may be useful to know the fetal Rh blood type because this identifies those Rh-negative infants who are not at risk. In many cases this can be accomplished by direct Rh typing of fetal RBCs obtained via cordocentesis. Alternatively, molecular biology techniques can be used to determine the Rh genotype in DNA obtained from amniocytes or chorionic villus samples (Daniels et al., 2004). Recently, methods have been developed to perform such analysis of free fetal DNA in maternal blood (Gonenc et al., 2015). When the fetus is found to be Rh negative, no further maternal monitoring or fetal blood studies are necessary.

An increase in the maternal titer of immunoglobulin (Ig) G anti-D indicates maternal sensitization but does not accurately predict the severity of fetal hemolysis. Ultrasonographic signs of hydrops are a relatively late sign of fetal anemia, typically not developing until the hemoglobin content of the fetal blood has fallen to below 7 g/dL for several days. Doppler assessment of peak velocity in the fetal middle cerebral artery (MCA) reflects

the lower viscosity of anemic blood and has become the standard method of screening fetuses for fetal anemia.

Hydrops occurs as early as 20 weeks of gestation. Doppler measurement of the peak velocity in the MCA can begin as early as 18 weeks but more commonly is initiated at approximately 24 weeks, then repeated every 1 to 2 weeks. When the MCA Doppler value exceeds 1.5 multiples of the mean (MoM) (A zone) between 24 and 35 weeks of gestation, cordocentesis is typically performed to measure the hematocrit, fetal blood type, DAT, reticulocyte count, and total bilirubin level. Morbidity from cordocentesis is less than 2%, and cordocentesis should be performed with blood available for intrauterine transfusion if necessary. The donor blood should be type O, Rh(D) negative, cytomegalovirus negative, and less than 72 hours from collection; extended crossmatch is often performed with maternal blood type. Irradiation is mandatory, and many centers also perform leukoreduction. The transfusion is generally administered at approximately 20 mL/kg estimated fetal weight with a target hematocrit of 40%–50%. Neonatal exchange transfusion, amniocentesis, selective early induction of delivery, and intrauterine fetal blood transfusions have all contributed to the declining neonatal death rate from Rh incompatibility.

Administration of intravenous immune globulin (IVIG) to neonates with Rh hemolytic disease was tested as a means of reducing the need for exchange transfusion. The proposed salutary mechanism of action was reduced removal of antibody-coated erythrocytes through Fc receptor blockade. However, randomized trials in the Netherlands (Smits-Womtkems et al., 2011) and Brazil (Santos et al., 2013) showed clearly that IVIG was not effective and can lead to increased hemolysis on the basis of other antibodies introduced in the IVIG preparation (Christensen et al., 2015a).

ABO

Hemolysis resulting from ABO incompatibility is like Rh hemolytic disease in that maternal anti-A or anti-B antibodies cross into the fetal circulation and react with A or B antigens on the surface of the fetal RBC. The ABO locus is on chromosome 9 at 9q34.1-q34.2. In mothers with type A or type B blood, naturally occurring anti-B and anti-A isoantibodies are typically IgM antibodies and consequently do not cross the placenta to affect fetal RBCs. In contrast, the anti-A and anti-B alloantibodies present in mothers with type O blood typically also include IgG antibodies that can cross the placenta and affect the fetal erythrocytes. For this reason, ABO hemolytic disease of the fetus and newborn is largely limited to type O mothers carrying type A or type B fetuses. The presence of IgG anti-A or anti-B antibodies in type O mothers also explains why hemolysis caused by ABO incompatibility frequently occurs during the first pregnancy without prior sensitization. ABO incompatibility is present in approximately 12% of pregnancies, although evidence of fetal RBC sensitization by positive direct antiglobulin testing of fetal RBCs is found in only 3% of births, and less than 1% of births are associated with significant hemolysis. A and B antigens are present in many tissues besides RBCs, and therefore only a fraction of anti-A or anti-B antibody crossing the placenta actually binds to fetal erythrocytes, the remainder being absorbed by other fetal tissues.

In suspected cases of ABO incompatibility, it is essential to exclude other antibodies and other nonimmune causes of hemolysis such as glucose 6-phosphate dehydrogenase (G6PD) deficiency or hereditary spherocytosis. In most cases, pallor and jaundice are minimal. Hepatosplenomegaly is uncommon. Laboratory features include evidence of minimal to moderate hyperbilirubinemia and, occasionally, some degree of anemia. The DAT is sometimes negative,

although the indirect antiglobulin test (neonatal serum plus adult group A or group B RBCs) result is more commonly positive. This paradox is related to the fact that fetal RBCs, compared with adult erythrocytes, have less type-specific antigen on their surface (Voak and Williams, 1971). The peripheral blood smear is characterized by marked spherocytosis that is similar to that seen in hereditary spherocytosis.

Hemolysis in ABO incompatibility is usually mild, presenting with some degree of hyperbilirubinemia but not with anemia and not with hydrops. However, ABO hemolytic disease has been described as the most often found explanation for extreme hyperbilirubinemia and kernicterus (Johnson et al., 2009). Whereas most cases, even severely affected cases, are treated effectively with phototherapy, occasionally exchange transfusion with group O Rh-compatible RBCs is used. Additional follow-up at 2 to 3 weeks of age to check for anemia in these infants is essential.

Kell

Antibody to Kel 1 (K) is a significant cause of hemolytic disease of the newborn (Denomme, 2015; Karagol et al., 2012). This system is the third most common cause of hemolytic disease of the fetus and newborn, after ABO and Rh disease. The *KEL* gene is located on chromosome 7 (7q33). The Kell system was named for the woman in whom this antibody was first detected. The Kell antigen is the first RBC-specific blood group antigen to be expressed during the burst-forming unit–erythroid stage of RBC development. As a consequence, when maternal antibody is expressed to this antigen, it can target early erythroid progenitors and create what appears to be a hypoproliferative anemia. It appears hypoproliferative because the reticulocyte count is commonly low or normal, and hyperbilirubinemia is not as severe as otherwise expected. Thus a fetus/neonate with anti-K hemolytic disease will typically have severe anemia without reticulocytosis or hyperbilirubinemia. This situation can initially be confused with fetal blood loss, such as fetomaternal transfusion. Antibody to Kel 1 is found in about 1 per 1000 pregnant women. Many of these women developed this antibody after blood transfusion.

Kidd

The Kidd antigen is a urea transporter on the surface of RBCs (Ferrando et al., 2008; Thakral et al., 2010). This antigen transports urea out of and into erythrocytes, maintaining the RBC shape and osmotic stability. The three common variants in this transporter are termed *Jka*, *Jkb*, and *Jk3*. *Jka* and *Jkb* differ from each other only in one base pair. The gene encoding the Kidd glycoproteins is located on chromosome 18 (18q11-q12). The Kidd antigen was named after Mrs. Kidd, who was found to have antibodies against a then unknown RBC antigen that her cells lacked but that were expressed on the RBCs of her neonate. Maternal anti-Kidd antibody can produce hemolytic disease of the fetus and newborn, but it is not typically severe enough to cause hydrops or to require intra-uterine transfusion or exchange transfusion. However, it can be a cause of hazardous hyperbilirubinemia.

Duffy

The RBC surface antigens that constitute the Duffy blood group are glycoproteins that are the attachment site for the malarial pathogen *Plasmodium vivax*. The Duffy antigen has two main forms (Fya and Fyb), which differ in only a single amino acid (Hughes et al., 2007; Meny, 2010). Other rare Duffy variants are termed *Fy3*, *Fy4*, *Fy5*, and *Fy6* (but only *Fy3* appears to be of clinical significance). The Duffy blood group was named for a

patient with hemophilia who had received many blood transfusions and developed anti-RBC antibodies. The Duffy locus is on chromosome 1 at position q22-q23. The null variant of the Duffy antigen is termed *Fy(a-b-)* and is the result of a single base pair change in the promotor at the site of binding for GATA-1, a hematopoietic transcription factor. This null variant does not express either Fya or Fyb on its RBCs and is found in more than 95% of West Africans, where it likely is an adaptation to the environmental pressure of malaria, as it seems to reduce the binding of *P. vivax* to erythrocytes. Specifically, RBCs that lack the Duffy antigens are relatively resistant to invasion by *P. vivax*. Maternal antibody to the Duffy antigens has been reported as a rare cause of hemolytic disease of the fetus and neonate. It is typically a mild condition.

MNS

After the ABO blood groups were discovered by Landsteiner in 1900, experiments were performed to identify other blood groups (Reid, 2009). The MNS system was the second blood group identified. The MN and S antigens are glycoproteins on the RBC surface. M and N are glycoprotein A, and S and s are glycoprotein B. Antigens S and s differ by only one amino acid. During erythrocyte development, glycoprotein A is detected shortly after the Kell antigen. The genes encoding the MNS antigens are located on chromosome 4 (4q28.2-q13.1). Rare MNS antigens have resulted from mutations in the glycoprotein A and glycoprotein B genes. Antibodies to the MNS antigens account for only 5% or less of cases of hemolytic disease of the fetus and newborn. Anti-M and anti-N are not considered to be a cause of transfusion reactions. Most neonates affected with anti-MNS hemolytic disease have hyperbilirubinemia that is manageable with phototherapy. Cases where exchange transfusions were used have been reported.

Maternal Disease

Maternal autoimmune hemolytic anemia or lupus erythematosus during pregnancy is sometimes associated with passive transfer of IgG antibody to RBC antigens. The diagnosis is suggested by the presence of neonatal hemolytic disease, a positive DAT, absence of Rh or ABO incompatibility, and antiglobulin-positive hemolysis in the mother (Lewin and Bussell, 2015). Treatment with prednisone in the mother may reduce both maternal hemolysis and the risk of neonatal morbidity. As in other cases of neonatal hemolysis, treatment is focused on prevention of severe hyperbilirubinemia.

Nonimmune-Mediated, Acquired Hemolytic Disease of the Neonate

Cytomegalic inclusion disease, toxoplasmosis, syphilis, bacterial sepsis, and DIC can all be associated with hemolytic anemia. In most of these conditions, some degree of thrombocytopenia also exists, and often hepatosplenomegaly is present. In cases of bacterial sepsis, both the direct and the indirect bilirubin levels may be elevated. The mechanism of hemolysis associated with infection can involve intravascular destruction of RBCs when they are tethered on fibrin strands. Broken erythrocytes can create erythrocyte fragments or deformed cells called *schistocytes*.

Hereditary Hemolytic Anemias Caused by Red Blood Cell Cytoskeletal Mutations

Hereditary Spherocytosis

Hereditary spherocytosis (HS) is a heterogeneous disorder in which abnormalities of RBC structural proteins lead to loss of erythrocyte

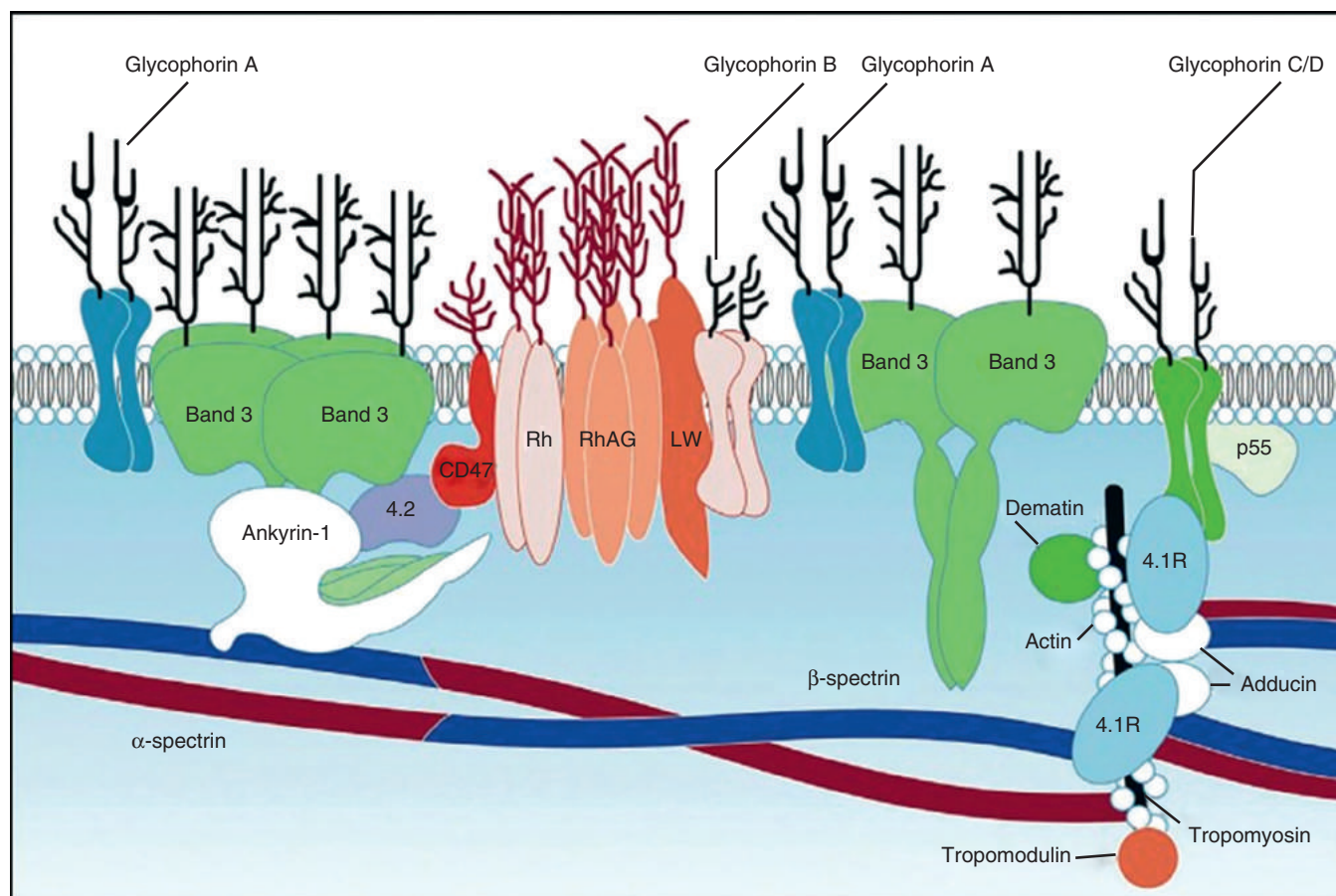
membrane surface area, resulting in spherical, hyperdense, poorly deformable RBCs (see Fig. 81.8) with a shortened life span (Perrotta et al., 2008; Sangerman et al., 2008; Christensen and Henry, 2010; Sheffield and Christensen, 2011; Gallagher, 2013; Christensen et al., 2015c). It occurs worldwide and affects individuals from all racial and ethnic groups. Individual pediatricians encounter HS uncommonly, but hospitals and healthcare systems with large delivery services deal with this condition regularly, particularly in white neonates of northern European ancestry, in whom the condition can be as frequent as 1 in 1000 to 2000 births. Early

suspicion and diagnosis of HS allow appropriate management, including provision of anticipatory guidance to parents, which can reduce the risk of adverse outcomes.

The loss of membrane surface area in HS erythrocytes is due to defects in various erythrocyte membrane proteins: ankyrin 1, band 3, β -spectrin, α -spectrin, and protein 4.2 (Table 81.5 and Fig. 81.13). Numerous mutations in the genes encoding these membrane proteins have been described. Destruction of poorly deformable HS erythrocytes in the spleen is the primary cause of hemolysis in patients with HS.

TABLE 81.5 Erythrocyte Membrane Proteins Involved in Hereditary Spherocytosis

| Protein | Gene | Chromosomal Location | Percentage of Hereditary Spherocytosis Cases | Typical Severity | Inheritance |
|--------------------|---------------|----------------------|--|------------------|---------------------|
| Ankyrin 1 | <i>ANK1</i> | 8p11.2 | 40–50 | Mild to moderate | Autosomal dominant |
| Band 3 | <i>SLC4A1</i> | 17q21 | 20–35 | Mild to moderate | Autosomal dominant |
| β -Spectrin | <i>SPTB</i> | 14q23-24.1 | 15–30 | Mild to moderate | Autosomal dominant |
| α -Spectrin | <i>SPTA1</i> | 1q22-23 | <5 | Severe | Autosomal recessive |
| Protein 4.2 | <i>EPB42</i> | 15q15-21 | <5 | Mild to moderate | Autosomal recessive |



• **Fig. 81.13** The red cell membrane, showing the subunits described to have genetic defects as the basis for congenital abnormalities in erythrocyte shape and predisposing to neonatal hemolytic jaundice. (From Gallagher PG. Disorders of erythrocyte metabolism and shape. In: Christensen RD, ed. *Hematologic Problems of the Neonate*. Philadelphia, PA: WB Saunders; 2000:224)

The clinical spectrum of HS during the perinatal period ranges from severe fetal anemia with hydrops fetalis to the asymptomatic neonate. This wide range is due, in part, to the various genes and specific mutations involved and also the presence or absence of coinherited conditions. For instance, HS coinherited with mutations or polymorphisms of genes involved in bilirubin uptake into hepatocytes (*SLC01B1*) or intrahepatic bilirubin conjugation (*UGT1A1*) can increase the risk of hazardous hyperbilirubinemia and kernicterus (Iolascon et al., 1998; Berardi et al., 2006; Korkmaz et al., 2011).

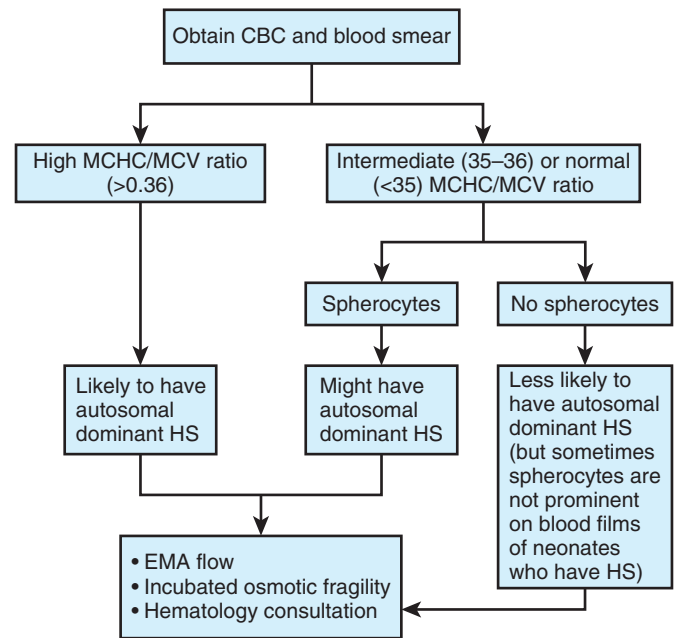
Jaundice is the most common presenting feature of HS in neonates (Delhommeau et al., 2000). In addition, the typically sluggish erythropoietic response of neonates often renders the reticulocyte count low relative to the degree of anemia, spherocytes are less often observed on the blood smear of neonates, and other markers of hemolysis seen in older patients, such as low haptoglobin levels (Chavez-Bueno et al., 2011), may be poor indicators of hemolysis in the neonate.

About 65% of neonates with HS have a parent with HS. When a parent has HS, it is very important that this information be placed prominently in the prenatal record and communicated verbally, before birth, to the physicians and the hospital staff who will be providing neonatal care. All parents with HS should be encouraged to communicate this information to their baby's physician before delivery. Failure to communicate this information sometimes occurs when the affected parent has been asymptomatic since splenectomy as a child and has all but forgotten about the condition and fails to consider that it might be problematic for the newborn. It can be helpful to specifically inquire of parents of anemic and/or jaundiced neonates about a family history of anemia, jaundice, splenectomy, or early gallstones.

One way to suspect HS in a jaundiced neonate is to obtain a complete blood count for interpretation of the RBC indices—in particular the MCHC and the MCV—and to examine the peripheral blood smear for the presence of spherocytes and polychromasia (Christensen et al., 2014e). Typically, a neonate with HS will have an elevated MCHC. Another way to estimate whether or not a neonate has HS takes advantage of the fact that in most neonates with HS the MCV is low. On the basis of this, the “neonatal HS ratio” can be calculated by division of the MCHC by the MCV. With use of this ratio, little overlap is seen between normal neonates and those later proven to have HS. In the Intermountain Healthcare database, a neonatal HS ratio greater than 0.36 indicates that HS is present with 97% sensitivity, more than 99% specificity, and more than 99% negative predictive value (Yaish et al., 2013).

Fig. 81.14 reviews our recommendations for how to evaluate a neonate for HS when a parent is known to have HS. Since the common forms of HS are inherited in an autosomal dominant manner, each child born to a parent with HS has a 50% chance of inheriting the disorder. The key element in the algorithm is to treat the neonate as if he or she has HS until proven otherwise. This includes, in addition to obtaining erythrocyte indices, peripheral smear examination, and reticulocyte count, adhering to the AAP guidelines for bilirubin monitoring in the birth hospital, aggressive phototherapy when indicated, and a follow-up bilirubin check no later than 24 hours after the hospital discharge.

Fig. 81.15 reviews our recommendations for evaluating a jaundiced neonate for HS when neither parent has HS. There is no need to wait until the neonate is several months old, or until significant anemia develops, to begin the evaluation. If these algorithms are followed, the diagnosis can often be made during the birth hospitalization.



• **Fig. 81.14** Evaluation of a neonate, during the birth hospitalization, whose parent has hereditary spherocytosis. *CBC*, Complete blood count; *EMA*, eosin 5-maleimide; *HS*, hereditary spherocytosis; *MCHC*, mean corpuscular hemoglobin concentration; *MCV*, mean corpuscular volume.

The presence of spherocytes on a peripheral blood smear is helpful when one is considering the diagnosis of HS (see Fig. 81.8; Christensen et al., 2014e), but up to one-third of neonates with HS do not have spherocytes identified prominently on their blood smear. Some neonates with band 3 deficiency have “pincered” RBCs (Jarolim et al., 1996). A subset of neonates with DAT-negative ABO incompatibility severe enough to produce spherocytes on a peripheral smear has been described. In most cases the differentiation from HS is clear (family history, maternal/infant blood group analysis, etc.), but additional testing such as elution of anti-A or anti-B from neonatal erythrocytes or detection of free anti-A or anti-B IgG antibody in neonatal serum (indirect antiglobulin test) can clarify the diagnosis.

When the diagnosis of HS is in doubt, eosin 5-maleimide (EMA) binding or osmotic fragility testing can be helpful (King and Zanella, 2013; Christensen et al., 2014a). EMA binding is a flow cytometry–based test that measures the relative amount of fluorescently labeled EMA dye bound to band 3 and Rh-related proteins in the erythrocyte membranes. In HS, the reduction in the levels of band 3 and other membrane proteins leads to decreased fluorescence intensity. In the Primary Children’s Hospital Hematology Clinic, EMA flow has outperformed other diagnostic tests for HS in newborns.

Table 81.6 lists laboratory evaluations we judge as helpful in diagnosing HS in a neonate. Sequencing of the relevant genes can be undertaken as a confirmatory test, available in a few reference laboratories, when desired (Christensen et al., 2013). We reserve genetic sequencing for cases with no family history and a severe HS phenotype so as to make a clear diagnosis, allowing appropriate therapy, and to provide parents and family members with genetic information for counseling regarding recurrence risk.

Phototherapy should reduce the bilirubin level of jaundiced neonates with HS, and it is the mainstay of treatment in the first

• **Fig. 81.15** Evaluation of a neonate with problematic jaundice where the cause is unclear. Not all neonates who receive phototherapy for 2 days or more have hemolytic jaundice. However, if hemolytic jaundice is suspected, this algorithm for stepwise evaluation of the cause might be useful. *CBC*, Complete blood count; *DAT*, direct antiglobulin test; *EMA*, eosin 5-maleimide; *G6PD*, glucose 6-phosphate dehydrogenase; *HS*, hereditary spherocytosis; *MCHC*, mean corpuscular hemoglobin concentration; *MCV*, mean corpuscular volume; *RBC*, red blood cell.

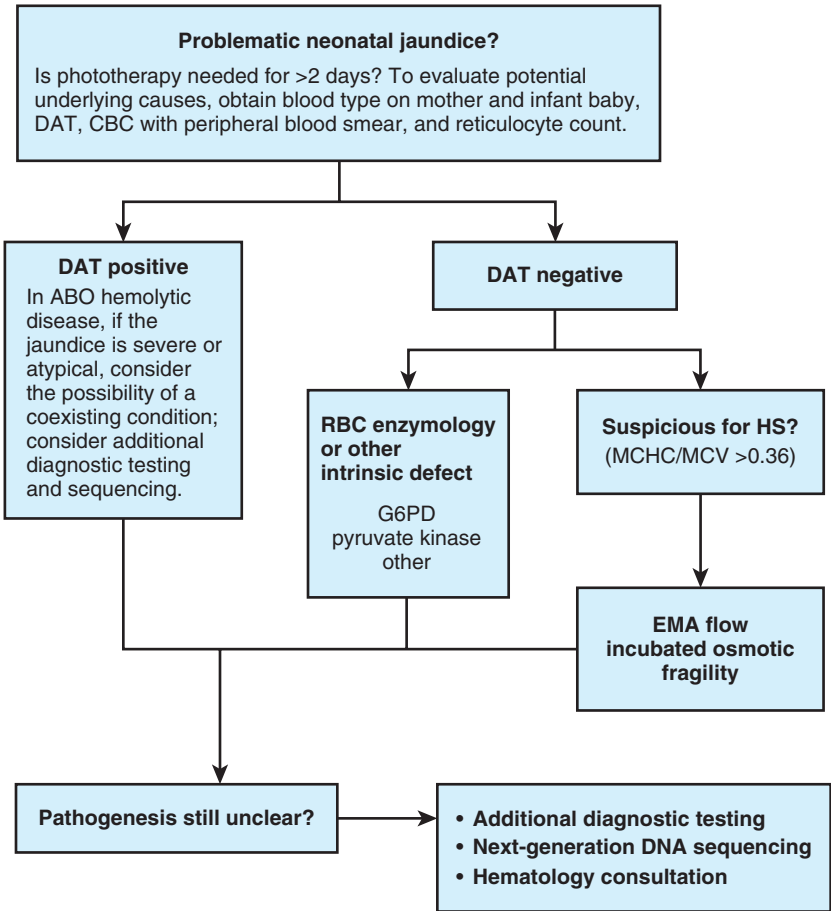


TABLE 81.6 Laboratory Evaluation for Hereditary Spherocytosis in a Jaundiced Neonate

| HS Ratio (MCHC/MCV) | EMA Flow ^a | Incubated Osmotic Fragility | DNA Sequencing ^b |
|---|--|--|---|
| Neonates with HS will generally have a high MCHC and a low MCV, giving an elevated ratio (>0.36). | EMA dye binds stoichiometrically to band 3 and Rh-related membrane proteins. Decreased fluorescence intensity of EMA-tagged erythrocytes caused by loss of membrane proteins is seen in HS. Decreased EMA binding is also seen in other disorders, such as hereditary pyropoikilocytosis and congenital dyserythropoietic anemia II. | HS erythrocytes are more susceptible to osmotic lysis than normal erythrocytes because of decreased membrane surface area. Incubation overnight stresses the already fragile HS erythrocyte, accentuating the defect. Spherocytes from any cause, including ABO incompatibility, will yield a positive osmotic fragility test. | Not needed to diagnose most cases of HS. However, it can establish the diagnosis in difficult cases. Consider sequencing of relevant genes when family history is negative and severe DAT-negative hemolysis is idiopathic. |

^aAvailable in many reference laboratories.
^bAvailable in selected hematology/genetics reference laboratories.
DAT, Direct antiglobulin test; *EMA*, eosin 5-maleimide; *HS*, hereditary spherocytosis; *MCHC*, mean corpuscular hemoglobin concentration; *MCV*, mean corpuscular volume; *Rh*, rhesus.

few days after birth (Maisels and McDonagh, 2008; Dennerly et al., 2001; Johnson and Bhurtani, 2011). When a bilirubin level is found to be in the high or high-intermediate risk zone (>75th percentile reference interval), phototherapy should be provided immediately. An exchange transfusion followed by intensive phototherapy should follow AAP guidelines (Maisels et al., 2009).

When signs of anemia appear, packed erythrocyte transfusions are helpful. Longitudinal studies indicate that transfusion requirements abate in most patients by 1 year of age. The few patients who remain transfusion dependent, typically those with severe anemia in utero or immediately after birth, have severe HS. Erythropoiesis-stimulating agents (recombinant Epo [rEpo] or darbepoetin) have sometimes been used as an alternative or adjunct

to transfusion (Tchernia et al., 2000; Neuman-Laniec et al., 2002; Schiff et al., 2003). The rationale for rEpo treatment relates to the relative hypoplastic phase of erythropoiesis during the first few weeks to months after birth. This phase might be related to the abrupt fall after birth from the highly stimulated erythropoiesis during fetal life, the switch of Epo production from the liver to the kidney, the switch from fetal to adult hemoglobin, or a lower serum level of Epo in infants compared with older children. Patients with moderate or severe HS should receive folate supplementation to prevent complications of folic acid deficiency. Splenectomy is rarely undertaken in the first year of life. Because hemolysis abates in most patients, careful symptomatic management is prudent, with transfusion therapy as indicated.

Hereditary Elliptocytosis

Hereditary elliptocytosis is an autosomal dominant clinically heterogeneous group of disorders caused by mutations of the RBC membrane cytoskeletal proteins, usually α -spectrin or protein 4.1 (Da Costa et al., 2013; Soderquist and Bagg, 2013; Swierczek et al., 2013; King et al., 2015). These weaken skeletal protein interactions and increase erythrocyte mechanical fragility. Heterozygotes usually exhibit elliptocytes on the blood smear, but in most instances hemolysis is well compensated for by reticulocytosis. Homozygotes or compound heterozygotes may have sufficient weakening of the cytoskeleton to cause significant hemolysis accompanied by striking abnormalities in RBC morphology.

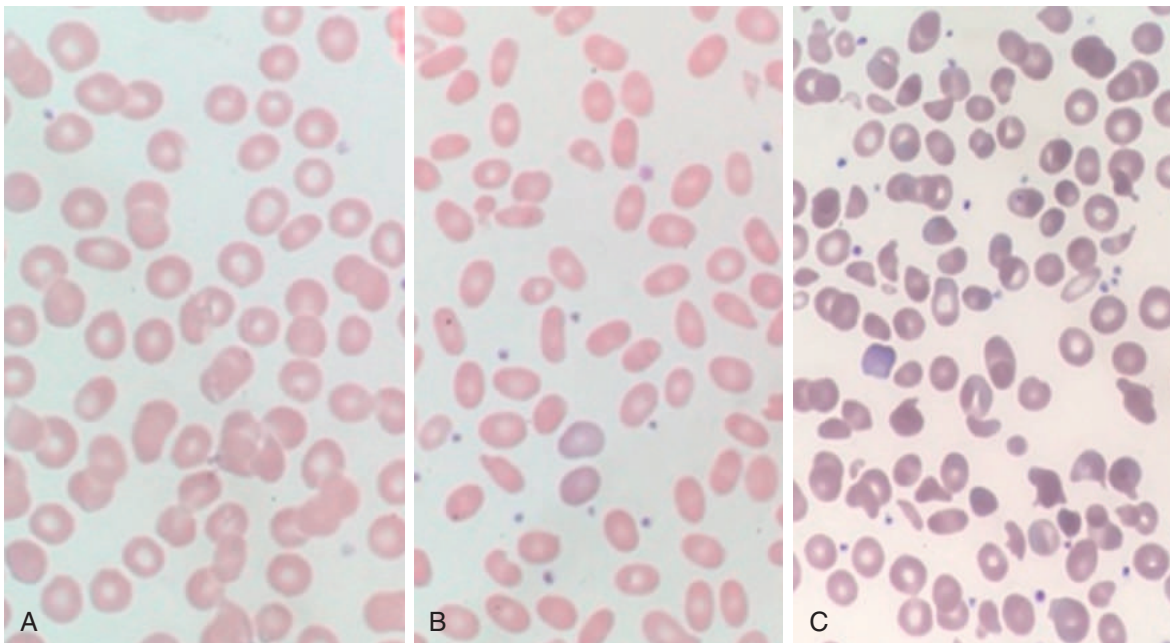
Most neonates with autosomal dominant hereditary elliptocytosis have minimal problems with hemolysis, and they go on to have asymptomatic elliptocytosis. In some neonates, RBC fragility is abnormal, and jaundice and anemia are problematic, but as HbF levels decline postnatally, fragility lessens, hemolysis disappears, and RBC morphology undergoes a transition from poikilocytosis to elliptocytosis.

Hereditary Pyropoikilocytosis

Hereditary pyropoikilocytosis (HPP) occurs in an autosomal recessive fashion, generally when one parent has hereditary elliptocytosis with a mutation in the gene encoding α -spectrin (*SPTA1*) on chromosome 1 at 1q23.1 and the other parent is hematologically normal but is a “silent carrier” of a relevant mutation in the same gene (Swierczek et al., 2013). The molecular basis is typically compound heterozygosity involving a missense mutation causing a severe spectrin dimer self-association defect inherited from the parent with hereditary elliptocytosis, in *trans* with an hypomorphic mutation of *SPTA1*^{LELY}, known as α -spectrin *Lelly*. *SPTA1*^{LELY} is typically asymptomatic and is a relatively high-frequency polymorphism. However when coinherited with other mutations in *SPTA1*, this can underlie an α -spectrin deficiency giving rise to a phenotype much severer than hereditary elliptocytosis. Clinical findings of neonates with HPP include significant hyperbilirubinemia, hemolytic anemia that can require periodic RBC transfusions (Ramos et al., 2007; Christensen et al., 2014d), and a propensity to develop parvovirus-induced aplastic crisis. Fig. 81.16 shows blood smears from an asymptomatic father who had a few elliptocytes, a hematocrit of 40%, and a reticulocyte count of 1%, a mother who had hereditary elliptocytosis, a hematocrit of 32%, and a reticulocyte count of 7.4%, and the baby, who had HPP with a hematocrit of 22% and a reticulocyte count of 9%.

Hereditary Hemolytic Anemias Caused by Red Blood Cell Enzyme Abnormalities

Hyperbilirubinemia, anemia, and hydrops fetalis can result from inherited erythrocyte enzymopathies. The two most commonly encountered such disorders are G6PD deficiency and pyruvate kinase (PK) deficiency.



• **Fig. 81.16** Photomicrographs of blood from (A) the father, (B) the mother, and (C) the neonate. The father had a few elliptocytes, the mother had many elliptocytes, and the neonate had a wide variety of abnormal shapes, including microspherocytes, schistocytes, helmet cells, and elliptocytes.

Glucose 6-Phosphate Dehydrogenase Deficiency

G6PD deficiency is an X-linked disorder affecting millions of people throughout the world, particularly in Mediterranean countries, the Middle East, Africa, and Asia. G6PD deficiency is thought to have become common because it provides a measure of protection against malaria. In most G6PD-deficient people, hemolysis and anemia are present only episodically, precipitated by infection, exposure to medications that are potent oxidants, or exposure to other agents, such as fava beans, naphthalene, or certain petrochemical-derived substances. Rarely, hemolytic anemia is chronic rather than episodic. The clinical heterogeneity of G6PD deficiency is due to the different mutations, usually single amino acid substitutions, which lead to altered enzyme function (Luzzatto et al., 2016).

Normal RBCs contain abundant amounts of reduced glutathione (GSH), a sulfhydryl-containing tripeptide that serves as an intracellular antioxidant, neutralizing oxidant drug metabolites and activated oxygen species. G6PD-deficient RBCs have a limited capacity to regenerate GSH from oxidized glutathione. In the absence of GSH, hemoglobin is vulnerable to oxidant injury, and denatured globin precipitates as Heinz bodies that bind to the cell membrane, alter its structure and function, and lead to hemolysis (see Fig. 81.10).

The variant G6PD A⁻ is responsible for nearly all of the G6PD deficiency seen in Africans (present in approximately 10% of African Americans). It is due to Val68Met and Asn126Asp, which affect the stability of the enzyme, causing an accelerated decline in activity during the life span of the RBC. Only in the oldest RBCs does enzyme activity decline to low enough levels to create vulnerability to oxidant hemolysis. For this reason hemolysis is typically mild and self-limited. Favism is not seen in African Americans with G6PD A⁻. In contrast, in Asians and individuals of Mediterranean or Middle Eastern descent, G6PD deficiency decreases the enzyme activity in young as well as old RBCs, and thus hemolysis is usually more severe.

The gene for G6PD is located on the X chromosome. All of the RBCs of G6PD-deficient males are affected by the enzyme deficiency, whereas a variable fraction of RBCs of G6PD-deficient females are enzyme deficient, depending on the degree of lyonization. Thus hemolysis caused by G6PD deficiency occurs mainly in males.

The diagnosis of G6PD deficiency in a neonate is suggested by DAT-negative hemolytic anemia, where the RBCs sometimes have distinctive features (Christensen et al., 2014e), including bite and blister types caused by splenic removal of Heinz bodies (see Fig. 81.10). Measurement of enzyme activity may not reveal the deficiency in African Americans immediately after a hemolytic episode, because the population of enzyme-deficient cells has been eliminated. Repeat of the assay at a later date is often necessary. Identification of specific G6PD mutations by DNA analysis is available from reference hematology laboratories.

In the neonatal period the major manifestation of G6PD deficiency is hyperbilirubinemia. Jaundice is not typically present at birth, with clinical onset between day 2 and day 3 (Kaplan et al., 2016). Most infants with hyperbilirubinemia caused by G6PD deficiency are of Mediterranean, Middle Eastern, or Asian descent.

The severity of jaundice differs widely. Data from the USA Kernicterus Registry from 1992 to 2004 indicate that more than 30% of kernicterus cases are associated with G6PD deficiency. These observations have raised the question of whether testing for G6PD deficiency should be included in all newborn screening programs (Kaplan et al., 2016).

Therapy for neonatal hemolysis and hyperbilirubinemia resulting from G6PD deficiency includes (1) phototherapy or exchange transfusion to prevent kernicterus, (2) RBC transfusion for symptomatic anemia, (3) removal of potential oxidants that may be contributing to hemolysis, and (4) treatment of infections using agents that do not themselves initiate hemolysis.

Pyruvate Kinase Deficiency

PK deficiency is an autosomal recessive disorder occurring in all ethnic groups (Grace et al., 2015). It is the most common defect of the Embden–Meyerhof pathway, but it is rare in comparison with G6PD deficiency. The overall incidence in the United States is perhaps one case in 20,000 births, but the frequency is much higher in certain groups where consanguinity is common (Christensen et al., 2011d). PK is one of the two RBC enzymes that generate adenosine triphosphate. Nonerythroid cells have other ways to generate adenosine triphosphate, and therefore clinical abnormalities in PK deficiency are limited to RBCs. More than 180 different mutations have been found in *PKLR*, the gene that encodes PK in erythrocytes (Zanella et al., 2007). The variations in the severity of jaundice and anemia among individuals with PK deficiency reflect this genetic diversity.

PK deficiency should be considered in the differential diagnosis of a neonate with unexplained Coombs negative, nonspherocytic hemolytic jaundice when neither parent has a history of jaundice and anemia, particularly when the RBC morphology of the neonate is consistent with PK deficiency (see Fig. 81.11).

Neonates with PK deficiency have jaundice and go on to have anemia. RBC transfusions for anemia are occasionally required but not typically during the first 2 weeks. Splenectomy can reduce the rate of hemolysis but should be avoided in infancy and early childhood because of the high risk of infection.

Neonatal Hemolysis Associated With Unstable Hemoglobins

Certain mutations in the gamma globin gene *HBG1* on chromosome 11 and on the alpha globin gene *HBA1* on chromosome 16 can render the hemoglobin molecule unstable, resulting in insoluble hemoglobin precipitates in erythrocytes (Glader, 2013). These precipitates are recognized by a supra vital stain and are termed *Heinz bodies*. When Heinz bodies are removed from erythrocytes in the spleen, bite cells, blister cells, and other schistocytic forms are seen, and hemolytic jaundice and anemia occur. Hemoglobin Hasharon is due to an Asp47His mutation in the alpha globin gene (*HBA1*) and results in unstable binding with the gamma chain in the production of HbF. After the gamma to beta switch, this variety of hemolytic anemia ceases. In a somewhat similar fashion, a Trp130Gly mutation in the gamma globin gene (*HBG1*) results in hemoglobin F Poole, which renders HbF unstable. As with hemoglobin Hasharon, once gamma chain synthesis has been replaced by beta chain synthesis, the hemolytic anemia ceases. In the neonatal period these conditions look very similar to G6PD deficiency, with similar blood films and clinical manifestations, but with normal G6PD enzyme levels; however, hemolysis from hemoglobin F Poole and hemoglobin Hasharon remits completely in the first month or so (Glader, 2013).

Neonatal Hemolysis Associated With Alpha or Gamma Thalassemia

Beyond infancy the predominant hemoglobin tetramer is hemoglobin A, which is composed of two alpha globin chains and two beta globin chains. To appreciate the hemoglobinopathies that can

occur in a neonate, it is helpful to understand the normal developmental changes that occur in globin synthesis during fetal and neonatal life (Glader, 2013). Embryonic hemoglobins are composed of zeta and epsilon chains. The transition from zeta to alpha globin chains is complete by the end of the first trimester. Epsilon chains disappear more slowly and are replaced first by gamma chains to form HbF (which is $\alpha_2\gamma_2$) and next by beta chains to form hemoglobin A. The various possible combinations of these different globin chains form a number of different hemoglobin tetramers that are characteristically found in embryonic, fetal, and postnatal life.

HbF is the major hemoglobin found in fetuses after the first trimester. Its replacement by adult hemoglobin A begins before birth, such that 60%–90% of the hemoglobin in the normal term infant is hemoglobin F. After birth, gamma chain synthesis declines rapidly as beta chain synthesis increases, so most newly formed hemoglobin is hemoglobin A. As RBCs made before birth are replaced postnatally, the percentage of hemoglobin F declines rapidly, reaching a level of approximately 5% by 6 months of age. Disorders of beta globin, such as sickle cell disease and beta thalassemia major, are not clinically apparent until several months of age, when the switch from hemoglobin F to hemoglobin A synthesis reveals the defect. In contrast, gamma globin mutations are evident in the neonate and then disappear as gamma globin synthesis wanes.

Thalassemias are due to absent or deficient synthesis of a normal globin chain, leading to a relative excess of the complementary or partner chain (Brancaleoni et al., 2016). For example, alpha thalassemias are due to diminished synthesis of alpha globin chains, leading to an excess of beta chains (or, in the fetus, of gamma chains). The excess beta chains form tetramers (hemoglobin H) that are unstable and can lead to hemolysis beyond infancy. The excess gamma chains also form tetramers (hemoglobin Bart's) that have an increased affinity for oxygen but do not cause hemolysis. In beta thalassemia, excess alpha globin chains accumulate, forming aggregates that injure the cell membrane, leading to hemolysis. In addition, the decrease in overall production of hemoglobin produces microcytic hypochromic erythrocytes.

Alpha Thalassemia

Neonatologists should be aware of the fetal/neonatal manifestations of alpha thalassemia. The more severe forms of alpha thalassemia are found in Southeast Asians, less commonly in individuals of Mediterranean origin, and are rare in Africans (Vichinsky, 2013). The molecular basis for alpha thalassemia involves deletion of one or more of the four alpha globin genes. Hemoglobin Constant Spring can also behave like a mild form of alpha thalassemia (He et al., 2016). The clinical severity of alpha thalassemia is determined by how many of the four alpha globin genes are absent. An infant can inherit none, one, or two alpha globin genes from each parent, giving rise to the following four clinical syndromes (Vichinsky, 2013):

1. *One-gene deletion. Silent carrier.* Deletion or nonfunction of one of the four alpha globin genes has no clinical or hematologic abnormalities. State metabolic screens sometimes identify small amounts of hemoglobin Bart's (gamma chain tetramers).
2. *Two-gene deletion. Alpha thalassemia trait.* Deletion or nonfunction of two of the four alpha globin genes, in *cis* (Asians) or *trans* (Africans), is associated with mild microcytic anemia, without hemolysis or reticulocytosis. These neonates will almost invariably have hemoglobin Bart's identified on their state hemoglobin screen.

3. *Three-gene deletion. Hemoglobin H disease.* When three of the four alpha globin genes are deleted or nonfunctional, a mild to moderate hemolytic anemia occurs, often aggravated by oxidant stresses just as in G6PD deficiency. The erythrocytes are hypochromic and microcytic and contain inclusions of hemoglobin H when appropriate staining is performed. Hemoglobin H Constant Spring disease can be a particularly severe syndrome, with up to one-third of neonates and infants requiring regular transfusions.

4. *Four-gene deletion. Homozygous alpha thalassemia.* Lack of all four alpha globin genes is associated with a severe intrauterine hemolytic anemia and hydrops fetalis, with massive hepatosplenomegaly and, in almost all instances, fetal demise (Bellini et al., 2015). The RBCs are very hypochromic, fragmented, and bizarre in shape, and erythroblastosis is present.

The diagnosis of the alpha thalassemia syndromes can be made during the newborn period by correlation of the clinical and hematologic appearance of the child with the amount of hemoglobin Bart's (tetramers of gamma chains). The large amount of hemoglobin Bart's found in the erythrocytes of homozygotes for alpha thalassemia contributes to the clinical severity of the syndrome because the increased oxygen affinity of this hemoglobin impairs oxygen release to the tissues. DNA-based diagnostic tests are available for prenatal diagnosis, which is often performed when a pregnancy at risk for a fetus with homozygous alpha thalassemia is identified.

Neonates with the silent carrier state or with an alpha thalassemia trait need no treatment for their condition. However, it can be helpful to determine the thalassemia status of other family members because this can inform genetic counseling (and prenatal diagnosis if indicated). Parents of neonates with hemoglobin H disease should be instructed to avoid oxidants that can cause hemolysis (the same list that is given to patients with G6PD deficiency). Although these infants are usually only mildly anemic, they can have severe episodes of hemolysis during infections or with exposure to oxidants. Fetuses with homozygous alpha thalassemia who are not spontaneously aborted are usually stillborn. A few affected children have been born alive and resuscitated or supported with in utero transfusion before delivery. A few have undergone bone marrow transplant, but the long-term outcome for such infants is quite uncertain.

Gamma Thalassemia

Large deletions within the beta globin gene cluster may remove both gamma globin genes as well as delta and beta globin genes. The resulting gamma–delta–beta thalassemia is lethal in the homozygous state but in the heterozygote produces a transient but moderately severe microcytic anemia in the newborn (Glader, 2013). Over the first few months the anemia resolves to a variable extent without specific therapy, and eventually the hematologic picture is that of a beta thalassemia trait. Several different gamma–delta–beta deletions have been reported.

Neonatal Polycythemia/Hyperviscosity and Methemoglobinemia

Polycythemia/Hyperviscosity

The definition of neonatal polycythemia is a statistical issue. The definition is met when the hematocrit, blood hemoglobin concentration, or RBC count (or all three) exceeds the 95th percentile upper reference interval for gestational age and postnatal age (Henry and

Christensen, 2015). Sometimes the upper reference interval is set at the 97.5th percentile. The higher cut-point for defining polycythemia is the most common for older children and adults. As detailed earlier in this chapter, the difference is that the adult reference intervals are generally derived from healthy adult volunteers, while the neonatal reference intervals are derived from clinically ordered tests on patients; thus the neonatal interval charges are more likely to contain abnormal (both low and high) values. On that basis a somewhat more restrictive definition (>95th percentile rather than >97.5th percentile) has become the convention.

Altitude relative to sea level is a significant consideration in defining polycythemia of adults and nonneonatal children. This is because at very high altitudes the relatively less oxygen (O₂) in inspired air results in a marrow compensation to produce higher hematocrit, blood hemoglobin concentrations, and RBC count. These physiologic adjustments compensate for the lower O₂ availability in the inspired air and permit a normal delivery of oxygen to tissues. Consequently, the hematocrit “cutoff” level that defines a patient as being polycythemic is much higher among those living at high altitude than it is among those living at sea level. This difference in altitude is less important, perhaps not important at all, in defining polycythemia of most neonates. Yancey et al. (1992) reported no differences in umbilical cord blood arterial or venous oxygen saturation values for births at 5900 feet above sea level (Fort Carson, Colorado) versus 87 feet above sea level. Reference intervals for hematocrit in newborns in Salt Lake City hospitals (Christensen et al., 2009; Jopling et al., 2009) (about 5000 feet above sea level) are essentially the same as those for newborns at sea level. However, perhaps at very high altitudes (>10,000 feet) fetal hematocrits are slightly higher, and thus for very high altitude nurseries, unique reference intervals might be needed to accurately define neonatal polycythemia.

In adult populations the definition of polycythemia depends on sex, because statistically, groups of males have higher hematocrits, hemoglobin levels, and RBC counts than women. Although some small studies suggested that male fetuses and neonates had higher hematocrits than their female counterparts, larger studies of matched populations of male and female newborns indicate no differences in hematocrit or hemoglobin level or RBC counts between the sexes. Therefore one set of reference ranges will suffice for both males and females.

It is important for clinicians to recognize that polycythemia is not synonymous with hyperviscosity and that not every neonate with polycythemia also has hyperviscosity or will benefit from a reduction transfusion procedure. Viscosity is the property of a liquid to resist changes in shape; for example, honey is more viscous than water. When the whole blood viscosity measurement of a neonate exceeds the appropriate 95th percentile upper reference interval for age, hyperviscosity is diagnosed. That laboratory-based definition of neonatal hyperviscosity implies that a Clinical Laboratory Improvement Amendments–approved clinical laboratory is available to perform a whole blood viscosity measurement and can perform this with a reasonable time and at reasonable cost. For some NICUs this is indeed the case, as it is for us in Salt Lake City, where ARUP Laboratories perform whole blood viscosity testing every day (Christensen et al., 2014b). However, when whole blood viscosity testing is not convenient, hyperviscosity can be inferred when a neonate with polycythemia has signs of hyperviscosity (Box 81.3). When signs of hyperviscosity are obvious, in a polycythemic neonate, obtaining a whole blood viscosity measurement might not be particularly important. But when clinical signs are subtle or absent, yet the hematocrit is greater than the 95th

• BOX 81.3 Clinical Signs of Hyperviscosity in a Neonate With Polycythemia

Plethora
Cyanosis
Tachycardia
Tachypnea
Hypotonia/lethargy
Weak suck/poor feeding
Tremulousness
Hypoglycemia
Hypocalcemia
Jaundice

percentile upper limit, hyperviscosity measurements can influence the decision about the risk–benefit analysis for reduction transfusion.

Neonatal polycythemia is usually caused by one of two conditions: increased intrauterine erythropoiesis or fetal hypertransfusion. Other causes seen in older children, such as arterial hypoxemia (cyanotic heart disease, pulmonary disease), abnormal hemoglobins, or hypersecretion of Epo by tumors, are rare, and primary polycythemia or polycythemia vera is virtually nonexistent in neonates. In normal term infants, delayed clamping of the umbilical cord for 3 minutes or more can sometimes lead to transfer of a sufficient amount of fetal blood so as to cause polycythemia. Placental insufficiency and chronic intrauterine hypoxia, as seen typically in SGA infants, can lead to an increase in Epo-mediated erythropoiesis.

As the hematocrit increases, blood viscosity increases exponentially. Oxygen transport, which is determined by both hemoglobin levels (i.e., oxygen-binding capacity) and blood flow, is maximal in the normal hematocrit range. At low hematocrits, oxygen transport is limited by reduced oxygen-binding capacity, whereas at higher hematocrits, reduction in blood flow secondary to hyperviscosity may similarly limit oxygen transport. At any given hematocrit, expansion of the blood volume beyond the normal level distends the vasculature, decreases peripheral resistance, and increases blood flow and, ultimately, oxygen transport.

Most polycythemic infants have no symptoms, particularly if the polycythemia becomes apparent only on routine neonatal screening. Symptoms, when present, are usually attributable to hyperviscosity and poor tissue perfusion or to associated metabolic abnormalities, such as hypoglycemia and hypocalcemia. Early signs include plethora, cyanosis, lethargy, hypotonia, poor suck and feeding, respiratory distress, and tremulousness. Complications can include cardiorespiratory distress, seizures, necrotizing enterocolitis, and renal failure. Because the elevated RBC mass increases the catabolism of hemoglobin, hyperbilirubinemia is common.

Methemoglobinemia

Methemoglobin (metHb) is derived from the oxidation of hemoglobin iron, whereby the iron is in the ferric (Fe³⁺) rather than the ferrous (Fe²⁺) state (Glader, 2013). Oxidized iron renders hemoglobin much less able to either bind or release oxygen. Thus when the concentration of metHb rises significantly, oxygen transport is reduced. Normally, small amounts of metHb are formed from hemoglobin constantly during the process of releasing

oxygen. The metHb that formed is then reduced through the action of erythrocyte nicotinamide adenine dinucleotide (NADH)-dependent metHb reductase, which is also called *cytochrome b5 reductase*. Thus normally the levels of metHb seldom exceed 1% of total hemoglobin. A second metHb reductase, dependent on NADPH as a cofactor, is also present in RBCs. This enzyme has little function under normal physiologic conditions, but it is greatly activated by the presence of certain redox compounds, such as methylene blue, forming the basis for the clinical treatment of methemoglobinemia.

Normal individuals can acquire methemoglobinemia after exposure to oxidizing chemicals. Neonates are particularly susceptible to this because HbF is more readily oxidized to the ferric state than is hemoglobin A and also because erythrocyte NADH-dependent metHb reductase activity is low during the first few months. Merely marking of the diapers of newborns with aniline dyes has caused methemoglobinemia. Drugs such as prilocaine, administered before birth to provide local anesthesia, can produce methemoglobinemia in both the mother and the infant. Although in most infants no increase in metHb levels follows the use of lidocaine–prilocaine cream (EMLA cream) to provide analgesia during circumcision, a few case reports of visible cyanosis caused by methemoglobinemia in infants treated with this cream have appeared (Tran and Koo, 2014). Perhaps the best known agent causing methemoglobinemia is nitrite, either present *de novo* in ingested material or generated by administration of nitric oxide to treat pulmonary hypertension (Davidson et al., 1998). Nitrates can be converted to nitrite by the action of intestinal bacteria. It is for this reason that well water or foods with a high nitrate content (e.g., cabbage, spinach, beets, carrots) can produce methemoglobinemia in infants (Glader, 2013). Accumulation of nitrate in the intestinal tracts of infants with diarrhea and acidosis or symptomatic dietary protein intolerance is thought to underlie the transient methemoglobinemia that occurs in these conditions.

Congenital methemoglobinemia can be the result of either inherited disorders of hemoglobin structure or a severe deficiency of NADH-dependent metHb reductase activity. The inherited abnormalities of hemoglobin structure that give rise to methemoglobinemia are known collectively as the *hemoglobin M disorders*. These are rare autosomal dominant defects caused by point mutations that alter a single amino acid in the structure of normal globin. The altered conformation favors the persistence of the ferric rather than the ferrous form of heme iron. Two of the mutations affect the alpha globin chain, three affect the beta globin chain, and two affect the gamma globin chain. Only the alpha and gamma globin chain mutations are associated with neonatal methemoglobinemia. Neonatal methemoglobinemia is transient when produced by one of the two gamma globin chain mutations, hemoglobin FM Osaka (Hayashi et al., 1980) or hemoglobin FM Fort Ripley (Glader, 1989), because the normal developmental switch from fetal to adult hemoglobin eliminates all but a trace of the mutant hemoglobin. Hemoglobin M heterozygotes inheriting alpha or beta globin mutations have lifelong cyanosis, but they are usually asymptomatic. The homozygous state is incompatible with life.

NADH-dependent metHb reductase deficiency is a rare autosomal recessive disorder. Heterozygotes are asymptomatic and do not have methemoglobinemia unless challenged by drugs or chemicals that cause methemoglobinemia. Homozygotes have lifelong metHb levels of 15%–40% and are cyanotic but otherwise asymptomatic unless exposed to toxic agents.

The cardinal clinical manifestation of methemoglobinemia is cyanosis not resulting from cardiac or respiratory disease. Cyanosis present at birth suggests hereditary methemoglobinemia, whereas that appearing suddenly in an otherwise asymptomatic infant is more consistent with acquired methemoglobinemia. The blood is dark and, unlike deoxygenated venous blood, does not turn red when exposed to air. Rapid screening for methemoglobinemia can be done by placing a drop of blood on filter paper and then waving the filter paper in air to allow the blood to dry. Deoxygenated normal hemoglobin turns red, whereas metHb remains brown. More accurate determination of metHb levels is accomplished in the blood gas laboratory by cooximetry or in the clinical laboratory with use of a spectrophotometer. Cyanosis is first clinically evident when metHb levels reach approximately 10% (1.5 g/dL), but symptoms attributable to hypoxemia and diminished oxygen transport do not appear until the levels reach 30%–40% of the total hemoglobin level. Death occurs at levels of 70% or greater. Methemoglobinemia is not associated with anemia, hemolysis, or other hematologic abnormalities.

Treatment with intravenously administered methylene blue (1 mg/kg as a 1% solution in normal saline) is indicated when metHb levels are greater than 15%–20%. Doses greater than 1 mg/kg should be avoided, because they may be toxic. The response to methylene blue is both therapeutic and diagnostic. MetHb levels decrease rapidly, within 1 to 2 hours, if methemoglobinemia is caused by a toxic agent or by a deficiency of NADH-dependent metHb reductase. In contrast, the hemoglobin M disorders do not respond to methylene blue. Reappearance of methemoglobinemia after an initial response to methylene blue suggests a deficiency of NADH-dependent metHb reductase or the persistence of an occult oxidant.

Treatment Considerations

Treatment of Anemia

The best practices in neonatal transfusion medicine are only beginning to be defined (Christensen et al., 2014c). Methods of preventing early anemia in VLBW neonates have been tested, such as delayed clamping of the umbilical cord or umbilical cord milking (stripping) and drawing the blood for initial laboratory blood studies of VLBW infants from otherwise discarded blood in the umbilical cord, not directly from the neonate. Guidelines to reduce phlebotomy-related losses of blood from VLBW neonates and the selected use of erythropoiesis-stimulating agents have also been favorably tested as complementary anemia-prevention approaches.

Regardless of the mechanism responsible for anemia of prematurity, exogenous Epo administered to preterm infants accelerates effective erythropoiesis (Aher and Ohlsson, 2014a, 2014b; Ohls et al., 2015). Metaanalysis of studies evaluating the use of “early” Epo administration and the use of “late” Epo administration to prevent and treat anemia of prematurity reveals a positive effect on decreasing transfusion requirements in preterm infants. In addition, beneficial neurodevelopmental effects of recombinant Epo administration have been reported in preterm infants (Ohls et al., 2013, 2014, 2016).

Pharmacokinetic studies of darbepoetin, a long-acting erythropoietic stimulator, have been conducted among neonates with anemia of prematurity, with the speculation that less frequent dosing and cost savings might render darbepoetin a more attractive alternative than rEpo for treatment of anemia of prematurity.

(Warwood et al., 2005, 2006a, 2006b; Patel and Ohls, 2015). Following subcutaneous and intravenous dosing, darbepoetin has a shorter terminal half-life in neonates than in adults. Intravenous dosing appears to be as effective as subcutaneous dosing.

One very basic issue that remains unsettled is at what level to keep the hemoglobin concentration during the NICU stay (Henry et al., 2015). It is not clear whether to keep an NICU patient's hemoglobin level as high as it would be in utero (often requiring multiple transfusions to do so) or to permit it to fall to considerably lower values, attempting to avoid or minimize transfusions. Attempts have been made to define the best hemoglobin range for NICU patients, but study findings are discordant. In a single-center study, Bell et al. (2005) randomized 100 neonates with birthweights below 1300 g (average birth weight 956 g) to maintain the hematocrit in a "higher range" versus a "lower range." Those with the hematocrit kept in the lower range received fewer transfusions (average of two fewer per patient) but may have been more likely to develop periventricular leukomalacia. In contrast, the Premature Infants in Need of Transfusion (PINT) study, a larger multicenter study involving 451 extremely low birth weight (ELBW) neonates (average birthweight 770 g), concluded that neonates randomized to having their hematocrit kept in the lower range had fewer transfusions but similar neurodevelopmental outcomes (Kirpalani et al., 2006). Longer-term follow-up of the Iowa cohort indicated that the restricted transfusion strategy resulted in better neurodevelopmental outcomes (McCoy et al., 2011; Nopoulos et al., 2011). Shorter-term (18- to 22-month) follow-up of the PINT study participants suggests that higher hemoglobin thresholds for transfusion may be beneficial to neurocognitive outcomes. These concepts are currently being tested by the Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network with the Transfusion of Prematures (TOP) trial, where a planned 1824 infants will be randomized to have higher versus lower transfusion thresholds with a primary outcome of death or neurodevelopmental impairment at 22 to 26 months.

Various neonatal transfusion guidelines have been used in the last 2 decades, and research, including the TOP trial, is ongoing to determine the optimal strategy for administering RBC transfusions to preterm and term neonates. The strategy developed by the University of Utah and Intermountain Healthcare is shown in Table 81.7. When one is considering a transfusion in a preterm infant with a low hematocrit, it should be determined if the infant

needs an immediate increase in oxygen delivery to tissues. If so, then treatment consists of a transfusion of packed RBCs. If there is no evidence that an immediate increase in oxygen delivery is necessary, then treatment with erythropoiesis-stimulating agents might be considered. As the process of stimulating erythropoiesis requires at least 1 week to significantly impact the reticulocyte count and may not appreciably increase the hemoglobin concentration during that time, the infant should continue to be observed for signs consistent with anemia.

A method of reducing erythrocyte transfusions among a subset of preterm neonatal patients is to begin the administration of rEpo or darbepoetin to those with low hematocrits after the first 3 weeks of life. rEpo certainly stimulates erythropoiesis in such patients, although its combination with additional folate, iron, vitamin E, and vitamin B₁₂ may be superior to recombinant Epo alone. Haiden et al. (2006) achieved significantly greater success in preterm infants weighing less than 800 g (38% of infants not transfused) when vitamin B₁₂ at a dosage of 21 mg/kg per week subcutaneously was added to a regimen of Epo, iron, vitamin E, and folate. When combined with limited phlebotomy-related blood losses, this therapy shows promise in ELBW infants.

Another method of reducing erythrocyte transfusions to ill neonates is to delay clamping of the umbilical cord. A delay of 60 seconds can result in improved iron status, fewer transfusions, and perhaps superior neurodevelopmental outcomes. Placenta transfusions can be expedited by cord milking or stripping. The amount of blood in a 30-cm segment of umbilical cord is estimated in Fig. 81.7. Typically, stripping a segment of cord, then letting it fill, and restripping it about three or four times can be accomplished in about 15 seconds. This generally accomplishes a transfusion of a similar volume of fetal blood as would be transfused with delayed cord clamping for 60 seconds (Al-Wassia et al., 2015; Katheria et al., 2015).

Yet another method of reducing or postponing early erythrocyte transfusions among ELBW neonates is to draw the blood for the initial laboratory tests from the placenta not from the neonate (Christensen et al., 2011c; Baer et al., 2013; Christensen et al., 2014c; Carroll and Christensen, 2015; Henry et al., 2015). The initial blood tests of an ELBW neonate on admission to the NICU can include a blood culture, complete blood count, tests for type and crossmatch, metabolic screen, and tests for blood gas, electrolyte, and glucose levels. Sometimes other studies such as coagulation tests are also performed at or shortly following NICU admission.

TABLE 81.7

Intermountain Healthcare/University of Utah Guidelines for Red Blood Cell Transfusions for Newborns

These guidelines provide an “indication” where transfusions are generally thought to have benefits outweighing risks. The guidelines do not mandate that a transfusion must be ordered for all neonates with this “indication”; rather the guidelines are intended as reminders to consider a transfusion under those circumstances. Any order for blood products should always be accompanied by a note in the medical record stating the clinical reason for the transfusion.

| Condition | Indication | Treatment |
|--|--------------------------|----------------------------|
| ECMO, cyanotic heart disease, pulmonary hypertension | HGB <12 g/dL or HCT <36% | 15 to 20 mL/kg in 2 to 4 h |
| Ventilator or CPAP | HGB <10 g/dL or HCT <30% | |
| Supplemental O ₂ | HGB <8 g/dL or HCT <24% | |
| Stable in room air | HGB <7 g/dL or HCT <21% | |

CPAP, Continuous positive airway pressure; ECMO, extracorporeal membrane oxygenation; HCT, hematocrit; HGB, hemoglobin; O₂, oxygen.

The total blood volume needed for these baseline NICU laboratory studies can be 4 to 5 mL or more. In a 400-g to 500-g neonate, this can exceed 10% of the total blood volume.

The need for early transfusions in the NICU can be reduced by careful attention to phlebotomy blood volumes required during the first few days following delivery. Transfusions given during the first week or first 2 weeks are principally to replace phlebotomy-related blood losses for laboratory tests. Use of laboratory methods that minimize blood loss will reduce the need for early transfusions. Such methods include point-of-care monitors, point-of-care analyzers, and a concerted effort to use the smallest amount of blood possible for the laboratory studies needed (Widness et al., 2005).

Treatment of Hyperviscosity

Treatment of hyperviscosity is by isovolumetric partial exchange transfusion to reduce the RBC mass without inducing hypovolemia. When polycythemia is present but hyperviscosity is not, treatment is probably unnecessary. The purpose of partial exchange is to alleviate the signs of hyperviscosity, such as plethora, hypoglycemia, tachypnea, and tachycardia. Partial exchange transfusion is not likely to alleviate long-term neurodevelopmental delay found in some neonates with hyperviscosity. The reason for this relates to the underlying causes of neonatal hyperviscosity. When it is due to intrauterine hypoxia, with a resulting elevation in Epo concentration and a consequent increase in erythrocyte production, the effect of the hypoxia can have long-term adverse neurodevelopmental consequences that are unaltered by reduction transfusions. Also, another treatment concern is that reduction transfusion may increase the risk of necrotizing enterocolitis.

Our treatment guidelines are as follows:

- Diagnose polycythemia on the basis that the vascular (not a capillary) hematocrit or hemoglobin concentration exceeds the 97.5th percentile upper reference interval for the gestational and postnatal age.
- Diagnose hyperviscosity on the basis that the whole blood viscosity measurement exceeds the 97.5th percentile upper reference interval for the gestational age. If a whole blood viscosity measurement is not available, diagnose hyperviscosity on the basis of polycythemia *plus* signs of hyperviscosity—plethora, hypoglycemia, and tachycardia/tachypnea—with no other explanation.
- Consider partial exchange transfusion (also known as *isovolemic reduction transfusion*) to alleviate the signs of hyperviscosity.
- Perform the reduction transfusion by simultaneously withdrawing 10 mL of blood per kilogram while in another vessel (not arterial) infusing for each kilogram 10 mL of lactated Ringer solution or normal saline, which was prewarmed by placing the syringe containing the intravenous solution near the neonate under the radiant warmer. If the prerelation hematocrit exceeds 75%, use 15 mL/kg rather than 10 mL/kg as the exchange volume.

Suggested Readings

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Neonatal Transfusion

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KEY POINTS

- The decision of when to administer red blood cell (RBC) transfusion may have an impact on long-term neurocognitive outcomes and may potentially influence short-term morbidity such as necrotizing enterocolitis.
- The optimal thresholds at which to transfuse RBCs are uncertain, although tolerance of hemoglobin values below the lower transfusion thresholds evaluated in randomized trials is not recommended.
- There is wide variation in the targeting of platelet thresholds, and it is unclear if prophylactic platelet transfusions in preterm neonates prevent serious hemorrhage.
- Prophylactic plasma transfusion is likely to have a minimal effect on prevention of intracranial bleeding in preterm infants, and newer measures such as thromboelastography may provide more precise data to guide the administration of plasma transfusion.
- Delaying clamping of the umbilical cord and minimizing phlebotomy-related blood losses are important cotreatment strategies in minimizing RBC transfusion exposure.

Overview

Blood transfusion is essential to modern neonatal intensive care and can be lifesaving, particularly for critically ill neonates or infants undergoing surgery. Blood components are necessary to carry and deliver oxygen to tissues, provide adequate preload to the heart to support cardiac output, and maintain a balance between hemostasis and coagulation to prevent both bleeding and thrombosis. In this chapter, specific blood component therapy and special circumstances in neonatal transfusion medicine will be reviewed.

Red Blood Cell Transfusion

Component

Red blood cells (RBCs) are the most commonly transfused component of whole blood. They are produced by centrifugation from whole blood or, less frequently, acquired directly from a donor by apheresis. Various storage solutions, such as citrate–phosphate–dextrose–adenine (CPDA-1) and additive–glucose–mannitol (e.g., AS-1, AS-3), contain anticoagulants and preservatives used to maintain RBCs at 4°C during storage. AS-1 and AS-3 units contain mannitol and adenine, which are associated with diuresis and renal toxicity respectively. Therefore RBCs stored in AS-1 and AS-3 should not be used for large-volume (≥ 20 mL/kg) transfusions.

Preparation

Preparation of RBCs for transfusion begins with donor assessment and ends with transfusion into the neonate. The goal is to assure blood safety and maximize efficacy and response to transfusion. Accurate RBC component and recipient identification are imperative for safety. More than 300 blood group antigens, from 35 blood group systems, have been discovered on RBCs ([Fasano and Chou, 2016](#)). Among these, the ABO and D (also known as rhesus [Rh]) blood groups are the most important in determining the compatibility of allogenic RBC transfusion. Infants can have four ABO blood types containing the corresponding A or B antigens on the RBC surface: group A, group B, group AB, or group O (no A or B antigens). In addition, infants can be D antigen positive or D antigen negative. Patient testing of the plasma or serum from either the infant or the mother must include ABO and D typing of their RBCs and a screen for unexpected RBC antibodies (indirect antiglobulin test). Before non-group type O RBCs are issued, the infant's plasma or serum is tested to detect passively acquired maternal anti-A or anti-B isohemagglutinins, which usually do not develop until more than 4 months of age. Importantly, crossmatch compatibility testing and repeated ABO and D typing, as is required for all patients older than 4 months, may be omitted during any hospitalization for an infant younger than 4 months, as long as any of the following criteria are met: (1) the antibody screen is negative; (2) the transfused RBCs are group O, ABO identical, or ABO compatible; or (3) the RBCs are either D negative or the same D type as those of the patient. Testing for the isohemagglutinins must also include the antiglobulin phase of testing at 37°C (body temperature). In the presence of an immunoglobulin

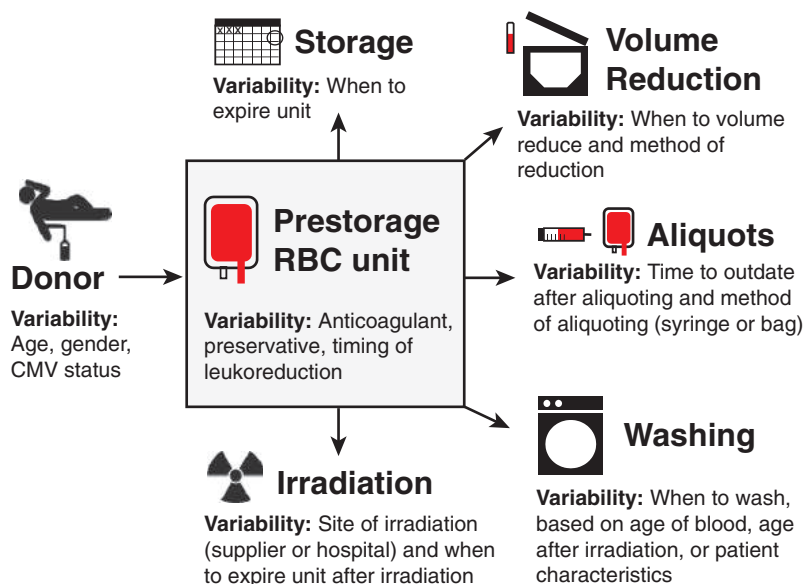
G (IgG) antibody, crossmatched, ABO-compatible RBCs are administered until the acquired antibody is no longer detected. Once RBC units have been properly selected, sterile aliquots from the parent unit are produced to more accurately provide volumes of RBCs dosed on the basis of the patient's weight. This can reduce the risk of transfusion-associated circulatory overload and also limit donor exposure–related infectious risks by repeatedly using the same RBC unit (Fung et al., 2013). Data suggest that 20 mL/kg transfusions in very low birth weight (VLBW) infants, compared with 10 mL/kg transfusions, lead to a greater increase in hematocrit without respiratory compromise (Paul et al., 2002). Although the optimal dose and duration of RBC administration are not known, transfusions should not run for longer than 4 hours. There is substantial variability in how blood centers and blood banks prepare RBCs (Fig. 82.1). This includes strategies to prevent cytomegalovirus (CMV) transmission (Josephson et al., 2014; AABB, *Clinical Transfusion Medicine Committee* et al., 2016), how and when RBCs are irradiated (Patel et al., 2015), when RBCs are unwashed (Keir et al., 2016), and what age of RBC units to use. The exclusive use of RBC transfusions from CMV-seronegative donors in VLBW infants has been shown to be associated with a very low estimated risk of transfusion-transmitted CMV infection of between 0.0% and 0.3% per unit of CMV-seronegative and leukoreduced blood (Josephson et al., 2014). Historically, the risk of transfusion-transmitted CMV (TT-CMV) infection from leukoreduced transfusions from CMV-untested donors has been reported to be higher than that from approaches using both CMV-negative donors and leukoreduction (Vamvakas, 2005). In the opinion of the authors, the safest approach for transfusion of VLBW infants is to use leukoreduced RBCs from CMV-negative donors. However, given the low risks of TT-CMV infection with improvements in modern leukoreduction techniques, use of leukoreduced blood from CMV-untested donors is an acceptably safe and low-risk alternative (Delaney et al., 2016; Strauss, 2016). Importantly, RBC transfusion

should not be delayed if CMV-negative blood is unavailable, and CMV-untested blood should be used given the relatively low risk of TT-CMV infection.

Many centers limit the age of RBCs transfused into neonates and infants (Fung et al., 2010; Josephson et al., 2015). The maximum storage duration depends on the type of storage solution, with 35 days for CPDA-1 units and 42 days for AS-1 and AS-3 units. Recent randomized trials have provided high-level evidence regarding the safety of using stored, older RBC units. The Age of Red Blood Cells in Premature Infants (ARIP) trial randomized VLBW infants to receive fresh blood (mean 5 days) versus standard issue blood (mean 15 days) and found no difference in a composite of mortality or morbidity between groups (Fergusson et al., 2012). Similar trials in adults have produced concordant findings (Lacroix et al., 2015; Steiner et al., 2015). However, the age of RBC units may not account for other donor RBC characteristics, such as storage solutions and irradiation (Patel and Josephson, 2013).

Indications

Common indications for RBC transfusion include anemia, bleeding, and cardiorespiratory compromise. Extremely preterm infants are among the most highly transfused populations in medicine, with more than 90% of extremely low birth weight infants weighing 1000 g or less at birth receiving at least one RBC transfusion during their neonatal intensive care unit (NICU) stay (Maier et al., 2000). In addition, 60% of VLBW infants will undergo RBC transfusions during hospitalization. Studies of RBC transfusion approaches in this population have yielded mixed results. Two clinical trials have compared the efficacy of RBC transfusion using liberal (high hemoglobin threshold) versus conservative (low hemoglobin threshold) transfusion strategies (Bell et al., 2005; Kirpalani et al., 2006), and two trials are ongoing, the Transfusion of Prematures (TOP) trial (Kirpalani et al., 2016) and the Effects



• **Fig. 82.1** Vein-to-Vein Processing of Red Blood Cells. Variability among centers and blood banks in processing of red blood cells, from donation to preparation before transfusion into the neonate. CMV, Cytomegalovirus; RBCs, red blood cells. (Reprinted with permission from Patel RM, Meyer EK, Widness JA. Research opportunities to improve neonatal red blood cell transfusion. *Transfus Med Rev.* 2016;30:165–173.)

TABLE 82.1 Red Blood Cell Transfusion Thresholds for Preterm Infants in Randomized Trials^a

| | | Iowa Trial (Bell et al., 2005) | PINT Trial (Kirpalani et al., 2006) | TOP Trial | ETTNO Trial |
|-------------|-------|--------------------------------------|---|--------------|----------------|
| Liberal | Upper | 15.3 | 13.5 | 13.0 | 13.7 |
| | Lower | 10.0 | 8.5 | 10.0 | 9.3 |
| Restrictive | Upper | 11.3 | 11.5 | 11.0 | 11.3 |
| | Lower | 7.3 | 7.5 | 7.0 | 7.0 |

^aThresholds are hemoglobin values in grams per deciliter, and differences between upper and lower thresholds within each transfusion arm reflect the range based on an infant's respiratory illness severity and postnatal age. See the text for additional information on the trials.

ETTNO, Effect of Transfusion Thresholds on Neurocognitive Outcome of Extremely Low Birth Weight Infants; PINT, Prematures in Need of Transfusion; TOP, Transfusion of Prematures.

of Transfusion Thresholds on Neurocognitive Outcome of Extremely Low Birth Weight Infants (ETTNO) trial (ETTNO Investigators, 2012) (Table 82.1). In the Prematures in Need of Transfusion (PINT) trial, a lower hemoglobin transfusion threshold resulted in a nonsignificant increase in neurodevelopmental impairment at 18–21 months (odds ratio [OR] 1.74, 95% confidence interval [CI] 0.98–3.11), which was significantly higher in a post hoc analysis with a more inclusive measure of neurodevelopmental impairment (Whyte et al., 2009). As RBC transfusions are sources of parenteral iron, fewer RBC transfusions using lower hemoglobin transfusion thresholds without appropriate enteral iron supplementation may increase the risk of iron-deficiency anemia, which is associated with adverse long-term neurodevelopmental outcomes (Lozoff et al., 1991; Lozoff and Georgieff, 2006). Among participants followed up at 12 years of age in a trial in Iowa, those who were more liberally transfused as a result of higher hemoglobin transfusion thresholds had lower brain volumes when compared with those randomized to lower hemoglobin thresholds with conservative transfusion (Nopoulos et al., 2011). Recent trends suggest an increasing use of conservative transfusion thresholds to decrease RBC exposure (Ekhaguere et al., 2016), mirroring patient blood management approaches in adults designed to minimize blood exposure (Shander et al., 2012). The outcomes of these studies are discussed later.

For more mature preterm and term infants, transfusion approaches are largely based on expert opinion owing to the lack of randomized trials. In select populations of infants, such as those undergoing surgery, hematocrit thresholds of 40% or higher may be desired (Goobie et al., 2016). By contrast, asymptomatic preterm infants may tolerate a hemoglobin level of 7.0–7.5 g/dL before needing RBC transfusion (Table 82.1). In neonates in whom there is not an ongoing source of blood loss, iron supplementation or erythropoiesis-stimulating agents may be sufficient to help restore RBC volume. Additional information to guide RBC transfusion will likely to be provided by ongoing large, multicenter trials that are evaluating the relative risks and benefits of conservative versus liberal RBC transfusion thresholds in neonates. However, additional studies are needed to guide appropriate thresholds in term infants, particularly among neonates undergoing surgery (Goobie et al., 2016), including cardiac surgery (Wilkinson et al., 2014) and those

TABLE 82.2 Risk of Transfusion-Transmitted Infections in the United States

| Infection | Estimated Risk |
|------------------|---|
| HIV | 1 in 2,135,000 |
| Zika | None reported in United States |
| Ebola | None reported in United States |
| CMV ^a | Historically estimated risk up to 1%–3% for leukoreduced RBC transfusions from CMV-untested donors, although likely substantially lower (Seed et al., 2015). For RBC products from CMV-negative donors, estimated risk of 0% (95% CI 0.0%–0.3%) |
| Hepatitis B | 1 in 277,000 |
| West Nile virus | 1 in 350,000 |
| Malaria | 1 in 1,000,000 to 5,000,000 |

^aEstimates of cytomegalovirus (CMV) infection risk are based on data from Josephson CD, Callendo AM, Easley KA, et al. Blood transfusion and breast milk transmission of cytomegalovirus in very low-birth-weight infants: a prospective cohort study. *JAMA Pediatr.* 2014;168:1054–1062; and Seed CR, Wong J, Polizzotto MN, Faddy H, Keller AJ, Pink J. The residual risk of transfusion-transmitted cytomegalovirus infection associated with leucodepleted blood components. *Vox Sang.* 2015;109:11–17.

CI, Confidence interval; CMV, cytomegalovirus; HIV, human immunodeficiency virus; RBC, red blood cell.

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receiving extracorporeal membrane oxygenation support (Jackson et al., 2014).

Risks of Red Blood Cell Transfusion

The risks of blood component therapy, including RBC transfusion, are reviewed here. However, many of the immunologic and nonimmunologic risks discussed are based on studies in adult transfusion recipients and have not been adequately studied in the neonatal population.

Immunologic Complications

Historically, the primary risk of blood transfusion has been infection. However, current estimates of the risk of transfusion-transmitted infections suggest current donor selection and postdonation diagnostic testing strategies reduce the risk to below 1 in 100,000 and often below 1 in 1,000,000 for most infections (Table 82.2; Bihl et al., 2007). Acute immunologic complications of transfusion include hemolytic transfusion reactions, immune-mediated platelet destruction, febrile nonhemolytic reactions, allergic reactions, anaphylaxis, and transfusion-related acute lung injury (TRALI) (Food and Drug Administration, 2014). Formation of antibodies to ABO blood group antigens (anti-A and anti-B IgM and IgG types) typically occurs after 3 to 4 months of age (Fong et al., 1974). Therefore transfusion reactions related to ABO blood group incompatibility are less likely to occur in neonates. Delayed immunologic complications include delayed hemolytic reactions, alloimmunization to white blood cells, RBCs, and platelet antigens in blood components, posttransfusion purpura, and transfusion-associated graft-versus-host disease. Transfusion-related immunomodulation is a potential entity described in adults that involves

immunosuppressive effects of blood transfusion, which may be beneficial in solid-organ transplant but also can potentially increase the risk of infection and malignancy (Vamvakas and Blajchman, 2007). However, many of the studies on transfusion-related immunomodulation (TRIM) were done in an era before leukoreduction, and TRIM has not been investigated in neonates.

Transfusion-Related Acute Lung Injury

Although TRALI has not been well studied in neonates and infants, it is one of the most severe complications of blood component transfusion, largely caused by substances in plasma and platelet components, but can also occur after RBC transfusion. The cause of TRALI is grouped into immune and nonimmune types. In adults, immune TRALI is estimated to occur in 1 per 5000 transfusions, with case-fatality rates of 6%–9% among those affected (Bux, 2005). However, the incidence and risks of TRALI in neonates are unclear. Immune-TRALI is suspected to be due to substances within transfused blood products that elicit an immune response, such as HLA and granulocyte-binding alloantibodies. Nonimmune TRALI is thought to occur following stored platelet and RBC transfusions although is more benign than immune TRALI and is thought to be mediated by biologically active lipids.

Nonimmunologic Complications

Infection

Viral, bacterial, parasitic, or prion infections are possible from blood transfusion. Donor screening by history questionnaire, infectious testing, and donor deferral substantially reduces the risk of these infections but does not entirely eliminate them. Additionally, there is the potential for yet undiscovered infections to be transmitted through transfusion, as occurred with human immunodeficiency virus (HIV) among hemophilia patients in the 1980s (Hilgartner, 1987). Donor screening based on guidance from the Food and Drug Administration (FDA) (Circular of Information Task Force, 2016) involves testing the donor's ABO group and Rh type, including testing for a weak D antigen. Infectious testing is based on FDA-licensed tests and involves screening patients for HIV, hepatitis B virus, hepatitis C virus, Human T-lymphotropic virus I/II, West Nile virus, syphilis, and *Trypanosoma cruzi*, the cause of Chagas disease. For HIV, hepatitis C virus, and West Nile virus, nucleic acid testing is performed. Donors can also be screened for CMV, which can be much higher in more southern geographic locales and lower in more temperate and cooler regions, causing a wide variation in seroprevalence of donors of 40%–80% in different regions of the United States. This circumstance leads to variation among blood centers in the use of a CMV donor testing strategy to prevent CMV transmission.

Emerging pathogens such as Zika virus and Ebola virus highlight the residual risk of infections (Motta et al., 2016), including those that are yet to be identified. This is particularly relevant given global travel and the effect of epidemics such as dengue (Stramer et al., 2012) and chikungunya (Gallian et al., 2014) on transfusion medicine. Pathogen reduction offers an alternative that has the potential to increase the safety of the blood supply and has been recently approved by the FDA in the United States for use with plasma and platelet transfusions (Snyder et al., 2015). Clinical trials evaluating pathogen-reduction technologies for whole blood and RBCs are ongoing (Snyder et al., 2015). Pathogen inactivation works by combining ultraviolet irradiation with photosensitizers, such as psoralen, that damage pathogen nucleic acids, preventing replication and host infection. This can work for a wide range of viruses, bacteria, parasites, and other pathogens. In addition, this

technology can be used to reduce the risk for emerging infections, particularly when donor testing strategies are not available or fully implemented. However, pathogen reduction does not inactivate all pathogens, such as hepatitis A virus and hepatitis E virus. In addition, pathogen reduction is contraindicated for infants treated with certain phototherapy devices, because of the potential reaction between the psoralen additive and ultraviolet light from phototherapy that can lead to skin erythema (Circular of Information Task Force, 2016).

Transfusion-Associated Circulatory Overload

Large-volume (>20 mL/kg) RBC transfusions may place the infant at risk of transfusion-associated circulatory overload (TACO) that can lead to congestive heart failure and pulmonary edema. Patients with severe anemia without acute bleeding may require slow correction of anemia with RBC transfusion to reduce the risk of TACO. Additionally, certain subsets of neonates, such as those with fetal anemia and hydrops fetalis, may need isovolumetric RBC transfusion using an exchange transfusion. In this procedure, RBCs without whole blood reconstitution with plasma are administered, and whole blood is removed from the patient. Whole blood exchange transfusion for hemolytic disease of the fetus and newborn is discussed later in this chapter.

Hypothermia

Large-volume RBC transfusions can increase the risk of hypothermia because of RBC unit storage at 4°C. Thus blood warmers in the setting of large-volume or massive transfusion are indicated. The possibility of hypothermia resulting from transfusion should also be monitored in operative transfusions, when patients may have acute bleeding requiring rapid large-volume transfusions, coupled with potential cold stress from the operating room environment.

Metabolite Derangements

Electrolyte abnormalities can occur following RBC transfusion because of additive solutions in donor RBCs that contain mannitol (AS-1), a diuretic, and citrate (CPDA-1, AS-3), a calcium chelator, which can lead to hypocalcemia. This is particularly of concern in infants receiving massive transfusion or exchange transfusion. Additional risks include changes in pH and hyperkalemia or hypokalemia, particularly among patients with impaired renal function in the setting of massive transfusion. Washing of RBCs may reduce the risk of hyperkalemia.

Fatal Transfusion Reactions

Although rare, fatal reactions can result from any of the complications previously discussed and should be reported to the FDA. Historically, acute hemolysis from ABO-incompatible transfusions was the leading cause of deaths from blood transfusion (Sazama, 1990), although these are now uncommon because of improvements in blood banking practices and the lack of antibodies to blood group antigens in neonates in the first few months of life (Fong et al., 1974). Currently, TRALI is the most frequent cause of acute transfusion fatality among all blood recipients, accounting for 37% of deaths. However, there is uncertainty if this complication occurs in neonatal patients (Food and Drug Administration, 2012). Transfusion-transmitted bacterial sepsis is the third most common cause of reported transfusion-related fatalities, with more than two-thirds of infections caused by gram-negative organisms. Although a number of organisms have been reported, *Klebsiella pneumoniae*, *Escherichia coli*, and *Staphylococcus epidermidis* are the

three most common organisms, all attributed to platelet transfusion (Hillyer et al., 2003; Niu et al., 2006).

Platelet Transfusion

Component

Two types of platelet units are available in the United States, platelet concentrates (known as *random-donor* or *whole blood–derived platelets*) and apheresis platelets, known as *single-donor platelets*. Platelet concentrates are produced from whole blood drawn from a donor, whereas single-donor platelets are collected via an apheresis technique that returns the remaining whole blood components to the donor. These two methods of platelet collection yield distinctly different numbers of platelets per unit. One platelet concentrate contains approximately 7×10^{10} platelets, in contrast to one single-donor apheresis platelet concentrate with 3×10^{11} to 6×10^{11} platelets per unit. Pooling of five to eight platelet concentrates from different donors is required to equal the same amount of platelets in an apheresis platelet unit. Platelets have a short shelf life (limited to 5 days) and are stored at room temperature and preserved with constant, gentle agitation.

Indications

Platelet transfusions, typically dosed at 10 to 15 mL/kg, are nearly always administered prophylactically in the setting of thrombocytopenia, defined as a platelet count of less than 150,000/ μ L, or in response to severe bleeding. Thrombocytopenia is a common occurrence in neonates requiring intensive care, affecting an estimated 18%–35% of neonates on NICU admission and close to 70% of extremely low birth weight infants (Sparger et al., 2016). However, only a fraction of these infants receive platelet transfusions, estimated to be used in 5%–9% of infants cared for in US NICUs. There is considerable debate as to the appropriate prophylactic platelet transfusion thresholds for neonates, with wide variation in practices (Josephson et al., 2009; Cremer et al., 2011). Only one randomized trial in preterm infants evaluated the use of platelet transfusion to maintain a platelet count of 150,000/ μ L or greater, compared with standard practice, and found no significant difference in the severity of intraventricular hemorrhage (IVH) between treatment arms (Andrew et al., 1993). A more recent multicenter observational study of VLBW infants in six US NICUs found wide variation in platelet transfusion thresholds for transfusion, ranging from 10,000/ μ L to 139,000/ μ L in the first week of life and from less than 10,000/ μ L to more than 150,000/ μ L after the first week of life. The most common thresholds were 80,000/ μ L to 89,000/ μ L in the first 7 days of life and 40,000/ μ L to 49,000/ μ L after the first 7 days of life. The authors found platelet transfusion, after controlling for the severity of thrombocytopenia, was not associated with a lower risk of IVH. Complicating the assessment of platelet threshold and risk of bleeding are contributors to platelet dysfunction, such as nonsteroidal antiinflammatory medications, acidosis, or concomitant coagulopathy (Setzer et al., 1982), that can influence the risk of bleeding.

Risks

One of the major risks of platelet transfusions is bacteria sepsis, although this is relatively uncommon, with an estimated occurrence of 1 in every 100,000 platelet transfusions. Approximately 1 in every 3000 platelet units contains clinically relevant bacteria

concentrations (Snyder et al., 2015). Currently in the United States it is required that all platelet products be bacterially tested before release from the blood donor center. Pathogen inactivation is a potential approach to decrease this residual risk even further, although the effects of this approach on transfusion of platelets into immature neonates require additional study given the still relatively low risks of platelet transfusion–associated sepsis.

Plasma and Cryoprecipitate Transfusion

Components

Plasma, is the aqueous, acellular portion of whole blood and includes albumin, the most abundant of the plasma proteins, along with complement (predominantly C3), enzymes, transport molecules, Igs (γ -globulins), and coagulation factors. The coagulation factors in plasma include (1) fibrinogen; (2) factor XIII; (3) von Willebrand factor; (4) factor VIII, primarily bound to its carrier protein von Willebrand factor (\sim 100 ng/mL); and (5) vitamin K–dependent coagulation factors II (prothrombin), VII, IX, and X. Plasma products, fresh frozen plasma (FFP), or F24 plasma is mainly produced from whole blood and less frequently from plasmapheresis collections. FFP is frozen within 6 to 8 hours of collection, while F24 plasma is frozen within 24 hours of collection. Cryoprecipitate is an insoluble precipitate that is formed by the thawing of FFP and then the refreezing of it in 10 to 15 mL of plasma within 1 hour. This produces a product with high concentrations of factor VIII (80–150 U/unit), von Willebrand factor (100–150 U/unit), fibrinogen (\sim 250 mg/unit), factor XIII (150–250 U/unit), and fibronectin (\sim 2 mg/mL). Cryoprecipitate can be stored at temperatures of -18°C or lower and can be maintained for up to 1 year.

Indications

Plasma transfusions are used in up to 15% of NICU patients for a variety of indications, most often to treat or prevent bleeding in the setting of coagulopathy or disseminated intravascular coagulation (Keir and Stanworth, 2016). Additionally, plasma transfusion may be used as a colloid to increase intravascular volume, including situations in which low plasma oncotic pressure is suspected or patients have not responded to crystalloid therapy. However, there is wide variation in the use of plasma transfusion, and this may be related to variation in the assessment of coagulation and treatment of abnormal coagulation function studies (Chaudhary and Clarke, 2008), with increasing coagulation testing associated with more frequent use of plasma transfusion (Catford et al., 2014). The treatment of abnormal coagulation tests with plasma transfusion is often used in preterm infants, despite the lack of evidence to support an association between abnormal coagulation values in the first week of birth and serious hemorrhage, including IVH (Christensen et al., 2014). No contemporary clinical trials exist to guide the use of plasma transfusion in neonates, particularly as it relates to the prevention of bleeding. One randomized trial compared the prophylactic use of plasma compared with dextrose infusion or gelatin plasma (Gelo-fusine) and found no beneficial effect of early plasma transfusion with regard to death or cranial ultrasound scan abnormality (Northern Neonatal Nursing Initiative [NNNI] Trial Group and Elbourne, 1996). Cryoprecipitate is used to treat or prevent bleeding in infants with acquired hypofibrinogenemia (fibrinogen level <100 or 150 mg/dL) or as part of a massive transfusion protocol, as we discuss later in this chapter.

A single unit of cryoprecipitate usually contains 15 to 20 mL, and infants are typically transfused with 2 mL/kg or 0.5 unit for the average term infant weighing 3.5 kg.

Risks

The risks of plasma transfusions include TRALI, as mentioned previously, and infection. In addition, two studies have reported an increased risk of thrombosis among neonates (Maruyama et al., 2012) and children (Puetz et al., 2012) who received plasma transfusion. In the study by Maruyama et al. (2012), a total dose of FFP of more than 50 mL/kg in the first 5 days after birth was associated with an increased risk of venous thrombosis (adjusted OR of 5.9, 95% CI 1.1–41.8). Potentially, the use of thromboelastography, which measures multiple aspects of clot formation, strength, and fibrinolysis, may provide a more precise measure of coagulation to better guide the use of plasma transfusion in neonates (Sewell et al., 2016).

Special Circumstances

Massive Transfusion

Massive transfusion may be required in neonates with large-volume blood loss. On the basis of evidence from adults and children, the use of massive transfusion protocols with balanced ratios of blood products (RBCs, plasma, platelets, cryoprecipitate) may decrease the adverse effects of massive transfusion, particularly on the ability of the neonate to maintain hemostasis (Diab et al., 2013). In addition, the risks of transfusion previously discussed, such as metabolic complications or TACO, are increased in massive transfusion and require close monitoring. The appropriate ratio of blood products is uncertain, but studies (Hendrickson et al., 2012; Diab et al., 2013) have suggested use of a 1 : 1 ratio of RBCs and FFP, with additional alternating use of platelet and cryoprecipitate transfusions. In adults, the use of whole blood among trauma patients requiring massive transfusion has been evaluated and may be associated with better outcomes (Repine et al., 2006), but this has not been sufficiently evaluated in preterm infants. The use of fresh whole blood for cardiopulmonary bypass priming has no advantage over the use of a combination of products during surgery for congenital heart disease, with whole blood priming associated with an increased length of stay and more fluid overload (Mou et al., 2004).

Exchange Transfusion

The indications for exchange transfusion include severe hyperbilirubinemia, often in the setting of hemolytic disease of the fetus and newborn with ABO or Rh incompatibility or other minor RBC antigen–antibody incompatibilities with the mother, as well as anemia with fluid overload (e.g., hydrops fetalis) or hemochromatosis. Exchange transfusion is a relatively uncommon treatment following the introduction of Rh₀(D) immune globulin prenatally administered to the mother and intensive phototherapy for the infant. In one series, only 5 of 111,009 infants in a cohort from California NICUs received exchange transfusion for severe hyperbilirubinemia (bilirubin level >30 mg/dL) (Newman et al., 2003), and more recent data estimate this may be as low as 1.9 per 100,000 live births (Bhutani et al., 2016). For infants with severe hyperbilirubinemia, exchange transfusion is the most effective method of removing bilirubin rapidly, while simultaneously removing and

diluting out the offending antibody and RBC antigen target for the antibody. Typically, a double-volume exchange transfusion is recommended (Thayil and Milligan, 2006). This involves removing twice the estimated amount of circulating blood volume (70–80 mL/kg for term infants, 90–100 mL/kg for preterm infants) and replacing it with RBCs reconstituted with plasma, to a target hematocrit of the infant, in 1 to 3 hours. The RBCs should be leukoreduced, and, if they are going to a fetus for intrauterine transfusion, they should be irradiated. Small volumes, 5 mL/kg or less, with absolute aliquot volumes ranging from 5 to 20 mL, are exchanged serially, with each exchange occurring in several minutes. This procedure replaces approximately 85% of native circulating RBCs.

The most common risks following exchange transfusion are hypocalcemia (estimated to occur in 29%–38% of infants), thrombocytopenia (38%–44%), and infection (Keenan et al., 1985). Less common risks include catheter-related complications, thrombosis, bleeding, hypothermia, necrotizing enterocolitis, and cardiac arrhythmia. The historical risk of death from exchange is reported to be 3 per 1000 procedures (0.3%), although the current modern-era risk is difficult to estimate given the infrequent use of this therapy and differences in patient populations of infants undergoing exchange transfusion. In addition to double-volume exchange transfusions, partial exchange transfusions involve removing whole blood from a patient and replacing this with an equal volume of colloid or crystalloid. This is often done in response to polycythemia (hematocrit ≥65%–70%), although the benefit of partial exchange transfusions in reducing complications from polycythemia has not been well established (Ozek et al., 2010).

Intravenous Immune Globulin

Intravenous immune globulin (IVIG) therapy is a therapy using pooled Ig from multiple donors, often several hundred. In neonates, the most common indication is treatment of hemolytic disease of the fetus and newborn that is refractory to intensive phototherapy (AAP Subcommittee on Hyperbilirubinemia, 2004). IVIG works by blocking Fc receptors in the splenic reticuloendothelial system from destroying RBCs or platelets that are bound to maternal circulating antibodies, although the exact mechanisms are not clear. Treatment with IVIG decreases the risk of exchange transfusion and, among those receiving exchange transfusion, decreases the risk of subsequent exchange transfusion (Alcock and Liley, 2002). However, the effect of IVIG in studies with a low risk of bias shows no clear benefit (Louis et al., 2014). Overall, IVIG is well tolerated and has been extensively studied in the treatment of sepsis (Ohlsson and Lacy, 2013). However, hypotension is a common risk, and the risk can be reduced by decreasing the infusion rate (Katz et al., 2007). In addition, rare risks, including viral infection (Fasano, 1995) and necrotizing enterocolitis (Figueras-Aloy et al., 2010), have been reported, although in a systematic review and metaanalysis, no serious adverse effects of IVIG were identified (Ohlsson and Lacy, 2013).

Neonatal Alloimmune Thrombocytopenia

Neonatal alloimmune thrombocytopenia (NAIT) is a rare disorder that can lead to severe bleeding, including intracranial hemorrhage, in the fetus or neonate. Thrombocytopenia results from maternal alloimmunization against paternally derived platelet antigens, most commonly human platelet antigen (HPA)-1a (Shulman et al., 1962), although more than 27 different HPAs have been associated

with NAIT. NAIT is estimated to occur in approximately 1 per 1000 live births (Dreyfus et al., 1997). Typically, the mother is negative for HPA-1a and often, but not always, positive for anti-HPA-1a antibodies (Kjeldsen-Kragh et al., 2007). The fetus is HPA-1a positive, inheriting the gene from the father. Pregnant women become sensitized to the platelet antigen, resulting in transplacental passage of antiplatelet antibodies that cause immune-mediated destruction of platelets and thrombocytopenia. Severe hemorrhage is estimated to occur in 10 per 100,000 neonates, commonly occurring before birth (Kamphuis et al., 2014). Therefore prenatal IVIG treatment has been investigated and has been found to increase fetal platelet counts (Bussel et al., 1988), although the beneficial effect on reducing intracranial hemorrhage is unclear. If NAIT is diagnosed in an infant, future pregnancies typically undergo close monitoring, with testing of the father and, if indicated, the fetus. After birth, treatment involves transfusion using platelets negative for the offending paternal antigen obtained from the mother and washed or from a random donor. IVIG may also be used to decrease antibody-mediated platelet clearance. For most infants with NAIT, thrombocytopenia resolves over time as the passive antibody degrades (Bassler et al., 2008). Additional information about NAIT is given in Chapter 80.

Decreasing the Need for Red Blood Cell Transfusion

Anemia is nearly universal in extremely preterm infants (Widness, 2008), largely owing to phlebotomy-related blood losses (Rosebraugh et al., 2013). The effect of erythropoiesis-stimulating agents, such as erythropoietin and darbepoetin, on reducing RBC transfusion and donor exposure has been modest (Aher and Ohlsson, 2014; Ohlsson and Aher, 2014), although recent studies show promising beneficial effects of erythropoiesis-stimulating agents on long-term neurocognitive outcome in extremely preterm infants (Ohls et al., 2013, 2014), and additional large, multicenter trials are ongoing (Juul et al., 2015). Strategies to reduce phlebotomy-related blood losses (Carroll and Widness, 2012) are important aspects of patient blood management. Placental transfusion from delayed cord clamping or cord milking (Rabe et al., 2012) is another strategy to decrease the need for RBC transfusion (Rabe et al., 2009) and improve outcomes (Ghavam et al., 2014; Andersson et al., 2015) and should be considered as part of an institutional transfusion protocol.

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83

Neonatal Leukocyte Physiology and Disorders

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KEY POINTS

- The neonate and the young infant depend primarily on the innate immune system for host defense. With limited prior exposure to infectious and environmental antigens, the adaptive immune arm is still in a phase of structural and functional development.
- Neutropenia is frequently encountered during the neonatal period.
- Neonatal neutrophils show a wide range of functional deficiencies in movement, phagocytosis, and microbial killing.
- With the exception of a few deficiencies, neonatal monocytes and macrophages are functionally comparable to their counterparts from adults.
- Neonatal T-cell and B-cell populations are still developing. Several adaptive mechanisms, such as the presence of B1 cells that can function without assistance from T cells and the production of immunoglobulins with polyspecific antigen binding, are unique to the neonate and partially mitigate the deficiencies in adaptive immunity.
- The innate lymphoid cells are a recently described, exciting new subset in innate immunity that are likely to play a major role during the neonatal period and early infancy.

This chapter presents an overview of neonatal leukocyte physiology and quantitative and qualitative disorders of leukocytes. Topics include the normal physiology and defects associated with neonatal hematopoiesis, neutrophils, monocytes, lymphocytes, dendritic cells (DCs), and innate lymphoid cells (ILCs) (Fig. 83.1). Novel therapeutic approaches are also discussed.

Neutrophil Physiology and Function

Ontogeny

Neutrophils are an important line of defense in the cellular innate response (see Fig. 83.1). The life cycle of a neutrophil can conceptually be divided into three phases, representing time spent in (1) marrow, (2) blood, and (3) tissues. The earliest neutrophilic precursors, myeloblasts, promyelocytes, and myelocytes, are capable of cell division and thus are referred to as the *neutrophil proliferative pool* (NPP). In later stages of maturation, neutrophils lose their ability for cell division. These metamyelocytes, bands, and segmented

neutrophils continue to differentiate in situ and constitute the neutrophil storage pool (NSP). In adults the NSP is a sizeable reservoir of neutrophils that can be rapidly mobilized into the bloodstream when needed. However, the NSP is relatively much smaller in the midgestation fetus and preterm infant and can be readily exhausted during sepsis. In adult rats, the NSP contains about 6×10^9 cells per kilogram. In contrast, the rat NSP at 19 days' gestation contains only about 0.9×10^9 cells per kilogram, which expands marginally to 1.2×10^9 cells per kilogram at term (21 days). Unlike in fetal and newborn rodents, in whom the liver and spleen house a significant fraction of the NPP and NSP (Erdman et al., 1982), neutrophil production and storage in human neonates occur primarily in the bone marrow (Calhoun et al., 1996).

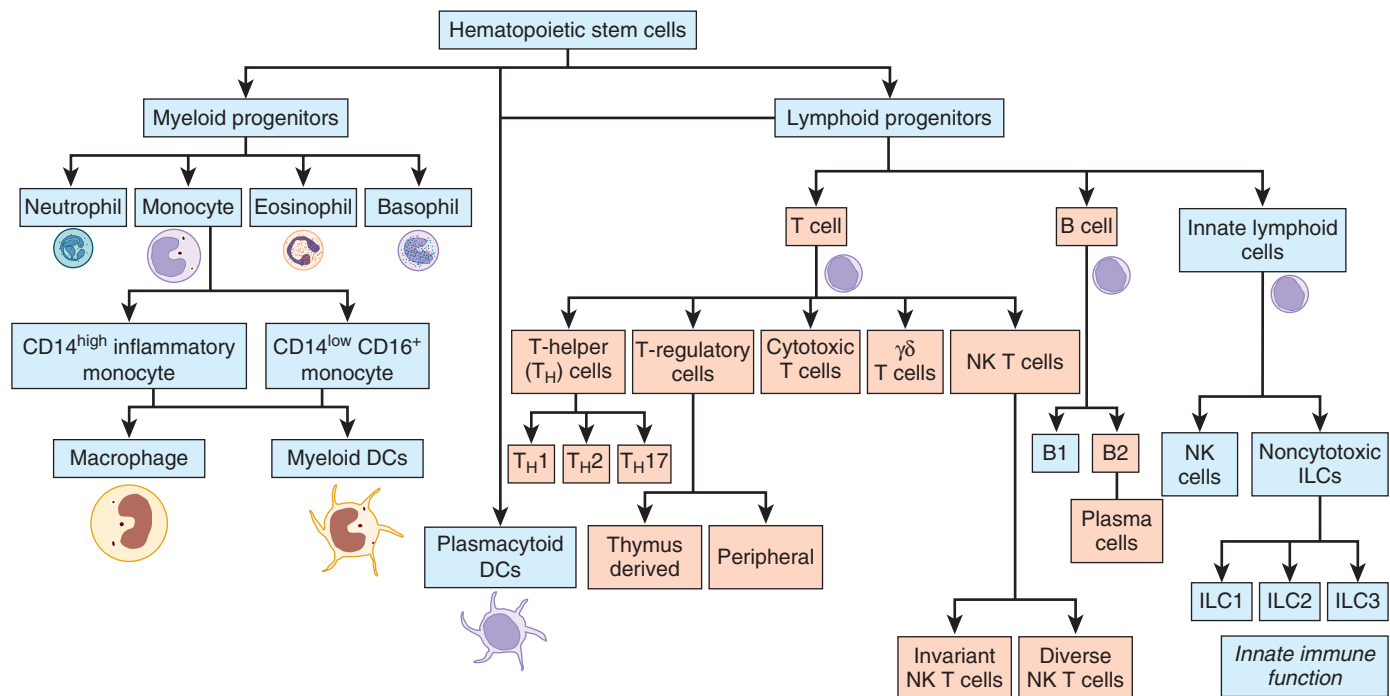
Circulating and Marginated Blood Neutrophil Pools

Circulating neutrophils are distributed into two compartments of approximately equal size, the circulating and marginated pools. As the name suggests, neutrophils in the circulating compartment freely circulate in the bloodstream, whereas those in the marginated compartment are transiently attached to the endothelium. In healthy adults, the circulating and marginated pools both contain approximately 0.4×10^7 cells per kilogram (Bishop et al., 1968). The marginated cells can move into the circulation for short periods of 30 to 45 minutes after strenuous crying or exercise or following administration of epinephrine or corticosteroids (Steel et al., 1971; Christensen and Rothstein, 1979).

Neutrophils remain in the circulation for a few hours (half-life of about 6.3 hours) and then migrate into the tissues. During infection, neutrophil trafficking into the tissues is increased. In fetal sheep, intra-amniotic exposure to endotoxin caused an initial drop in circulating neutrophil counts due to tissue emigration, which was followed by a gradual increase over the next 6 days. The length of time that neutrophils spend in the tissues and their subsequent fate are not well understood.

Neutrophil Heterogeneity

There is now evidence for the existence of different neutrophil subsets with distinct molecular markers. Details of differences in function are still emerging. Three molecular markers are highlighted next:



• **Fig. 83.1** Leukocyte Populations in the Neonate and the Young Infant. Hematopoietic stem cells differentiate along the myeloid and lymphoid lineages to ultimately give rise to leukocyte populations that participate in the innate (blue background) or adaptive (orange background) immune responses. DCs, Dendritic cells; ILC, innate lymphoid cell; NK, natural killer.

- Olfactomedin 4 (OLFM4) is a glycoprotein that has been suggested to act as a tumor suppressor and has recently been identified in specific granules of approximately 25% of circulating human neutrophils. Expression of OLFM4 could negatively regulate the efficiency of bacterial killing in a subset of neutrophils (Clemmensen et al., 2012).
- The surface glycoprotein CD177 (NB1) is a 55-kDa glycosylphosphatidylinositol-anchored receptor that is expressed at various levels on circulating neutrophils. Several distinct functions have recently been attributed to CD177, including high-affinity binding to platelet–endothelial cell adhesion molecule 1 and the ability to associate with the serine protease PR3. During infection, CD177⁺ neutrophil subsets may show increased tissue infiltration as aided by the associated cell surface PR3 (Hu et al., 2009).
- Interleukin-1 (IL-1)-positive neutrophils are believed to undergo reverse transendothelial migration from the tissues to enter the bloodstream and may be involved in the systemic dissemination of inflammation (Buckley, 2006).

Neonatal Neutropenia

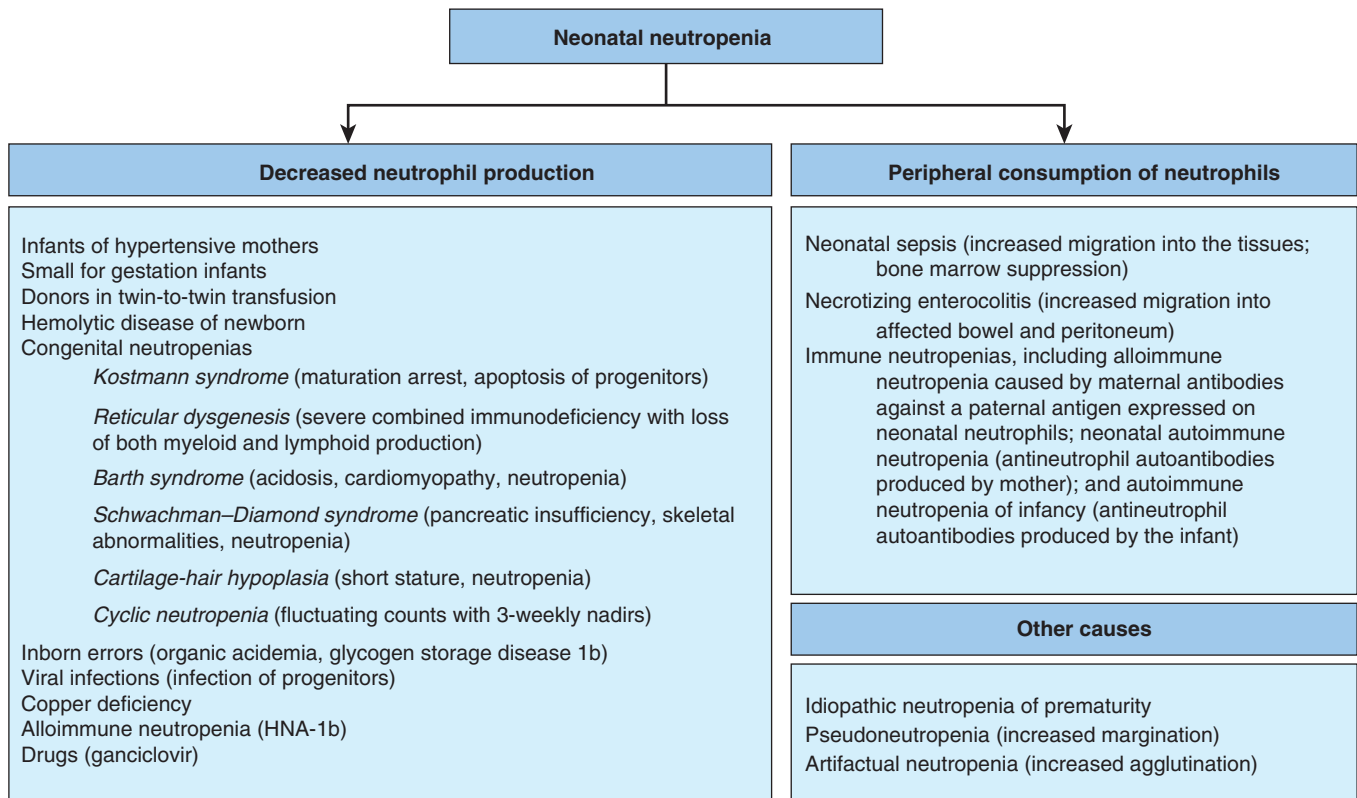
Statistically, neutropenia is defined as an absolute neutrophil count (ANC) less than two standard deviations below the mean value or below the fifth percentile for postnatal age. Manroe et al. (1979) established reference values for ANCs in term and preterm infants during the first 28 days of life for both healthy infants and those with perinatal complications. Mouzinho et al. (1994) studied serial white blood cell counts in healthy preterm very low-birthweight (VLBW) infants to investigate whether this patient cohort had neutrophil counts different from those found in previous studies

in which cohorts consisted mostly of term infants. They detected a wider range of the ANC, mostly resulting from a downward shift of the lower boundary, especially during the first 60 hours of life. However, there was no difference in absolute total immature neutrophil counts or in the ratio of immature neutrophil counts to total neutrophil counts. Schmutz et al. (2008) showed that the ANC peaked at 6–8 hours for neonates born at 28 weeks' gestation or later but at 24 hours for those born before 28 weeks' gestation. The 5th and 95th percentiles for ANCs at 72–240 hours among neonates born at more than 36 weeks' gestation were 2700/μL and 13,000/μL, respectively; for neonates born at 28 to 36 weeks' gestation they were 1000/μL and 12,500/μL respectively; and for neonates born at less than 28 weeks' gestation they were 1300/μL and 15,300/μL respectively. In that study, ANCs were higher in neonates born after a prolonged period of labor than in those born by elective cesarean delivery. Female infants also had higher ANCs, averaging about 2000/μL more than their male counterparts.

In neutropenic infants the pathophysiologic mechanisms include exhaustion of myeloid progenitors, inadequate response of the progenitor cells to proliferative or maturational signals, and increased usage and destruction. Fig. 83.2 highlights the causes of neutropenia in the newborn period. Some of the more frequently encountered causes of neonatal neutropenia are discussed in the following sections.

Sepsis-Induced Neutropenia

Neonates with overwhelming sepsis often develop neutropenia, which illustrates some of the differences between adult and neonatal neutrophils. Neonates have fewer neutrophil progenitors and a diminished precursor storage pool, so neutrophils are easily depleted in stress conditions (Levy, 2007). Following experimental sepsis



• **Fig. 83.2** Causes of Neonatal Neutropenia. HNA, Human neutrophil antigen.

with group B streptococci, adult rats respond with a transient decrease in circulating neutrophil counts, followed by significant neutrophilia associated with a twofold to threefold increase in the progenitor pool (colony-forming unit–megakaryocyte) and an increase in the proliferative rate to 75% of the maximal capacity (Christensen et al., 1982, 1983). In contrast, neonatal rats under the same conditions had a decrease of 50% of their progenitor pool and failed to increase their myeloid proliferative rate, which, as discussed previously, was already at near maximal levels. Most important, during experimental sepsis, neonatal rats had further depletion of their already reduced NSP reserves by almost 80%, compared with a decline of 33% in adult rats.

Alloimmune Neonatal Neutropenia

Alloimmune neonatal neutropenia occurs as a result of maternal sensitization to neutrophil antigens present on the infant's neutrophils (paternally acquired) that are not present on the maternal neutrophils, with subsequent production of immunoglobulin G (IgG). Neutrophil-specific antibodies are found in the maternal and infant sera, but the mother has a normal neutrophil count. Alloimmune neonatal neutropenia is estimated to occur at a frequency of 3% of live births (Curnette, 1993). The antigens most commonly involved in the United States are HNA-1a, HNA-1b, and Human Neutrophil Antigen (HNA)-2a. Because the antibodies are IgG, which crosses the placenta, peripheral blood counts show profound neutropenia. The condition is self-limiting and typically resolves within 6 to 7 weeks, during which time the neonate is susceptible to infections, mostly cutaneous in nature. Most infections are mild, although infants with profound neutropenia are at risk of life-threatening infections and should be monitored closely (Maheshwari et al., 2002a).

Maternal Hypertension–Associated Neutropenia

One of the most common and well-described causes of transient neonatal neutropenia is maternal hypertension. Neonatal neutropenia is inversely related to the birthweight and gestational age and directly related to the severity of the hypertension. Infants of hypertensive mothers seem to have decreased production of neutrophils, but the cause is uncertain. Several studies have demonstrated a decrease in the numbers of neutrophil progenitor cells, decreased cycling of these cells, a relatively normal NPP and NSP, and the absence of a “left shift” (Koenig and Christensen, 1989). Studies show conflicting evidence for the risk of infection in these infants (Doron et al., 1994; Mouzinho et al., 1994; Paul et al., 1999), although the risk is probably low because neutropenia resolves in most cases within 72 hours, and almost always in 5 to 7 days (Koenig and Christensen, 1991; Tsao et al., 1999).

Treatment of Neonatal Neutropenia

Recombinant granulocyte colony-stimulating factor (G-CSF) and granulocyte–macrophage colony-stimulating factor (GM-CSF) are often used to treat neonatal neutropenia. G-CSF stimulates neutrophil production, maturation, and release from the marrow and also reduces neutrophil apoptosis (Calhoun and Christensen, 2000). GM-CSF generates both granulocyte and macrophage colonies from precursor cells (Burgess and Metcalf, 1980). G-CSF is the primary systemic regulator of the circulating neutrophil concentrations (Calhoun and Christensen, 2000). GM-CSF may not play a major role in the steady state but is induced in inflamed tissues (Hamilton and Anderson, 2004).

Recombinant G-CSF or GM-CSF has been used in neonatal sepsis with conflicting results (Gillan et al., 1994; Schibler et al., 1998; Bedford Russell et al., 2001). In a meta-analysis of five

studies, G-CSF was shown to reduce mortality (odds ratio 0.17, 95% confidence interval [CI] 0.03–0.70) (Bernstein et al., 2001). However, this protective effect was no longer significant when nonrandomized studies were excluded. The role of GM-CSF in prevention or treatment of neonatal sepsis is also unclear (Cairo et al., 1999; Carr et al., 1999; Bilgin et al., 2001). Carr et al. (2003) reviewed the efficacy and safety of G-CSF/GM-CSF in the treatment or prophylaxis of neonatal sepsis. In preterm infants with suspected sepsis, G-CSF or GM-CSF did not reduce mortality at 14 days after the start of therapy (relative risk [RR] of death 0.71, 95% CI 0.38–1.33). However, in a subgroup of 97 infants with sepsis and neutropenia (ANC <1700/ μ L), there was a significant reduction in mortality (RR 0.34, 95% CI 0.12–0.92; number needed to treat 6, 95% CI 3–33).

Recombinant G-CSF is highly effective in correcting immune-mediated neutropenia. Along with its effects on neutrophil production, G-CSF may decrease antigen expression on neutrophils, rendering them less vulnerable to circulating antibodies (Rodwell et al., 1996), and also improve neutrophil function (de Haas et al., 1994). Similarly, G-CSF is also effective in many patients with congenital neutropenias (Christensen and Calhoun, 2004; Zeidler, 2005). Kostmann syndrome presents in early infancy with low ANC, often less than 200/ μ L, and recurrent bacterial infections. These patients have mutations in the neutrophil elastase gene *ELANE* (Dale et al., 2000). G-CSF can reduce the need for antibiotics and hospitalization (Zeidler, 2005) and can improve survival in these patients. Cyclic neutropenia is another rare hematologic disorder that may be modified by the use of G-CSF. Cyclic neutropenia is characterized by regular drops in ANC to levels as low as 250/ μ L at 3-week intervals (Dale and Hammond, 1988). Although the marrow may look normal during periods of higher ANC, it shows a characteristic “maturation arrest” of myeloid cells during or just before the onset of severe neutropenia. G-CSF treatment can be used to raise neutrophil counts during periods of severe neutropenia (Schmitz et al., 1995). These patients may not respond to GM-CSF (Schmitz et al., 1996).

Some premature infants can show idiopathic neutropenia that can be severe (sometimes ANC <500/ μ L) and prolonged but resolves spontaneously (La Gamma et al., 1995). These infants may be neutropenic at or shortly after delivery and can remain neutropenic for several weeks. Blood and bone marrow studies indicate decreased neutrophil production, although the cause remains unclear. In one study, G-CSF treatment induced a rapid rise in ANC in such patients, indicating that the marrow contains an adequate neutrophil reserve (Juul and Christensen, 2003).

G-CSF is administered intravenously or subcutaneously at a dosage of 5–10 μ g/kg per day. The response to G-CSF therapy and a rise in ANC usually occurs within 24–48 hours. In an occasional patient, G-CSF therapy will not raise blood neutrophil counts. In such a patient, G-CSF doses can be increased in increments of 10 μ g/kg at 7- to 14-day intervals if the ANC remains below 1000/ μ L (Zeidler et al., 2000; Maheshwari et al., 2002b). Doses can be reduced or withheld once the ANC exceeds 5000/ μ L. G-CSF treatment is usually tolerated in neonates without adverse effects. Long-term G-CSF therapy in congenital neutropenias has been associated with splenomegaly, thrombocytopenia, osteoporosis, myelodysplastic syndrome/leukemia, and development of anti-G-CSF antibodies (Taniguchi et al., 1993; Yakisan et al., 1997).

Intravenous immunoglobulin (IVIG) is effective in about 50% of infants with alloimmune and autoimmune neutropenia (Huizinga et al., 1990; Cartron et al., 1991; Yoshida et al., 1991). IVIG can mobilize neutrophils from the NSP, although repeated doses may

be needed for a sustained effect (Bussel et al., 1988; Christensen et al., 1991). While steroids are generally not effective in alloimmune neutropenia (Buckwold and Emson, 1959; Lalezari, 1984), they may raise the ANC for short periods in autoimmune neutropenia of infancy. Exchange transfusions are generally not effective in immune-mediated neutropenia (Hinkel et al., 1986).

Monocyte Physiology and Dysfunction

Ontogeny

Embryonic macrophages are found among hematopoietic cells in the yolk sac at 3–6 weeks' gestation. The fetal liver becomes the primary site of hematopoiesis from 6 weeks until midgestation, and the bone marrow then becomes the lifelong center of blood cell production (Palis and Yoder, 2001). Monocytes are present in high proportions in the early hematopoietic tissues, with approximately 70% of hematopoietic cells at 4.5 weeks' gestation morphologically identifiable as monocytes. This proportion falls to 1%–2% during the next 6 weeks as erythroid cells become predominant. The precursors of monocytes, monoblasts, and promonocytes continue to be present in the fetal liver; however, intravascular monocytes are not observed until the fifth month of gestation. Circulating monocytes do not appear with regularity until hematopoiesis is first established in the bone marrow after the 10th week of gestation.

During ontogeny, macrophages in the fetal liver express CD11b as early as 12 weeks' gestation. The classic monocyte marker, CD14, does not appear until about 15–21 weeks' gestation. CD14 expression on circulating mononuclear cells is equivalent in cord blood and adult peripheral blood. CD11a, CD11b, and CD11c are expressed in lower densities on cord blood monocytes than on adult cells. There is also lower expression of class II major histocompatibility complex (MHC) antigens HLA-DR, HLA-DP, and HLA-DQ on neonatal monocytes compared with adult monocytes. The density of these class II MHC antigens has been correlated with antigen-presenting capacity of monocytes in vitro, although the effect of this deficiency on neonatal host defense is not clear. Other important monocyte markers are the receptors for the Fc moiety of IgG (Fc γ R) and the Toll-like receptors (TLRs). Fc γ R receptors are important in the process of monocyte and macrophage phagocytosis of microbes and antibody-dependent cytotoxicity. Monocytes constitutively express the high-affinity receptor Fc γ RI (CD64) and Fc γ RII (CD32). TLRs play a critical role in the recognition of microbial pathogens. Term neonatal monocytes express normal basal levels of TLR2 and TLR4 but show reduced tumor necrosis factor (TNF) release in response to stimulation with a range of TLR agonists (Levy, 2005; Viemann et al., 2005; Sadeghi et al., 2007). Cord blood monocytes of preterm neonates, however, have lower TLR4 expression than adult peripheral blood monocytes (Forster-Waldl et al., 2005).

Macrophage colony-stimulating factor (M-CSF) is a hematopoietic growth factor that regulates the proliferation, differentiation, and functional activation of monocytes. Normally detected in human serum, M-CSF plays an important role in enhancing the effector functions of monocytes and macrophages. Serum M-CSF levels are increased in cord blood and rise further during the neonatal period.

Circulating Monocytes

Term infants show a relative monocytosis that persists through the neonatal period. Although there is some disagreement about normal

blood monocyte counts in neonates, we have described normal ranges of absolute monocyte counts (AMCs) using data from more than 62,000 blood counts (Christensen et al., 2010). In this cohort, blood monocyte concentrations increased almost linearly between 22 and 42 weeks' gestation. Monocyte concentrations also increased during the first 2 weeks postnatally. These data are consistent with previous kinetic studies in human fetuses that show a similar maturational increase in the concentrations of monocyte precursors (Linch et al., 1982; Porcellini et al., 1983). In neonates, monocytosis has been associated with prematurity, blood transfusions, albumin infusions, and theophylline therapy. Monocytosis has also been described in infants with congenital infections such as candidiasis and syphilis (Karayalcin et al., 1977; Wolach et al., 1991), and in association with immune-mediated neutropenia (Bux et al., 1991). In contrast, monocytopenia is not seen frequently in neonates, except in growth-restricted preterm infants who may have low monocyte counts as part of an overall suppression of all leukocyte lineages (Wirbelauer et al., 2010). Recently, we showed that a fall in AMCs can be a useful diagnostic marker of necrotizing enterocolitis (NEC) in VLBW infants (Remon et al., 2014). In this study, we compared blood counts obtained at onset of feeding intolerance with the last available counts obtained before the onset of symptoms. In a given infant with feeding intolerance, a drop in AMC of more than 20% indicated NEC with sensitivity of 0.70 (95% CI 0.57–0.81) and specificity of 0.71 (95% CI 0.64–0.77). The negative predictive value was 88%, indicating that the test may be valuable for exclusion of the diagnosis of NEC in infants with feeding intolerance due to other causes. Despite modest diagnostic accuracy, AMC is a convenient tool because the information is already available at no extra cost in complete blood counts from automated hematology analyzers.

Monocyte Subsets

Increasing evidence indicates that peripheral blood monocytes are a heterogeneous population comprised of “classic” CD14⁺CD16[−] monocytes, which express C-C chemokine receptor (CCR)2, CD64, CD62L and represent nearly 80%–90% of all blood monocytes. The remaining fraction comprises the “nonclassic” CD14^{low}CD16⁺ monocytes that lack CCR2 (Geissmann et al., 2003). Both subsets express the receptor for fractalkine, CX3C chemokine receptor (CX₃CR)1, but CD14^{low}CD16⁺ monocytes characteristically express higher levels. CD16⁺ monocytes are composed of at least two populations with strikingly distinct functions. Monocytes that express CD16 and CD14 (CD14⁺CD16⁺) also express the Fcγ receptors CD64 and CD32, have phagocytic activity, and produce TNF and interleukin (IL)-1β in response to lipopolysaccharide (LPS). In contrast, monocytes that express CD16 but very low levels of CD14 (CD14^{dim}CD16⁺) lack the expression of other Fc receptors, are poorly phagocytic, and do not produce TNF or IL-1β in response to LPS (Grage-Griebenow et al., 2001). This subset of “resident” monocytes patrol blood vessels in the steady state and extravasate during infection with *Listeria monocytogenes* or during tissue healing (Geissmann et al., 2008).

Function

Monocytes are capable of directed movement (chemotaxis) in response to chemoattractants produced by bacteria or by host cells at the site of injury or invasion. The chemotactic capabilities of neonatal and adult peripheral blood monocytes have been compared, and chemotaxis was found to be less pronounced in neonates than in adults (Table 83.1).

TABLE 83.1 Function and Phenotype of Adult Versus Neonatal Mononuclear Cells

| Cells | Function/Phenotype | Adult | Neonate |
|----------------------|-----------------------------|-------|---------|
| Monocytes | Chemotaxis | ↑ | ↓ |
| | Phagocytosis | ↑ | ↓ |
| | Adhesion | ≈ | ≈ |
| | Respiratory burst | ≈ | ≈ |
| Dendritic cells | Expression of CD83, CD86 | ↑ | ↓ |
| | Mixed lymphocyte reaction | ↑ | ↓ |
| | IL-12 (p40) production | ↑ | ↓ |
| | IL-12 production | ↑ | ↓ |
| | IL-10 production | ↓ | ↑ |
| Natural killer cells | Expression of CD8, CD57 | ↑ | ↓ |
| | Expression of ICAM-1, CD161 | ↑ | ↓ |
| | Cytolytic activity | ↑ | ↓ |

ICAM-1, Intercellular adhesion molecule1; IL, interleukin.

During an acute infection, circulating monocytes become activated and migrate into the tissues. During this process, monocytes adhere to the endothelium through the interaction of the integrins (CD11a, CD11b, CD11c, and CD18) expressed on the monocyte cell membrane, with ICAM-1 or ICAM-2 on the endothelial surface. Finally, the activated monocyte moves through the endothelium to the site of inflammation or infection. Preliminary studies demonstrate that the levels of monocyte adhesion molecule expression are comparable in neonate and adult peripheral blood (Schibler, 2000). The CD11b–CD18 complex (macrophage 1 antigen/complement receptor 3) promotes monocyte trafficking to the sites of infection by binding ICAM-1 and is also involved in the recognition of opsonized microbial pathogens.

Antimicrobial activity of monocytes includes oxygen-dependent mechanisms such as the respiratory burst, which through a complex series of reactions forms highly reactive hydroxyl radicals that damage host and microbial membranes. The ability of fetal and neonatal monocytes to kill pathogens (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, and *Candida albicans*) is generally comparable to that of the monocytes in adult peripheral blood (Table 83.1), although isolated preterm monocytes may show weaker superoxide production and degranulation in vitro than monocytes from term neonates.

On exposure to microbial antigens, monocytes and macrophages become activated by collaborative actions of soluble recognition proteins, including CD14 and TLRs, and produce several cytokines and chemokines contributing to the inflammatory process. IL-1β, interferon (IFN)-α, and TNF are synthesized at similar levels in adults and neonates. Kaufman et al. (1999) detected lower TNF production in LPS-stimulated adherent monocytes from preterm infants compared with monocytes from term infants; however, no difference was seen in the production of IL-1β or IL-6. They also found lower expression of CD11b and CD18 adhesion receptor subunits in preterm monocytes. Although TLR expression of term neonatal monocytes is similar to that of adult monocytes, there are important functional differences. Levy et al. (2004) reported that cord blood monocytes are less sensitive to TLR ligands than adult monocytes for TNF induction. The innate immune responses of neonatal monocytes to TLR agonists may be biased toward high

IL-6 levels but low TNF levels in vitro because of distinct neonatal cellular (monocyte) and humoral (serum) factors, and such a pattern was also evident in vivo (Angelone et al., 2006).

Developmental Defects in the Phagocytic Immune System in Neonates

The immaturity of the phagocytic immune system predisposes neonates to increased morbidity and mortality during bacterial sepsis. This impairment is attributed to developmental deficiencies in both the innate immune system and the adaptive immune systems (see Fig. 83.1). In the following section, we use the term *phagocyte* to refer mainly to neutrophils and the monocyte/macrophage lineage. Although immature DCs also show phagocytic activity, there is limited information about these cells in the neonate. To function efficiently the phagocyte system depends on the presence of adequate numbers of phagocytes in circulation, the ability to respond to signals from the sites of inflammation, the ability of phagocytes to migrate to these sites, and the capability of phagocytes to ingest and kill invading microorganisms.

Several studies show important differences in TLR expression on neonatal versus adult leukocytes. The basal expression of TLR2 and TLR4 in neutrophils and monocytes from healthy adults and term neonates is similar (Levy, 2007). During sepsis, neonates showed a sustained upregulation of TLR2 on monocytes but not on neutrophils, and there was no change in TLR4 expression on monocytes or neutrophils (Viemann et al., 2005). In VLBW infants, monocytes express TLR4 at much lower levels than in mature infants and adults and show significantly lower LPS-stimulated IL-1 β , IL-6, and TNF release in vitro (Forster-Waldl et al., 2005). Other studies have shown that stimulation of newborn monocytes with LPS produced a significant decrease in the expression of MyD88, supporting the premise of impaired TLR4-mediated signaling (Marodi, 2006). Belderbos et al. (2009) compared TLR-induced cytokine responses of whole blood leukocytes from cord blood from healthy term neonates, neonatal venous blood at the age of 1 month, and adult venous blood. On TLR4 activation, neonatal leukocytes (both at birth and at 1 month of age) showed a skewed pattern of cytokine expression, with low levels of T-helper (T_H) type 1–polarizing cytokines such as IL12p70 and IFN- α , and more of the antiinflammatory cytokine IL-10. In contrast, cytokine responses to TLR3, TLR7, and TLR9 matured by 1 month of age.

The immaturity of neonatal host defense is also characterized by profound deficiencies in quantitative and qualitative phagocytic effector cell function, particularly during stress or bacterial sepsis. The initial step in mounting a host defense response is directed migration, or chemotaxis (*chemo* refers to a chemical substance, *taxis* refers to rearrangement) of activated phagocytes toward the site of microbial invasion. Such movement occurs along concentration gradients of chemoattractants, which may include bacterial products such as *N*-formyl-L-methionyl-L-leucylphenylalanine (f-MLP), leukotriene B₄, complement products such as C5a, or chemokines. At the site of inflammation, the phagocytes ingest and kill the pathogens by oxygen-dependent and oxygen-independent mechanisms. Neutrophils from both preterm and term neonates show numerous qualitative abnormalities, including decreased deformability and impaired functions, including chemotaxis, phagocytosis, adherence, bacterial killing, aggregation, and oxidative metabolism. In contrast to neutrophils, neonatal monocytes are more comparable in function to the monocytes from adults.

GM-CSF increases adult neutrophil oxidative metabolism by augmenting superoxide anion production. Additionally, GM-CSF increases chemotaxis, promotes phagocytosis of *Staphylococcus aureus*, and augments neutrophil aggregation by the increased expression of surface adhesion molecules (Gasson et al., 1984; Weisbart et al., 1985). GM-CSF is not a direct stimulant of neonatal neutrophil function, but cord blood leukocytes show increased superoxide production if primed with GM-CSF before exposure to f-MLP or opsonized zymosan particles (Cairo et al., 1989).

The diminished inflammatory response of neonatal neutrophils results in a high incidence of microbial invasion. Significant defects in the upregulation of surface-active glycoprotein receptors (C3bi) and reduced aggregation also predispose the neonate to impaired response to bacterial infection. C3bi expression has been compared in adult and neonatal neutrophils and was found to be significantly less in neonatal neutrophils when stimulated by f-MLP– or zymosan-activated serum. However, cord blood neutrophils incubated with GM-CSF demonstrated a significant induction of C3bi expression. Also, a significant increase in C3bi expression was seen when cord blood neutrophils were pretreated with GM-CSF and subsequently stimulated with the calcium ionophore A23187. The upregulation of C3bi receptor by GM-CSF also appears to correlate with enhancement of neutrophil aggregation. GM-CSF also primes neutrophils for increased neutrophil aggregation following agonist (f-MLP) stimulation.

G-CSF and TNF have also been reported to modulate the function of adult and neonatal neutrophils in a manner similar to that for GM-CSF. Priming cord blood neutrophils with G-CSF or TNF and subsequent stimulation with f-MLP induces the expression of C3bi receptors and enhances bacterial and phagocytic activity and superoxide generation.

Leukocytes Contributing to Acquired Immunity

Lymphocytes form critical components of the acquired immune system and constitute 20% of blood leukocytes. There are two broad categories: (1) T lymphocytes, which mature in the thymus and subsequently seed peripheral lymphatic organs, including the spleen and lymph nodes, and (2) B lymphocytes, which are produced in the bone marrow, mature in secondary lymphoid organs, and subsequently differentiate into antibody-secreting plasma cells. The following sections outline the development and function of T and B lymphocytes in the neonate.

T-Lymphocyte Physiology and Function

Ontogeny

T-cell precursors undergo initial differentiation and maturation in the thymus, an organ that arises from the third branchial arch at approximately 6 weeks' gestation (Vacchio et al., 2016). Hematopoietic stem cells destined to become T-cell precursors move to the thymus by 9 weeks' gestation, initially entering the subcapsular cortical areas, and then undergo a period of differentiation as they move from the cortex to the medulla of the thymus. Lymphoid progenitors entering the cortical area do not express the T-cell receptor (TCR) or the coreceptors CD4 and CD8 and are therefore referred to as *double-negative thymocytes*. These cells genetically rearrange α and β genes to generate a TCR with unique antigen specificity. In the next stage of differentiation, this newly rearranged TCR is expressed on the cell surface along with *both* CD4 and

CD8, and the cells are termed *double positive*. T cells with TCRs that recognize MHC bound to microbial peptides are “positively selected.” Following the double-positive stage, T cells lose expression of either CD4 or CD8 to become “single positive,” and during this stage, T cells that recognize “self-peptides” are negatively selected to undergo apoptosis. At the end of this differentiation process, mature T cells that enter the medulla are either CD4⁺ or CD8⁺ and can bind host MHC but only when it is not bound to self-antigens. Approximately 98% of thymocytes are eliminated during this process.

T-Cell Receptor Repertoire

The TCR is composed of two different protein chains that are associated with CD3, a protein involved in signal transduction. Most T cells carry a TCR composed of α and β chains, but in about 5% of all T cells, the TCR comprises γ and δ chains instead. Each TCR protein chain contains an immunoglobulin-like extracellular region with a variable (V) domain at its N-terminal and a constant (C) domain at the C-terminal end. The variable domains contain hypervariable, complementary-determining regions (CDRs). The third CDR (CDR3) imparts affinity for a specific processed antigen (Zemlin et al., 2002).

TCR diversity arises from genetic recombination of DNA encoding for variable (V), diversity (D), and joining (J) gene segments in individual T cells using recombination activating gene (RAG)1 and RAG2 recombinases or gene conversion using cytidine deaminases. These recombinations create a diverse pool of T cells with a wide repertoire of antigen specificity.

T-Cell Subtypes

T cells are broadly categorized as CD4⁺, CD8⁺, or T cells that are neither CD4⁺ nor CD8⁺. CD4⁺ T cells recognize antigens bound to class II MHC molecules and are further classified into T_h cells and T-regulatory cells, commonly referred to as T_{regs}.

T-Helper Cells

Naïve CD4⁺ cells differentiate into three distinct effector T_h subsets: T_h1, T_h2, and T_h17 (Zhu et al., 2010). T_h1 cells are inflammatory cells that interact with mononuclear phagocytes and provide cellular immunity against intracellular pathogens and virus-infected cells. These cells produce IL-2, IFN- γ , TNF, IL-13, and GM-CSF. T_h2 cells associate with B cells and defend against helminths and play a role in allergic reactions through regulation of antibody isotype switching. These cells produce IL-4, IL-5, IL-9, IL-10, IL-12, and IL-13 (Saito et al., 2010). T_h17 cells protect against infections, where they take on a T_h1-like effector function to promote pathogen clearance by enhancing neutrophil recruitment to sites of infection, and also by activating macrophages. These cells can also play a pathogenic role in allergic, autoimmune, and other chronic inflammatory diseases (Weaver et al., 2006; Ivanov et al., 2007; Cosmi et al., 2008). These cells produce cytokines such as IL-17A, IL-17F, IL-21, IL-22, and IL-26.

Regulatory T Cells

T_{regs} are derived from naïve T cells (Bluestone and Abbas, 2003). The transcriptional regulator forkhead box P3 (FoxP3) plays a critical role in the development of T_{regs} and is often used as a biomarker for these cells. T_{regs} downregulate the immune response by expressing inhibitory cytokines (IL-10, transforming growth factor β), cytotoxic molecules, modulators of cyclic AMP, and cytokine competition to limit effector and/or antigen-presenting cell function (Burt, 2013).

Two populations of T_{regs} have been identified: thymus-derived T_{regs} (tT_{regs}) and peripherally derived T_{regs} (pT_{regs}). tT_{regs} develop in the thymus, whereas pT_{regs} develop in the periphery from naïve T cells (Abbas et al., 2013). CD4⁺CD25⁺FoxP3⁺ T_{regs} are important contributors to the control of the immune response in the fetus/neonate. In the fetus, CD4⁺ T cells have a predisposition to differentiate into tolerogenic T_{regs} that promote self-tolerance. During midgestation, CD4⁺CD25⁺FoxP3⁺ T_{regs} may constitute 15%–20% of all CD4⁺ cells in the lymphoid tissues (Cupedo et al., 2005; Michaelsson et al., 2006). T_{regs} represent less than 5% of all CD4⁺ cells in cord blood from term infants and in the adult peripheral blood (Michaelsson et al., 2006).

Cytotoxic T Lymphocytes

Following antigen presentation and activation, CD8⁺ T cells can differentiate into cytotoxic T lymphocytes (CTLs). These cells protect against intracellular pathogens and also play a role in graft rejection and tumor surveillance (Gromo et al., 1987; Liu et al., 1996). CTLs cause cytotoxicity by releasing pore-forming mediators (perforin/granzyme system) or by activating the Fas/Fas ligand-dependent apoptotic pathway (Kagi et al., 1996; Smyth et al., 2001).

$\gamma\delta$ T Cells

$\gamma\delta$ T cells are detectable in the fetal thymus and liver at 6–8 weeks' gestation. At 16 weeks' gestation, $\gamma\delta$ T cells constitute 10% of circulating T cells but the proportion gradually declines to about 3% at term (McVay and Carding, 1996; Holtmeier et al., 2001). Postnatally, these cells are present mainly on skin and mucosal surfaces. Although the role of $\gamma\delta$ T cells remains unclear, these cells respond to nonpeptide microbial metabolites, show cytotoxicity, and produce cytokines such as IFN- γ and TNF (Hintz et al., 2001; Yamashita et al., 2003). The cytotoxicity of neonatal $\gamma\delta$ T cells is significantly less than that of their counterparts from adults (Morita et al., 1994).

Natural Killer T Cells

Natural killer (NK) T cells are a subset of T cells that express TCR $\alpha\beta$ chains as well as a variety of NK cell markers (Rhost et al., 2012; Kumar and Delovitch, 2014). These cells recognize both exogenous and endogenous lipid antigens in the context of the MHC-like molecule CD1d. CD1d-restricted NK T cells are classified into two main subsets: type I or invariant NK T cells and type II or diverse NK T cells. Type I NK T cells express a conserved $\alpha\beta$ TCR and constitute less than 1% of T cells in cord blood (Bendelac et al., 1997; Lopez et al., 2014). These cells have been identified in the human fetal small intestine (Loh et al., 2014). Type II NK T cells are more abundant than type I NK T cells in humans and express relatively diverse TCR α and β chains.

Circulating T Cells

Term neonates have higher counts of CD4⁺ T cells in peripheral blood than adults. The CD4/CD8 ratio is high (up to 4.9:1) in neonates and declines to adult values (2:1) by 4 years of age (Panaro et al., 1991; Erkeller-Yuksel et al., 1992). In cord blood, 85% of the T cells express CD38 (compared with less than 5% in adults) but lack other markers of activation (Wilson, 1991). Nearly 80% of all T cells in cord blood display a naïve CD45RA phenotype, compared with less than half of the circulating T cells in adults (Pirenne et al., 1992; Hassan and Reen, 1993). The proportion of memory CD45RO T cells reaches adult levels only by the second decade (Pirenne et al., 1992).

Neonatal T-Lymphocyte Function

T cells form a critical component of the adaptive immune defense system in the neonate. Although term infants have higher T-cell counts in peripheral blood than adults (Walker et al., 2011), preterm neonates tend to have lower CD4⁺ and CD8⁺ lymphocyte counts than their term counterparts. This deficiency could, at least in part, place the preterm infant at risk of infection. In contrast to the CD4⁺ effector T cells, preterm infants have more T_{regs} in cord blood than do term infants or than adults do in peripheral blood (Correa-Rocha et al., 2012).

Studies suggest altered function of CD4⁺ T cells in neonates compared with adults. T_H1 cell function is decreased in human neonates. Neonatal CD4⁺ T cells secrete less IL-12 and IFN- γ and express less CD154 (CD40 ligand) on their surface than their counterparts from adults (Chen et al., 2006). Cord blood lymphocytes also show limited capacity to produce IL-17 (Schaub et al., 2008). Similarly, both preterm and term neonatal T_{regs} appear less functional than their adult counterparts. T_{regs} from neonates limit contact between DCs and conventional T cells, downmodulating the expression of costimulatory molecules by DCs. This results in decreased DC immunogenicity and impaired T-cell activation (Rueda et al., 2015).

T-cell signals from antigen presentation and T-cell–derived cytokines such as IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, and IFN- γ are required for B-cell proliferation, differentiation, and survival (Bishop and Hostager, 2001). T-cell and B-cell cross talk also involves receptor ligand pairs such as CD40 (on B cell)–CD40 ligand (on T cell), which is critical during B-cell immunoglobulin isotype switch, and B7–CD28, CD11a (lymphocyte function–associated antigen–1)–ICAM-1, and CD58–CD2 (Bishop and Hostager, 2001). Neonatal T cells show lower CD40 ligand expression than adults and are less efficient at providing humoral and CD40-dependent activation signals to B cells (Splawski et al., 1996; Nonoyama et al., 1998).

CTL function is also less efficient in the neonate compared with the adult (Toivanen et al., 1981). Neonatal CTLs express less perforin than their counterparts from adults. Circulating inhibitors, such as alpha fetoprotein and prostaglandins, have been implicated as the cause of impaired CTL activity in neonates (Lubens et al., 1982).

B-Lymphocyte Physiology and Function

Ontogeny

B-cell progenitors are derived from pluripotent hematopoietic cells in the bone marrow (Klein, 1983). The first recognizable B-cell progenitor is the large pre-B cell, which is characterized by the presence of cytoplasmic μ heavy chains. Pre-B cells can be identified in the fetal liver as early as 7 weeks' gestation and in the marrow by 12 weeks. Immature B cells undergo a selection process analogous to T cells to eliminate self-identifying clones (Nossal, 1986; Buhl et al., 2000). Once B cells begin to express surface IgM (sIgM), they are ready to leave the bone marrow to enter the peripheral circulation (Rudin and Thompson, 1998); sIgM⁺ B cells can be seen in the fetal liver by 9 weeks and in the bone marrow, peripheral blood, and spleen by 12 weeks. B cells with surface IgA, surface IgG, and surface IgD (sIgD) isotypes appear between 10 and 12 weeks (Gathings et al., 1977). IgM/IgD⁺ B cells populate the lymph nodes at 16 weeks. Plasma cells are first seen around 20 weeks' gestation. By 22 weeks, the proportion of B cells in the spleen, peripheral blood, and marrow is similar to that in adults (Gathings et al., 1977; Bofill et al., 1985). The bone marrow

becomes the exclusive site for B-cell maturation by 30 weeks' gestation.

B-Cell Subtypes

In the fetus and neonate, most B cells express CD5, a T-cell antigen, on the cell surface. These CD5⁺ B cells have been referred to as *B1 cells* to differentiate them from the conventional B2 cells seen in adults. These B1 cells show greater capacity for bone marrow–independent self-renewal, display an unusual CD11b⁺ sIgM^{high} sIgD^{low} phenotype, and show constitutive expression of the transcription factor signal transducer and activator of transcription 3 (Hardy and Hayakawa, 1994; Karras et al., 1997). These cells are the predominant B-cell type during fetal life and show a distinctive anatomic localization in the spleen and the peritoneal cavity (Antin et al., 1986; Bhat et al., 1992). In adults, CD5 expression can be found on about 25%–35% of all B cells and on less than 5% of mononuclear cells in peripheral blood. In contrast, CD5⁺ B cells represent 90% of all B cells in cord blood. The proportion falls to 75%–80% during infancy and approaches adult levels only by late adolescence (Antin et al., 1986; Bhat et al., 1992).

The role of B1 cells in the fetus is still unclear. Their unique localization, their broad polyspecific specificities, and the restricted immunoglobulin repertoire suggests a role in innate immunity rather than in adaptive immunity (Hardy and Hayakawa, 1994). Unlike follicular B2 cells, which respond to protein antigens and show immunoglobulin heavy chain class switching and affinity maturation, B1 cells respond mainly to T-cell–independent carbohydrate antigens (Hardy, 2006; Dorshkind and Montecino-Rodriguez, 2007).

Circulating B Cells

Cord blood contains more B cells than adult peripheral blood, although the ratio of B cells to circulating T cells is similar (Paloczi, 1999). B-cell counts peak at 3–4 months and then decline gradually to adult levels by 6 years of age (Ugazio et al., 1974). Preterm infants have B-cell counts comparable to those of term infants, although the number may be smaller in growth-restricted infants (Thomas and Linch, 1983). Unlike adults, most B cells in cord blood express activation markers such as CD25, CD23, and transferrin receptor (Durandy et al., 1990).

Neonatal B-Lymphocyte Function

Immunoglobulin Production

The fetus and the neonate are capable of mounting an antibody response to antigenic challenges, although they may not be able to respond to all the antigens in a vaccine or may show other deficiencies such as a delayed isotype switch (D'Angio et al., 1995). Overall, postnatal age seems to be a better predictor of antibody response than the gestational age. Both preterm and term infants immunized with diphtheria toxoid in the first 10 days after birth showed weaker antibody responses than similarly immunized adults, but the response was more robust when the vaccine was administered at 1–2 months of age (D'Angio et al., 1995). Similarly, premature infants respond poorly to the hepatitis B vaccine in early infancy but show antibody responses comparable with those of their term counterparts in later infancy (Golebiowska et al., 1999).

Immunoglobulin Repertoire

Immunoglobulin diversity is generated by DNA recombination involving various V, D, or J gene segments, giving rise to a large number of V(D)J permutations (Oltz, 2001). Additional diversity

is generated by imprecise gene segment joins, addition of extra nucleotides to the splice junction of the VDJ joins, and somatic mutations (Kelsoe, 1999; Neuberger, 2005).

In the fetus and neonate, the immunoglobulin repertoire is relatively limited. During early gestation and midgestation, the heavy chain V (V_H) gene segments located closest to the J segments are utilized preferentially, resulting in shorter CDR3 regions than in adults. This altered architecture of the antigen-binding site has been postulated to allow greater polyspecificity of antigen binding, although at a cost of lower antibody affinity (Schroeder et al., 1987; Casali and Schettino, 1996). The utilization of V_H segments spreads out more evenly with increasing gestation but does not reach adult levels (Choi et al., 1995). In general, the antibody response in neonates is of low affinity and restricted to the IgM isotype. Somatic mutation of the heavy and light immunoglobulin variable region genes and the selection of higher affinity antibody-producing B cells is also limited at birth and increases slowly after 10 days of age (Ridings et al., 1997).

Serum Immunoglobulin Levels

At birth, most of the Igs present in serum are derived from transplacental transfer of maternal IgG (particularly IgG1 and IgG3) during the third trimester (McNabb et al., 1976; Palfi et al., 1998). Serum IgG levels in term neonates (up to 1000 mg/dL) may be similar to or higher than those in maternal serum, although preterm infants, who did not receive the maternal antibody, have lower IgG levels (Kohler and Farr, 1966; Hobbs and Davis, 1967). During early infancy, Ig levels fall because of normal turnover to reach a nadir of 300–500 mg/dL at 3–5 months of age, when the infant starts producing increasing amounts of his or her own. This nadir is reached earlier and is typically lower in preterm infants (Ballow et al., 1986).

Dendritic Cell Physiology and Function

DCs are specialized mononuclear cells with highly developed antigen-presenting capabilities that play a pivotal role in humoral and cellular immunity by initiating T-cell responses. DCs have a distinct morphology and are noted for their irregular shape with veil-like ruffled cell membranes (lamellipodia), many pseudopods, and numerous membrane processes. Microscopic examination of DCs reveals prominent mitochondria, endosomes, and lysosomes within the cytoplasm, which are necessary for the processing of antigen. The morphology of DCs in cord blood and adult peripheral blood is identical, although cord blood contains a lower proportion of DCs (0.5%) than adult blood (1%).

Ontogeny

Most DCs are derived from pluripotent hematopoietic stem cells differentiating along myeloid or lymphoid lineages. An exception may be the *follicular DCs*, a nonmigratory population of DCs located in the paracortical areas of the spleen and lymph nodes, which may be of mesenchymal origin (Kapasi et al., 1998).

Dendritic Cell Subtypes

In humans, DCs are usually classified into the following subsets (Ziegler-Heitbrock et al., 2010):

1. *Myeloid DCs (mDCs)* express typical myeloid antigens CD11c, CD13, CD33, and CD11b and are homologous to mouse CD11c⁺ “classic” or “conventional” DCs. In humans, mDCs

may be split into CD1c⁺ and CD141⁺ fractions (Jongbloed et al., 2010; Mittag et al., 2011). CD1c⁺ mDCs are the major population of human mDCs in blood (1% of mononuclear cells), tissues, and lymphoid organs. In lymph nodes, CD1c⁺ DCs are found as “interdigitating cells” of T-cell areas. The expression of CD141 (thrombomodulin) is seen on approximately 10% of human blood mDCs (0.1% of mononuclear cells). These cells show enhanced ability to take up necrotic cells, sense viral nucleic acids with TLR3 and TLR8, and cross-present antigen to CD8⁺ T cells (Jongbloed et al., 2010).

2. *Plasmacytoid DCs (pDCs)* typically lack myeloid antigens and are distinguished by expression of CD123 (IL-3 receptor), CD303 (C-type lectin domain family 4, member C), and CD304 (neurophilin). These cells are not related to plasma cells; the name reflects subtle lymphoid features and abundant secretory properties. In quiescent tissues, there are few pDCs, but they may constitute up to 20% of MHC class II–positive cells in lymph nodes and are rapidly recruited to both sites during inflammation. Further, pDCs express high levels of TLR7 and TLR9 and release type 1 IFN in response to viruses (Liu and Nussenzweig, 2010).
3. *CD14⁺ DCs* are a third subset of CD11c⁺ DCs found in tissues and lymph nodes. Originally described as “interstitial DCs,” these cells are more monocyte-like than the other mDCs and may arise from classic CD14⁺ monocytes (Haniiffa et al., 2012).
4. *Langerhans cells and microglia* are two specialized self-renewing DC populations found in stratified squamous epithelium and the brain parenchyma, respectively.

DC populations in blood include pDCs and CD1c⁺ and CD141⁺ mDCs; these cells are immature forms of those found in tissues and lymph nodes. Most epithelial tissues contain “migratory” DC populations that acquire antigen and migrate via the afferent lymphatics to lymph nodes, where they present antigens to T cells. In the steady state, tissues contain CD1c⁺ mDCs, CD141⁺ mDCs, and CD14⁺ DCs but few pDCs. Lymphoid tissue contains blood-derived nonmigratory “lymphoid” or “resident” DCs and some migratory DCs from the tissues (Segura et al., 2012).

Mature DCs express high levels of CD80 and CD83 on the cell membrane. Several agents have been shown to increase CD80 and CD83 expression on DCs, including TNF, bacterial endotoxin, and monocyte-derived inflammatory signals. Interactions with other cells also can induce maturation of DCs, as demonstrated by T-cell–mediated cross-linking of CD40.

Morphology

DCs express numerous surface molecules commonly found on mononuclear cells, MHC molecules, CD4, CD45 isoforms, adhesion molecules (ICAM-1, ICAM-2, ICAM-3, and leukocyte function–associated antigen 1), and Fc receptors (FcγRI [CD64] and FcγRII [CD32]). Maturation of DCs is commonly associated with high expression of CD80 and CD83. On stimulation, DCs upregulate the costimulatory molecules CD40, CD80, and CD86. Cord blood DCs express lower levels of ICAM-1 (CD54), MHC class I (HLA-ABC) and MHC class II (HLA-DR), CD80, and CD40 antigens than DCs in adult peripheral blood (Goriely et al., 2001; Hunt et al., 1994). Liu et al. (2001) compared monocytes from cord blood and adult peripheral blood for the capacity to generate DCs; cord blood monocytes generated fewer DCs, which also showed lower MHC class II expression. The endocytotic ability and ability to stimulate CD3⁺ T cells were also reduced in cord blood DCs.

Function

The activation of TLR pathways has been shown to mediate DC maturation. Krutzik et al. (2005) demonstrated that TLR activation of monocytes induces rapid differentiation to DCs that could be expanded by TLR-mediated upregulation of GM-CSF. Cord blood monocyte-derived DCs showed lower expression of the maturation markers CD83 and CD86 and produce less IL-12 (p40, p70) than adult DCs (Goriely et al., 2001; Krumbiegel et al., 2007; Belderbos et al., 2009). In mixed leukocyte culture with adult CD4⁺ T cells, neonatal DCs induced significantly lower levels of IFN- γ but higher levels of IL-10 than did adult DCs (Table 83.1). Furthermore, neonatal DCs also performed poorly as accessory cells for T-cell mitogenic responses (Hunt et al., 1994).

Innate Lymphoid Cell Physiology and Function

Innate lymphoid cells (ILCs) include the classic cytotoxic NK cells and the recently described noncytotoxic ILC populations (Artis and Spits, 2015). These cells are characterized by a classic lymphoid cell morphology but, unlike the adaptive T and B cells, do not show antigen specificity and function as a part of the innate immune system.

Natural Killer Cell Physiology and Function

NK cells have the ability to lyse target cells and secrete immunomodulatory cytokines (Robertson and Ritz, 1990). These cells make up 10% of peripheral blood lymphocytes and are characterized by the lack of the lymphocyte marker CD3 but expression of CD56 (Farang et al., 2002).

Ontogeny

NK cells develop from the committed lymphoid progenitors in the aortogonadomesonephron and later within the fetal liver (Godin and Cumano, 2002). Lineage-specific NK-cell progenitors are first identified in the fetal thymus and later in the bone marrow (Miller et al., 1992; Shibuya et al., 1993; Carlyle and Zuniga-Pflucker, 1998). Human NK-cell development requires early growth factors such as fms-like tyrosine kinase (Flt)-3 ligand or Kit ligand and later IL-15.

Natural Killer Cell Subtypes

NK cells can be classified on the basis of the surface density of CD56 into CD56^{bright} NK cells and CD56^{dim} NK cells. Early NK cells are CD56^{bright}, which then undergo further differentiation into CD56^{dim} NK cells. The CD56^{dim} NK cells are the more cytotoxic of the two categories and represent 90% of the NK-cell population. These cells express CD16 (Fc γ RIII) and show antibody-dependent cellular cytotoxicity (Leibson, 1997).

Function

NK cells express a repertoire of classes of receptors that bind to MHC class I or MHC class I-like molecules that regulate whether NK cells will be activated or inhibited (Moretta et al., 2001; Farang et al., 2002). When NK cells fail to interact with the MHC class I molecules and the activating receptor is activated, NK-cell-mediated cell lysis will ensue, as occurs in the case of infection (Moretta et al., 2005). After activation, lysosome-like vesicles containing perforin, serine esterases, and sulfated proteoglycans

are secreted toward the target cell. Perforin forms pores in the target cell, leading to osmotic lysis. The serine esterases, including granzymes, induce apoptosis (Robertson and Ritz, 1990; Berthou et al., 1995; Spaeny-Dekking et al., 2000; Moretta et al., 2005; Pao et al., 2005). After stimulation with cytokines, CD56^{bright} NK cells produce positive feedback loops by secreting IFN- γ , TNF, and GM-CSF. These cytokines help to recruit macrophages and other antigen-presenting cells for more efficient control of the infection (Cooper, 2001a, 2001b).

Neonatal NK cells show functional immaturity, which leads to an impaired immune system. Cord blood NK cells show lower expression of CD8 and CD57, two markers associated with NK cell maturation (Dalle et al., 2005; Table 83.1). Cord blood NK cells also express less ICAM-1 and CD161, which are involved in endothelial adhesion and activation, respectively, than adult NK cells (Dalle et al., 2005). Neonatal NK cells also show less cytolytic activity compared with adult NK cells, which results in impaired clearance of intracellular pathogens (Dalle et al., 2005; Wynn et al., 2009).

Noncytotoxic Innate Lymphoid Cell Physiology and Function

Ontogeny

Similarly to NK cells, noncytotoxic ILCs are also derived from the common lymphoid progenitor that differentiates into precursors committed to the various subsets. Recent studies have confirmed that NK cells and noncytotoxic helper ILCs belong to distinct cell lineages (Constantinides et al., 2014; Klose et al., 2014).

Innate Lymphoid Cell Subtypes

Noncytotoxic ILCs are characterized by a classic lymphoid cell morphology, express IL-2 receptor α and IL-7 receptor α , but unlike the adaptive T and B cells, ILCs do not exhibit antigen specificity. There are three groups of noncytotoxic ILCs: group 1 ILCs (ILC1s), group 2 ILCs (ILC2s) and group 3 ILCs (ILC3s) including lymphoid tissue inducer (LTi) cells. These subsets are defined on the basis of differential requirements for transcription factors during development, expression of effector cytokines, and the acquisition of other distinct effector functions (Spits et al., 2013).

The three ILC subsets function in the absence of antigen specificity but exhibit remarkable functional similarity with the T_h cell subsets, T_h1, T_h2, and T_h17, respectively, in cytokine expression and effector function (Artis and Spits, 2015). ILC1s express the T-bet transcription factor and produce T_h1-associated cytokines such as IFN- γ and TNF to protect against intracellular bacteria and parasites. ILC2s express the transcription factor GATA-binding protein 3 and produce T_h2-associated cytokines (including IL-4, IL-5, IL-9, and IL-13) and/or the epidermal growth factor receptor ligand amphiregulin. These cells promote type 2 inflammation seen during tissue repair, allergic disorders, and antihelminth immunity. ILC3s express the transcription factor RAR-related orphan receptor γ T, have a cytokine signature similar to that of T_h17 cells, and produce IL-17A, IL-17F, IL-22, GM-CSF, and TNF, to promote antibacterial immunity, chronic inflammation, or tissue repair. ILC3s comprise two subsets that can be distinguished on the basis of their expression of the chemokine receptor CCR6. CCR6⁺ ILC3s encompass CD4⁺ and CD4⁻ LTi cells. The CCR6⁻ ILC3 population may be further categorized on the basis of the expression patterns of natural cytotoxicity receptors.

Function

There is limited information on ILCs in the fetus and neonate. ILC2 cells are detectable in cord blood and may be present in higher proportions in male neonates than in female neonates (Forsberg et al.; 2014). In a murine model of gastroschisis, organs exposed to amniotic fluid were shown to contain high numbers of ILC2 and ILC3 and eosinophilia, which was reversed by the administration of anti-IL5 antibody (Frascoli et al., 2016). In another study, ILC3s were implicated as a source of increased IL-17 levels seen in patients with preeclampsia, and

gestational/chronic diabetes was associated with ILC3s (Barnie et al., 2015).

ILC3s are abundant at mucosal surfaces, particularly in the gastrointestinal tract. There is evidence that the development and function of some subsets of ILC3s are induced in response to microbial colonization and are involved in enhancing B-cell IgA production (Magri et al., 2014). In addition, there is evidence that IL-22 production by ILCs plays an important role in containing and shaping the composition of the intestinal microbiota (Sonnenberg et al., 2011).

Summary

Immaturity of neonatal neutrophils, monocytes, lymphocytes, DCs, and ILCs predisposes newborns to an increased incidence and/or severity of infectious complications. An increased understanding

of the genetic mechanisms responsible for these defects will provide insight regarding future treatment strategies to prevent and/or treat serious or overwhelming infection in the newborn.

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Suggested Readings

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Neonatal Indirect Hyperbilirubinemia and Kernicterus

JON F. WATCHKO

KEY POINTS

- Early clinical jaundice or rapidly developing hyperbilirubinemia is often a sign of hemolysis, the differential diagnosis of which commonly includes immune-mediated disorders, red cell enzyme deficiencies, and red cell membrane defects.
- Knowledge of the maternal blood type and antibody screen is critical in identifying non-ABO alloantibodies in the maternal serum that may pose a risk for severe hemolytic disease of the newborn.
- Knowledge of the hour-specific predischARGE bilirubin measurement and the infant's gestational age in weeks is critical to determining appropriate timely post-birth hospitalization follow-up and evaluation.
- Hyperbilirubinemia in late-preterm neonates (34^{0/7}–36^{6/7} weeks' gestation) is more prevalent, more pronounced, and more protracted than in their term counterparts, and these immature neonates are more vulnerable to bilirubin-induced brain injury.
- Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a noteworthy cause of bilirubin encephalopathy worldwide. Clinicians everywhere must have a high index of suspicion for G6PD deficiency in neonates whose genetic heritage derives from Africa, the Middle East, the Mediterranean region, and Asia.

Hyperbilirubinemia is the most common clinical condition requiring evaluation and treatment in the newborn and a frequent reason for hospital readmission during the first week of life. Although generally a benign, postnatal, transitional phenomenon, a few neonates develop marked potentially hazardous bilirubin levels that can pose a direct threat of serious brain injury (Watchko and Tiribelli, 2013). Acute bilirubin encephalopathy (ABE) may ensue and evolve into kernicterus (chronic bilirubin encephalopathy), a permanent disabling neurologic condition classically characterized by (1) the movement disorders of dystonia and/or choreoathetosis, (2) hearing loss caused by auditory neuropathy spectrum disorders, and (3) oculomotor pareses (Watchko and Tiribelli, 2013).

Total serum bilirubin (TSB) is the measure of albumin-bound bilirubin, whereas the small circulating fraction not bound to albumin or other serum proteins is indexed by the unbound or “free” (Bf) bilirubin level. There is a keen interest in circulating Bf, its measurement, and its ability to predict bilirubin-induced

neurologic injury. Indeed, Bf is the vehicle of bilirubin's biologic effects in the brain. However, bilirubin-induced neurotoxicity depends on a complex interaction between the level and duration of central nervous system (CNS) Bf exposure and the innate cellular characteristics of the developing CNS that may predispose or protect against bilirubin-induced neuronal injury, and the clinical laboratory measurement of circulating Bf is not generally available (Watchko and Tiribelli, 2013). As a result, clinicians must rely on the TSB and the bilirubin/albumin (B/A) ratio, an imperfect surrogate of circulating Bf, to index the risk for ABE and drive treatment decisions.

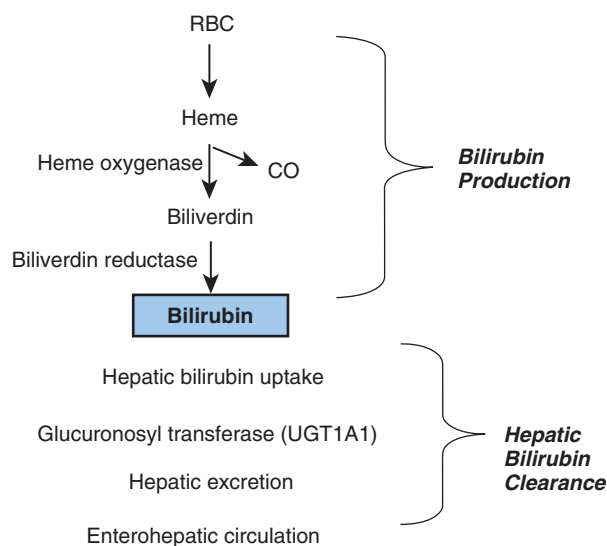
The genesis of neonatal hyperbilirubinemia reflects the interplay of developmental red blood cell (RBC), hepatic, and gastrointestinal immaturities that result in an imbalance favoring bilirubin production over hepatic enteric bilirubin clearance (Fig. 84.1). The equation below summarizes the interactions among the rates of bilirubin production (a), the enterohepatic circulation of bilirubin (b), and bilirubin elimination (c), in determining the TSB at any postnatal time point *t*, where TSB₀ is the cord blood TSB (Valaes, 2001):

$$TSB_t = TSB_0 + \sum [a(t) + b(t) - c(t)]^{\Delta t}$$

A variety of clinical conditions can increase the bilirubin load or decrease bilirubin clearance and thereby contribute to neonatal hyperbilirubinemia in any given infant (Box 84.1). In a small fraction of neonates, a constellation of conditions may lead to hazardous levels of hyperbilirubinemia that pose a neurotoxic risk. Accelerated RBC turnover (hemolysis) plays a pivotal role in increasing the risk for subsequent severe hyperbilirubinemia and in potentiating the risk of bilirubin neurotoxicity. Treatment interventions are therefore recommended at a lower bilirubin level whenever hemolysis is present. Caretakers of neonates must therefore be attuned to identifying hemolytic disorders in the jaundiced neonate.

Hemolytic Disease—Increased Hepatic Bilirubin Load

The causes of hemolysis in the neonatal period can be broadly grouped into three major categories: (1) *heritable* defects in red cell metabolism, membrane structure, or hemoglobin; (2) *acquired* disorders; and (3) *immune*-mediated mechanisms (Box 84.1).



• **Fig. 84.1** Schematic of Bilirubin Production and Hepatic Bilirubin Clearance in Neonates. Heme, produced largely by the breakdown of red blood cells (RBCs), is catabolized by heme oxygenase to produce an equimolar amount of carbon monoxide (CO) and biliverdin; the latter is reduced to unconjugated bilirubin by biliverdin reductase. Unconjugated bilirubin is taken up by the hepatocyte via facilitated diffusion, bound to glutathione S-transferase (ligandin), and conjugated with glucuronic acid by hepatic uridine diphosphate-glucuronosyltransferase 1A1 (UGT1A1). Conjugated bilirubin is excreted into bile via multidrug resistance protein-2, a portion of which may be deconjugated by intestinal β -glucuronidase and reabsorbed into the portal circulation, enhancing the hepatic bilirubin load (enterohepatic circulation).

Red Cell Membrane Defects

Of the many red cell membrane defects that lead to hemolysis, only hereditary spherocytosis, elliptocytosis, stomatocytosis, and infantile pyknocytosis manifest themselves in neonates (Tuffy et al., 1959; Oski, 1993; Caprari et al., 1997). Establishing a diagnosis of these disorders is often difficult because newborns normally exhibit a marked variation in red cell membrane size and shape (Oski, 1982a, 1993; Zipursky et al., 1983; Stockman, 1988). Spherocytes, however, are not often seen on RBC smears of hematologically normal newborns and when prominent suggest a diagnosis of hereditary spherocytosis, as does an elevated mean corpuscular hemoglobin concentration (MCHC >36.5 – 37 g/dL) or MCHC to mean corpuscular volume (MCV) ratio (MCHC/MCV >0.36) (Christensen et al., 2015). Given the likelihood of autosomal dominant inheritance, a positive family history can often be elicited. The diagnosis of hereditary spherocytosis can be confirmed using the incubated osmotic fragility test coupled with fetal red cell controls or eosin-5-maleimide flow cytometry (Christensen et al., 2015). One must rule out symptomatic ABO hemolytic disease by performing a direct Coombs test as infants so affected can manifest prominent microspherocytosis (Becker et al., 1993). Moreover, hereditary spherocytosis and symptomatic ABO hemolytic disease can occur in the same infant and result in anemia and severe hyperbilirubinemia (Trucco and Brown, 1967).

Hereditary elliptocytosis and stomatocytosis are rare but reported causes of hemolysis in the newborn period (Oski, 1993). Infantile pyknocytosis is a transient red cell membrane abnormality manifesting itself during the first few months of life. The pyknocyte, an

• BOX 84.1 Causes of Indirect Hyperbilirubinemia in Neonates

A. Increased Hepatic Bilirubin Load

- Hemolytic disease—immune mediated (positive direct Coombs test)
 - Rhesus isoimmunization
 - ABO incompatibility
 - Minor blood group incompatibility
- Hemolytic disease—red blood cell enzyme abnormalities
 - Glucose-6-phosphate dehydrogenase deficiency
 - Pyruvate kinase deficiency
- Hemolytic disease—red blood cell membrane defects
 - Hereditary spherocytosis
 - Elliptocytosis
 - Stomatocytosis
 - Pyknocytosis
- Hemolytic disease—hemoglobinopathies
 - Alpha-thalassemia
 - Gamma-thalassemia
- Extravascular blood (e.g., cephalohematoma)
- Polycythemia
- Enhanced enterohepatic bilirubin circulation
 - Intestinal obstruction, pyloric stenosis
 - Ileus, meconium plugging, cystic fibrosis
 - Breast-milk feeding

B. Decreased Hepatic Bilirubin Clearance

- Prematurity including late-preterm gestation
- Hormonal deficiency
 - Hypothyroidism
 - Hypopituitarism
- Impaired hepatic bilirubin uptake
 - Patent ductus venosus
 - SLC01B1 gene polymorphisms
- Disorders of bilirubin conjugation—UGT1A1 gene variants
 - Crigler–Najjar syndrome type I
 - Crigler–Najjar syndrome type II (Arias disease)
 - Gilbert disease
- Enhanced enterohepatic circulation
 - Intestinal obstruction, pyloric stenosis
 - Ileus, meconium plugging, cystic fibrosis
 - Breast-milk feeding

SLC01B1, Solute carrier organic anion transporter 1B1; UGT1A1, uridine diphosphate glucuronosyltransferase 1A1.

irregularly contracted red cell with multiple spines, can normally be observed in premature infants where as many as 5% of red cells may manifest this morphologic variant (Tuffy et al., 1959). In newborns affected with infantile pyknocytosis, however, up to 50% of red cells exhibit the morphologic abnormality, associated with anemia, a reticulocytosis, and hyperbilirubinemia that can be severe enough to require control by exchange transfusion (Tuffy et al., 1959). Red cells transfused into affected infants become pyknocytic and have a shortened life span, suggesting that an extra corpuscular factor mediates the morphologic alteration (Tuffy et al., 1959; Keimowitz and Desforjes, 1965; Ackerman, 1969). Whatever the mechanism underlying infantile pyknocytosis, the disorder tends to resolve after several months of life. Pyknocytosis may also occur in other conditions, including glucose-6-phosphate dehydrogenase (G6PD) deficiency and hereditary elliptocytosis, and these must be excluded before a diagnosis of infantile pyknocytosis is made.

Red Cell Enzyme Deficiencies

The two most common red cell enzyme defects that can lead to hyperbilirubinemia in the neonatal period are G6PD (Beutler, 1994; Valaes, 1994, 2000; MacDonald, 1995; Kaplan et al., 1998, 2004; Luzzatto, 2003) and pyruvate kinase deficiency (Mentzer, 2003; Rider et al., 2011). Of these, pyruvate kinase deficiency is far less frequent; it is an autosomal recessive disorder largely confined to populations in which consanguinity is prevalent, including newborns of Amish descent and other isolated communities (Christensen et al., 2010, 2011; Rider et al., 2011). Pyruvate kinase deficiency often presents with anemia, reticulocytosis, and severe hyperbilirubinemia (Osaki 1982a; Mentzer, 2003). Its importance derives from the fact that a full third of affected infants require exchange transfusion to control their hyperbilirubinemia (Matthay and Mentzer, 1981), and kernicterus is a real risk (Osaki et al., 1964; Christensen et al., 2011; Rider et al., 2011). The diagnosis of pyruvate kinase deficiency is often difficult, as the enzymatic abnormality is frequently not simply a quantitative defect but may involve abnormal enzyme kinetics or an unstable enzyme that decreases in activity as the red cell ages. The diagnosis of pyruvate kinase deficiency should be considered whenever marked hyperbilirubinemia and a picture of nonspherocytic, Coombs negative hemolytic anemia is observed.

Glucose-6-Phosphate Dehydrogenase Deficiency

G6PD deficiency is an X-linked enzymopathy affecting hemizygous males, homozygous females, and a subset of heterozygous females (via X chromosome inactivation) and is an important cause of hazardous hyperbilirubinemia and kernicterus worldwide, including the United States. Although most prevalent in Africa, the Middle East, East Asia, and the Mediterranean, G6PD deficiency has evolved into a global neonatal problem as a result of past and present migration patterns, the slave trade, and intermarriage (Beutler, 1994; Valaes, 1994, 2000; Kaplan et al., 1998, 2004). It is a noteworthy contributor to endemic rates of bilirubin encephalopathy in several developing countries (e.g., Nigeria, where ~3% of neonatal hospital admissions evidence bilirubin encephalopathy) (Oguniesi et al., 2007) and accounts for a substantial and disproportionate number of neonates with kernicterus in the United States Pilot Kernicterus registry (20.8% of all reported cases) (Bhutani et al., 2004; Johnson et al., 2009). The majority of these kernicterus cases have been in African-American neonates (Watchko, 2010), an at-risk population given G6PD deficiency prevalence rates in the United States of 12.2% for African-American males and 4.1% for African-American females (Chinevere et al., 2006). Other subgroups at risk for G6PD deficiency include newborns of East Asian, Greek, Italian (especially Sardinia and Sicily), and Middle Eastern descent (Kaplan et al., 2004).

In this regard, G6PD is remarkable for its genetic diversity (more than 370 variants have been described) (Beutler, 2008), and those mutations seen in the United States include among numerous others the (1) African A variants, a group of double site mutations all of which share the adenine(A)376guanine (G) variant (also known as G6PD A+ when expressed alone; a nondeficient variant) coupled most commonly with the G202A mutation (G202A;A376G, known as G6PD A-) but on occasion with the thymine (T)968cytosine (C) variant (T968C;A376G; also known as G6PD Betica), or the G680T mutation (G680T;A376G); (2) the Mediterranean (C563T) mutation; (3) the Canton (G1376T) mutation; and (4) the Kaiping (G1388A) variant (Beutler, 1994; Lin et al., 2005).

G6PD is critical to the redox metabolism of red blood cells, and G6PD deficiency may be associated with acute severe hemolysis

• BOX 84.2 Agents Producing Hemolysis in Patients With Glucose-6-Phosphate Dehydrogenase Deficiency

Antimalarials

Pamaquine
Pentaquine
Plasmoquine
Primaquine
Quinacrine
Quinine
Quinocide

Sulfonamides

Sulfacetamide
Sulfamethoxazole
Sulfanilamide
Sulfamethoxypyridazine
Sulfapyridine
Sulfisoxazole
Trisulfapyrimidine

Sulfones

Nitrofurans
Furaltadone
Furazolidone
Nitrofurantoin
Nitrofurazone
Thiazolesulfone

Antipyretics and Analgesics

Acetophenetidin
Acetylsalicylic acid
Aminopyrine
Antipyrone
p-Aminosalicylic acid

Others

Ascorbic acid
Chloramphenicol
Chloroquine
Aniline dyes
Dimercaprol
Fava beans
Methylene blue

Nalidixic Acid

Naphthalene^a (used in mothballs)
Naphthoquinones^a (used in mothballs)
Paradichlorobenzenes (moth repellent, car freshener, bathroom deodorizer)
Phenylhydrazene
Probenecid
Quinidine

Tolbutamide

Vitamin K, water-soluble analogues
Menadione diphosphate
Menadione sodium disulfate

Infection

Sepsis
Urosepsis
Necrotizing enterocolitis

^aAssociated with most severe and numerous hemolytic episodes.

Adapted from Osaki FA, Nalman JL. Hematologic Problems in the Newborn, 2nd ed. Philadelphia, PA: WB Saunders; 1972 and from Valaes F. Severe neonatal jaundice associated with glucose-6-phosphate dehydrogenase deficiency: Pathogenesis and global epidemiology. *Acta Paediatr Suppl.* 1994;394:58–76.

in newborns following exposure to oxidative stress. Reported hemolytic triggers in G6PD deficiency are outlined in Box 84.2. A sudden, often rapid exponential rise in TSB to potentially hazardous levels may occur and result in kernicterus that may not always be preventable (Valaes, 1994; Kaplan et al., 1998, 2004; Watchko, 2010). Severe jaundice rather than anemia may predominate in the clinical presentation (Valaes, 1994; Kaplan et al., 2008a). In some neonates, G6PD deficiency and hepatic uridine diphosphate-glucuronosyltransferase 1A1 gene (*UGT1A1*) polymorphisms of Gilbert syndrome that limit hepatic bilirubin conjugation combine to significantly increase the risk of hyperbilirubinemia (Kaplan et al., 1997). These variants include the promoter variant *UGT1A1**28 (Valaes 1994, 2000; Kaplan et al., 1997, 2004) and coding sequence variant *UGT1A1**6 (Huang et al., 2002a). Kaplan et al. (1997) have demonstrated a dose-dependent genetic interaction between the *UGT1A1**28 promoter variant and G6PD deficiency that substantially increases neonatal hyperbilirubinemia risk. Details regarding this icterogenic genetic interaction and other aspects of *UGT1A1* gene variants in neonates are described later under Hepatic Bilirubin Conjugation. Coexistent nongenetic factors may also impact hyperbilirubinemia risk in G6PD-deficient neonates, as

shown in those who are also late-preterm and breastfed (Kaplan et al., 2006).

Caretakers must have a high index of suspicion for G6PD deficiency in populations at increased risk (Mediterranean region, Africa, the Middle East, Asia) and in particular the African-American neonate with significant hyperbilirubinemia (Watchko, 2010). Although there has been discussion on the potential utility of screening for G6PD deficiency in the United States, no consensus has emerged on whether or how best to screen, and point-of-care testing during birth hospitalization is not routinely practiced (Watchko et al., 2013). One targeted screening program at a US hospital with a large at-risk population, however, demonstrates the feasibility and utility of identifying G6PD-deficient newborns (Nock et al., 2011). Reports from abroad (e.g., Israel, Singapore, and Taiwan) (Padilla and Therrell, 2007; Kaplan et al., 2008b) show that point-of-care G6PD screening strategies are associated with reductions in the prevalence of severe hyperbilirubinemia and kernicterus.

Heritable Causes of Hemolysis—Hemoglobinopathies

Defects in hemoglobin structure or synthesis are rare disorders that infrequently manifest themselves in the neonatal period. Of these, the α -thalassemia syndromes are the most likely to be clinically apparent in newborns. Each human diploid cell contains four copies of the α -globin gene, and thus four α -thalassemia syndromes have been described reflecting the presence of defects in 1, 2, 3, or 4 α -globin genes. Silent carriers have one abnormal α -globin chain and are asymptomatic. α -Thalassemia trait is associated with two α -thalassemia mutations, is not associated with hemolysis in newborns, and is common in black populations and detected by a low MCV of less than $95 \mu\text{m}^3$ (normal infants $100\text{--}120 \mu\text{m}^3$) (Schmaier et al., 1973). Hemoglobin H disease results from the presence of three thalassemia mutations and can cause hemolysis and anemia in neonates (Pearson, 1982). Homozygous α -thalassemia (total absence of α -chain synthesis) results in profound hemolysis, anemia, hydrops fetalis, and almost always stillbirth or death in the immediate neonatal period.

The pure β -thalassemias do not manifest themselves in the newborn period, and the γ -thalassemias are (1) incompatible with life (homozygous form), (2) associated with transient mild-to-moderate neonatal anemia if one or two genes are involved that resolves when β -chain synthesis begins, or (3) in combination with impaired β -chain synthesis, associated with severe hemolytic anemia and marked hyperbilirubinemia (Oort et al., 1981).

Acquired Causes of Hemolysis

Acquired causes of hemolysis comprise a miscellaneous group of disorders, which include among others the (1) microangiopathic hemolysis associated with disseminated intravascular coagulation or hemangiomas and (2) infection (bacterial sepsis or congenital infections) (Oski, 1982a). The mechanism(s) underlying the hemolytic process in the latter is not fully understood but may also serve as a hemolytic trigger in G6PD deficiency.

Immune-Mediated Hemolytic Disease

Immune-mediated disorders are the most common cause of hemolysis in neonates and should be suspected when there is (1) a heterospecific mother–infant pair where the infant expresses a

red cell antigen(s) foreign to the mother, (2) the presence of a maternal antibody directed to the infant RBC antigen, (3) and a positive direct Coombs test in the neonate indicating maternal antibody bound to the infant RBC.

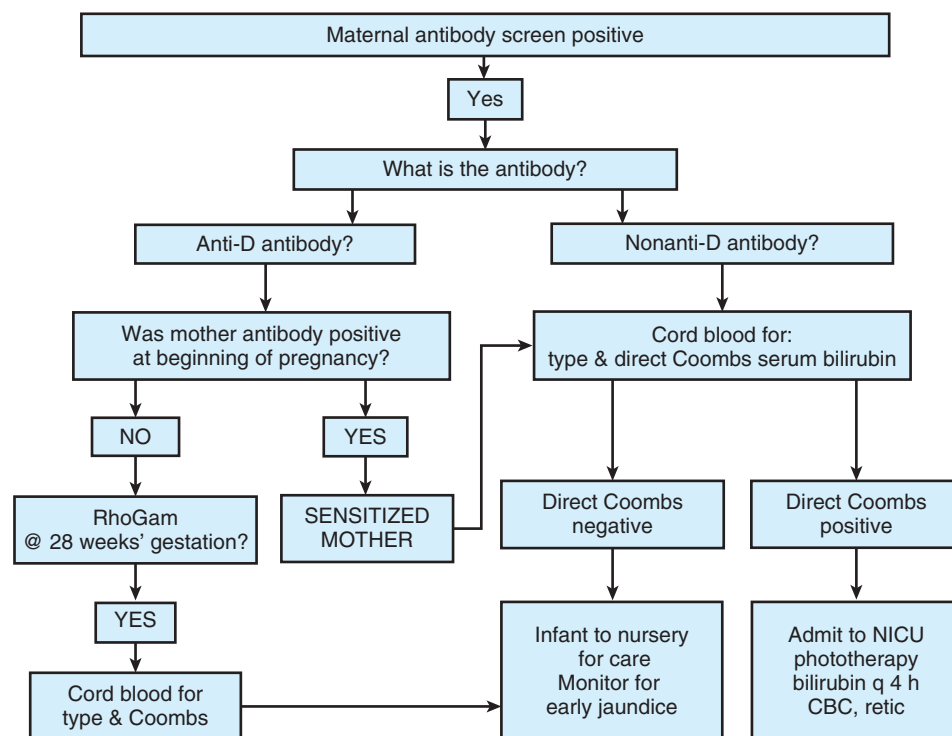
Non-ABO Alloantibodies

A priority in evaluating every newborn is knowledge of the maternal blood type and the maternal antibody screen routinely performed at maternal registration upon pregnancy diagnosis to identify non-ABO alloantibodies in the maternal serum that may pose a risk for hemolytic disease in the newborn. In addition, Rhesus (Rh)-D negative women who have a negative antibody screen at registration will have a repeat screen at 24–28 weeks' gestation before Rhogam (RhD-immunoglobulin, manufactured by CSL Biehring AG, Bern, Switzerland) administration and another screen at delivery along with a type and Coombs test on the infant to determine the need for postpartum Rhogam.

In addition to the classic Rhesus hemolytic disease of the newborn (HDN) secondary to Rh-D isoimmunization, alloantibodies directed to non-D Rhesus antigens and a broad range of non-Rhesus blood group (minor) antigens are seen. Increasingly the latter two categories comprise a clinically relevant proportion of HDN. The incidence of clinically significant sensitization to the minor blood group antigens as determined by metaanalysis approximates 1:330 pregnancies (Solola et al., 1983). Only a review of the maternal antibody screen and the direct Coombs test on the infant will uncover such cases. Fig. 84.2 outlines an approach to the evaluation and management of the neonate born to a mother with a positive antibody screen. A blood type and direct Coombs test are indicated at delivery (cord or infant blood) for all infants born to women with a positive antibody screen.

Interpreting the results of the maternal antibody screen by neonatal caregivers is critical in identifying mothers who carry a non-ABO alloantibody, several of which can cause moderate-to-severe HDN. Table 84.1 outlines several clinical scenarios in which the maternal antibody screen is positive, accompanied by the likely clinical explanation for the positive screen (Watchko, 2015). The only scenario shown that does not indicate maternal sensitization is that secondary to Rhogam administration. The latter positive anti-D maternal antibody screen must be distinguished from the occurrence of late Rh-D sensitization by confirming that the mother was anti-D antibody negative before Rhogam administration and that she did indeed receive Rhogam. At times, the infant will also have a positive direct Coombs test secondary to maternal Rhogam administration (Bowman et al., 1978; Judd, 2001; Maayan-Metzger et al., 2001). This finding is generally not thought to indicate a hemolytic risk (Bowman et al., 1978; Judd, 2001; Maayan-Metzger et al., 2001; Dillon et al., 2011), albeit one recent case report suggesting in rare circumstances it may (Cohen et al., 2014). The latter has yet to be confirmed (Watchko, 2014b).

It is also important to note that Rh-D positive infants delivered to Rh-D negative women during the first isoimmunized pregnancy (conversion from negative to positive maternal antibody titer in that pregnancy) are at an approximately 20% risk of developing HDN requiring treatment, including the possibility of an exchange transfusion (Goplerud et al., 1973). An infant born of a pregnancy during which maternal antibody conversion occurs will by definition carry the foreign antigen and may have a positive direct Coombs test. Such infants are at risk of HDN, and should be monitored closely for severe hyperbilirubinemia with serial TSB measurements and not discharged early from the birth hospital.



• **Fig. 84.2** An Approach to the Evaluation and Management of the Neonate Who Is Born to a Mother With a Positive Antibody Screen. Care must be taken to (1) distinguish passive anti-D as a result of Rhogam administration from sensitization to anti-D in the context of a positive maternal antibody screen to anti-D and (2) identify all non-D alloantibodies. CBC, Complete blood count; NICU, neonatal intensive care unit. (Courtesy of Kalyani Vats and Jon F. Watchko, Magee-Womens Hospital. See also text and Table 84.1.)

TABLE 84.1

Interpreting Maternal Antibody Status in Rhesus-D Negative Women at Delivery

| Maternal Antibody Status at Beginning of Pregnancy | Maternal Antibody Status at 24–28 Weeks Prior to Rhogam | Was Rhogam Administered? | Maternal Antibody Status at Delivery | Maternal Antibody | Diagnosis | Infant at Risk for Hemolytic Disease of the Newborn |
|--|---|--------------------------|--------------------------------------|-------------------|-------------------------------------|---|
| Negative | Negative | Yes | Positive | Anti-D | Passive anti-D; Rhogam effect | Unlikely ^a |
| Negative | Negative | No | Positive | Anti-D | Late sensitization to Rh-D | Yes |
| Negative | Positive | No | Positive | Anti-D | Early sensitization to Rh-D | Yes |
| Positive | Positive | No | Positive | Anti-D | Sensitized pregnancy to Rh-D | Yes |
| Negative | Negative | Yes | Positive | Non-D antibody | Late sensitization to non-D antigen | Yes |

^aAt times, the infant will also have a positive direct Coombs test secondary to maternal Rhogam administration (Bowman et al., 1978; Judd 2001; Maayan-Metzger et al., 2001). This finding is generally not thought to indicate a hemolytic risk (Bowman et al., 1978; Judd 2001; Maayan-Metzger et al., 2001) albeit reports suggest in rare circumstances it may (Dillon et al., 2011; Cohen et al., 2014). The latter has yet to be confirmed (Watchko and Triulzi, 2014).

From Watchko JF. Common hematologic problems in the newborn nursery. *Pediatr Clin North Am.* 2015; 62:509–524 with permission.

ABO Hemolytic Disease

Hemolytic disease related to ABO incompatibility is generally limited to mothers who are blood group O and infants of blood group A or B (Naiman, 1982; Ozolek et al., 1994). Although this association exists in approximately 15% of pregnancies, only a fraction of infants born in this context will develop significant hyperbilirubinemia (Naiman, 1982; Ozolek et al., 1994). Despite

the difficulty in predicting its development, symptomatic ABO hemolytic disease does occur. The diagnosis should be considered in infants who develop marked jaundice in the context of ABO incompatibility that is generally accompanied by a positive direct Coombs test and prominent microspherocytosis on red cell smear (Naiman, 1982). The hyperbilirubinemia seen with symptomatic ABO hemolytic disease is often detected within the first 12–24

hours of life (“icterus praecox” [Halbrecht, 1944]). Although usually controlled with intensive phototherapy alone (Naiman, 1982), a few affected infants develop hyperbilirubinemia to levels requiring exchange transfusion (Mollison, 1983). Routine screening of all ABO-incompatible cord blood has been recommended in the past and remains common practice in many nurseries (Leistikow et al., 1995). The current literature (Quinn et al., 1988; Ozolek et al., 1994; Leistikow et al., 1995), however, suggests that such routine cord blood screening is not warranted given the low yield and cost, consistent with the position of the American Association of Blood Banks (Judd et al., 1990). This recommendation assumes that universal prebirth hospitalization discharge bilirubin screening is used to help assess the risk of subsequent severe hyperbilirubinemia (Maisels et al., 2009b). A blood type and Coombs test are indicated in the evaluation of any newborn with early and/or clinically significant jaundice.

Infants born of ABO-incompatible mother–infant pairs who have a negative direct Coombs test appear to be at no greater risk for developing hyperbilirubinemia than their ABO-compatible counterparts (Ozolek et al., 1994), and the development of significant hyperbilirubinemia in such neonates should prompt an evaluation for a cause other than isoimmunization (Herschel et al., 2002). Similarly, group A or B infants born to incompatible group B or A mothers are not likely to manifest symptomatic ABO hemolytic disease, and less than 1% will have a positive direct Coombs test (Ozolek et al., 1994).

Decreased Hepatic Bilirubin Clearance

Hepatic Bilirubin Uptake

During intrauterine life, fetal bilirubin is removed by the placenta, metabolized, and excreted by the maternal liver. Blood flow through the hepatic arteries develops only in the first week of extrauterine life, and the ductus venosus may remain partially patent for several days, allowing blood to bypass the liver. Unconjugated bilirubin also appears to be a substrate for the solute carrier organic anion transporter 1B1 (SLCO1B1), a sinusoidal transporter that facilitates the hepatic uptake of a broad range of endogenous substrates and xenobiotics in an adenosine triphosphate-independent fashion (Cui et al., 2001). It follows that delayed developmental expression of *SLCO1B1* and the presence of nonsynonymous *SLCO1B1* gene variants may impact hepatic bilirubin uptake and bilirubin metabolism of neonates (Huang et al., 2004; Daood and Watchko, 2006). These factors may contribute to a delay in the plasma clearance of bilirubin.

Hepatic Bilirubin Conjugation

The bilirubin-conjugating capacity of infants is dependent on the activity of hepatic uridine diphosphate-glucuronosyltransferase 1A1 (UGT1A1), which is developmentally expressed at 0.1% of adult levels at 17–30 weeks’ gestation, increasing to 1% of adult values between 30 and 40 weeks’ gestation, and reaching adult levels only at 14 weeks of postnatal life (Kawade and Onishi, 1981; Coughtrie et al., 1988). This graded upregulation of hepatic UGT1A1 activity over the first few days of life may be induced by TSB itself and is noted following birth, regardless of the newborn’s gestational age. Induction of *UGT1A1* is also enhanced by phenobarbital via the phenobarbital responsive enhancer module (PBREM) in the *UGT1A1* gene promoter element. A nonsynonymous polymorphism of the *UGT1A1* PBREM (T3279G) is

associated with an increased risk of hyperbilirubinemia (Sugatani et al., 2002).

In addition to the developmentally modulated postnatal transition in hepatic bilirubin UGT1A1 activity, there are congenital inborn errors of *UGT1A1* expression, commonly referred to as the indirect hyperbilirubinemia syndromes (Valaes, 1976). These include the Crigler–Najjar type I and II (Arias) syndromes and Gilbert syndrome (Table 84.2). Infants with Crigler–Najjar type I have complete absence of bilirubin UGT1A1 activity and are at significant risk for bilirubin encephalopathy and its neurodevelopmental sequelae (Crigler and Najjar, 1952; Strauss et al., 2006). Inherited in an autosomal recessive pattern, the type I syndrome has marked genetic heterogeneity (Clarke et al., 1997). Currently, at least 85 different genetic mutations have been identified in Crigler–Najjar type I syndrome, typically nonsense or “stop” mutations in nature (Clarke et al., 1997).

Phototherapy is the mainstay of treatment for infants and children with Crigler–Najjar type I syndrome, although neonates may develop hazardous hyperbilirubinemia necessitating exchange transfusion. Liver transplantation is the only current definitive therapeutic intervention for this disorder (Shevell et al., 1987). Human hepatocyte transplantation holds promise to enhance hepatic UGT1A1 activity as an alternative approach that could obviate the need for liver transplantation. The ultimate treatment for this inborn error of bilirubin *UGT1A1* expression will reside in the development of an effective gene therapy strategy (Roy-Chowdhury et al., 2001; Bortolussi et al., 2014).

In contrast, the Arias syndrome, typified by more moderate levels of indirect hyperbilirubinemia as well as low but detectable hepatic bilirubin UGT1A1 activity, appears in the majority of cases to be mediated by missense mutations in the *UGT1A1* gene (Clarke et al., 1997). Phenobarbital can be trialed to induce residual UGT1A1 activity. These rare but important clinical syndromes must be included in the differential diagnosis of prolonged marked indirect hyperbilirubinemia.

Gilbert Syndrome

Gilbert syndrome, originally described at the turn of the century (Gilbert and Lereboullet, 1901), is far more common and characterized by mild, chronic, or recurrent unconjugated hyperbilirubinemia in the absence of liver disease or overt hemolysis (Gourley, 1994). Hepatic *UGT1A1* activity is reduced at least 50% in affected subjects, and more than 95% of their total serum bilirubin is unconjugated (Gourley, 1994). Gilbert syndrome affects approximately 9% of the population, and its genetic basis in Caucasians and African Americans is an abnormal *UGT1A1* promoter element (Bosma et al., 1995). More specifically, the variant promoter contains a two base-pair addition (TA) in the TATAA promoter element, giving rise to 7 (A[TA]₇TAA) rather than the more usual 6 (A[TA]₆TAA) repeats and is termed *UGT1A1**28. The extra TA repeat (A[TA]₇TAA) impairs proper message transcription and accounts for a reduced UGT1A1 activity (Bosma et al., 1995). As the repeat number increases, UGT1A1 activity decreases (Beutler et al., 1998). Subjects with Gilbert syndrome are homozygous for the *UGT1A1**28 variant promoter, providing a unique genetic marker for this disorder (Bosma et al., 1995).

Investigators had long speculated that Gilbert syndrome might contribute to indirect hyperbilirubinemia in the newborn period (Valaes, 1976; Odell, 1980; Oski, 1984), and clinical reports show that neonates with *UGT1A1**28 have accelerated jaundice, decreased fecal excretion of bilirubin monoglucuronides and diglucuronides

TABLE
84.2**Congenital Nonhemolytic Unconjugated Hyperbilirubinemia: Clinical Syndromes**

| Characteristic | SEVERITY | | |
|------------------------------------|---|---|--|
| | Marked Crigler–Najjar Type I | Moderate Crigler–Najjar Type II | Mild Gilbert Syndrome |
| Steady-state serum total bilirubin | >20 mg/dL | <20 mg/dL | <5 mg/dL |
| Range of bilirubin values | 14–50 mg/dL | 5.3–37.6 mg/dL | 0.8–10 mg/dL |
| Total bilirubin in bile | <10 mg/dL (increased with phototherapy) | 50–100 mg/dL | Normal |
| Conjugated bilirubin in bile | Absent | Present (only monoglucuronide) | Present (50% monoglucuronide) |
| Bilirubin clearance | Extremely decreased | Markedly decreased | 20–30% of normal |
| Hepatic bilirubin uptake | Normal | Normal | Reduced |
| Bilirubin UGT1A1 activity | None detected | None detected | Decreased |
| Genetics | Autosomal recessive | Heterogeneity of defect distinctly possible | Genetic polymorphisms: 1. Thymine-adenine (TA) ₇ and (TA) ₈ repeats in the <i>UGT1A1</i> promoter region. 2. G211A (Gly71Arg) <i>UGT1A1</i> coding sequence mutation identified in the Asian populations. 3. Linkage disequilibrium between (TA) ₇ /(TA) ₇ and T-3279G PBREM <i>UGT1A1</i> promoter polymorphisms |

PBREM, Phenobarbital responsive enhancer module; *UGT1A1*, uridine diphosphate glucuronosyltransferase 1A1 isoenzyme.
Adapted, updated, and modified from Valaes T. Bilirubin metabolism. Review and discussion of inborn errors. *Clin Perinatol*. 1976;3:177.

(Bancroft et al., 1998), and modestly elevated postnatal TSB levels (Roy-Chowdhury et al., 2002). Others have failed to demonstrate a clinically significant effect of *UGT1A1**28 alone on peak TSB. However, the coupling of the *UGT1A1* promoter variant A(TA)₇TAA with icterogenic conditions, e.g., G6PD deficiency and hereditary spherocytosis, markedly increases a newborn's risk for severe hyperbilirubinemia (Kaplan et al., 1997; Iolascon et al., 1998). Several studies clearly demonstrate that the A(TA)₇TAA promoter variant is prevalent in breastfed infants who develop prolonged neonatal indirect hyperbilirubinemia (Maruo et al., 1999; Monaghan et al., 1999; Roy-Chowdhury et al., 2002). In Asian populations the nucleotide 211 guanine to adenine mutation (G71R) in the coding sequence of *UGT1A1* (*UGT1A1**6) appears to underlie the Gilbert phenotype and contribute to neonatal hyperbilirubinemia risk (Huang et al., 2002b, 2004; Huang, 2005). These studies taken together demonstrate that Gilbert syndrome is a contributing factor to neonatal jaundice.

Enhanced Enterohepatic Circulation

Intestinal reabsorption of bilirubin excreted into the intestine is enhanced in neonates, adding to their hyperbilirubinemia risk (Poland, Odell, 1971). Conjugated bilirubin, as either the monoglucuronide or diglucuronide, is unstable and can be spontaneously or enzymatically hydrolyzed back to unconjugated bilirubin via intestinal β-glucuronidase and readily reabsorbed through the mucosa. Two other factors accelerating the deconjugation of bilirubin glucuronides in the newborn intestine are the mildly alkaline pH of the proximal intestine, which facilitates nonenzymatic hydrolysis, and the predominance of monoglucuronides as the main excretion form of bilirubin in the first few days of life. Neonatal absorption

is also enhanced by the lack of intestinal flora, which metabolize conjugated bilirubin to the water-soluble and readily excretable breakdown products urobilin and stercobilin.

Other Clinically Relevant Icteric Conditions

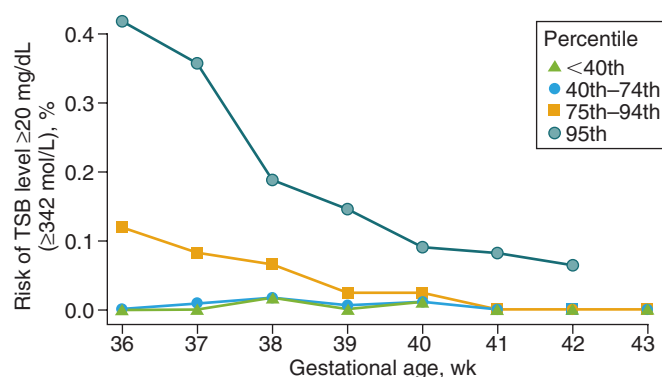
Certain demographic, environmental, and genetic risk factors, among a myriad of icterogenic contributors, merit special clinical attention in addition to hemolytic conditions. Many have been characterized as major risk factors for the development of severe hyperbilirubinemia in the 2004 American Academy of Pediatrics (AAP) clinical practice guideline on hyperbilirubinemia management in infants of greater than 35 weeks' gestation (Box 84.3) (American Academy of Pediatrics, 2004). Although each holds the potential to be a singularly important contributor to an infant's hyperbilirubinemia, more often multiple risk factors are observed in combination (Newman et al., 2000; Huang et al., 2004) in marked hyperbilirubinemia. Indeed, in infants with peak TSB levels greater than or equal to 25 mg/dL (428 μmol/L), 88% had a least two and 43% had three or more identified risk factors in one report (Newman et al., 2000), and 58% with peak TSB greater than or equal to 20 mg/dL (342 μmol/L) had at least two risk factors in another (Huang et al., 2004). Data derived from hyperbilirubinemia risk instruments that incorporate several factors support the multifactorial pathogenesis of marked hyperbilirubinemia (Newman et al., 2000, 2005) and highlight the importance of two in particular: (1) late-preterm gestational age and (2) exclusive breastfeeding (Newman et al., 2000, 2005; Keren et al., 2008). These contributors and others of notable clinical impact are reviewed in further detail later.

• BOX 84.3 Major Risk Factors for Development of Severe Hyperbilirubinemia in Infants of Greater Than 35 Weeks' Gestation

Gestational age 35–36 weeks
 Exclusive breastfeeding, particularly if nursing is not going well and weight loss is excessive
 Blood group incompatibility with positive direct antiglobulin test, other known hemolytic disease (e.g., G6PD deficiency)
 East Asian race
 Jaundice observed in the first 24 hours
 Cephalohematoma or significant bruising
 Previous sibling received phototherapy
 PredischARGE TSB or TcB level in the high-risk zone (>95% on Bhutani nomogram)

G6PD, Glucose-6-phosphate dehydrogenase; TcB, transcutaneous bilirubin; TSB, total serum bilirubin.

Adapted from American Academy of Pediatrics. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. *Pediatrics*. 2004;114:297–316.



• **Fig. 84.3** Risk of developing a total serum bilirubin (TSB) greater than or equal to 20 mg/dL (342 μ mol/L) as a function of gestational age (weeks) and percentile-based TSB measured at less than 48 hours using the Bhutani nomogram. (Modified from *Arch Pediatr Adolesc Med*. 2005;159(2):117, Copyright © 2005, American Medical Association. All rights reserved.)

Late-Preterm Gestation

Late-preterm ($34^{0/7}$ – $36^{6/7}$ weeks' gestation) infants evidence lower UGT1A1 enzyme activity (Kawade and Onishi, 1981; Kaplan et al., 2005) and a slower increase in UGT1A1 enzyme activity than their term counterparts during the first week of life (Kawade and Onishi, 1981). This exaggerated hepatic immaturity contributes to the greater prevalence, severity, and duration of neonatal jaundice in late-preterm infants (Watchko, 2006b). Indeed there is an approximately eightfold increased risk of developing a TSB of greater than or equal to 20 mg/dL (342 μ mol/L) in infants born at 36 weeks' gestational age (5.2%) as compared with those born at 41 or greater than or equal to 42 weeks' gestation (0.7% and 0.6%, respectively) (Newman et al., 1999). This gestational age effect is even more evident when examined as a function of hour-specific TSB risk zones using the Bhutani nomogram (Bhutani et al., 1999), as shown in Fig. 84.3 (Newman et al., 2005). The reported difficulty in visually assessing jaundice in late-preterm newborns (Keren et al., 2009) underscores the importance of routine birth hospitalization TSB or transcutaneous bilirubin (TcB) screening in this at-risk cohort (Maisels et al., 2009b).

Late-preterm neonates, because of their immaturity, often demonstrate less effective sucking and swallowing and may have difficulties achieving consistent nutritive breastfeeding (Wang et al., 2004), phenomena that may predispose to varying degrees of lactation failure. Suboptimal feeding was the leading reason for discharge delay during birth hospitalization in late-preterm neonates in one recent study (Wang et al., 2004). Pediatricians, therefore, need to be alert to the potential of suboptimal breast-milk feeding in late-preterm neonates and not be misled by the seemingly satisfactory breastfeeding efforts of late-preterm newborns during the birth hospitalization when limited colostrum volumes make it a challenge to adequately assess the effectiveness of breast-milk transfer (Neifert, 2001). Late-preterm infants who are breastfed merit timely post birth-hospitalization discharge follow-up and lactation support (Bhutani and Johnson, 2006; Watchko, 2006b; Maisels et al., 2009). Parental education (written and verbal) about neonatal jaundice and when to call the pediatrician is also important (American Academy of Pediatrics, 2004). A shortened hospital stay (<48 hours after delivery), although permitted for selected healthy term neonates, is not recommended for late-preterm neonates (American Academy of Pediatrics, 2007).

Late-preterm infants are disproportionately over-represented in the United States Pilot Kernicterus Registry, a database of voluntarily reported cases of kernicterus (Bhutani et al., 2004; Bhutani and Johnson, 2006; Johnson et al., 2009). Moreover, the registry demonstrates that late-preterm neonates show signs of bilirubin neurotoxicity at an earlier postnatal age than term newborns indirectly suggesting a greater vulnerability to bilirubin-induced brain injury (Bhutani and Johnson, 2006). Clinical hyperbilirubinemia management guidelines for late-preterm infants therefore recommend treatment at lower TSB thresholds than term newborns, a distinction that is an important component of the 2004 AAP practice guideline on neonatal jaundice (American Academy of Pediatrics, 2004). It is also important to note that (1) the management of late-preterm newborns born between $34^{0/7}$ and $34^{6/7}$ weeks' gestation is not addressed by the 2004 AAP guideline but is covered in a more recent approach to management (Maisels et al., 2012a), and (2) infants born between $37^{0/7}$ and $37^{6/7}$ weeks' gestation, although strictly defined as term, are characterized in the 2004 AAP guideline as medium to higher risk and are to be managed as "late-preterm" regarding phototherapy and exchange thresholds (American Academy of Pediatrics, 2004), reflecting the fact that neurotoxicity risk corresponds to gestational age.

Exclusive Breast-Milk Feeding

It is probably no coincidence that almost every reported case of kernicterus over the past three decades has been in a breastfed infant (Bhutani et al., 2004). As such, exclusive breast-milk feeding, particularly if nursing is not going well and weight loss is excessive, is listed as a major hyperbilirubinemia risk factor in the 2004 AAP practice guideline (American Academy of Pediatrics, 2004; Maisels et al., 2009a). What does the association between exclusive breast-milk feeding and kernicterus imply with respect to the etiopathogenesis of marked neonatal jaundice? Numerous studies have reported an association between breastfeeding and an increased incidence and severity of hyperbilirubinemia, both during the first few days of life and in the genesis of prolonged neonatal jaundice (Kivlahan and James, 1984; Linn et al., 1985; Maisels et al., 1986; Schneider, 1986; Hansen, 2001). A pooled analysis of 12 studies comprising over 8000 neonates showed a threefold greater incidence in TSB of greater than or equal to 12.0 mg/dL (205 μ mol/L) and

a sixfold greater incidence in levels of greater than or equal to 15 mg/dL (257 μ mol/L) in breastfed infants as compared with their formula-fed counterparts (Schneider, 1986). Others, however, report that if adequate breastfeeding is established and sufficient lactation support is in place breastfed infants should be at no greater risk for hyperbilirubinemia than their formula-fed counterparts (De Carvalho et al., 1982; Nielsen et al., 1987; Yamauchi and Yamanouchi, 1990; Rubaltelli, 1993). The later studies suggest that many breastfed infants who develop marked neonatal jaundice do so in the context of a delay in lactation or varying degrees of lactation failure. Indeed, an appreciable percentage of the breastfed infants who develop kernicterus have been noted to have inadequate intake and variable but substantial degrees of dehydration and weight loss (Maisels and Newman, 1995; Johnson et al., 2002; Bhutani et al., 2004). Inadequate breast-milk intake in addition to contributing to dehydration can further enhance hyperbilirubinemia by increasing the enterohepatic circulation of bilirubin and resultant hepatic bilirubin load (Maisels, 2000; Bertini et al., 2001). Breastfeeding-associated jaundice, however, is not associated with increased bilirubin production (Stevenson et al., 1980).

Lactation failure, however, is not uniformly present in affected infants, suggesting that other mechanism(s) may be operative in breastfeeding-associated jaundice. Breast-milk feeding may act as an environmental modifier for selected genotypes and thereby potentially predispose to the development of marked neonatal jaundice (Watchko et al., 2012). In one report the risk of developing a TSB greater than or equal to 20 mg/dL (342 μ mol/L) associated with breast-milk feeding was enhanced 22-fold when combined with expression of *UGT1A1**6 or *SLCO1B1**1b (Huang et al., 2004) and increased 88-fold when breast-milk feedings were combined with both *UGT1A1* and *SLCO1B1* variants (Huang et al., 2004). Several others have reported an association between prolonged (>14 days) breast-milk jaundice and expression of the *UGT1A1* gene promoter variant *UGT1A1**28 (Maruo et al., 1999; Monaghan et al., 1999). While recognizing the relationship between breast-milk feeding and jaundice, the benefits of breast-milk feeds far outweigh the related risk of hyperbilirubinemia. Cases of severe neonatal hyperbilirubinemia with suboptimal breast-milk feedings underscore the need for effective lactation support and timely follow-up examinations.

East Asian Ethnicity

Neonates of East Asian ethnicity encompassing the populations of mainland China, Hong Kong, Japan, Macau, Korea, and Taiwan demonstrate a higher incidence of hyperbilirubinemia than others (Newman et al., 1990) and an overall increased risk for a TSB of greater than or equal to 20 mg/dL (342 μ mol/L) (odds ratio [OR] 3.1, confidence interval [CI] 1.5–6.3) (Newman et al., 2000). As such, East Asian ancestry is listed as a major risk factor for severe hyperbilirubinemia in the 2004 AAP clinical practice guideline (American Academy of Pediatrics, 2004). Investigators have speculated as to the nature of this phenomenon, invoking potential population differences in the incidence of ABO hemolytic disease and G6PD deficiency as well as environmental exposures to Chinese materia medica among others (Ho, 1992). There is little doubt that G6PD deficiency is an important contributor to hyperbilirubinemia risk in East Asian newborns. Innate ethnic variation in hepatic bilirubin clearance (Ho, 1992) also contributes to the biologic basis of hyperbilirubinemia risk in Asian newborns, as revealed by genetic analysis of enzymatic variants that modulate bilirubin metabolism. Four different *UGT1A1* coding sequence

variants: (1) G211A (*UGT1A1**6), (2) C686A (*UGT1A1**27), (3) C1091T (*UGT1A1**73), and (4) T1456G (*UGT1A1**7) have been described in East Asian populations, each associated with a Gilbert syndrome phenotype (Huang et al., 2000, 2004). Of these, the *UGT1A1**6 variant is predominant, with an allele frequency of 11%–13% in East Asians (Huang et al., 2000) (as high as 30% in neonates with hyperbilirubinemia \geq 15 mg/dL [257 μ mol/L]) (Huang et al., 2002a) and an associated significant decrease in *UGT1A1* enzyme activity (Yamamoto et al., 1998). Hepatic *SLCO1B1* gene variants are also prevalent in East Asian populations (Huang et al., 2004; Kim et al., 2008) and the *SLCO1B1**1b variant was demonstrated to enhance neonatal hyperbilirubinemia risk (Huang et al., 2004). As noted in the section on breast-milk feeding earlier, coupling *UGT1A1* and *SLCO1B1* variants together enhances hyperbilirubinemia risk, one that is further increased when that infant is also exclusively breastfed (Huang et al., 2004).

Jaundice Observed in the First 24 Hours of Life

Jaundice appearing in the first 24 hours of life has long been regarded as an abnormal clinical finding and an indication for a serum bilirubin measurement (American Academy of Pediatrics, 2004). Approximately 2.8% of newborns will evidence jaundice within 18 hours and 6.7% within 24 hours of life (Newman et al., 2002). As contrasted with nonjaundiced newborns on the first day of life, newborns with overt jaundice in the first 24 hours of life are more likely to receive phototherapy (18.9% vs 1.7%; relative risk [RR] 10.1, 95% CI 4.2–24.4) and to develop a TSB greater than or equal to 25 mg/dL (428 μ mol/L) (OR 2.9, 95% CI 1.6–5.2) (Newman et al., 2002). Hemolytic disease, immune-mediated and otherwise, should be a diagnostic consideration in any infant with early clinical jaundice.

Cephalohematoma or Significant Bruising

Internal hemorrhage, ecchymosis, and other extravascular blood collections will enhance bilirubin production and the bilirubin load on the liver. Extravascular red cells have a markedly shortened life span and their heme fraction is quickly catabolized to bilirubin by tissue macrophages that contain heme oxygenase and biliverdin reductase (Odell, 1980). Thus cephalohematoma, subdural hemorrhage, massive adrenal hemorrhage, and marked bruising can be associated with increased serum bilirubin levels and typically manifest 48–72 hours following the extravasation of blood (Odell, 1980). This temporal pattern is consistent with the evolution of ecchymosis and bilirubin formation in situ and also accounts for why extravascular blood can cause prolonged indirect hyperbilirubinemia (Odell, 1980). An unusual but dramatic example of how extravascular blood can contribute to the genesis of hyperbilirubinemia is found in reports of marked jaundice associated with the delayed absorption of intraperitoneal blood in infants who received fetal intraperitoneal red cell transfusions (Wright et al., 1982; Rajagopalan and Katz, 1984). In one such case, 13 exchange transfusions were necessary to control the hyperbilirubinemia that resolved only when approximately 87 cc of packed red cell were evacuated from the intraperitoneal cavity (Wright et al., 1982). In this instance, the intraperitoneal blood hematocrit of 60% had the potential to contribute up to approximately 600 mg of bilirubin to the infant's bilirubin load over time. Although other causes of extravasation generally are not associated with such large amounts of sequestered blood, they can nevertheless contribute to the development of jaundice.

Previous Sibling Treated With Phototherapy

A history of a previous sibling treated with phototherapy is an identified risk factor for hyperbilirubinemia (Gale et al., 1990; Newman et al., 2000), in particular bilirubin concentrations greater than 15 mg/dL (257 μ mol/L) (Khoury et al., 1988). This relationship may reflect recurrent ABO or Rh hemolytic disease (Maisels, 1982) or exposure to a common environmental factor in addition to a shared genetic background (Gale et al., 1990). It is known that the recurrence rate of ABO hemolytic disease is high: 88% in those infants of the same blood type as their index sibling, with almost two-thirds of the affected infants requiring treatment (Katz et al., 1982). An excess risk in siblings independent of hyperbilirubinemia risk factors expected to recur in sibships including breastfeeding, lower gestational age, and hemolytic disease, however, suggests that genetic effects play an important role (Khoury et al., 1988; Gale et al., 1990). Consistent with this hypothesis, there is a higher concordance level in TSB between monozygotic (identical) than dizygotic (fraternal) twins when controlled for confounders that modulate neonatal bilirubinemia (Ebbesen and Mortensen, 2003).

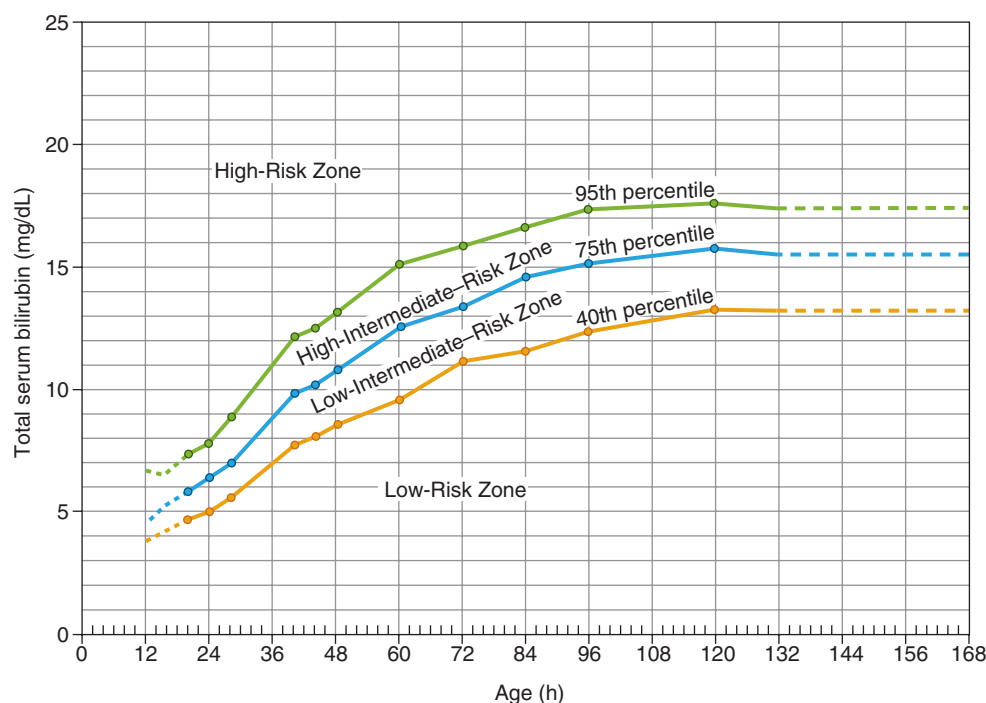
African-American Ethnicity

African-American neonates demonstrate a lower overall incidence of significant hyperbilirubinemia, a lower risk for TSB greater than 20 mg/dL, comprise approximately 12% of the US population, and yet account for more than 25% of kernicterus cases in the United States (Watchko, 2010). An explanation for this discordancy has recently emerged (Wickremasinghe et al., 2013). Using a large neonatal cohort, Wickremasinghe et al. (2013) reported that black infants, despite having a reduced risk of TSB greater than 20 mg/dL, actually have a fourfold increased risk for a TSB greater than

or equal to 30 mg/dL than other groups. Black race is not protective against hazardous (>30 mg/dL) hyperbilirubinemia, levels that pose the greatest risk of bilirubin-induced brain injury. G6PD deficiency is a plausible contributor to this pattern, but no effect modification by sex expected in an X-linked disorder was observed (Wickremasinghe et al., 2013). Regardless, black race can no longer be considered a factor associated with decreased hyperbilirubinemia risk (American Academy of Pediatrics, 2004), and black infants must be monitored for hyperbilirubinemia at least as closely as other neonates (Wickremasinghe et al., 2013). Some would caution that African-American neonates with overt jaundice (male or female) merit special attention and follow-up because such infants represent exceptions, a subset of which might be G6PD deficient. Consistent with this assertion, Kaplan et al. (2004) reported that 48.4% of G6PD-deficient African-American neonates developed a TSB greater than 75% (high-intermediate) and 21.9% a TSB greater than 95% (high) risk zones on the Bhutani nomogram, respectively. In contrast, Keren et al. (2008) report that neither the presence of a G6PD mutation or overt clinical jaundice was associated with the development of significant hyperbilirubinemia in black infants or black male infant cohorts.

Combining Clinical Risk Factor Assessment With PredischARGE Bilirubin Measurement

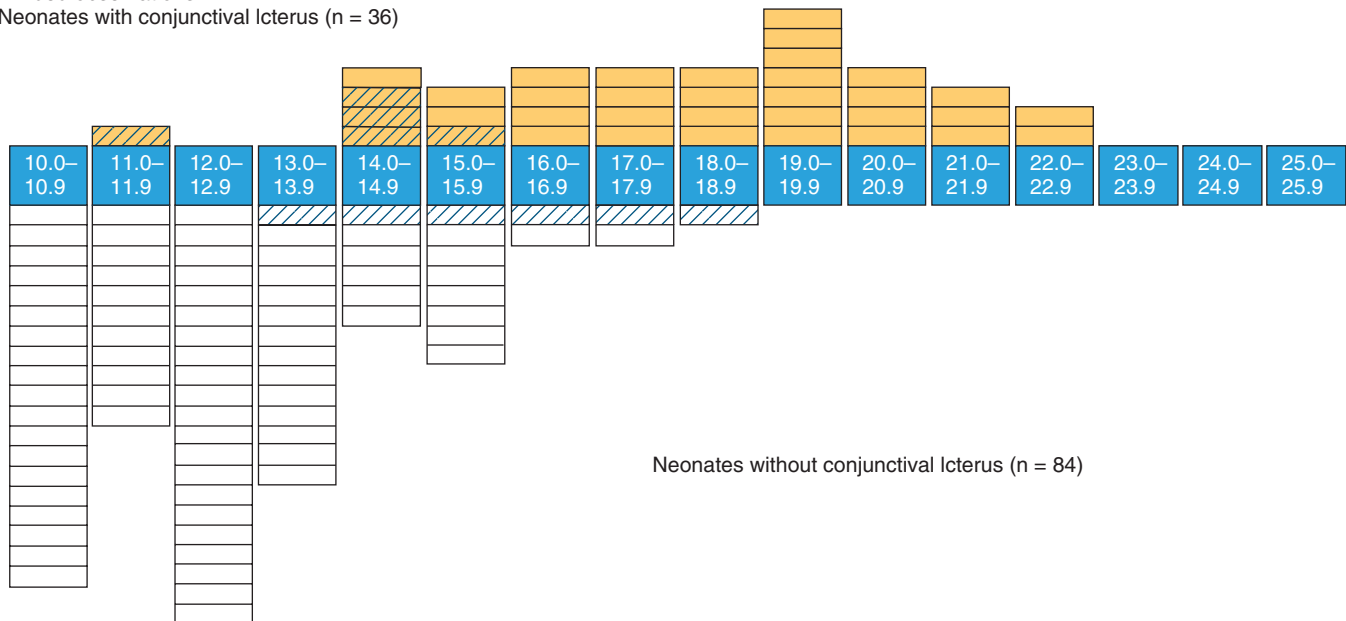
Several clinical studies show that combining clinical risk factor analysis with a birth hospitalization predischARGE measurement of TSB or TcB significantly improves the prediction of subsequent hyperbilirubinemia risk (Newman et al., 2005; Keren et al., 2008; Maisels et al., 2009b). An hour-specific prebirth hospitalization discharge TSB or TcB level in the high-risk zone (>95%) using the Bhutani nomogram (Bhutani et al., 1999) (Fig. 84.4) is a



• **Fig. 84.4** Nomogram for Designation of Hyperbilirubinemia Risk Based on Hour-Specific Bilirubin Value. (Adapted from Bhutani V, Johnson L, Sivieri EM, et al. Predictive ability of a predischARGE hour-specific serum bilirubin for subsequent significant hyperbilirubinemia in healthy term and near-term newborns. *Pediatrics*. 1999;103:6–14.)

Blinded observations

Neonates with conjunctival icterus (n = 36)



• **Fig. 84.5** Plot of individual neonates (each block = one subject) as a function of total serum bilirubin (mg/dL) and presence (top) or absence (bottom) of conjunctival icterus. Blinded observations of two clinicians; concordant examinations in open bars; the 11 discordant examinations are indicated by hatched bars. (Adapted from Azzuqa A, Watchko JF. Bilirubin concentrations in jaundiced neonates with conjunctival icterus. *J Pediatr*. 2015;167:840–844, with permission.)

major risk factor for severe hyperbilirubinemia (Bhutani et al., 1999; 2000) (Box 84.3), and the clinical factors most predictive of hyperbilirubinemia risk when combined with the risk zone characterization are lower gestational age and exclusive breastfeeding (Newman et al., 2000, 2005; Keren et al., 2008; Maisels et al., 2009a). Coupling predischarge TSB or TcB measurement with gestational age is central to the recommendations for management and follow-up outlined in an update with clarifications to the 2004 AAP clinical practice guideline on the management of hyperbilirubinemia in the newborn infant ≥ 35 weeks' gestation (Maisels et al., 2009a) (see *Clinical Efforts at Kernicterus Prevention* later). Recently published data further suggest that predischarge bilirubin screening is associated with a reduction in the incidence of TSB greater than or equal to 25 mg/dL (428 $\mu\text{mol/L}$) (Eggert et al., 2006; Kuzniewicz et al., 2009; Sgro et al., 2016) possibly by increasing the use of phototherapy (Kuzniewicz et al., 2009).

Clinical Evaluation of Jaundice

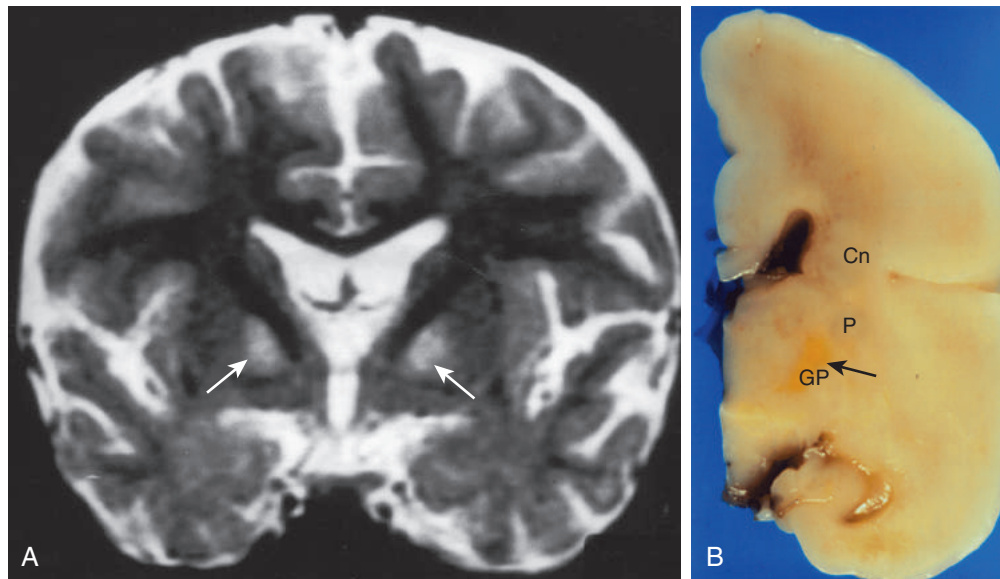
Jaundice is the visible manifestation in the skin of elevated serum concentrations of bilirubin. Although most adults are jaundiced when TSB levels exceed 2.0 mg/dL (34 $\mu\text{mol/L}$), neonates characteristically do not appear jaundiced until the TSB exceeds 5.0–7.0 mg/dL (86–120 $\mu\text{mol/L}$). Some degree of jaundice develops in approximately 85% of neonates (Keren et al., 2009), and chemical hyperbilirubinemia, defined as a TSB greater than or equal to 2.0 mg/dL (34 $\mu\text{mol/L}$), is virtually universal in newborns during the first week of life. Jaundice in neonates becomes evident first on the face and progresses in a cephalocaudal fashion, with increasing hyperbilirubinemia, as classically characterized by Kramer (1969). Recent studies, however, suggest that the pattern and intensity of jaundice during the birth hospitalization may not be as reliable an indicator of hyperbilirubinemia degree as previously thought

(Moyer et al., 2000; Keren et al., 2009). Therefore the AAP cautions that “visual estimation of bilirubin levels can lead to errors” (American Academy of Pediatrics, 2004). It is currently recommended that a TcB or TSB measurement complement the clinical assessment for jaundice in every neonate during the birth-hospitalization to assist in hyperbilirubinemia detection and neonatal management (Maisels et al., 2009b).

The absence of jaundice has excellent negative predictive value (99%) for developing a TSB that merits phototherapy (Keren et al., 2009); jaundice limited to the face and upper chest likely predicts a TSB of less than 12.0 mg/dL (Moyer et al., 2000). More recent observations suggest that the presence of conjunctival icterus may be a sign of significant hyperbilirubinemia, often associated with elevations in TSB greater than 14.9 mg/dL [255 $\mu\text{mol/L}$] and greater than 95% on the Bhutani nomogram (Azzuqa and Watchko, 2015) (Fig. 84.5). Others, while confirming a strong correlation with elevated bilirubin levels, also noted conjunctival icterus in neonates at lower bilirubin levels (Maisels et al., 2016). The AAP recommends that parents who detect conjunctival icterus should call their physician, who in turn should evaluate the infant, including a bilirubin measurement.

Kernicterus—Chronic Bilirubin Encephalopathy

Chronic bilirubin encephalopathy (CBE) defines the permanent clinical sequelae of bilirubin toxicity that become evident in the first year of life and is synonymous with the term “kernicterus” (American Academy of Pediatrics, 2004; Shapiro, 2012). These include the extrapyramidal movement disorders of dystonia and/or choreoathetosis, hearing loss caused by auditory neuropathy spectrum disorders, and the eye movement abnormality of paresis of upward gaze (Perlstein, 1960; Shapiro, 2012). The CNS sequelae reflect the regional topography of bilirubin-induced neuronal damage



• **Fig. 84.6** (A) Coronal T2-weighted magnetic resonance image at the level of the basal ganglia in one infant is shown on the left, demonstrating bilateral, symmetric high-intensity globus pallidus (GP) signals (arrows). (B) Deep orange-yellow staining of the GP of the coronal section at postmortem in another neonate. Note unstained putamen (P) and caudate nucleus (Cn). These findings illustrate the selective vulnerability and regional nature of kernicterus and concordance of neuroimaging and neuropathology in this disorder. (A, Reprinted with permission of Blackwell Publishing Asia, from Shah Z, Chawla A, Patkar D, Pungaonkar S. MRI in kernicterus. *Australasian Radiology* 2003;47:55–57; permission conveyed through Copyright Clearance Center, Inc. B, Reprinted with permission from *Monographs in Clinical Pediatrics*. 2000;11:78. Available at www.tandf.co.uk.)

distinguished by remarkably selective involvement of the globus pallidus, subthalamic nucleus, the CA2–CA3 sectors of the hippocampus, the reticular portion of the substantia nigra, the red nuclei, dentate nuclei and Purkinje cells of the cerebellum, and select brainstem nuclei (Ahdab-Barmada, 1983, 2000; Ahdab-Barmada and Moossy, 1984). Infants with chronic bilirubin encephalopathy often demonstrate abnormal bilateral, symmetric, high-signal intensity on T2-weighted magnetic resonance imaging (MRI) of the globus pallidus and subthalamic nucleus, consistent with the neuropathology of kernicterus (Fig. 84.6) (Shapiro, 2012; Wisnowski et al., 2014).

Originally described in the context of severe hyperbilirubinemia secondary to Rh hemolytic disease (Evans and Polani, 1950; Vaughan et al., 1950), kernicterus has also been reported in other hemolytic conditions (e.g., hereditary spherocytosis and pyruvate kinase deficiency), G6PD deficiency, premature neonates, and in otherwise healthy term ($\geq 37^{0/7}$ weeks) and late-preterm ($34^{0/7}$ – $36^{6/7}$ weeks) gestation breastfed infants without hemolysis (Maisels and Newman, 1995; Bhutani et al., 2004). Population-based kernicterus incidence estimates for term neonates in developed countries range from approximately 0.4–2.7 per 100,000 (Maisels et al., 2012a); higher rates have been reported for (1) preterm newborns (Watchko and Claassen, 1994; Morioka et al., 2016) and (2) infants born in developing countries where kernicterus is a serious endemic problem: e.g., Nigeria, where approximately 3% of neonatal hospital admissions evidence bilirubin encephalopathy (Oguniesi et al., 2007; Zipursky, 2009).

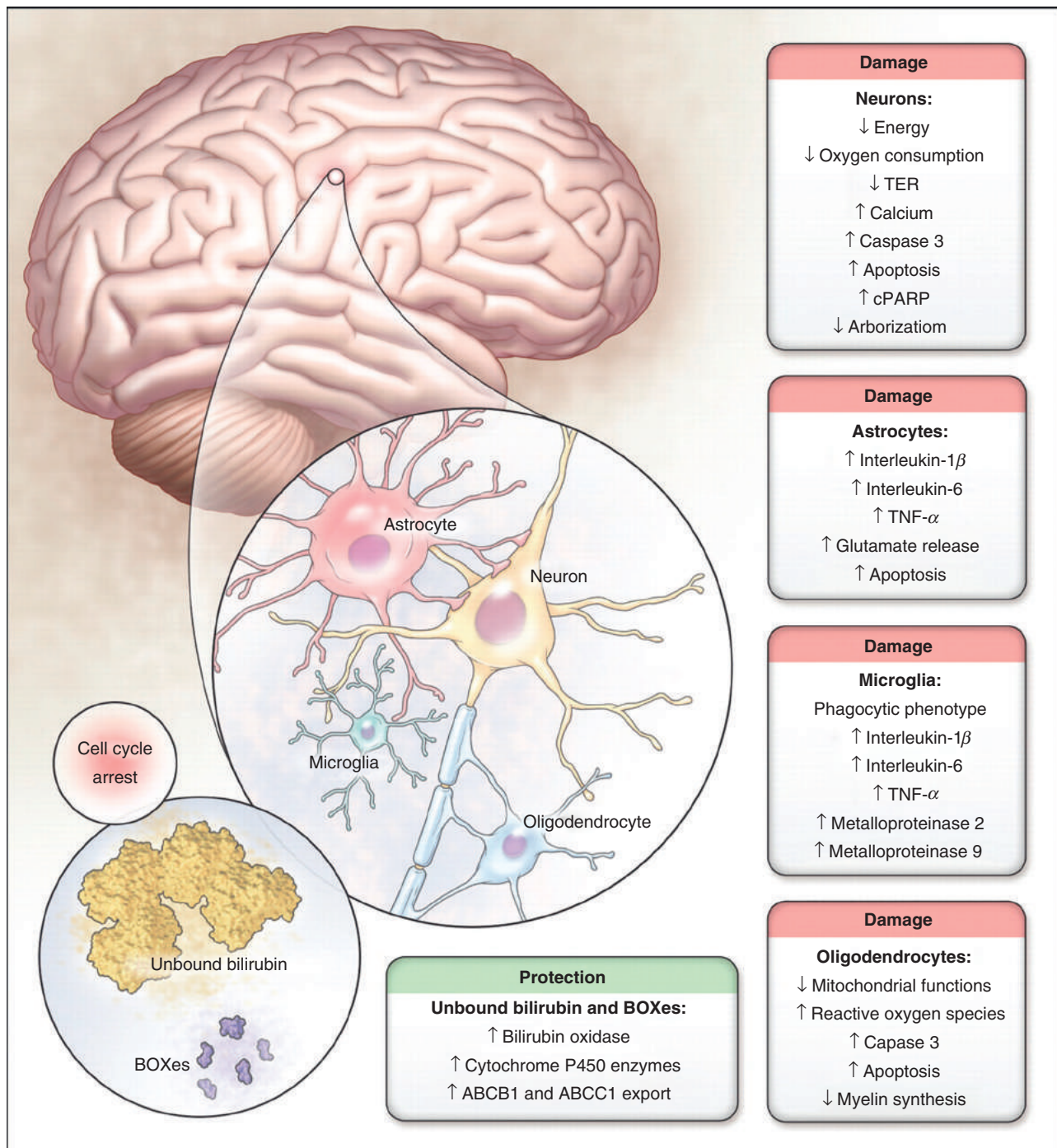
Bilirubin toxicity usually does not become clinically overt until high TSB levels have been established for several hours. Acute bilirubin encephalopathy defines an encephalopathic state during the first days of postnatal life characterized by a constellation of abnormal clinical signs typically progressive in their severity. In term

and late-preterm gestation infants, stupor (lethargy), hypotonia, and poor sucking are seen in the initial phase (Volpe, 2008; Shapiro, 2012). These nonspecific signs are seen in numerous clinical contexts but in a hyperbilirubinemic infant should raise the possibility of early ABE. Clinical signs of intermediate to advanced stages of ABE are increasingly more specific to bilirubin-induced neurotoxicity and herald a marked increased risk for permanent injury (Volpe, 2008; Shapiro, 2012). These include hypertonia often manifested by retrocollis and opisthotonus, fever, and high-pitched cry. Inability to feed and apnea may ensue (Johnson et al., 2009). Infants less than 34 weeks' gestation less frequently show these classic abnormal neuromotor signs. Recurrent apnea and desaturations may be the only clinical manifestations of ABE in preterm infants during the neonatal period, if any appear at all (Amin, 2004; Amin et al., 2014).

Although a preponderance of the literature suggests that once an infant demonstrates advanced signs of ABE, then permanent damage to the CNS has occurred (Jones et al., 1954; Van Praagh, 1961); more recent reports suggest that at least some such infants if treated aggressively may escape unscathed (Harris et al., 2001; Hansen et al., 2009). The latter support the AAP recommendation for immediate exchange transfusion in any infant who is jaundiced and manifests the signs of intermediate to advanced stages of ABE (hypertonia, arching, retrocollis, opisthotonus, fever) even if the TSB is falling (American Academy of Pediatrics, 2004).

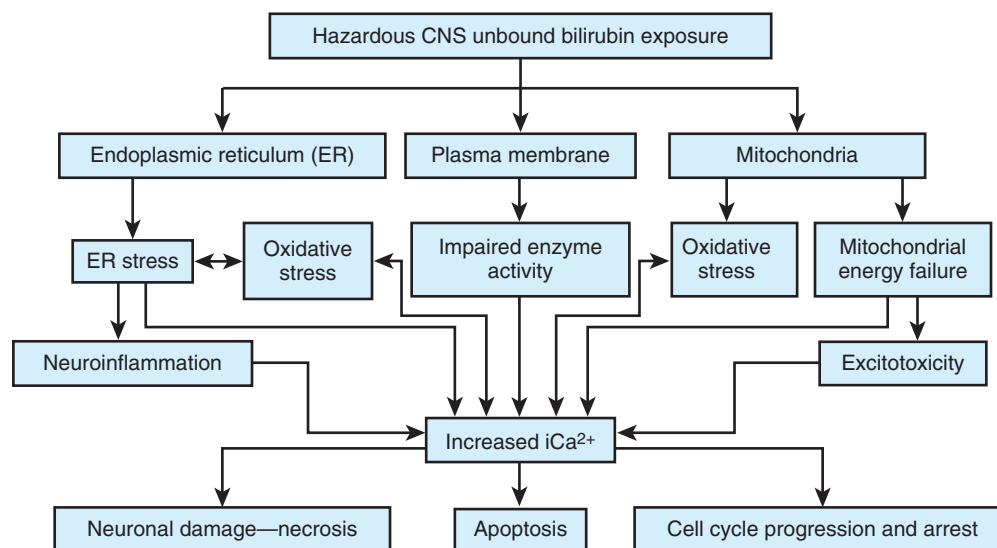
Molecular Pathogenesis

Although no one disputes the neurotoxic potential of bilirubin, the cellular and molecular events attendant to bilirubin-induced neurotoxicity have been only partially characterized, and there is little agreement as to which, from a mechanistic standpoint, may be the most clinically relevant. Fig. 84.7 highlights the multiple



• **Fig. 84.7** Cell Types and Metabolic Processes Affected by Bilirubin in the Central Nervous System.

The main effects of bilirubin on neurons are decreased oxygen consumption and increased release of calcium and caspase 3, resulting in apoptosis. There is also decreased dendritic and axonal arborization. A similar pattern is observed in oligodendrocytes with increased apoptosis, impairment of the redox state (oxidative stress), and reduced synthesis of myelin. Microglia react to toxic injury associated with bilirubin by increased release of proinflammatory cytokines and metalloproteinase activity as cells manifest a phagocytic phenotype. A similar proinflammatory pattern is observed in astrocytes, with enhanced release of glutamate and apoptosis. At the same time, cells may reduce the intracellular concentration of bilirubin either by extruding the pigment through the ABC transporters and/or by increasing the formation of the less toxic products through bilirubin oxidation products (BOXes) and/or cytochrome P450 enzymes (1a1 and 1a2 in particular). These responses are protective, whereas all others result in cell damage; this suggests that once the intracellular concentration of bilirubin exceeds a toxic threshold (still to be defined) the polymorphic metabolic cascade leading to neurotoxicity ensues. *ABCB1*, ATP-binding cassette B1 transporter; *ATP-binding cassette C1 transporter*; *cPARP*, cleaved poly(adenosine diphosphate-ribose) polymerase; *TNF- α* , tumor necrosis factor α ; *TER*, transcellular resistance. (From Watchko JF, Tiribelli C. Bilirubin-induced neurologic damage – mechanism and management approaches. *N Engl J Med*. 2013;369:2025. Copyright © (2013) Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.)



• **Fig. 84.8** Schematic of Several Hypothesized Pathophysiologic Mechanism(s) in Bilirubin-Induced Neuronal Injury. Hazardous unbound bilirubin exposure in the central nervous system (CNS) exerts direct effects at level of the plasma membrane, mitochondria, and/or endoplasmic reticulum (ER), leading to ER stress, oxidative stress, impaired enzyme activity, and mitochondrial energy failure, culminating in neuroinflammation, excitotoxicity, and increased intracellular calcium levels (iCa^{2+}). If CNS free bilirubin exposure is of sufficient degree and/or duration than irreversible neuronal damage, i.e., necrosis, and/or cell cycle arrest may ensue.

reported effects of bilirubin on neurons and glia cells (Watchko and Tiribelli, 2013). Bilirubin-induced neuronal cell injury probably reflects the adverse effects of hazardous unbound (“free”) unconjugated bilirubin concentrations on plasma and mitochondrial and/or endoplasmic reticulum membranes (Fig. 84.8) (Watchko, 2006a, 2016; Brites and Brito, 2012; Watchko and Tiribelli, 2013). These membrane perturbations, in turn, precipitate untoward cellular events that culminate in increased intracellular calcium concentrations (iCa^{2+}). Downstream events triggered by increased iCa^{2+} may include the activation of proteolytic enzymes, apoptosis, necrosis, as well as abnormalities of cell cycle progression including cell cycle arrest. Bilirubin-induced excitotoxicity, neuroinflammation, and oxidative stress have all been hypothesized to play important roles in potentiating bilirubin-induced neuronal injury whereas innate cellular characteristics of the CNS may predispose or protect against bilirubin-induced neuronal injury (Watchko and Tiribelli, 2013). Prematurity, hemolysis, and sepsis play key roles in enhancing bilirubin neurotoxicity risk (Watchko, 2004; Watchko et al., 2009).

Preterm Neonates and Low-Bilirubin Kernicterus

Preterm newborns are more vulnerable to kernicterus secondary to CNS immaturity and concurrent adverse clinical conditions that may potentiate bilirubin neurotoxicity. There remains some uncertainty, however, on how to quantify that risk and when to intervene with phototherapy or exchange transfusion in preterm neonates (Hansen, 1996; Maisels, 2000; Maisels and Watchko, 2003). In this regard, low-bilirubin kernicterus, i.e., bilirubin-induced neuronal damage at TSB levels generally thought to be nonhazardous (i.e., those below double volume exchange transfusion thresholds) continue to occur in preterm neonates. The CNS bilirubin exposure, however, is neurotoxic, suggesting either (1) an albumin problem, i.e., an abnormally low serum albumin and/or impaired albumin-bilirubin binding that results in a hazardous unbound unconjugated bilirubin concentration, and/or (2) a

vulnerable neuronal pool resulting from cellular immaturity coupled with antecedent or concurrent insults that potentiate bilirubin neurotoxicity (Govaert et al., 2003; Watchko, Maisels, 2014; Watchko, 2016). Examples of the latter are infection/inflammation, including chorioamnionitis and necrotizing enterocolitis and comorbid CNS injuries including hypoxic-ischemic encephalopathy, intraventricular hemorrhage, and periventricular leukomalacia (Govaert et al., 2003; Watchko 2014; 2016). Oftentimes both an albumin problem and a vulnerable neuronal pool are evident in a given neonate with low bilirubin kernicterus, suggesting this is a two-hit or multihit phenomenon (Watchko 2014, 2016).

Important causes of hypoalbuminemia include leakage of albumin into the extravascular, extracellular interstitial space reported in association with several conditions in sick preterm newborns and significant albumin loss secondary to fetal perinatal hemorrhage. Marked neonatal hypoalbuminemia can be seen in (1) fetal-maternal transfusion, (2) the donor twin in twin-twin transfusion syndrome (TTTS) (Verbeek et al., 2013a) and the twin anemia-polycythemia sequence (TAPS) (Verbeek et al., 2013b), and (3) neonatal anemia associated with malformations of the placenta and cord (Oski, 1982b). Whenever anemia is present in the immediate neonatal period, measurement of the albumin concentration is prudent.

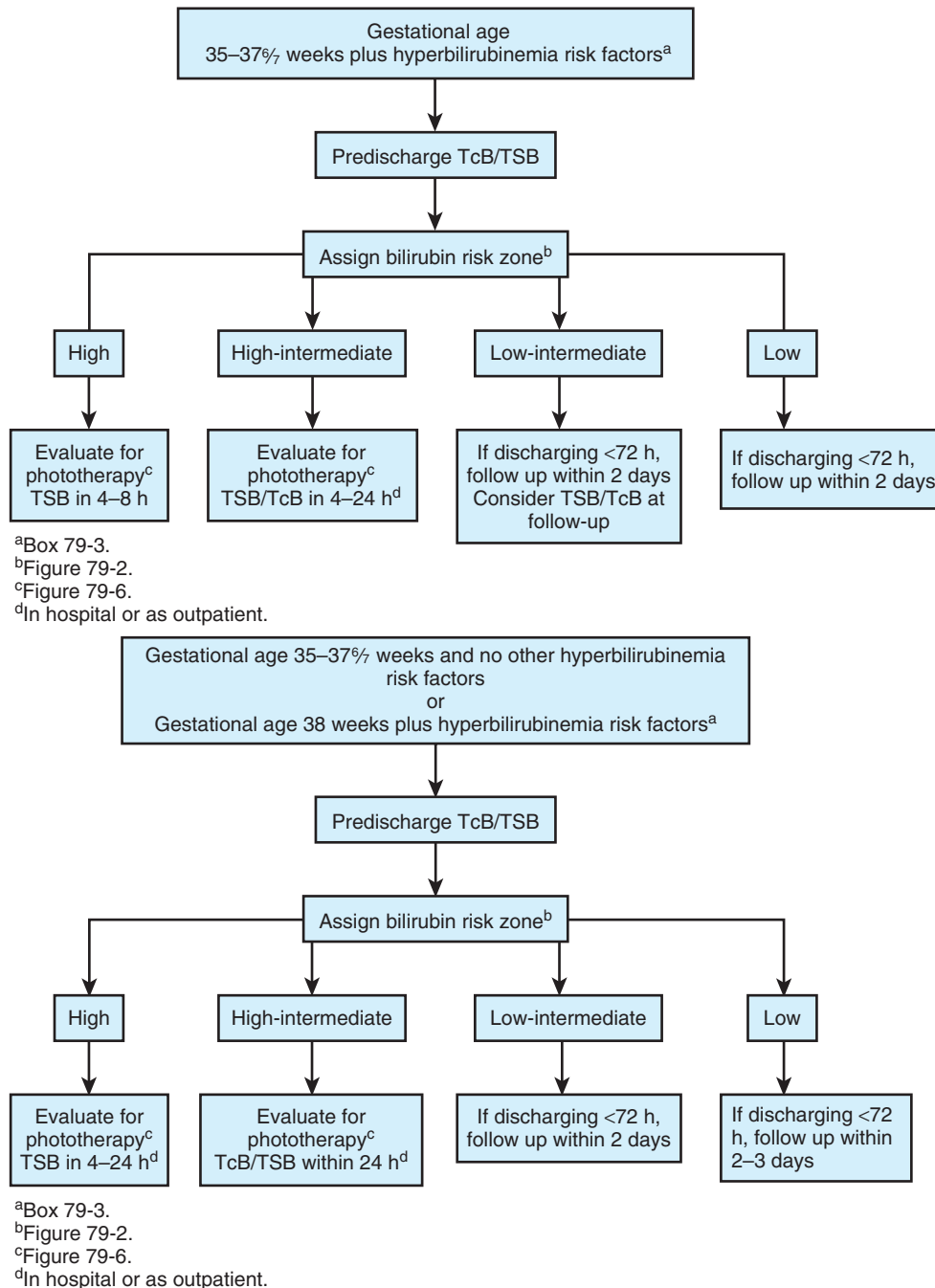
Clinical Efforts at Kernicterus Prevention

The key elements to preventing kernicterus in term and late-preterm neonates are (1) hyperbilirubinemia risk assessment, (2) appropriate and timely birth-hospitalization follow-up, and (3) timely and effective treatment of marked hyperbilirubinemia with phototherapy and/or exchange transfusion. Support of successful breastfeeding is also an important part of hyperbilirubinemia control. A TSB or TcB measured 18–36 hours after birth interpreted according to the infant’s age in hours using the Bhutani hour-specific nomogram (see Fig. 84.4) significantly improves the prediction of subsequent severe hyperbilirubinemia (Newman et al., 2005;

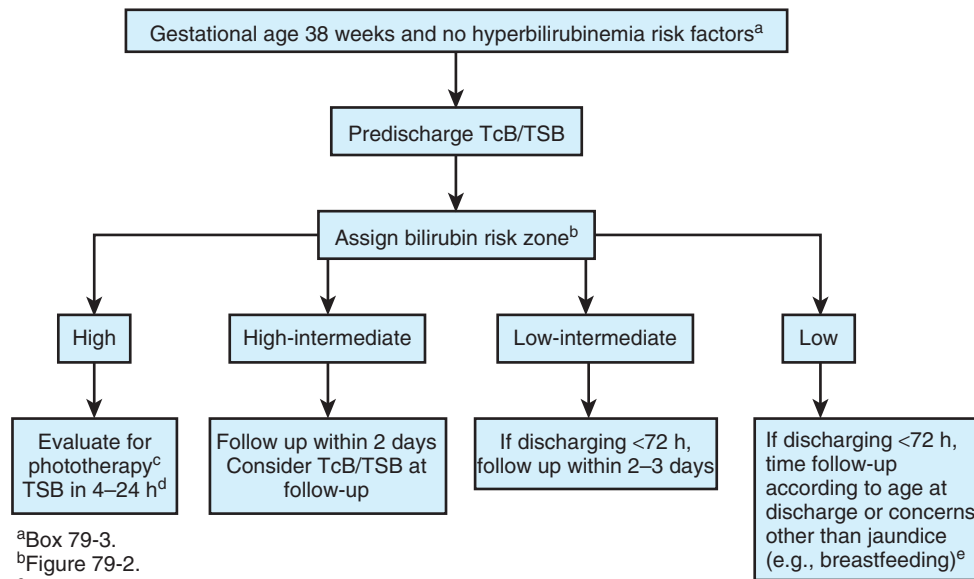
Keren et al., 2008; (Maisels et al., 2009a). As outlined in Fig. 84.9 (Maisels et al., 2009b) the bilirubin risk zone, gestational age at birth, and hyperbilirubinemia risk factors (Box 84.3) combine to assess the risk of subsequent severe hyperbilirubinemia and formulate a plan for birth hospitalization management and timely postbirth hospitalization follow-up (see Fig. 84.9). The AAP has developed a resource kit for hospitals and clinicians to help provide breastfeeding support and manage the jaundiced newborn (www.aap.org/bookstore), and a web-based program developed at Stanford accessible at www.bilitool.org is a practical instrument for plotting hour-specific TSB/TcB measurements.

Treatment Considerations

Phototherapy and exchange transfusion are the mainstays of intervention for neonatal hyperbilirubinemia and current AAP phototherapy, and exchange transfusion treatment thresholds for infants greater than or equal to 35 weeks' gestation are shown in Figs. 84.10–84.11, respectively (American Academy of Pediatrics, 2004). Tables 84.3–84.6 illustrate a range of TSB levels for intervention in varying circumstances for preterm neonates. Phototherapy is generally quite effective and capable of controlling the bilirubin levels in almost all infants, with the exception of the occasional



• **Fig. 84.9** Algorithms Providing Recommendations for Management and Follow-Up According to Pre-discharge Bilirubin Measurements, Gestational Age at Birth, and Other Risk Factors for Subsequent Hyperbilirubinemia. TcB, Transcutaneous bilirubin; TSB, total serum bilirubin.

^aBox 79-3.^bFigure 79-2.^cFigure 79-6.^dIn hospital or as outpatient.^eFollow-up recommendations can be modified according to level of risk for hyperbilirubinemia. Depending on the circumstances, in infants at low risk, later follow-up can be considered.

• Fig. 84.9, cont'd

TABLE 84.3 Guidelines for the Use of Phototherapy and Exchange Transfusion in Low Birth Weight Infants Based on Birthweight^a

| Birthweight (g) | Phototherapy ^c | Total Bilirubin Level (mg/dL [μ mol/L] ^b) Exchange Transfusion ^d |
|-----------------|---------------------------|---|
| ≤1500 | 5–8 (85–140) | 13–16 (220–275) |
| 1500–1999 | 8–12 (140–200) | 16–18 (275–300) |
| 2000–2499 | 11–14 (190–240) | 18–20 (300–340) |

^aNote that these guidelines reflect ranges used in neonatal intensive care units. They cannot take into account all possible situations. Lower bilirubin levels should be used for infants who are sick (e.g., presence of sepsis, acidosis, hypoalbuminemia) or have hemolytic disease.

^bConsider initiating therapy at these levels. Range allows discretion based on clinical conditions or other circumstances. Note that bilirubin levels refer to total serum bilirubin concentrations. Direct reacting or conjugated bilirubin levels should not be subtracted from the total.

^cUsed at these levels and in therapeutic doses, phototherapy should, with few exceptions, eliminate the need for exchange transfusions.

^dLevels for exchange transfusion assume that bilirubin continues to rise or remains at these levels despite intensive phototherapy.

From Maisels MJ. Jaundice. In: Avery GB, Fletcher MA, MacDonald MG (eds), *Neonatology: Pathophysiology and Management of the Newborn*. Philadelphia, PA: J.B. Lippincott; 1999, pp. 765–819.

TABLE 84.4 Bilirubin/Albumin Ratio Trial Phototherapy and Exchange Transfusion Criteria^a

| BW (g) | PHOTOTHERAPY | | | | EXCHANGE TRANSFUSION | | | |
|-----------|---------------|------------------|-----------|------------------|----------------------|------------------|-----------|------------------|
| | Standard Risk | | High Risk | | Standard Risk | | High Risk | |
| | TSB | B/A ^b | TSB | B/A ^b | TSB | B/A ^b | TSB | B/A ^b |
| <1000 | 5.8 | 2.3 | 5.8 | 2.3 | 9.9 | 3.9 | 9.9 | 3.9 |
| 1000–1250 | 8.7 | 3.5 | 5.8 | 2.3 | 12.8 | 5.1 | 9.9 | 3.9 |
| 1250–1500 | 11.1 | 3.7 | 8.7 | 2.9 | 15.2 | 6.1 | 12.8 | 5.1 |
| 1500–2000 | 12.8 | 4.2 | 11.1 | 3.7 | 16.9 | 6.8 | 15.2 | 6.1 |
| 2000–2500 | 14.0 | 4.6 | 12.8 | 4.2 | 18.1 | 7.2 | 16.9 | 6.8 |

^aAt 48 hours of postnatal age and older.^bB/A, Bilirubin/albumin ratio (mg/g); BW, birthweight; TSB, total serum bilirubin (mg/dL).

High risk: asphyxia, hypoxemia, acidosis, hemolysis, neurologic deterioration (sepsis, meningitis, intracranial hemorrhage >grade 2).

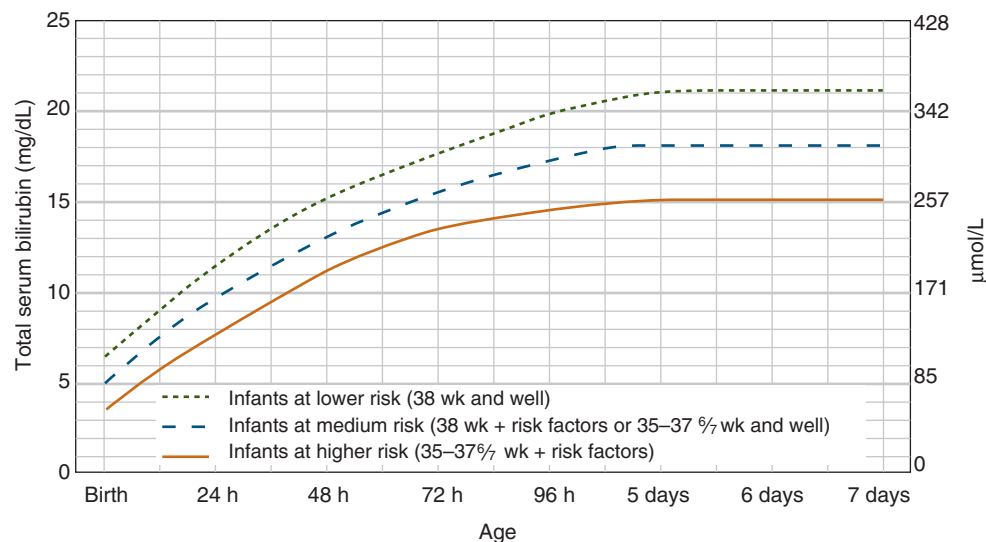
From Hulzebos CV, Dijk PH, van Imhoff DE, et al. The bilirubin albumin ratio in the management of hyperbilirubinemia in preterm infants to improve neurodevelopmental outcome: a randomized controlled trial – BARTrial. *PLoS One*. 2014;9:e99466.

infant with severe hemolysis. Indeed, a rising TSB on phototherapy should raise the concern that the neonate has significant hemolysis, and preparations for exchange should be made if the TSB is rising close to the exchange threshold.

Phototherapy

The most effective phototherapy units deliver output in the blue–green region of the visible spectrum, including the commercially available special blue fluorescent tubes and increasingly light-emitting diode (LED) units (Ennever, 1990; Ebbesen et al., 2016). Special blue fluorescent tubes are labeled F20 T12/BB or

PL52/20W, and they provide much greater irradiance than regular blue tubes (labeled F20T12/B). Special blue tubes and LED units are particularly effective because they provide light with wavelengths that penetrate the skin well and are absorbed maximally by bilirubin (Ennever, 1990; Ebbesen et al., 2016). Phototherapy effectiveness can be further enhanced by increasing the irradiance and the surface area exposed (Maisels, 1996, 2001; Maisels and McDonagh, 2008). Irradiance increases dramatically as the distance between the light source and infant decreases, and this effect is most significant when special blue tubes are used (Maisels and Watchko, 2003). The efficacy of phototherapy is also closely related to the surface area



• **Fig. 84.10** Guidelines for Phototherapy in Hospitalized Infants of Greater Than 35 Weeks' Gestation.

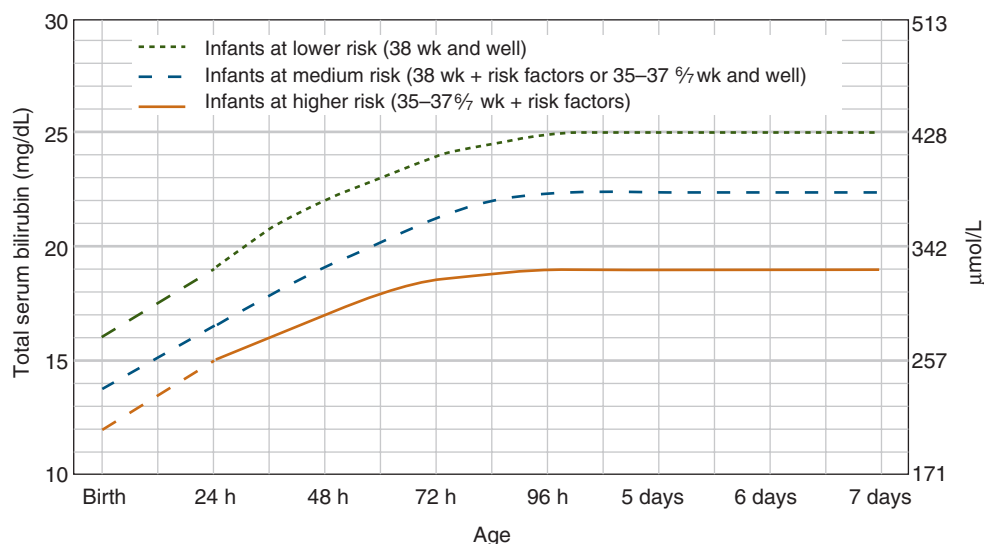
- Use total bilirubin. Do not subtract direct reading or conjugated bilirubin.
- The lines for lower, medium, and higher risk refer to risk for neurotoxicity.
- Risk factors for neurotoxicity—isoimmune hemolytic disease, glucose-6-phosphate dehydrogenase deficiency, asphyxia, significant lethargy, temperature instability, sepsis, acidosis or albumin of less than 3.0 g/dL (if measured).
- For well infants 35–37% weeks can adjust total serum bilirubin (TSB) levels for intervention around the medium-risk line. It is an option to intervene at lower TSB levels for infants closer to 35 weeks and at higher TSB levels for those closer to 37% weeks.
- It is an option to provide conventional phototherapy in hospital or at home at TSB levels 2–3 mg/dL (35–50 mmol/L) below those shown, but home phototherapy should not be used in any infant with risk factors.
- These guidelines refer to the use of intensive phototherapy, which should be used when the TSB exceeds the line indicated for each category. Infants are designated as “higher risk” because of the potential negative effects of the conditions listed on albumin binding of bilirubin (8–10), the blood–brain barrier (11), and the susceptibility of the brain cells to damage by bilirubin (11).
- Intensive phototherapy implies irradiance in the blue-green spectrum (wavelengths of ~430–490 nm) of at least 30 $\mu\text{W}/\text{cm}^2$ per nm (measured at the infant's skin directly below the center of the phototherapy unit) and delivered to as much of the infant's surface area as possible. Note that irradiance measured below the light source center is much greater than that measured at the periphery. Measurements should be made with a spectroradiometer specified by the manufacturer of the phototherapy system.
- If the total serum bilirubin does not decrease or continues to rise in an infant who is receiving intensive phototherapy, this strongly suggests the presence of hemolysis.

(Adapted from American Academy of Pediatrics. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. *Pediatrics*. 2004;114:297–316.)

of the infant exposed to the phototherapy lights (Maisels and Watchko, 2003). Clinical studies comparing intermittent versus continuous phototherapy in the past have produced conflicting results, but in many circumstances phototherapy does not need to be continuous. As long as the serum bilirubin level is being controlled, phototherapy can be interrupted during feeding or short parental visits.

Recent studies show that phototherapy converts bilirubin to more polar photoisomers quite rapidly that account for about 10% of TSB by 15 minutes of treatment, increasing to 20%–25% of TSB by 2 hours of exposure (Mreihil et al., 2010, 2015). In theory these polar stereoisomers should be less able to cross the blood–brain barrier and enter the CNS (Mreihil et al., 2010, 2015), reducing the risk for neurotoxicity. Preliminary in vitro evidence also suggests that compared with unconjugated bilirubin, bilirubin photoisomers have no direct adverse effect on neuronal cell viability (Jasprova et al., 2016).

Significant complications associated with phototherapy are exceptionally rare. The National Institute of Child Health and Human Development (NICHD) Neonatal Network study on aggressive versus conservative phototherapy for infants with extremely low birth weight (<1000 g), however, merits comment in this regard (Morris et al., 2008). Table 84.7 outlines the aggressive and conservative phototherapy treatment guidelines and exchange transfusion thresholds used in the Network study. Their data suggest that aggressive phototherapy may be preferred for infants of 751–1000 g birthweight because of significant neurodevelopmental benefit, including a reduction in athetosis and severe hearing loss (Morris et al., 2008). However, their findings also raised concerns for aggressive phototherapy use in infants with birthweights of 501–750 g because of a higher mortality rate in that subgroup; i.e., increased mortality may offset any potential neurologic benefits of aggressive treatment in these smallest of infants (Morris et al., 2008). Although it is unclear why phototherapy might increase



• **Fig. 84.11** Guidelines for Exchange Transfusion in Infants of Greater Than 35 Weeks' Gestation.

- Immediate exchange transfusion is recommended if infant shows signs of acute bilirubin encephalopathy (hypertonia, arching, retrocollis, opisthotonos, fever, high-pitched cry) or if total serum bilirubin (TSB) is greater than or equal to 5 mg/dL (85 μ mol/L) above these lines.
- The lines for lower, medium, and higher risk refer to risk for neurotoxicity.
- Risk factors for neurotoxicity—isoimmune hemolytic disease, glucose-6-phosphate dehydrogenase (G6PD) deficiency, asphyxia, significant lethargy, temperature instability, sepsis, acidosis.
- Measure serum albumin, and calculate bilirubin/albumin (B/A) ratio (see legend below).
- Use total bilirubin. Do not subtract direct reacting or conjugated bilirubin.
- During birth hospitalization, exchange transfusion is recommended if the TSB rises to these levels despite intensive phototherapy. For readmitted infants, if the TSB level is above the exchange level, repeat TSB measurement every 2–3 hours, and consider exchange if the TSB remains above the levels indicated after intensive phototherapy for 6 hours.

The following B/A ratios can be used together with but in not in lieu of the TSB level as an additional factor in determining the need for exchange transfusion.

| Risk Category | B/A RATIO AT WHICH EXCHANGE TRANSFUSION SHOULD BE CONSIDERED | |
|---|--|---|
| | TSB (mg/dL)/ Alb (g/dL) | TSB (μ mol/L)/ Alb (μ mol/L) |
| Infants $\geq 38\frac{6}{7}$ wk | 8.0 | 0.94 |
| Infants $35\frac{6}{7}$ – $36\frac{6}{7}$ wk and well or $\geq 38\frac{6}{7}$ wk if higher risk or isoimmune hemolytic disease or G6PD deficiency | 7.2 | 0.84 |
| Infants $35\frac{6}{7}$ – $37\frac{6}{7}$ wk if higher risk or isoimmune hemolytic disease or G6PD deficiency | 6.8 | 0.80 |

- If the TSB is at or approaching the exchange level, send blood for immediate type and crossmatch. Blood for exchange transfusion is modified whole blood (red cells and plasma) crossmatched against the mother and compatible with the infant.

(Adapted from American Academy of Pediatrics. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. *Pediatrics*. 2004;114: 297–316.) Alb, Albumin.

mortality in this birthweight cohort, speculation focuses on greater light penetration deep into subcutaneous tissues via thin gelatinous skin and possible oxidative injury to cell membranes (Maisels et al., 2012b; Vreman et al., 2004).

Perhaps the most noticeable clinical complication encountered during the use of phototherapy is that associated with the presence of direct hyperbilirubinemia or cholestatic jaundice. When infants with direct hyperbilirubinemia are exposed to phototherapy, they may develop a dark, greyish-brown discoloration of the skin, serum, and urine (the “bronze baby syndrome”) (Rubaltelli et al., 1983, 1996). The pathogenesis of this syndrome is unknown but is not

related to copper porphyrins as previously hypothesized (McDonagh, 2011). Although few deleterious consequences of the bronze baby syndrome have been described, there are case reports of infants with this syndrome who developed kernicterus (Clark et al., 1976; Grobler and Mercer, 1997; Maisels, 2000). Impaired binding of bilirubin to albumin has been detected in three infants with this syndrome (Kopelman et al., 1972; Ebbesen, 1982) and in infants with cholestasis (Ebbesen, 1982). If there is a need for phototherapy, the presence of direct hyperbilirubinemia should not be considered a contraindication to its use, particularly in the sick newborn, and, as a rule, the direct serum bilirubin level should not be subtracted

TABLE 84.5 Guidelines for Exchange Transfusion in Low-Birth Weight Infants Based on Total Bilirubin (mg/dL) and Bilirubin/Albumin Ratio (mg/g)^a

| Birthweight (g) | <1250 | 1250–1499 | 1500–1999 | 2000–2499 |
|----------------------------|-------|-----------|-----------|-----------|
| Standard risk | 13 | 15 | 17 | 18 |
| Or bilirubin/albumin ratio | 5.2 | 6.0 | 6.8 | 7.2 |
| High risk ^b | 10 | 13 | 15 | 17 |
| Or bilirubin/albumin ratio | 4.0 | 5.2 | 6.0 | 6.8 |

^aExchange transfusion at whichever comes first.^bRisk factors: Apgar less than 3 at 5 minutes; PaO₂ less than 40 mmHg at greater than 2 hours, pH less than 7.15 at greater than 1 hour; birthweight less than 1000 g, hemolysis; clinical or central nervous system deterioration; total protein less than or equal to 4 g/dL or albumin less than or equal to 2.5 g/dL.From Ahlfors CE. Criteria for exchange transfusion in jaundiced newborns. *Pediatrics*. 1994;93:488–494.

from the total bilirubin concentration when decisions are made about initiating phototherapy or an exchange transfusion. In infants who develop the bronze baby syndrome, exchange transfusion should be considered if phototherapy does not lower the TSB. Because of the paucity of data, however, firm recommendations cannot be made. Rarely, purpuric bullous eruptions have also been described in infants with severe cholestatic jaundice receiving phototherapy.

Exchange Transfusion

Exchange transfusion occupies a unique place in the history of neonatal jaundice because it was the first intervention to permit effective control of severe hyperbilirubinemia and prevent kernicterus. In addition to the immediate control of hyperbilirubinemia, an exchange transfusion in immune-mediated hemolytic disease also achieves (1) the removal of antibody-coated red blood cells (a source of “potential” bilirubin), (2) the correction of anemia (if present), and (3) the removal of maternal antibody. A “double volume” exchange refers to an exchange of twice the neonate’s blood volume, or approximately 170–200 mL/kg, and removes approximately 110% of circulating bilirubin (extravascular bilirubin enters the blood during the exchange) but only 25% of total body bilirubin. The exchange transfusion is much less efficient in the removal of total body bilirubin because the majority of the infant’s bilirubin is in the extravascular compartment (Valaes, 1963). Postexchange bilirubin levels are approximately 60% that of preexchange levels, but the rapid (~30 min) reequilibration of bilirubin between the vascular and extravascular compartments produces a rebound of serum bilirubin levels to 70%–80% pre-exchange levels (Brown et al., 1957).

Exchange transfusions are most readily performed via the umbilical vein using a 5- or 8-French umbilical catheter inserted just far enough to obtain free flow of blood (usually no more than the distance between the xiphoid process and umbilicus). The “push-pull” method with a single syringe and special four-way stopcock assembly permits a single operator to complete the procedure (Fig. 84.12; (Watchko, 2000). Given that the efficacy of a double volume exchange is a direct function of the mass of albumin exchanged (Valaes, 1963) the ideal replacement fluid should have both a high plasma volume and a high albumin concentration to optimize the amount of bilirubin-free albumin

TABLE 84.6 Suggested Use of Phototherapy and Exchange Transfusion in Preterm Infants Less Than 35 Weeks Gestational Age

| Gestational Age (wk) | PHOTOTHERAPY | EXCHANGE TRANSFUSION |
|----------------------|---|-------------------------------|
| | Initiate Phototherapy Total Serum Bilirubin (mg/dL) | Total Serum Bilirubin (mg/dL) |
| <28% | 5–6 | 11–14 |
| 28%–29% | 6–8 | 12–14 |
| 30%–31% | 8–10 | 13–16 |
| 32%–33% | 10–12 | 15–18 |
| 34%–34% | 12–14 | 17–19 |

This table reflects the authors’ recommendations for operational or therapeutic total serum albumin (TSB) thresholds—bilirubin levels at, or above which, treatment is likely to do more good than harm. These TSB levels are not based on good evidence and are lower than those suggested in recent UK and Norwegian guidelines.

The wider ranges and overlapping of values in the exchange transfusion column reflect the degree of uncertainty in making these recommendations.

Use the lower range of the listed TSB levels for infants at greater risk for bilirubin toxicity: e.g., (1) lower gestational age; (2) serum albumin levels less than 2.5 g/dL; (3) rapidly rising TSB levels, suggesting hemolytic disease; and (4) those who are clinically unstable. When a decision is being made about the initiation of phototherapy or exchange transfusion, infants are considered to be clinically unstable if they have one or more of the following conditions: (1) blood pH less than 7.15; (2) blood culture positive sepsis in the previous 24 hours; (3) apnea and bradycardia requiring cardiorespiratory resuscitation (bagging and or intubation) during the previous 24 hours; (4) hypotension requiring pressor treatment during the previous 24 hours; and (5) mechanical ventilation at the time of blood sampling.

Recommendations for exchange transfusion apply to infants who are receiving intensive phototherapy to the maximal surface area but whose TSB levels continue to increase to the levels listed.

For all infants, an exchange transfusion is recommended if the infant shows signs of acute bilirubin encephalopathy (hypertonia, arching, retrocollis, opisthotonos, high-pitched cry), although it is recognized that these signs rarely occur in very low birth weight infants.

Use total bilirubin. Do not subtract direct reacting or conjugated bilirubin from the total.

For infants less than or equal to 26 weeks’ gestation, it is an option to use phototherapy prophylactically starting soon after birth.

Use postmenstrual age for phototherapy: e.g., when a 29% week neonate is 7 days old, use the TSB level for 30% weeks.

Discontinue phototherapy when TSB is 1–2 mg/dL below the initiation level for the infant’s postmenstrual age.

Discontinue TSB measurements when TSB is declining and phototherapy is no longer required. Measure the serum albumin level in all infants.

Measure irradiance at regular intervals with an appropriate spectroradiometer.

The increased mortality observed in infants who weigh less than or equal to 1000 g who are receiving phototherapy suggests that it might be prudent to use less intensive levels of irradiance in these infants. In such infants, phototherapy is almost always prophylactic—it is used to prevent a further increase in the TSB, and intensive phototherapy with high irradiance levels usually is not needed. In infants who weigh less than or equal to 1000 g it is reasonable to start phototherapy at lower irradiance levels. If the TSB continues to rise, additional phototherapy should be provided by increasing the surface area exposed (phototherapy above and below the infant, reflecting material around the incubator). If the TSB, nevertheless, continues to rise, the irradiance should be increased by switching to a higher-intensity setting on the device or by bringing the overhead light closer to the infant. Fluorescent and light-emitting diode light sources can be brought closer to the infant, but this cannot be done with halogen or tungsten lamps because of the danger of a burn.

On the other hand, in the first National Institute of Child Health and Human Development (NICHD) trial, irradiance levels were quite low, yet there was a 19% increase in mortality in infants who weighed less than 1000 g who received phototherapy ($P = .112$). One alternative to using lower irradiance is to decrease the length of exposure by using intensive phototherapy for a short period. Another option is to consider intermittent (cyclical) phototherapy. In one recent preliminary study, infants who weighed less than 1000 g who received phototherapy for 15 minutes in the hour had mean peak bilirubin levels (6.5 ± 1.6 mg/dL) that were virtually identical to those receiving continuous phototherapy (6.3 ± 1.3 mg/dL).

From Maisels MJ, Watchko JF, Bhutani VK, et al. An approach to the management of hyperbilirubinemia in the preterm infant less than 35 weeks of gestation. *J Perinatol*. 2012;32:660–664, with permission (footnotes modified).

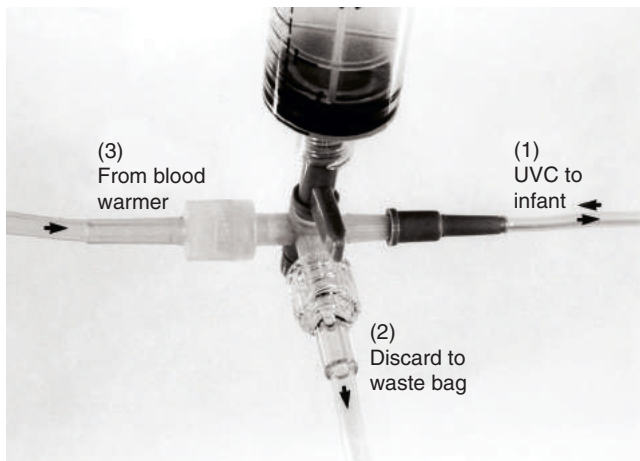
TABLE 84.7 Guidelines for Initiating Phototherapy and Exchange Transfusions—NICHD Neonatal Research Network Trial^a

| Birth Weight | AGGRESSIVE MANAGEMENT | | CONSERVATIVE MANAGEMENT | |
|--------------|-----------------------|----------------------|-------------------------|----------------------|
| | Phototherapy Begins | Exchange Transfusion | Phototherapy Begins | Exchange Transfusion |
| 501–750 g | ASAP after enrollment | ≥13.0 mg/dL | ≥8.0 mg/dL | ≥13.0 mg/dL |
| 751–1000 g | ASAP after enrollment | ≥15.0 mg/dL | ≥10.0 mg/dL | ≥15.0 mg/dL |

^aEnrollment expected within the period 12–36 hours after birth, preferably between 12 and 24 hours.

ASAP, As soon as possible; NICHD, National Institute of Child Health and Human Development.

From Morris BH, Oh W, Tyson JE, et al. Aggressive vs. conservative phototherapy for infants with extremely low birth weight. *N Engl J Med*. 2008;359:1885–1896.



• **Fig. 84.12** Special Four-Way Stop Cock Assembly. (1) Male adapter to umbilical venous line (UVC); (2) female adapter to waste bag; and (3) attachment to blood bag and warmer. The stop cock handle points to the port that is open to the syringe, and the stop cock handle is rotated in a clockwise fashion when correctly assembled (e.g., first, withdraw aliquot from infant; second, discard to waste container; third, draw fresh blood from bag, and then fourth, infuse into infant to complete one cycle). (From Watchko JF. Exchange transfusion in the management of neonatal hyperbilirubinemia. In: Maisels MJ, Watchko JF (eds), *Neonatal Jaundice*. Amsterdam: Harwood Academic; 2000, pp. 169–176.)

introduced into the infant's circulation. Accordingly, reconstituted whole blood, i.e., packed red blood cells mixed with fresh frozen plasma to a hematocrit approximating 40%, is preferred. The adult fresh frozen plasma ensures a high albumin concentration and the hematocrit of 40% a high plasma volume. Reconstituted whole blood should be less than 72 hours old and devoid of the offending antigen in the case of immune-mediated hemolytic disease.

Although the risk for graft-versus-host disease following an exchange transfusion is extremely rare, blood for exchange transfusion should be irradiated. The blood should be warmed to body temperature by a blood/fluid warmer. The actual exchange should be performed slowly in aliquots of 5–10 cc/kg body weight with each withdrawal-infusion cycle approximating 3-minute duration (Aranda and Sweet, 1977). Using this approach, a double volume exchange should take approximately 1.5 ± 0.5 hours and avoids deleterious hemodynamic changes (Aranda and Sweet, 1977).

During the exchange, the infant's vital signs should be monitored closely, including electrocardiogram, respiration, oxygen saturation, temperature, and blood pressure. Supplemental calcium gluconate administration during the exchange transfusion has little effect on serum ionized calcium (Maisels et al., 1974; Ellis et al., 1979;

Wieland et al., 1979), and too rapid infusion of calcium may cause bradyarrhythmias or cardiac arrest. If symptomatic hypocalcemia develops, temporary cessation of the procedure will allow recovery toward normal calcium levels as the citrate (which binds calcium) is metabolized by the liver. Postexchange studies should include bilirubin, hemoglobin, platelet count, ionized calcium, serum electrolytes, and serum glucose.

The unintended consequences of exchange transfusion include cardiovascular, hematologic, gastrointestinal, biochemical, and infectious hazards among others (Watchko, 2000). Previously reported overall mortality rates associated with exchange transfusion ranged from 0.3 to 0.95 per 100 procedures (Hovi et al., 1985; Keenan et al., 1985), and significant morbidity (apnea, bradycardia, cyanosis, vasospasm, thrombosis) was observed in 6.7% of infants who received exchange transfusion in the NICHD collaborative phototherapy study (Keenan et al., 1985). These rates, however, may not be generalizable to the current era if, like most procedures, frequency of performance is an important determinant of risk and experience with exchange transfusion is decreasing (Newman and Maisels, 1992). It is quite possible that the mortality (and morbidity) for this now infrequently performed procedure might be higher than previously reported. On the other hand, none of the reports before 1986 included contemporary monitoring capabilities such as pulse oximetry. Jackson (1997) reported a 2% overall mortality rate (2/106) associated with exchange transfusions between 1980 and 1995. There was a 12% risk of serious complications attributable to exchange transfusion in ill infants (Jackson, 1997). Moreover, in infants classified as ill with medical problems in addition to hyperbilirubinemia, the incidence of exchange transfusion-related complications leading to death was 8% (Jackson, 1997). There were no procedure-related deaths in 81 healthy infants (Jackson, 1997). Symptomatic hypocalcemia, bleeding related to thrombocytopenia, catheter-related complications, and apnea-bradycardia requiring resuscitation were common serious morbidities observed in this study, suggesting that exchange transfusion should be performed by experienced individuals in a neonatal intensive care unit with continuous monitoring (including pulse oximetry) prepared to respond to these adverse events. Finally, although the risk is now very low, the risks per tested unit for known transfusion-transmitted viruses in the United States are as follows: for the human immunodeficiency virus, 1:1,467,000; for the hepatitis C virus, 1:1,149,000; and for the hepatitis B virus, 1:282,000 (Carson et al., 2012).

Intravenous Immunoglobulin

Intravenous immunoglobulin (IVIG) inhibits hemolysis in immune-mediated hemolytic disease possibly by blocking Fc receptors

(Hammerman et al., 1996). Although earlier studies and a systematic review suggested that IVIG administration to infants with Rh or ABO hemolytic disease reduces the need for exchange transfusion (Rübo et al., 1992; Dağoglu et al., 1995; Voto et al., 1995; Hammerman et al., 1996; Gottstein and Cooke, 2003), more recent investigations do not (Smits-Wintjens et al., 2011; Santos et al., 2013; Louis et al., 2014). In addition, there have been anecdotal reports of an association of necrotizing enterocolitis with the administration of IVIG (Navarro et al., 2009; Figueras-Aloy et al., 2010; Krishnan and Pathare, 2011; Kara et al., 2013), although this may be product specific (Krishnan and Pathare, 2011, 2012). The AAP recommends the administration of IVIG in immune-mediated hemolytic disease if the TSB is rising despite intensive phototherapy and the TSB level is within 2–3 mg/dL (34–51 $\mu\text{mol/L}$) of the exchange level (American Academy of Pediatrics, 2004).

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Suggested Readings

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85

Congenital Malignant Disorders

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KEY POINTS

- The spectrum of malignancies in neonates differs from that in children.
- Malignancies in neonates are often associated with a genetic predisposition.
- Neuroblastoma and leukemia are the most common malignancies in neonates.
- Cancer treatment in neonates poses unique challenges, including the likelihood of significant late effects.
- Congenital solid tumors are often diagnosed in utero by ultrasonography.

Neonatal malignancies differ in incidence, clinical behavior, and heritable features from cancers seen in older children. While acute leukemia is the most common malignancy in young children, most neonatal tumors are solid tumors, many of which are detected prenatally during routine ultrasonography. Some childhood malignancies that carry excellent prognoses, such as acute lymphoblastic leukemia (ALL), are often fatal in neonates. In contrast, neuroblastoma, which responds poorly to treatment in older children, can spontaneously regress in newborns.

Treatment of cancer in the neonatal period presents special challenges. Among these are differences in drug metabolism in newborns, the sensitivity of rapidly growing normal tissues to chemotherapeutic agents and radiation, and the increased possibility of late effects, including neurocognitive sequelae, impaired reproductive capacity, growth disturbances, and secondary malignancies. The epidemiology, etiology, and diagnosis of neonatal malignancy are reviewed here, followed by a discussion of commonly encountered malignancies.

Epidemiology, Etiology, and Diagnosis of Neonatal Malignancy

Epidemiology: Incidence and Mortality

Neonatal tumors are rare, with an incidence of 1 per 27,500 live births in the United States; they compose 2% of childhood malignancies (Moore et al., 2003). Although trend analyses suggest that the incidence of malignancy in the pediatric population may

be increasing (Linabery and Ross, 2008), a number of factors affect incidence rates, including improvements in molecular methods of diagnosis, changes in population characteristics, screening fetal ultrasonography practices, and case ascertainment by cancer registries (Spector and Linabery, 2009).

The most common malignancy in infants is neuroblastoma, followed by leukemia, central nervous system (CNS) tumors, retinoblastoma, and germ cell tumors (Linabery and Ross, 2008). Female and male infants have similar cancer incidence rates, but white infants have significantly higher rates than those reported in African-American infants for all histologic types. The distribution of the major types of cancers in newborns, infants, and children is depicted in Table 85.1. Incidence rates for the most common types of malignancy in infants are shown in Table 85.2.

The mortality rates for infants with cancer exceed those for older children, even among identical diseases (Ries et al., 1999). Despite cure rates exceeding 85% for children older than 1 year with a diagnosis of ALL, newborns with ALL have cure rates of less than 50% (Pieters et al., 2007). Poorer survival patterns for infants are also seen with rhabdomyosarcoma and CNS tumors, including primitive neuroectodermal tumor (PNET), atypical teratoid/rhabdoid tumor (ATRT), and ependymoma (Ries et al., 1999). Two notable exceptions are neuroblastoma, for which the 5-year survival rate in newborns with disseminated disease is more than 90%, and infantile fibrosarcoma, for which cure rates in newborns often exceed those achieved in older children or adults.

Etiology

Genetic Predisposition Syndromes and Congenital Defects

The cause of cancer in children is multifactorial, involving both genetic and environmental factors. However, in neonates, predisposing genetic factors more often play an important role. An acquired or inherited abnormality of a cancer-predisposing gene that is critical during embryogenesis underlies some cases of neonatal cancer, and the malignant transformation of normal cells results from the activation or suppression of these cancer-predisposing genes. The retinoblastoma gene at 13q is an example of a constitutional chromosomal abnormality that results in a high risk of malignancy.

A number of defined hereditary conditions and genetic defects are associated with an increased incidence of specific neoplasms; these are listed in Table 85.3. Except for retinoblastoma,

TABLE 85.1 Distribution of the Major Types of Cancer in Newborns, Infants, and Children

| Malignancy | Newborns Younger Than 30 Days (%) | Infants Younger Than 1 Year (%) | Children Younger Than 15 Years (%) |
|-------------------------------------|---|---------------------------------------|--|
| Leukemia | 13 | 14 | 31 |
| Central nervous system tumors | 3 | 15 | 18 |
| Neuroblastoma | 54 | 27 | 8 |
| Lymphoma | 0.3 | 1 | 14 |
| Renal tumors | 13 | 11 | 6 |
| Sarcoma | 11 | 5 | 11 |
| Hepatic tumors | 0 | 3 | 1.3 |
| Teratoma | 0 | 6 | 0.4 |
| Retinoblastoma | 0 | 13 | 4 |
| Other | 5.7 | 5 | 6.3 |

From Reaman GH, Bleyer WA. Infants and adolescents with cancer: special considerations. In: Pizzo PA, Poplack DG, eds. *Principles and Practice of Pediatric Oncology*, 5th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2006:453 [chapter 15].

TABLE 85.2 Incidence of Malignant Tumors in US Infants Younger Than 1 Year

| Malignancy | Number | Proportion of Total (%) | Incidence Rate ^a |
|--|--------|----------------------------|--------------------------------|
| Neuroblastoma | 402 | 24 | 54.1 |
| Leukemia | 296 | 18 | 39.9 |
| Central nervous system tumors | 225 | 13 | 30.3 |
| Retinoblastoma | 196 | 12 | 26.4 |
| Germ cell tumors | 156 | 9 | 21.5 |
| Wilms tumor | 107 | 6 | 14.4 |
| Hepatoblastoma | 78 | 5 | 10.5 |
| Soft tissue sarcoma (nonrhabdomyosarcoma) | 76 | 5 | 10.2 |
| Rhabdomyosarcoma | 39 | 2 | 5.3 |

^aIncidence rate per 1,000,000 person-years, age adjusted to the 2000 US Standard population.

From Linabery AM, Ross JA. Trends in childhood cancer incidence in the U.S. (1992-2004). *Cancer*. 2008;112:416-432.

TABLE 85.3 Hereditary Conditions With Associated Tumors

| Syndrome | Gene | Locus | Inheritance Pattern | Most Common Tumors |
|---------------------------------------|-------------------------------|------------|--|---|
| Ataxia–telangiectasia | <i>ATM</i> | 11q22-q23 | Recessive | Leukemia Lymphoma |
| Beckwith–Wiedemann syndrome | <i>IGF2</i> | 11p15 | Some autosomal dominant imprinting | Wilms tumor Hepatoblastoma Adrenal cortical carcinoma Rhabdomyosarcoma |
| Bloom syndrome | <i>BLM</i> | 15q26 | Autosomal recessive | Leukemia |
| Congenital central hypoventilation | <i>PHOX2B</i> | 4p13 | Autosomal dominant | Neuroblastoma |
| Congenital mismatch repair deficiency | <i>MLH1, MSH2, MSH6, PMS2</i> | Multiple | Autosomal recessive | Any malignancy |
| Denys–Drash syndrome | <i>WT1</i> | 11p13 | Autosomal dominant | Familial Wilms tumor |
| Down syndrome | | Trisomy 21 | Sporadic | Leukemia |
| Familial adenomatous polyposis | <i>APC</i> | 5q22.2 | Autosomal dominant | Hepatoblastoma |
| Familial neuroblastoma | <i>ALK</i> | 2p23 | Autosomal dominant | Neuroblastoma |
| Fanconi anemia | <i>BRCA2, BRIP1, PALB2</i> | Multiple | Autosomal recessive | Leukemia Brain tumors Wilms tumor Neuroblastoma |
| Frasier syndrome | <i>WT1</i> intron 9 | 11p15 | Autosomal dominant | Wilms tumor |
| Gonadal dysgenesis | | 45X/46XY | X-linked? | Gonadoblastoma Germinoma |
| Gorlin syndrome | <i>PTCH2, PTCH1</i> | 1p33 | Autosomal dominant | Medulloblastoma Basal cell carcinoma |

TABLE 85.3 Hereditary Conditions With Associated Tumors—cont'd

| Syndrome | Gene | Locus | Inheritance Pattern | Most Common Tumors |
|--|---------------------------------------|------------|---------------------|---|
| Klinefelter syndrome | ? | XXY | Sporadic | Teratoma Leukemia Breast cancer |
| Li–Fraumeni syndrome | <i>TP53</i> | 17p13 | Autosomal dominant | Sarcoma Central nervous system tumor Breast cancer |
| Medulloblastoma predisposition | <i>SUFU</i> | 10q24 | Autosomal dominant | Medulloblastoma |
| Multiple endocrine neoplasia, type 2B | <i>RET</i> | 10q11 | Autosomal dominant | Medullary thyroid carcinoma Pheochromocytoma |
| Neurofibromatosis | <i>NF1</i> | 17q11.2 | Autosomal dominant | Glioma Leukemia (JMML) Sarcoma |
| Noonan syndrome | <i>PTPN11, HRAS, KRAS, BRAF, SOS1</i> | Multiple | Autosomal dominant | Transient myeloproliferative disorder Leukemia (JMML) Neuroblastoma |
| Perlman syndrome | <i>DIS3L2</i> | 2q37 | Autosomal recessive | Wilms tumor |
| Pleuropulmonary blastoma and ovarian sex-cord stromal tumor predisposition | <i>DICER1</i> | 14q32 | Autosomal dominant | Pleuropulmonary blastoma Cystic nephroma Ovarian sex-cord stromal tumors |
| Retinoblastoma | <i>RB1</i> | 13q14 | Autosomal dominant | Retinoblastoma Osteosarcoma Rhabdomyosarcoma |
| Rhabdoid tumor predisposition | <i>SMARCB1, ATRF</i> | Many | Autosomal dominant | Atypical teratoid/rhabdoid tumor Renal rhabdoid tumor Extrarenal rhabdoid tumor |
| Trisomy 18 | ? | Trisomy 18 | Sporadic | Wilms tumor |
| Turner syndrome | | X0 | Sporadic | Neuroblastoma |
| Von Hippel–Lindau syndrome | <i>VHL</i> | 3p26 | Autosomal dominant | Hemangioblastoma |
| WAGR syndrome | <i>WT1</i> | 11p13 | | Wilms tumor |
| Wiskott–Aldrich syndrome | <i>WAS</i> | Xp11.23 | X-linked | Non-Hodgkin lymphoma |
| X-linked lymphoproliferative disorders | <i>SAP</i> | Xq25 | X-linked | EBV lymphomas |

EBV, Epstein–Barr virus; JMML, juvenile myelomonocytic leukemia; WAGR, Wilms tumors, aniridia, genitourinary abnormalities, mental retardation.

Data from Orbach D, Sarnacki S, Brisse HJ, et al. Neonatal cancer. *Lancet Oncol.* 2013;14:e609–e620 and Jackson EM, Shaikh TH, Gururangan S, et al. High-density single nucleotide polymorphism array analysis in patients with germline deletions of 22q11.2 and malignant rhabdoid tumor. *Hum Genet.* 2007;122:117–127.

hepatoblastoma, and Wilms tumor, the neoplasms associated with these syndromes seldom manifest themselves in the neonatal period, but the associated abnormalities may be recognized early, allowing regular screening. A lack of a family history should not dissuade the clinician from investigating these syndromes, as both spontaneous germline mutations and parental mosaicism occur. The genetic defect in many of these neoplasms has been identified. For example, the *NF1* gene, located at 17q11.2, encodes a protein, neurofibromin, that normally acts as a guanosine triphosphatase-activating protein that downregulates the Ras signaling pathway. Children with neurofibromatosis1 (NF1) are at increased risk of developing juvenile myelomonocytic leukemia (JMML), a rare but aggressive myeloproliferative neoplasm that is currently only cured with hematopoietic stem transplant (Niemeyer and Kratz, 2008). In children with NF1 and JMML, the hematopoietic cells display loss of the wild-type *NF1* gene and duplication of the mutant allele, thus

resulting in the complete loss of the normal neurofibromin protein in the leukemia cells (Stephens et al., 2006). This promotes cell growth because there is no functional “off” switch.

A large number of childhood tumors occur in association with congenital defects. For instance, Down syndrome has an increased association with both leukemia and transient myeloproliferative disorders. Children with congenital aniridia have an increased incidence of Wilms tumor. While aniridia is found in only 1 in 75,000 persons, it is found in as many as 1 in 75 children with Wilms tumor. Children with abnormalities of the Wilms tumor 1 gene (*WT1*), located at chromosome band 11p13, also have an increased risk of developing Wilms tumor (Scott et al., 2006). Most individuals with constitutional *WT1* defects have associated phenotypic syndromes that include combinations of genitourinary abnormalities, renal dysfunction, and mental retardation. Beckwith–Wiedemann syndrome (BWS) and hemihypertrophy syndromes

are associated with several neoplasms. This syndrome is typified by macroglossia, gigantism, and abdominal wall defects; patients may also have visceromegaly, flame nevus, neonatal hypoglycemia, microcephaly, and retardation (Scott et al., 2006). Approximately 8% of infants with either the complete syndrome or the partial syndrome develop neoplasms, including Wilms tumor, adrenal cortical carcinoma, and hepatoblastoma—tumors of the same organs in which visceromegaly develops. Also reported are rhabdomyosarcoma, neuroblastoma, ganglioneuroma, and adenomas and hamartomas. BWS is linked with abnormalities of 11p15; this is the location of the insulin-like growth factor II gene (*IGF2*) and the tumor suppressor gene *H19* (Rahman, 2005).

Transplacental Tumor Passage

An exceedingly rare cause of cancer in neonates and infants is the transplacental passage of tumor cells from the mother. Rare cases of transplacentally transmitted cancer have been reported (Tolar et al., 2002). The malignancies transmitted include leukemia, melanoma, lymphoma, hepatic carcinoma, and lung cancer. The diagnosis of transplacentally acquired neoplasm usually occurs at birth but has been reported as late as age 8 months. The frequency of concurrent maternal malignancy in pregnant women is estimated at 1 per 1000 pregnancies (Pavlidis, 2002; Maruko et al., 2004), and alternative treatment plans or delays in treatment are options for pregnant women. That transplacental transmission is so rare is attributed to the protective function of the placenta.

Twin-to-Twin Transmission

The risk of development of leukemia is increased in a monozygotic twin. If one monozygotic twin has leukemia, the cotwin has an approximately 25% chance of developing leukemia, usually within weeks or months of the diagnosis in the sibling. A dizygotic twin on the other hand has only a slightly increased risk of developing leukemia. This increased incidence is likely due to in utero twin-to-twin transmission of a preleukemic clone rather than the simultaneous development of a shared germline mutation facilitating the later development of leukemia.

Environmental Factors

Environmental factors are probably less important in the development of neonatal cancer compared with their role in the development of cancer in older children and adults. Nonetheless, there is evidence that environmental influences, including radiation exposure, maternal medication use, and various environmental exposures, may affect the incidence of neonatal cancer (Moore et al., 2003).

Exposure to ionizing radiation during pregnancy is known to increase the risk of a number of tumors, including acute leukemia, in exposed offspring. There appears to be a dose–response relationship between the dose of ionizing radiation received by the fetus in utero and the subsequent development of cancer in childhood, with doses on the order of 10 milliGray sufficient to produce an increase in risk. Mixed evidence comes from atomic bomb survivors who were exposed to radiation in utero and from studies that followed Doll and Wakeford's study. Maternal exposure to ionizing radiation should be used sparingly and only for diagnostic purposes if required (Brent, 2014).

Maternal exposure to some drugs during pregnancy has been associated with the subsequent development of cancer in offspring. Maternal use of diethylstilbestrol has been strongly associated with the development of clear cell adenocarcinoma of the vagina and cervix in daughters born from those pregnancies. Some substances and exposures known to be teratogenic may also be carcinogenic

to offspring. Excessive maternal alcohol consumption may be linked to an increased risk of developing cancer in the newborn period, particularly acute myeloid leukemia (AML). The use of fertility drugs does not appear to increase the risk of cancer in the exposed offspring (Basatemur and Sutcliffe, 2008).

Environmental exposures of the mother or father to hydrocarbons, dyes, and other chemicals and solvents may be related to the development of neonatal tumors, but there is only a weak association for most of the risk factors identified (McCall et al., 2005). The association of neoplasms with other environmental factors, such as maternal use of tobacco, has not been conclusively proven (Momen et al., 2016).

Diagnosis and Evaluation

The diagnostic evaluation of a newborn suspected of having cancer is guided by the signs and symptoms of the disease. Symptoms of malignancy in neonates can be nonspecific, such as irritability, poor feeding, failure to thrive, and fever. Table 85.4 lists clinical features associated with the more common malignancies found in the neonatal period. Most neonatal tumors present as a mass at birth; often the mass has previously been identified by prenatal ultrasonography. Postnatal imaging with magnetic resonance imaging (MRI) is usually required to better delineate the lesion.

Laboratory and pathologic evaluations should be directed at making the diagnosis efficiently, sparing the newborn unnecessary procedures that could result in acute and chronic morbidity. Routine laboratory studies, including a complete blood count (CBC) and liver and renal function tests, should be performed. Urine catecholamine excretion should be measured when neuroblastoma is being considered. Serum alpha fetoprotein (AFP) and beta human chorionic gonadotropin (β -hCG) levels should be measured in infants suspected of having a germ cell tumor or teratoma; these can serve as tumor markers, although the normally elevated levels in infancy can complicate the interpretation of these values (Wu et al., 1981). A pediatric oncologist should be consulted. Surgeons and pathologists should submit biopsy tissue for histologic examination, immunoperoxidase staining, flow cytometry, cytogenetic analysis, and tumor banking.

Specific Neoplasms

Neuroblastoma

Overview

Neuroblastoma is the most common malignant tumor of infancy. It is of embryonal origin, derived from neural crest cells that have committed to the sympathoadrenal lines. The tumor can present in utero, in infancy, and in childhood; the age of presentation significantly affects the prognosis and treatment plans. Because, in part, of improvements in prenatal ultrasonography, neuroblastoma is now diagnosed in more children; neuroblastoma is diagnosed in approximately 100 children per year in North America prenatally or at age less than 3 months (Nuchtern, 2006).

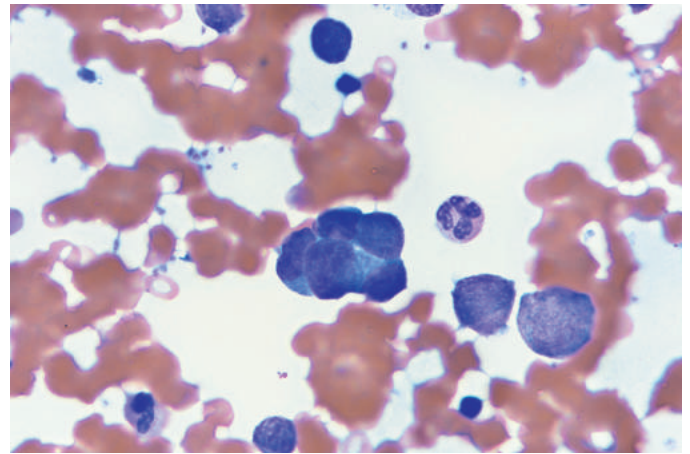
Etiology

Neuroblastoma can be associated with genetic disorders but does not have a single cause. The incidence is increased in patients with Turner syndrome, Noonan syndrome (in 50% of Noonan syndrome patients the *PTPN11* gene is mutated, which is associated with an increased risk of leukemia and neuroblastoma), and Costello

TABLE 85.4 Differential Diagnosis of Malignant and Nonmalignant Conditions in Infancy

| Feature | Malignancy | Nonmalignant Condition |
|---|--|---|
| Skin nodules | Neuroblastoma Acute leukemia Reticuloendothelioses | Congenital viral infections Vasculitis Fibromatosis Neurofibromatosis Xanthoma |
| Head and neck masses | Rhabdomyosarcoma Orbital Cervical Nasopharyngeal Neuroblastoma Lymphoma Infantile fibrosarcoma | Brachial cleft cyst Thyroglossal duct cyst Cystic hygroma Fibromatosis Hemangioma Abscess Cellulitis Reactive hyperplasia of cervical nodes Granulomatous lesions (e.g., atypical tuberculosis) |
| Abdominal or pelvic masses | Neuroblastoma Wilms tumor Sarcoma Malignant teratoma Lymphoma Germ cell tumor | Polycystic kidneys Hydronephrosis Benign teratoma Urinary retention Gastrointestinal duplication Intussusception Chordoma Meningomyelocele Horseshoe kidney Splenomegaly Hepatomegaly |
| Hepatomegaly | Neuroblastoma Acute leukemia Hepatoblastoma Reticuloendothelioses | Congenital viral infections Storage diseases Cavernous hemangioma Hemangioendothelioma |
| Signs/symptoms of increased intracranial pressure | Brain tumors Acute leukemia Retinoblastoma | Intracranial hemorrhage Communicating hydrocephalus Dandy–Walker malformation Vascular malformations |
| Anemia | Acute leukemia Neuroblastoma | Short-term or long-term blood loss Hypoproliferative anemia (nutritional, congenital) Dyserythropoietic anemias Hemolytic anemia Transient erythroblastopenia |
| Pancytopenia | Acute leukemia Neuroblastoma Retinoblastoma (disseminated) | Congenital viral infections Immune-mediated neutropenia and thrombocytopenia Congenital and acquired aplastic anemias |

Modified from Reaman GH, Bleyer WA. Infants and adolescents with cancer: special considerations. In: Pizzo PA, Poplack DG, eds. *Principles and Practice of Pediatric Oncology*, 5th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2006:455 [chapter 15].



• **Fig. 85.1** Clump of Neuroblastoma Cells Found in Bone Marrow Aspirate. (Courtesy of Kristie White, UCSF Assistant Professor of Pathology, San Francisco, CA.)

syndrome. While *NF1* mutations have been detected in neuroblastoma cell lines, patients with germline *NF1* mutations do not have a predisposition to neuroblastoma. In BWS and other overgrowth disorders, neuroblastoma is more common, and abdominal ultrasonography is recommended quarterly until age 8 years for early detection. Congenital central hypoventilation syndrome (specifically the *PHOX2B* mutation) and Hirschsprung disease are associated with increased risk of neuroblastoma and ganglioneuroblastoma. There are cases of familial neuroblastoma; in 80% of these cases the anaplastic lymphoma kinase receptor has been found to be mutated although there are no phenotypic abnormalities (Janoueix-Lerosey et al., 2008). In addition to germline mutations in the familial cases, somatic *ALK* mutations are found in up to 12% of sporadic neuroblastoma tumors (Mossé et al., 2008).

Presentation

In children, symptoms of neuroblastoma are often due to a mass effect in the compartment of tumor origin. Among all pediatric neuroblastoma cases, two-thirds of cases occur within the abdominal cavity; most of these occur in the adrenal glands. Abdominal distention is a common initial presentation. Neuroblastoma can occur anywhere along the sympathetic chain, however, and is sometimes incidentally found on chest X-rays. Neuroblastoma in the posterior mediastinum can present as bronchial obstruction. Neuroblastoma arising in the sympathetic paraspinal ganglia may invade the neural foramina, causing spinal cord compression with associated neurologic symptoms. Tumor cells can also rarely be found circulating on review of the peripheral blood smear (Fig. 85.1).

In the newborn, neuroblastoma presents commonly as an asymptomatic adrenal mass found on routine ultrasonography, but it most often manifests itself as stage 4S (or stage MS) neuroblastoma with hepatomegaly, seen in 65% of cases, followed by subcutaneous metastases, seen in 32% of cases. This metastatic pattern is different from that seen in older infants and children (Table 85.5). Metastases to the lungs, bones, skull, and orbit are rare in the newborn, although clumps of tumor cells are often found in the bone marrow. In the newborn the primary site of disease often cannot be identified or may be a small adrenal primary tumor. Liver involvement can cause massive hepatomegaly, which can be a cause of dystocia during vaginal delivery. This massive involvement can also cause abdominal distention, coagulopathy, heart failure, and life-threatening respiratory distress (Fig. 85.2).

TABLE 85.5 Sites of Metastatic Disease at Diagnosis for 81 Patients With Stage 4S Neuroblastoma, 133 Patients With Stage 4 Neuroblastoma Younger Than 1 Year, and 434 Patients With Stage 4 Neuroblastoma 1 Year or Older

| Sites of Metastases | Stage 4S | Stage 4 <1 year | Stage 4 ≥1 year | Total (%) |
|----------------------------|------------|-----------------|-----------------|-----------|
| Bone marrow ^{a,b} | 28 (34.6%) | 76 (57.1%) | 353 (81.3%) | 70.5 |
| Bone ^b | 0 | 65 (48.9%) | 296 (68.2%) | 55.7 |
| Lymph node | 7 (8.6%) | 38 (28.6%) | 155 (35.7%) | 30.9 |
| Liver ^{a,b} | 65 (80.2%) | 71 (53.4%) | 56 (12.9%) | 29.6 |
| Intracranial/orbit | 0 | 34 (25.6%) | 84 (19.6%) | 18.2 |
| Adrenal ^b | 5 (6.2%) | 18 (13.5%) | 26 (6.0%) | 7.6 |
| Skin ^b | 11 (13.6%) | 11 (8.3%) | 4 (0.9%) | 4.0 |
| Pleura | 0 | 6 (4.5%) | 16 (3.7%) | 3.4 |
| Lung | 0 | 3 (2.3%) | 18 (4.1%) | 3.2 |
| Peritoneum | 0 | 5 (3.8%) | 9 (2.1%) | 2.2 |
| Other | 0 | 5 (3.8%) | 7 (1.6%) | 1.9 |
| Central nervous system | 0 | 0 | 4 (0.9%) | 0.6 |

^aSignificant difference between the proportion of patients with stage 4S neuroblastoma with the site and the proportion of patients with stage 4 neuroblastoma younger than 1 year with the site ($P < .01$), only for those sites which are included in the definition of stage 4S neuroblastoma.

^bSignificant difference between the proportion of patients with stage 4 neuroblastoma younger than 1 year with the site and the proportion of patients with stage 4 neuroblastoma 1 year or older with the site ($P < .02$).

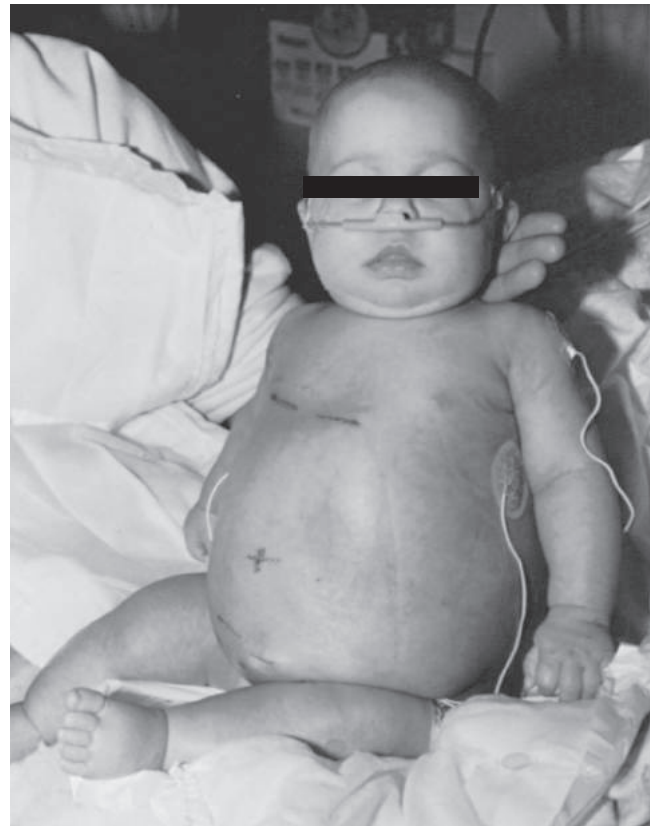
From DuBois S, Kalika Y, Lukens J, et al. Metastatic sites in stage IV and IVA neuroblastoma correlate with age, tumor biology, and survival. *J Pediatr Hematol Oncol*. 1999;21:181–189.

Subcutaneous skin nodules are typically bluish, and palpation of the nodules leads to transient erythema followed by blanching, presumably because of the vasoconstriction caused by the release of catecholamines from the tumor cell.

The neoplasm may also arise in the neck or pelvis. Involvement of the stellate ganglion may result in Horner syndrome, which includes ptosis of the upper eyelid, slight elevation of the lower eyelid, meiosis, narrowing of the palpebral fissure, anhidrosis, and enophthalmos (Fig. 85.3). Neuroblastoma arising from the paravertebral sympathetic ganglion has a tendency to grow into the intervertebral foramina, causing spinal cord compression and resultant paralysis. Careful periodic neurologic evaluation should be performed in a child with neuroblastoma in this region to evaluate the child for the onset of cord compression, which may necessitate emergency intervention with chemotherapy, surgery, or irradiation.

Unusual Presentations

Intractable diarrhea can be the sole presenting manifestation of neuroblastoma. Secretion of vasoactive intestinal peptide by the tumor has been postulated to be the cause of the diarrhea, which resolves following surgical removal of the tumor (Bourdeaut et al., 2009).



• **Fig. 85.2** Stage 4S neuroblastoma causing abdominal distention and respiratory distress secondary to hepatic infiltration.



• **Fig. 85.3** Horner syndrome in an infant with neuroblastoma arising from the left cervical sympathetic ganglion.

Opsoclonus and *myoclonus* (“dancing eyes, dancing feet”) are associated with neuroblastoma, although this presentation is only rarely seen in the neonatal period (Rudnick et al., 2001). Patients have rapid multidirectional eye movements (opsoclonus), myoclonus, and truncal ataxia (OMA) in the absence of increased intracranial pressure. The condition may be due to an autoimmune reaction, as the presence of antineuronal antibodies has been shown to be significantly more common in children with neuroblastoma and OMA than in case-controlled neuroblastoma patients (Antunes et al., 2000). Removal of the tumor usually results in a decrease in neurologic signs and symptoms, but the use of steroids is frequently required for complete resolution. In general, the prognosis

for survival of children with opsomyoclonus is excellent, although long-term neurologic deficits and learning delays are common and can be quite debilitating.

Maternal symptoms such as sweating, pallor, headaches, palpitations, hypertension, and tingling in the feet and hands during the eighth and ninth months of pregnancy may rarely be a sign of neuroblastoma in the fetus. The symptoms, which disappear after birth, are likely caused by fetal catecholamines entering the maternal circulation. Newborns with neuroblastoma whose mothers have experienced these symptoms typically receive a diagnosis of neuroblastoma shortly after birth or during the first few months of life.

Erythroblastosis with hepatosplenomegaly, severe jaundice, and an increase in the number of nucleated red blood cells is occasionally seen in newborns with neuroblastoma. Congenital neuroblastoma with metastases to the liver and placenta can be clinically indistinguishable from hydrops fetalis.

Catecholamine Secretion

A hallmark of neuroblastoma cells is the ability to store and secrete catecholamines. Patients with neuroblastoma usually have elevated urinary levels of norepinephrine as well as its biochemical precursors and their metabolites. More than 90% of patients have an elevated urinary excretion of vanillylmandelic acid (VMA) or homovanillic acid (HVA) or both. VMA and HVA determinations can be made on random urine samples when values are normalized for creatinine concentration. In the occasional case with no elevation of catecholamine levels, a 24-hour urine collection is necessary. Catecholamine secretion can be used not only as a diagnostic aid but also as a means to assess the response to therapy and to detect tumor recurrence. Thus urine catecholamine levels should be measured before surgical removal of the tumor or before initiation of therapy.

Diagnosis

Clinical evaluation should include a physical examination with particular attention paid to detecting an abdominal mass, hepatomegaly, lymphadenopathy, Horner syndrome, and skin lesions; a baseline neurologic examination is also performed. Laboratory evaluation should include a CBC, tests for urine levels of VMA and HVA, and tests for serum ferritin and lactate dehydrogenase. While the initial imaging study in an infant is often abdominal ultrasonography, additional imaging to better delineate the tumor and to evaluate the infant for metastatic disease is needed; this should include computed tomography (CT) or MRI of the primary lesion. MRI of the spine should be performed for paraspinal and posterior mediastinal lesions. An [^{123}I]metaiodobenzylguanidine (MIBG) scan is particularly important for diagnosis and follow-up. MIBG, a norepinephrine analogue specifically taken up by neuroblastoma in bone and soft tissue, serves as a sensitive modality (90% sensitive) for disease localization (Matthay et al., 2010). Bilateral bone marrow aspiration (along with bilateral bone marrow biopsy in patients older than 6 months) is also part of the initial evaluation.

Histologic evidence provides confirmation of the diagnosis of neuroblastoma. Tissue may be obtained from a primary lesion or a metastatic site. Because tumor-specific biologic information plays a critical role in risk classification and treatment recommendations, obtaining adequate tissue for biologic studies is essential.

Pathologic Classification

Neuroblastoma is made up of small round blue cells that are uniformly sized and contain dense, hyperchromatic nuclei and

TABLE 85.6 Features That Affect Prognosis in Neuroblastoma

| Feature | Favorable | Unfavorable |
|------------------------|--|---|
| Age at diagnosis | <18 months | >18 months |
| INRG stage | L1, L2, MS | M |
| <i>MYCN</i> status | Nonamplified | Amplified |
| Histologic appearance | Ganglioneuroma, ganglioneuroma maturing, ganglioneuroblastoma intermixed | Ganglioneuroblastoma nodular or neuroblastoma |
| DNA ploidy (DNA index) | >1 or <1 | 1 |
| Allelic status of 11q | Normal | 11q deletion or LOH at 11q or any segmental chromosome loss |

INRG, International Neuroblastoma Risk Group; LOH, loss of heterozygosity.

scattered cytoplasm with stroma around it. Immunohistochemistry is positive for neurofilament protein, synaptophysin, neuron-specific enolase, ganglioside GD2, and chromogranin A, which distinguishes it from the other small round blue cell tumors of childhood. The histopathologic appearance of neuroblastoma ranges from undifferentiated neuroblasts, to more mature ganglioneuroblastoma, to fully differentiated and benign ganglioneuroma. The most widely used morphologic classification system is based on the system proposed by Shimada et al. (1984) in which tumors are classified as favorable or unfavorable. It is based on the amount of stroma, degree of neuroblastic differentiation, and the mitosis–karyorrhexis index. Additional classification systems exist; the International Neuroblastoma Pathology Classification separates neuroblastoma into four categories: (1) neuroblastoma that is undifferentiated, poorly differentiated (<5% exhibiting differentiation), or differentiating (>5%); (2) ganglioneuroblastoma, intermixed; (3) ganglioneuroblastoma, nodular; and (4) ganglioneuroma.

Genetic Prognostic Factors: Tumor Biology

In addition to clinical factors and histology, a number of biologic factors have been shown to correlate with prognosis (Table 85.6). Genomic data currently used in risk classification schemes include the status of the *MYCN* oncogene, tumor cell DNA content (ploidy), and the allelic status of chromosome arms 1p, 11q, 14q, and 17q (Ambros et al., 2009). More recently, it has been found that any segmental chromosomal abnormality indicates a less favorable outcome.

Amplification of the *MYCN* oncogene is present in 16% of primary neuroblastomas and has been shown to correlate with poor prognosis independent of age, stage, and other genetic alterations (Thompson et al., 2016). Patients with stage 1, 2, or 4S disease demonstrate *MYCN* amplification only rarely; when present, it has been associated with rapid disease progression in these normally favorable stages. In a Children's Cancer Group study of stage 4 neuroblastoma in infants, the progression-free survival rate after 3 years was less than 10% in infants with tumors that demonstrated *MYCN* amplification, compared with 93% for those with single-copy tumors.

Total cellular DNA content also predicts response to therapy in infants with neuroblastoma (Look et al., 1991). Diploid DNA content is an unfavorable prognostic factor, particularly in infants younger than 12 months. Infants with hyperdiploid tumors have a significantly better response to therapy than those with diploid tumors. Diploidy often correlates with tumor *MYCN* amplification, although in rare cases of hyperdiploidy with *MYCN* amplification, the *MYCN* amplification portends an unfavorable outcome.

Tumor karyotype also influences outcome. Common genomic aberrations found in neuroblastoma include deletion at chromosomal region 1p36.3 or 11q23, deletion of 14q32, and unbalanced gain of the long arm of chromosome 17 (17q21 to 17qter) (Ambros et al., 2009). Loss of heterozygosity (LOH) of 1p occurs in up to 36% of primary tumors, and LOH at 11q23 is seen in 44% of primary neuroblastomas (Guo et al., 1999). Both are associated with poor outcomes, older age at presentation, and advanced-stage disease. Gain of 17q occurs in 60% of neuroblastomas and is associated with metastatic disease and unfavorable prognosis. Comprehensive genome-wide approaches such as comparative genomic hybridization are becoming increasingly useful in refining the prognostic accuracy of chromosomal alterations (Schleiermacher et al., 2007).

Staging

Neuroblastoma has traditionally been staged according to the International Neuroblastoma Staging System (INSS). Staging is based on age, disease site(s), and degree of surgical resection. New guidelines for a pretreatment risk classification system have been developed by the International Neuroblastoma Risk Group (INRG) Task Force and are being used in addition to the INSS summarized in Table 85.7 (Monclair et al., 2009); this system is undergoing evaluation in risk-based clinical trials. INRG stages include L1, localized tumor not involving vital structures (corresponds to INSS stages 1 and 2); L2, locoregional tumor with one or more image-defined risk factors (corresponds to INSS stage 3); M, metastatic disease (corresponds to INSS stage 4); and MS, metastatic disease in children younger than 18 months with metastases confined to skin, liver, and/or bone marrow (corresponds to INSS stage 4S). Two important differences in the INRG system compared with the INSS are that it is a radiologic rather than a surgical staging system and that the upper age limit for stage MS has been extended from 12 to 18 months.

TABLE 85.7 International Neuroblastoma Risk Group Staging System

| Stage | Definition |
|-------|---|
| L1 | Localized tumor not involving vital structures as defined by the list of image-defined risk factors ^a and confined to one body compartment |
| L2 | Locoregional tumor with the presence of one of more image-defined risk factors ^a |
| M | Distant metastatic disease (except stage MS) |
| MS | Metastatic disease in children younger than 18 months with metastases confined to the skin, liver, and/or bone marrow |

^aImage-defined risk factors are specific to each body compartment. For example, risk factors within the neck include tumor encasing the carotid and/or vertebral artery and/or internal jugular vein, tumor extending to the base of the skull, or tumor compressing the trachea. Data from Monclair T, Brodeur GM, Ambros PF, et al. The International Neuroblastoma Risk Group (INRG) staging system: an INRG Task Force report. *J Clin Oncol*. 2009;27:298–303.

Stage 4S (MS) comprises a unique group of patients with disseminated disease but a good prognosis. This combination occurs exclusively in infants. In this special group of patients, typical findings include a small primary tumor that does not cross the midline and remote spread involving the liver, skin, or bone marrow (<15% of marrow replacement by tumor), without radiographic evidence of skeletal metastases. There is lack of *MYCN* oncogene amplification in most INSS stage 4S tumors, in contrast to INSS stage 4 tumors (Seeger et al., 1985). Infants with INSS stage 4S disease have a very good prognosis despite having disseminated disease (5-year survival rate >90%); spontaneous regression occurs without cytotoxic therapy in approximately 50% of cases (Nickerson et al., 2000; Hero et al., 2008; Taggart et al., 2011).

Treatment

Treatment modalities for neuroblastoma include observation alone, surgery, chemotherapy, and radiation therapy or a combination of these. Patients with INSS stage 1 and INSS stage 2 neuroblastoma have a 96%–100% survival rate with surgery alone (Strother et al., 2012). Isolated adrenal masses, the more common presentation among infants with neuroblastoma diagnosed prenatally, can be monitored closely for spontaneous regression if a tumor diameter meets the size criteria and if urine VMA and HVA levels are decreasing (Nuchtern et al., 2012). Infants with INSS stage 3 and INSS stage 4 disease have a poorer survival, even with aggressive chemotherapy, although the outcome, with better than 70% surviving overall, is far better than the survival rate of 10%–20% reported for older children with disease of these stages (Schmidt et al., 2000).

The unpredictable course of neuroblastoma, with its occasional spontaneous maturation or regression, not only makes this tumor unusual but also requires careful assessment of clinical and biologic risk factors in planning therapy. The type and intensity of treatment are determined by identification of infants with relatively good, intermediate, and poor prognoses on the basis of stage, international pathology classification, Shimada histologic classification, ploidy, segmental chromosomal abnormalities, and *MYCN* amplification. Patients who have localized disease (L1 or L2) without amplification of *MYCN* have an excellent prognosis, and such patients should undergo surgical resection or partial resection, but they likely will not derive any additional benefits from postoperative chemotherapy or radiation therapy. An exception to this rule is in the case of spinal cord compression, in which prompt decompression with chemotherapy, laminectomy, or local irradiation may be used to preserve function. There is an increasing trend to use chemotherapy first, given the exquisite sensitivity of the tumor to chemotherapeutic agents, but a rapid deterioration in neurologic function should prompt alternative interventions. A neurosurgeon should be consulted early in the diagnosis. The combination of extensive laminectomy with postoperative irradiation should be avoided because later spinal deformity is almost inevitable. Infants with stage 3 and stage 4 disease are usually treated with a combination of chemotherapy and local surgery, with radiation therapy given only as necessary to eradicate residual disease. The active drugs that are most commonly used include cisplatin or carboplatin, etoposide, doxorubicin, cyclophosphamide, and vincristine. Infants with stage 4 disease with amplification of the *MYCN* oncogene have a very unfavorable prognosis; standard chemotherapy regimens are not sufficient for cure. In these high-risk patients, intensive chemotherapy followed by myeloablative therapy with stem cell support may offer additional benefit (Canete et al., 2009). In addition, the use of the differentiation agent isotretinoin and the

anti-GD2 antibody ch14.18 has been shown to improve outcome in patients with advanced-stage, high-risk neuroblastoma (Matthay et al., 2009; Yu et al., 2010).

Infants with INSS stage 4S disease have a highly favorable prognosis and may require minimal or no therapy. Because many patients undergo spontaneous regression, therapy should be directed toward supportive care, with use of chemotherapy and surgery restricted to relieving symptoms (De Bernardi et al., 2009). The main cause of death in these patients is massive hepatic involvement resulting in respiratory insufficiency or compromise of renal or gastrointestinal function. Symptomatic patients are treated with chemotherapy. When there is a risk of organ impairment due to tumor bulk not responding to initial chemotherapy, low-dose radiotherapy can be administered (450 centiGray given in three fractions; in some cases not all three fractions are needed) (Nickerson et al., 2000; Taggart et al., 2011).

Prenatal Diagnosis

Neuroblastoma is increasingly being detected prenatally by screening ultrasonography. Newborns with adrenal or other mass lesions detected prenatally should be evaluated by urine catecholamine levels (although this has a low specificity) and follow-up ultrasonography. Careful observation may be adequate for infants with localized tumors, which frequently regress.

Newborn Screening

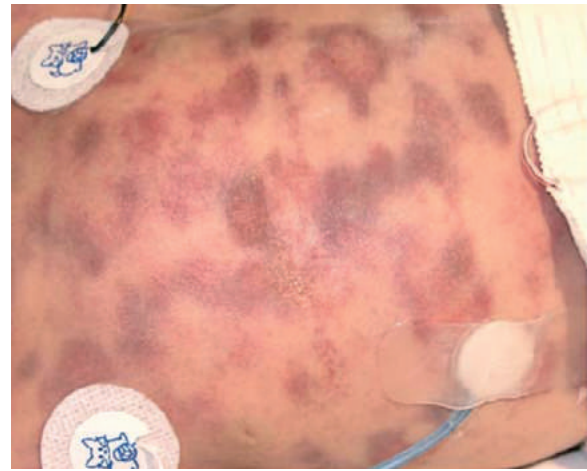
Newborn screening for neuroblastoma by the measurement of urine catecholamine levels has been studied in Japan and a number of other countries (Hiyama et al., 2008). It was hoped that early diagnosis of neuroblastoma would reduce the frequency of cases with poor prognosis from advanced-stage disease. Screening, however, has had a mixed impact on survival; neuroblastomas detected by screening almost always have favorable biologic features (Schilling et al., 2002), although screening in infants in Japan was shown to reduce overall mortality (Hisahige, 2014). Routine screening of infants for neuroblastoma is not currently recommended.

Congenital Leukemia

Epidemiology

Although leukemia is the second most common malignancy in infants, congenital leukemia, defined as leukemia diagnosed in the first 4 weeks of life, is quite rare. The incidence of leukemia in the first 3 months is approximately five cases per million (Bajwa et al., 2004). Two-thirds of congenital leukemia cases are classified as AML, in contrast to older infants and children, in whom ALL predominates. Congenital leukemia is associated with a high mortality with an overall survival rate at 24 months of only 20% (Van der Linden et al., 2009), which is due to the aggressive biology of these leukemias and age-related treatment complications.

The cause of leukemia is unclear. In infants and older children a number of factors are associated with the development of leukemia; these include genetic factors, environmental influences, and immunodeficiencies. Genetic epidemiologic studies of infant leukemia indicate that most, if not all, cases are initiated in utero and involve acquired, noninherited genetic rearrangements; chromosome band 11q23 (*MLL* rearrangement) is frequently involved. Leukemia-associated gene rearrangements have been retrospectively identified in archived newborn screen blood spots of children who subsequently developed leukemia (Hjalgrim et al., 2002; Taub et al., 2002; Wiemels et al., 2002). The Children's Oncology Group has reported a trend toward higher incidence of AML, but not



• **Fig. 85.4** Extensive Leukemia Cutis in a Newborn Infant. (From Zhang IH, Zane LT, Braun B, Maize Jr J, Zoger S, Loh M. Congenital leukemia cutis with subsequent development of leukemia. *J Am Acad Dermatol.* 2006;54:S22–S27 with permission from Elsevier.)

ALL, in infants of mothers who consumed larger amounts of naturally occurring topoisomerase 2 inhibitors, such as those in foods high in flavonoids and phytates (Spector et al., 2005).

Clinical Manifestations

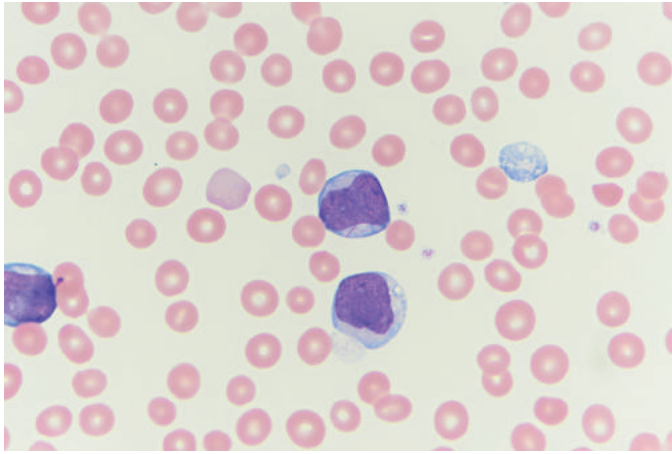
Clinical signs of leukemia may be evident at birth and include hepatosplenomegaly, petechiae, and ecchymoses. Myeloid leukemic cell infiltration of the skin (*leukemia cutis*) is present in 25%–30% of patients with congenital leukemia; a skin nodule may be the first clinical sign of leukemia (Fig. 85.4). Patients typically have multiple nodules that are freely movable over the subcutaneous tissue with a greenish-blue or dark pink discoloration of the overlying skin. It is important to perform flow cytometry and cytogenetic studies on the skin biopsy specimen; infants found to have an *MLL* rearrangement have a poor prognosis even in the absence of bone marrow involvement and should be treated aggressively (Zhang et al., 2006). When chloromas are present on the head or neck, imaging studies should be performed to assess the patient for the presence of intracranial or skull involvement. At birth many infants have respiratory distress from leukemic infiltration in the lungs. Severe respiratory distress may develop soon after birth from pulmonary hemorrhage secondary to thrombocytopenia. Some infants with leukemia may appear somnolent or have periodic apnea as a result of CNS leukostasis, caused by sludging of leukemic cells in blood vessels.

Laboratory Manifestations

Hemoglobin levels may be normal initially, but anemia soon develops as the normal postnatal decrease in red blood cell production is combined with the leukemic proliferation and expansion within the bone marrow. Total white blood cell counts (WBCs) may be normal or decreased, but leukocytosis is more often present. WBC counts of 150,000/mm³ to 250,000/mm³ or higher are common, and counts as high as 1,300,000/mm³ have been reported. There is usually a predominance of blast cells on the CBC differential. Auer rods, characteristic intracellular inclusions, may be present; they are pathognomonic of AML (Fig. 85.5).

Differential Diagnosis

A number of conditions can mimic congenital leukemia. Leukocytosis, hepatosplenomegaly, and thrombocytopenia can be seen



• **Fig. 85.5** Malignant Blast Cells With Auer Rods Present in Cytoplasm. This finding is pathognomonic of acute myelogenous leukemia. (Courtesy of Kristie White, UCSF Assistant Professor of Pathology, San Francisco, CA.)

in congenital infections such as syphilis, cytomegalovirus (CMV) infection, herpes simplex virus infection, toxoplasmosis, and bacterial sepsis. Congenital human immunodeficiency virus infection may rarely be confused with leukemia. Clonal B-cell expansion in such patients may cause lymphadenopathy. A marked but transient leukemoid reaction may occur during the newborn period in the setting of suspected or proven infection, particularly in very low birth weight neonates (Morag et al., 2008). Severe erythroblastosis fetalis can mimic leukemia. Affected infants usually have hepatosplenomegaly, large numbers of nucleated erythroblasts in the peripheral blood, and, occasionally, thrombocytopenia. Small infiltrates of extramedullary erythropoiesis in the skin are a rare manifestation, resembling leukemia cutis.

A transient leukemoid reaction (*transient myeloproliferative disorder*) occurs in many infants with Down syndrome. The leukemoid reaction usually resolves, but these infants sometimes require low-dose chemotherapy to reduce the risk of death because of end organ failure, and they are at higher risk of later development of acute leukemia (see later). Infants with neonatal neuroblastoma may have symptoms similar to those of congenital leukemia, with hepatomegaly and discolored skin nodules. CBCs are usually normal, without circulating blasts. Bone marrow biopsies and aspirates sometimes show clusters of neuroblastoma cells (see Fig. 85.1). Increased excretion of catecholamine metabolites and the presence of a primary tumor, most often intra-abdominal, are other clues that point to the diagnosis of neuroblastoma rather than leukemia.

Cellular Morphology and Immunophenotype

The bone marrow of a newborn with leukemia shows extreme hypercellularity and a marked predominance of immature cells, either myeloid or lymphoid. AML and ALL are differentiated on the basis of typical morphologic characteristics, such as the presence of granules or Auer rods (in AML), histochemical stains and immunophenotyping by flow cytometry, and chromosomal analysis. Terminal deoxynucleotidyl transferase, a DNA polymerase that catalyzes the polymerization of deoxynucleotides in thymocytes, is usually present in lymphoblasts but is only rarely present in myeloblasts. Myeloblasts are usually positive for myeloperoxidase, whereas lymphoblasts are not. AML is subclassified according to an international French–American–British (FAB) classification based on morphology and

TABLE 85.8 World Health Organization 2008 and Immunophenotypic Classification of Childhood Acute Leukemia

| WHO 2008 Classification | Antigen Expression ^a |
|---|-------------------------------------|
| Acute Lymphoblastic Leukemia | |
| B-cell acute lymphoblastic leukemia | HLA-DR, CD10, CD19, CD20, CD24 |
| T-cell acute lymphoblastic leukemia | CD2, CD5, CD7 |
| AML and Related Neoplasms | |
| AML with recurrent genetic abnormalities ^b AML with t(9;11)(p22;q23); <i>MLL3-MLL</i> | |
| AML, not otherwise specified | |
| AML with minimal differentiation (M0) | CD13, CD33, CD34 |
| AML without maturation (M1) | CD13, CD33, CD34 |
| AML with maturation (M2) | CD13, CD33, CD34 |
| Acute myelomonocytic leukemia (M4) | CD11b, CD13, CD14, CD15, CD33, CD34 |
| Acute monocytic leukemia (M5) | CD11b, CD13, CD14, CD15, CD33, CD34 |
| Acute erythroid leukemia (M6) | Glycophorin CD34 |
| Acute megakaryoblastic leukemia (M7) | CD34, CD41, CD42, CD61 |
| Myeloid Proliferations Related to Down Syndrome | |
| Transient myeloproliferative disorder | CD34, CD41, CD42, CD61 |
| Myeloid leukemia associated with Down syndrome | CD34, CD41, CD42, CD61 |

^aThe indicated diagnosis may express some or all of the indicated antigens.

^bOther genetic abnormalities omitted because of rarity in neonatal and infantile acute myeloid leukemia.

AML, Acute myeloid leukemia; WHO, World Health Organization.

Modified from the 2008 revision of the WHO classification of myeloid neoplasms and acute leukemia.

histochemistry (Table 85.8). The traditional FAB classification system has been integrated into the World Health Organization classification system, which incorporates additional information, such as genetic abnormalities, immunophenotype, and clinical features (Vardiman et al., 2009).

The immunophenotype, determined with a panel of fluorescently labeled monoclonal antibodies most often against cluster of differentiation (CD) antigens, is critical for differentiating AML and ALL (Craig and Foon, 2008). Myeloid leukemia cells are usually positive for CD13/CD33 antigens, which are markers of myeloid and monocytic differentiation. A notable exception is acute megakaryoblastic leukemia, which expresses the CD41/CD42 platelet glycoproteins and CD61, and is most commonly seen in patients with Down syndrome. Most neonatal and infant lymphoblastic leukemia cells exhibit an early precursor B-cell phenotype and often are CD1a, CD19, CD24, and CD15 positive and CD10 negative (Pui and Evans, 1999). In addition, coexpression of myeloid antigens is often present, suggesting that these leukemias arise from very immature lymphoid progenitors. Surface antigen expression is summarized in Table 85.8.

Genetics

A number of cytogenetic abnormalities have been found in association with congenital leukemia; many of these abnormalities

are independent prognostic indicators. The most frequent abnormalities involve disruptions of the mixed-lineage leukemia gene (*MLL*) at 11q23. Abnormalities of 11q23 are found in approximately 50% of infant AML cases and 70%–80% of infant ALL cases (Pui et al., 2003; Chowdhury and Brady, 2008; Van der Linden et al., 2009). These are nonhereditary, nonconstitutional abnormalities that occur in utero. In mice and in humans, the *MLL* protein positively regulates *HOX* genes, which are critical for hematopoietic development (Armstrong et al., 2002). Rearrangements in the *MLL* gene confer a poor prognosis, which worsens with decreasing age (Pieters et al., 2007). Gene expression analysis studies demonstrate a unique early hematopoietic progenitor genetic signature that distinguishes infant ALL with *MLL* rearrangements from ALL and AML in older children (Armstrong et al., 2002).

Treatment and Prognosis

The course of congenital leukemia is usually characterized by rapid deterioration and death from hemorrhage or infection. Although survival has been significantly prolonged in older children with leukemia, success has been limited in neonates with ALL. Infants with leukemia frequently present with hyperleukocytosis (blast cell count in excess of 100,000/mm³), which may result in sludging of blast cells in capillaries with resultant intracranial hemorrhage, respiratory distress, or tumor lysis syndrome, which is characterized by hyperkalemia, hyperphosphatemia, hypocalcemia, hyperuricemia, and acute renal failure. Disseminated intravascular coagulation is another common complication, especially with monocytic subtypes. Leukemic blast cells release procoagulants, causing a consumptive coagulopathy that places the neonate at risk of either bleeding or thrombotic sequelae and may be exacerbated by further leukemia cell lysis induced by chemotherapy.

Initial supportive care includes correction of metabolic and hemorrhagic complications. Transfusion of platelets and fresh frozen plasma is frequently required. Exchange transfusion is sometimes undertaken in infants to lower the WBC count and to correct metabolic abnormalities. Rasburicase, a recombinant urate oxidase enzyme, has been safely used in neonates with hyperuricemia (McNutt et al., 2006; Hobbs et al., 2010) but is contraindicated if glucose 6-phosphate dehydrogenase deficiency is suspected or diagnosed as precipitation of severe hemolysis and death have been reported (Zaramella et al., 2013).

Intensification of chemotherapy regimens have resulted in increased rates of remission and survival in both infants and older children. The contemporary chemotherapy regimens used in infants are similar to those used in older children and usually include daunorubicin, cytarabine, and etoposide, and outcomes for infants with AML are similar to those for older children, with overall survival rates of 65%–75% (Creutzig et al., 2012; Gamis et al., 2014).

Conversely, young infants with ALL fare worse than do older children with ALL, in whom chemotherapy results in a disease-free survival rate of more than 85%. Studies of infant ALL have reported rates of disease-free survival of 28%–47% (Pieters et al., 2007), which is a significant improvement from previous trials and has been associated with the introduction of hybrid regimens that combine elements from standard ALL and AML protocols, addressing the more primitive nature of *MLL*-rearranged ALL. Unfortunately, patients with congenital ALL continue to have higher relapse rates, with a disease-free survival rate of less than 20% (van der Linden et al., 2009).

Since CNS involvement is common in infants with AML and ALL, intrathecal chemotherapy is an important part of treatment. Radiation therapy, which can cause neurocognitive sequelae and secondary malignancies, is no longer routinely used. The use of hematopoietic stem cell transplant is controversial and remains an area of research in the treatment of congenital leukemia.

Transient Myeloproliferative Disorders and Leukemia in Patients With Down Syndrome

The incidence of acute leukemia in children with Down syndrome is 20-fold higher than in the general population. In Down syndrome children younger than 3 years, a rare megakaryoblastic subtype of acute leukemia predominates and is classified by the World Health Organization as myeloid leukemia associated with Down syndrome (ML-DS). ML-DS is characterized by *GATA1* transcription factor mutations and is distinct from conventional acute megakaryoblastic leukemia, which occurs in older non-Down syndrome patients, on the basis of molecular features and prognosis (Vardiman et al., 2009). The prognosis of myeloid leukemia associated with Down syndrome is favorable (Sorrell et al., 2012) and is associated with blast hypersensitivity to traditional chemotherapy agents such as cytarabine and daunorubicin (Taub et al., 1999).

Transient Myeloproliferative Disorder

Transient myeloproliferative disorder (TMD), which occurs in 4%–10% of neonates with Down syndrome, is clinically indistinguishable from AML (Malinge et al., 2009). While most cases of TMD occur in Down syndrome patients, TMD is also rarely seen in patients with no constitutional chromosomal abnormalities or with trisomy 21 mosaicism. TMD is a clonal disorder typically manifested by hepatomegaly, splenomegaly, and circulating myeloblasts. There may or may not be associated anemia or thrombocytopenia. In general, the blast count of the peripheral blood exceeds that of the bone marrow. Blast cells often have cell surface antigens characteristic of megakaryoblasts. Somatic mutations in *GATA1* have been detected in nearly all cases of TMD and myeloid leukemia associated with Down syndrome (Wechsler et al., 2002; Mundschau et al., 2003).

Most neonates with Down syndrome and TMD experience complete clinical and hematologic recovery without systemic therapy, usually within 3 months. The blast count slowly decreases in a period of 2 to 3 weeks, and the hemoglobin and platelet counts normalize. In some cases, however, spontaneous resolution does not occur, and the neonate may experience clinical deterioration manifested by progressive hepatosplenomegaly, hepatic dysfunction, coagulation disorder, ascites, and pleural or pericardial effusions. Approximately 20% of Down syndrome patients with TMD die, usually of hepatic or cardiopulmonary failure (Muramatsu et al., 2008). Treatment with low-dose cytarabine has been shown to reduce the risk of early death in the setting of high-risk TMD. The indications for starting treatment differ but generally include a WBC count of more than 50,000/mm³ to 100,000/mm³, platelet count of less than 100,000/mm³, renal or hepatic dysfunction, or cardiopulmonary compromise (Klusmann et al., 2008; Gamis et al., 2011). In some instances, complications from TMD occur in utero, and various degrees of hydrops fetalis can result.

Infants with Down syndrome and a history of TMD should undergo careful follow-up since TMD is considered to be a pre-leukemic syndrome: approximately 20% of neonates with TMD develop ML-DS within 4 years (Malinge et al., 2009). The administration of low-dose cytarabine does not appear to alter the risk of ML-DS.

Germ Cell Tumors

Germ cell tumors are neoplasms derived from primordial germ cells. They are a heterogeneous group of tumors, differing in site, age at presentation, histopathologic features, and malignant potential. They can occur in both gonadal and extragonadal sites (Horton et al., 2007). Normal pluripotent primordial germ cells migrate from the yolk sac to the embryo, and persistence and abnormal migration of these cells or lack of normal erasure of parental methylating imprinting is thought to lead to these tumors (Frazier et al., 2012).

Germ cell tumors make up 35%–40% of all neoplasms in the neonate, but only 5% of those contain a malignant component (Frazier et al., 2012). Benign germ cell tumors in the fetus and newborn are classified as either mature or immature teratomas (Isaacs, 2004). However, one or more of the germ layer derivatives may develop malignant characteristics. Germ cell tumors may arise in a variety of locations in the body, usually along the axial midline. Common sites in children include the pineal gland, neck, mediastinum, retroperitoneum, and sacrococcygeal region. In the neonatal period, most teratomas occur in the sacrococcygeal region (40% of all germ cell tumors), followed next by tumors in the neck. After puberty, teratomas most frequently occur in the gonads, particularly the ovary (Frazier et al., 2012). Yolk sac tumor (endodermal sinus tumor) is the most common malignant germ cell tumor in neonates and young children. In the neonate it most often occurs with a teratoma, often in the sacrococcygeal region (see later).

Pathology

By definition, teratomas contain tissue arising from all three layers of the embryonic disk. Ectodermal components, including glial tissue, are a major component of teratomas occurring at birth, in particular, sacrococcygeal tumors. There are often skin, hair, and tooth elements. Mesodermal components, including fat, bone, and muscle, also are present. Endodermal components include digestive tract tissue; this component generally forms a smaller portion of the tumor. The levels of hormonal markers, including serum AFP and β -hCG, are often elevated in the presence of malignant tissue within the teratoma and are useful to follow as therapy progresses. Elevated serum AFP level indicates the presence of immature endodermal sinus tissue or yolk sac elements, while elevated β -hCG level indicates the presence of embryonal carcinoma. Choriocarcinoma, which is rarely seen in newborns, manifests itself with an extremely elevated β -hCG level. Immature teratomas are defined and graded on a scale of 1 to 3 depending on the amount of embryonal tissue present (Frazier et al., 2012).

Evaluation

MRI or CT of the primary tumor is indicated to evaluate the extent of disease. Sacrococcygeal tumors should be imaged by MRI because of the possible involvement of the spinal cord. The entire abdomen is included in the imaging study to assess the extent of any local invasion, particularly involvement of the rectal wall. Chest imaging is performed to rule out metastasis. Baseline levels of serum AFP and β -hCG, which are normally elevated in newborns, are measured. Because of the variation in the levels at birth and the variation in the rates at which the levels decline to normal, these tumor markers can be difficult to interpret as measures of residual disease or recurrence. The half-life of AFP is 5 to 7 days; that of β -hCG is 24 to 36 hours.

Sacrococcygeal Teratomas

Sacrococcygeal teratomas are the most common solid tumors in newborns. The estimated incidence is 1 in 27,000 live births (Swamy et al., 2008). A minority are malignant: 10%–17% of sacrococcygeal teratomas contain yolk sac tumor (Isaacs, 2007). Malignancy is less common, only 7%–10%, in infants younger than 2 months (Frazier et al., 2012). Females are affected two to four times more frequently than males, but the malignancy rate is similar. Half of sacrococcygeal teratomas are diagnosed prenatally by ultrasonography. Polyhydramnios, nonimmune fetal hydrops, and dystocia have all been described in association with sacrococcygeal teratomas. Lack of prenatal diagnosis has been reported to carry a 20% risk of death in the first hour after birth secondary to hemorrhage (Swamy et al., 2008). Congenital anomalies, including genitourinary, hindgut, and lower vertebral malformations, are present in 15% of patients (Isaacs, 2007). In most cases the tumor manifests itself as a mass protruding between the coccyx and rectum; the mass may be quite large (Fig. 85.6). About 10% of these tumors are found only by rectal examination. Nearly all arise at the tip or inner surface of the coccyx.

Differential Diagnosis

Sacrococcygeal teratomas may be confused with meningocele, rectal abscess, pelvic neuroblastoma, pilonidal cyst, and a variety of very rare neoplasms that may occur in the sacral region. Most benign teratomas in this area produce no functional difficulties, even when marked intrapelvic extension is present. Bowel or bladder dysfunction, painful defecation, and vascular or lymphatic obstruction suggest that the lesion is malignant.

Treatment

Treatment of sacrococcygeal tumors is primarily surgical (Horton et al., 2007). The tumor should be radically excised as soon as possible because small, undifferentiated foci may proliferate and become aggressive. Removal of the entire coccyx is required. Failure to remove the coccyx carries a 30%–40% risk of local recurrence, which is sometimes accompanied by malignant elements. The survival rate for neonates with sacrococcygeal teratoma is 85% (Isaacs, 2007).

Sacrococcygeal teratomas diagnosed prenatally by ultrasonography (approximately 50% of cases) are associated with a worse outcome; the survival rate is only 30%–50% (Isaacs, 2004; Adzick, 2010). Fetal hydrops and prematurity are the main factors



• Fig. 85.6 Large Sacrococcygeal Teratoma in a Newborn Girl.

contributing to the low survival rate. If hydrops occurs before fetal pulmonary maturity, open fetal surgical intervention to debulk and devascularize the tumor is an option (Adzick, 2010).

Infants with sacrococcygeal teratoma containing malignant yolk sac elements are treated with surgery followed by chemotherapy with cisplatin, etoposide, and bleomycin. Immediate and late complications of this regimen can be significant and include hearing loss, pulmonary fibrosis, and secondary malignancy.

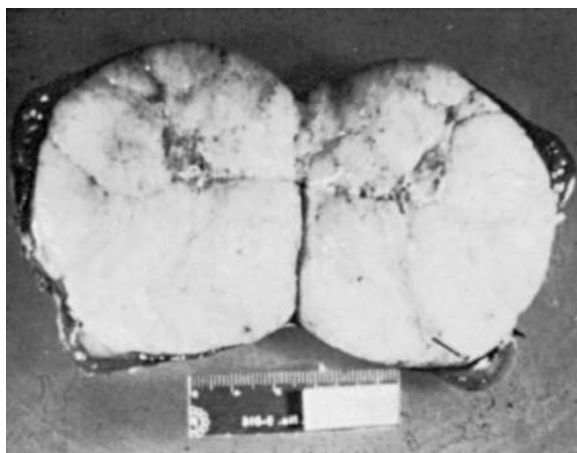
Renal Neoplasms

Approximately two-thirds of intra-abdominal masses in the neonatal period arise from the kidney. The vast majority of these neoplasms are nonmalignant and include polycystic or dysplastic kidney disease, hydronephrosis, renal vein thrombosis, and ectopic kidneys. The most common intrarenal neoplasm manifesting itself at birth is congenital mesoblastic nephroma (CMN), followed by Wilms tumor and nephroblastomatosis. Less common intrarenal neoplasms seen in the newborn period include rhabdoid tumor, clear cell sarcoma of the kidney, renal cell carcinoma, rhabdomyosarcoma, and lymphoma (Lowe et al., 2000). The typical clinical presentation is an asymptomatic abdominal mass detected on physical examination or by ultrasonography.

Congenital Mesoblastic Nephroma

CMN is the most common intrarenal neoplasm in the neonate. It usually behaves as a benign neoplasm, but the histopathologic subset, cellular CMN, occasionally recurs or metastasizes. The typical clinical presentation is an asymptomatic abdominal mass detected on physical examination or by ultrasonography, but hypertension due to a mass effect on the renal vasculature and hypercalcemia due to elevated renin levels are also common findings. The tumor infiltrates into normal renal parenchyma and is not encapsulated (Fig. 85.7). Specific sonographic features can help to differentiate CMN from Wilms tumor. The tumor may be diagnosed prenatally by ultrasonography, which reveals a greatly enlarged kidney distorted by the tumor. There is an increased incidence of polyhydramnios (71%) and premature labor (Glick et al., 2004).

Two histologic subtypes of CMN have been identified: the classic subtype and the cellular variant. The cellular variant usually manifests itself at an older age (>3 months) than the classic type (mean age at presentation of 1 month). Cytogenetic analysis of



• **Fig. 85.7** Congenital Mesoblastic Nephroma Compressing and Nearly Totally Replacing the Kidney.

the cellular variant shows a translocation $t(12;15)(p13;q25)$ that results in an ETV6–NTRK3 fusion protein that is identical to that found in infantile fibrosarcoma (Glick et al., 2004; Bayindir et al., 2009).

Complete surgical resection is usually an effective treatment for the classic form of CMN. Patients with the cellular variant are also treated with complete resection, but local and distant recurrences to the lungs and brain can occur. Positive surgical margins or tumor rupture during resection are risk factors for recurrence, which usually occurs within the first year following surgery. Resection and chemotherapy can successfully treat recurrent disease (Glick et al., 2004). The overall survival rate for CMN is 95%–98% (Isaacs, 2008).

Wilms Tumor

Wilms tumor, or nephroblastoma, is the most common intra-abdominal tumor of childhood, affecting 1 in 8000 children, but it is relatively rare in the neonatal period. In subsets of children with aniridia and hypospadias, the incidence is much higher. Wilms tumor is thought to arise from abnormal proliferation of metanephric cells, referred to as *nephrogenic rests*, without normal tubular and glomerular differentiation. Nephrogenic rests normally occur in 1% of newborn kidneys but often regress early in childhood.

Clinical Manifestations

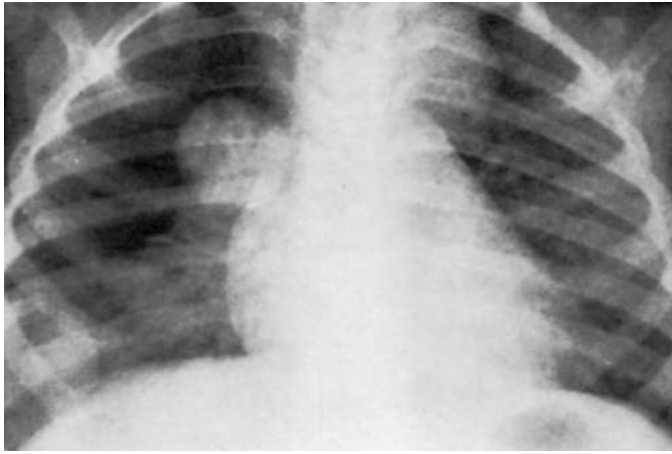
Wilms tumor arises from one or both kidneys, and most children present with an abdominal or flank mass. It seldom extends beyond the midline, even though it may grow downward beyond the iliac crest. In 5%–10% of all cases, tumors involve both kidneys. Gross hematuria is a rare presenting symptom, but microscopic hematuria is found in approximately 25% of cases. Wilms tumor is seldom diagnosed at birth or during the neonatal period. Characteristics associated with an earlier presentation include bilaterality, associated aniridia or hypospadias, and a family history (Pastore et al., 1988).

Hereditary Associations and Congenital Anomalies

While most cases of Wilms tumor are sporadic, a number of conditions are associated with an increased risk of developing the tumor. These include *WT1*-associated phenotypes caused by deletions and mutations of the *WT1* gene (11p13), overgrowth syndromes, and constitutional chromosomal disorders (Scott et al., 2006).

WAGR syndrome (Wilms tumor, aniridia, genitourinary abnormalities, mental retardation) predisposes to Wilms tumor because of a deletion in *WT1* and aniridia because of a deletion of *PAX6*, which are both encompassed within a deleted region at 11p13 (Breslow et al., 2003). From a clinical perspective, if an infant has aniridia, chromosome analysis should be undertaken. If a deletion of chromosome 11p13 is found, the child should be monitored for the development of Wilms tumor with serial renal ultrasound examinations. Wilms tumor develops in approximately half of these patients. Denys–Drash syndrome, caused by missense mutations within the *WT1* gene, classically describes the triad of Wilms tumor, genitourinary anomalies, and nephropathy. The genitourinary anomalies may be severe enough to result in pseudohermaphroditism.

Hemihypertrophy, which can be either isolated or associated with various genetic syndromes, is associated with an increased risk of the development of Wilms tumor. BWS, an overgrowth disorder caused by dysregulation of imprinted genes at chromosome 11p15, is associated with an increased risk of Wilms tumor, particularly bilateral disease. Between 1% and 8% of individuals



• Fig. 85.8 Pulmonary Metastases From Wilms Tumor.

with BWS develop Wilms tumor (Scott et al., 2006). Other characteristic clinical findings include macroglossia, anterior abdominal defects, ear creases and pits, neonatal hypoglycemia, and hemihypertrophy.

Between 1% and 2% of Wilms tumor cases occur within families, but the underlying cause of familial Wilms tumor is heterogenous and mostly unknown (Scott et al., 2006).

Prognostic Factors

Important prognostic factors include the histologic assessment, the extent of disease, and chromosomal abnormalities. The presence of anaplasia is an important predictor of adverse outcome, and patients who have tumors with diffuse anaplasia fare worse than those with focal anaplasia. The presence of tumor-specific loss of heterozygosity for chromosome arms 1p and 16q is associated with a worse prognosis (Grundy et al., 2005). Patients younger than 2 years have fewer relapses, especially of distant sites, than older children.

Evaluation and Staging

Clinical staging, which includes a CT scan of the abdomen and chest, is an important factor in predicting survival; tumors with more extensive spread carry a poorer prognosis. The most common sites of metastasis are the liver and the lungs (Fig. 85.8). Tumor thrombus is occasionally noted in the inferior vena cava.

Treatment

Patients with low-stage Wilms tumor have a cure rate of more than 90%. Patients with small tumors limited to the kidney can be cured with surgery alone. Patients with intermediate-risk Wilms tumor are treated with a short course (12 weeks) of chemotherapy with vincristine and dactinomycin. More extensive disease or high-risk features, such as anaplasia, require the addition of doxorubicin and possibly other chemotherapy agents. Radiation therapy is indicated in children with diffuse abdominal disease; tumor spillage, including percutaneous biopsies, which is not a recommended diagnostic approach for pediatric renal tumors; or nonresponsive pulmonary metastases. Even patients with metastatic disease have a good prognosis, with a 70% long-term overall survival rate. Recurrent disease, which can be local or may involve metastases to the liver, lungs, or brain, is treated with additional chemotherapy and radiation therapy.

Rhabdoid Tumor of the Kidney

Rhabdoid tumor of the kidney (RTK) is an uncommon tumor of children that is one of the most lethal neoplasms of early neonatal life, with a mortality rate exceeding 80%. RTK is the second most common malignant neoplasm of the kidney in neonates, after Wilms tumor. It has a predilection for males and for infants, with median age at diagnosis of 11 months. It is often widely metastatic at diagnosis; metastatic sites include lung, abdomen, lymph nodes, liver, bone, skin, and brain. Homozygous inactivating deletions or mutations of the *INI1* gene, located in chromosome band 22q11.2, are responsible for most rhabdoid tumors (Jackson et al., 2007) and can be detected immunohistochemically. The prognosis for infants with RTK is extremely poor. The survival rate at 2 years for infants with a diagnosis before 6 months of age is less than 15% (van den Heuvel-Eibrink et al., 2011). Virtually all patients with distant metastases will have a fatal outcome. Treatment modalities have included surgical resection, chemotherapy, and radiation therapy.

Persistent Nephrogenic Rests and Nephroblastomatosis

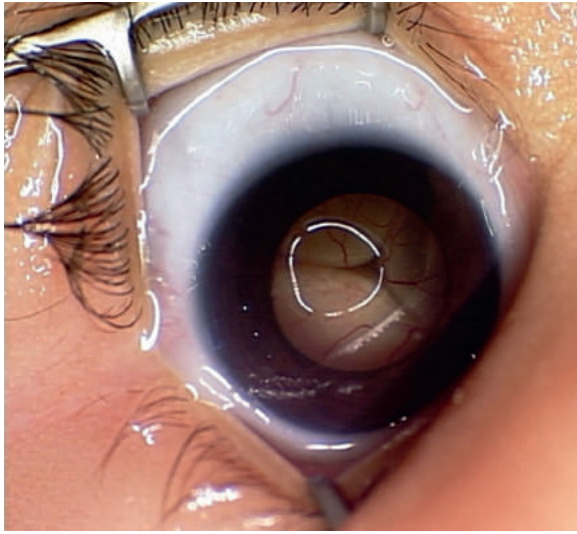
Nephrogenic rests are residual immature metanephric tissue within a fully developed mature kidney. They normally occur in 1% of newborn kidneys but typically regress during early childhood. Although benign, nephrogenic rests have been confused with Wilms tumor in the past and are believed to have the potential for neoplastic transformation to Wilms tumor. Histologically, nephrogenic rest cells may resemble Wilms tumor and occasionally present a diagnostic dilemma, although the presence of mitoses favors Wilms tumor. Radiographically, when imaged with MRI, nephrogenic rests tend to appear less heterogeneous, demonstrate less contrast enhancement, and are smaller than Wilms tumor. Bilateral or multicentric Wilms tumors are associated with a high incidence of synchronous nephrogenic rests. When nephrogenic rests are multifocal or diffuse, they are referred to as *nephroblastomatosis*. Children with massive bilateral nephroblastomatosis often respond to therapies for Wilms tumor (Hennigar et al., 2001).

Retinoblastoma

Retinoblastoma is a malignant ocular tumor that arises from embryonic retinal cells. It occurs in the setting of hereditary and nonhereditary disease. The incidence of retinoblastoma is approximately 1 in 18,000 live births; between 250 and 350 cases are diagnosed in the United States each year (Kiss et al., 2008; Gombos, 2012). Bilateral involvement, which occurs in the setting of heritable disease, is observed in 20%–35% of patients. While the incidence of heritable retinoblastoma is constant among various population groups, the incidence of sporadic, nonhereditary, unilateral retinoblastoma is increased in poorer, tropical, and subtropical regions (Kiss et al., 2008).

Genetics

Approximately one-third of patients have hereditary disease, which is often bilateral. Patients with hereditary disease have a germline mutation in the retinoblastoma gene, *RB1*, a tumor suppressor gene located on chromosome band 13q14. The mutation is inherited from a parent or occurs during embryonic development. There is autosomal transmission with high penetrance, approximately 90%. Patients with nonhereditary disease tend to have unilateral retinoblastoma. Their disease is the result of acquired somatic mutations in both *RB1* alleles.



• **Fig. 85.9** Retinoblastoma Filling the Orbit. (Courtesy of Bertil Damat and Andrew Kao, UCSF Ophthalmology, San Francisco, CA.)

Approximately 5% of retinoblastoma patients are born with a constitutional deletion of chromosome 13, 13q-. These patients have associated constitutional anomalies, including microencephaly, macrognathia, malformed ears and thumbs, hypertelorism, microphthalmia, ptosis, short stature, cleft palate, and developmental delay.

In children with the bilateral and hereditary form, diagnosis is often at 1 year of age as opposed to on average at 2 years of age in children with the nonhereditary form (Gombos, 2012). This is due in part to early screening initiated because of the family history. In rare instances, a family history of retinoblastoma may be lacking; hereditary bilateral retinoblastoma may result from germline mosaicism in the parent.

Clinical Manifestations

Patients with retinoblastoma commonly present with leukocoria ("cat's eye"), squinting, or strabismus caused by loss of vision in the affected eye. Multifocal retinal involvement is common, occurring in 84% of cases. Intraocular spread may fill the vitreous body by extension or seeding, whereas exophytic tumors arise from the outer retinal layer and cause retinal detachment (Fig. 85.9). Extraocular spread is seen in less than 15% of patients, usually occurring by direct invasion of the optic nerve and eventually leading to subarachnoid involvement and intracranial spread. In such cases the cerebrospinal fluid may contain tumor cells. Rarely, tumors may spread by invasion of the orbit or by hematogenous dissemination to bone and bone marrow. Children with bilateral retinoblastoma are at risk of tumor dissemination to the pineal gland, a condition known as *trilateral retinoblastoma*. All patients with retinoblastoma should be evaluated initially by brain MRI for this condition. Patients with bilateral retinoblastoma are typically evaluated by brain MRI annually until age 5 or 6 years. Retinoblastoma can be variably be detected in utero by ultrasonography or MRI in patients known to carry the mutated *RBI* gene (Gombos, 2012).

The diagnosis of retinoblastoma is made by fundoscopic examination and orbital ultrasonography performed with the patient under general anesthesia. MRI of the orbit is useful to determine tumor extent and optic nerve involvement. A lumbar puncture for cerebrospinal fluid cytology is performed if there is optic nerve

invasion, but more extensive evaluation with bone marrow biopsy or bone scan is not usually necessary. Tumors are staged according to the International Classification of Retinoblastoma, on the basis of tumor size and location and the extent of vitreous and subretinal seeding (Kiss et al., 2008).

Treatment

Because extraocular spread and death from dissemination are rare, the main goal of treatment is local control and preservation of vision. Surgical enucleation is used only when there is no chance for useful vision, if glaucoma is present, or if conservative measures fail to control the tumor. Small tumors confined to the retina can often be controlled with focal consolidative therapies such as cryotherapy and laser photocoagulation. Systemic chemotherapy with agents such as carboplatin, vincristine, and etoposide is frequently used concurrently with cryotherapy and laser therapy. Intra-arterial chemotherapy with agents such as melphalan can provide directed therapy, as can brachytherapy. External beam radiation therapy is effective, but because of the late effects of radiation on bone growth and the potential for second tumor induction, aggressive local therapy and systemic and/or intra-arterial chemotherapy are preferable. Decisions about management involve multidisciplinary discussion because of the various treatment modalities.

Prognosis

The prognosis for children with unilateral retinoblastoma is excellent, with cure rates of 85%–90%. However, patients with bilateral disease have a much lower long-term survival rate because of the high incidence of second malignancies, which may occur at any point in the life span. Patients with hereditary disease can develop secondary sarcomas in the area treated with radiation therapy; they are also at increased risk of developing sarcomas in other, nonirradiated areas. Local extension of retinoblastoma confers a poor prognosis, with survival rates of less than 10% with orbital extension or distant dissemination.

Central Nervous System Tumors

Incidence and Epidemiology

Congenital brain tumors are rare (<5% of childhood CNS tumors), with an incidence of approximately one to three per million live births (Lasky et al., 2008; Hwang et al., 2012). They are defined as brain tumors identified up to 2 months of life. The incidence is likely underreported as 30% of neonates with brain tumors are delivered stillborn. Most brain tumors in infants are supratentorial, in contrast to pediatric CNS tumors. Half are gliomas, including astrocytomas. Primitive neuroectodermal tumors and medulloblastomas also occur. Atypical teratoid or rhabdoid tumor of the CNS is associated with a high mortality rate. In general, brain tumors presenting in the perinatal period carry a very poor prognosis. Mortality from congenital CNS tumors has been reported to be from 72%–93% (Hwang et al., 2012). Discussion with the family regarding prognosis and alternatives to therapy should be undertaken prenatally if CNS tumors are identified.

Clinical Manifestations

Macrocephaly is often the first sign of a congenital CNS tumor if the tumor has not been identified in utero. Signs and symptoms of increased intracranial pressure (ICP) may be present. In infants these include a bulging fontanelle, split sutures, or rapidly increasing head size. Poor feeding, vomiting, lethargy, and irritability can

also be symptoms of increased ICP. Fundoscopic examination may or may not show papilledema. Loss of developmental milestones may be detected in older infants and children. Specific neurologic abnormalities include Parinaud syndrome (impaired upward gaze secondary to increased pressure in the dorsal midbrain), cranial nerve palsies, and nystagmus. Head tilting can occur in patients with posterior cerebellar masses secondary to cervical root irritation.

Treatment

Treatment depends on the pathologic characteristics of the tumor, which usually cannot be ascertained until after birth and further imaging. An important component to therapy in neonates is usually surgical resection of the tumor. The degree of surgical resection is the single most important predictor of survival (Lasky et al., 2008). Complete resection is often not possible because tumors in infants tend to be large, highly malignant, and invasive. They are also highly vascular, making it difficult to remove the tissue without significant morbidity. Many patients also require ventriculoperitoneal shunt placement for relief of hydrocephalus.

Radiation therapy, a backbone of treatment for older children with malignant brain tumors, is avoided if possible in young infants because infants experience devastating late effects, including neurocognitive deficits and growth impairment. Adjuvant chemotherapy can play a role in treatment; this may allow necessary radiation therapy to be delayed until the child is older. Most tumor types are treated similarly to childhood CNS tumors, although the efficacy of these treatments is not clear especially in light of the limitations of radiation therapy.

Sarcomas

Soft tissue sarcomas are rarely seen in newborns. The most commonly diagnosed soft tissue sarcoma in the neonatal age group is infantile or congenital fibrosarcoma, which is classified as a low-grade nonrhabdomyosarcoma soft tissue sarcoma. The incidence in infants between age 1 month and age 12 months is five cases per million infants (Ries et al., 1999). In general, infantile fibrosarcoma is treated by complete surgical excision, although neoadjuvant chemotherapy with a variety of agents has been successfully used for tumor shrinkage, with subsequent reduction in the morbidity related to radical surgical procedures (Russell et al., 2009). The cure rates for infantile fibrosarcoma, with surgery alone or with chemotherapy and surgery, approach 100% (Kurkchubasche et al., 2000; Loh et al., 2002).

The initial evaluation of a patient with a congenital fibrosarcoma includes imaging of the primary tumor by MRI or CT and a chest CT scan. Diagnosis is made by biopsy of the lesion. The chemotherapy regimens used successfully for treatment of this tumor include vincristine, dactinomycin, and cyclophosphamide, as well as etoposide and ifosfamide. Doxorubicin, while efficacious, is generally avoided because of the risk of cardiac toxicity. The duration of therapy depends on the size, location, and response of the tumor, but the general goal is to reduce the tumor size to maximize the chances of surgical local control. Radiation therapy is usually avoided to spare the infant the associated late effects of poor growth and secondary cancers.

Rhabdomyosarcoma (RMS), the most common soft tissue sarcoma in children, is rarely seen in neonates: less than 1% of RMS cases are diagnosed in the first month of life. Congenital RMS often involves the genitourinary tract and is frequently of the embryonal subtype. Congenital embryonal RMS appears to

be associated with a specific translocation, $t(2;8)(q35;q13)$ (Meloni-Ehrig et al., 2009).

Histiocytosis

The histiocytoses are a diverse group of disorders characterized by the accumulation and proliferation of cells derived from the monophagocytic system—macrophages and dendritic cells (Isaacs, 2006). These disorders have been categorized into three classes (Table 85.9). Taken as a whole, histiocytoses can occur throughout life but peak incidence is in 1- to 3-year-old patients. Class I disorders, which are dendritic cell related, include Langerhans cell histiocytosis (LCH) (formerly known as *histiocytosis X*, *Letterer–Siwe disease*, *Hand–Schüller–Christian disease*, and *eosinophilic granuloma*) and pure cutaneous histiocytosis. Class II disorders, which involve macrophage-derived cells, include familial hemophagocytic lymphohistiocytosis (FHL) and infection-associated hemophagocytic syndrome (IAHS). Class III disorders are malignant disorders of mononuclear phagocytes and include acute monocytic leukemia, malignant histiocytosis, and histiocytic lymphoma. In addition to these three classes, other rare, noncategorized histiocytoses can occur in newborns, in particular, juvenile xanthogranuloma (JXG). The pathophysiology of the histiocytic disorders appears to be related to abnormal regulation of histiocyte activation resulting in cell proliferation and cytokine production (Isaacs, 2006).

The most common histiocytic disease seen in the fetus and neonate is LCH. About half of newborns with LCH have disease confined to the skin, while the other half have disseminated disease with resultant organ dysfunction, usually involving bone marrow, liver, or lung. Presenting symptoms of LCH in neonates can include skin lesions, hepatosplenomegaly, lymphadenopathy, and respiratory distress. Histologically, both cutaneous and disseminated LCH are characterized by granuloma-like lesions. Diagnosis is made by biopsy; Langerhans cells are positive by immunohistochemistry for CD1a and S-100.

The course of LCH is unpredictable. The pure cutaneous form of LCH usually resolves spontaneously in 2 to 3 months, whereas disseminated LCH carries a poorer prognosis, with 57% mortality (Minkov et al., 2005); prognosis depends on level of organ involvement, age of diagnosis, and progression of disease. To complicate matters, newborns with skin lesions may not develop the symptoms of disseminated disease for several weeks to months. Disseminated LCH is usually treated with chemotherapy, including vinblastine and prednisone. Recently, genetic findings in LCH include *BRAF* V600E mutations, which have been treated effectively with vemurafenib (BRAF inhibitor); use in pediatrics is being studied (Haroche et al., 2013).

FHL and IAHS are also seen in the newborn period. FHL has an incidence of approximately 1 in 50,000 liveborn children (Henter et al., 2007) and is more common in neonates than IAHS. It is alternatively known as *primary hemophagocytic lymphohistiocytosis*. The overall survival rate for newborns with FHL is only 9% unless a stem cell transplant is performed; the survival rate for newborns with IAHS is 59% (Isaacs, 2006). Newborns with these disorders commonly have fever, hepatosplenomegaly, and cytopenias. Other symptoms include liver dysfunction, neurologic symptoms, hypertriglyceridemia, elevated serum ferritin levels, and hypofibrinogenemia. Diagnostic criteria are listed in Box 85.1. Hemophagocytosis is found in the bone marrow in 76% of patients. Natural killer cell and cytotoxic T-cell activity is reduced or absent. Thirty percent of patients with FHL harbor constitutional mutations in the *PRF1* gene, which encodes an essential protein for cellular

TABLE 85.9 Classification of Childhood Histiocytoses

| Feature | Class I | Class II | Class III |
|--------------------------------------|--|---|--|
| Diseases included | Langerhans cell histiocytosis ^a | IAHS; FEL; grouped together as the hemophagocytic lymphohistiocytoses | Malignant histiocytosis; acute monocytic leukemia; true histiocytic lymphoma |
| Cellular characteristics | Langerhans cells with cleaved nuclei and Birbeck granules seen by electron microscopy; cell surface antigens include S100 and CD1a; cells mixed with various proportions of eosinophils; multinucleated giant cells sometimes seen | Morphologically normal, reactive macrophages with prominent erythrophagocytosis; process involves entire reticuloendothelial system | Neoplastic cellular proliferation of cells exhibiting characteristics of macrophages or dendritic cells or their precursors; localized or systemic |
| Proposed pathophysiologic mechanisms | Immunologic stimulation of a normal antigen-presenting cell—the Langerhans cell—in an uncontrolled manner | Histiocytic reaction secondary to an unknown antigenic stimulation (FEL) or to an infectious agent (IAHS), with erythrophagocytosis possibly reflecting foreign antigens absorbed on erythrocytes or activation of macrophages by excess lymphokine production due to abnormal immunoregulation | Neoplasm; clonal autonomous uncontrolled proliferative process |

^aPreviously known as histiocytosis X and its related syndromes of eosinophilic granuloma, Hand–Schüller–Christian disease, and Letterer–Siwe disease.

FEL, Familial erythrophagocytic lymphohistiocytosis; IAHS, infection-associated hemophagocytic syndrome.

From Ladisch S, Jaffe ES. The Histiocytoses. In: Pizzo PA, Poplack DG, eds. *Principles and Practice of Pediatric Oncology*, 5th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2006:772 [chapter 26].

• BOX 85.1 Diagnostic Criteria for Hemophagocytic Lymphohistiocytosis

The diagnosis can be established if:

1. The patient has a molecular diagnosis consistent with hemophagocytic lymphohistiocytoses (pathologic mutations of *PRF1*, *UNC13D*, *STXBP2*, *RAB27A*, *STX11*, *SH2D1A*, or *BIRC4*).
2. The patient has five of the following eight diagnostic criteria:
 - a. Temperature $\geq 38.5^{\circ}\text{C}$
 - b. Splenomegaly
 - c. Cytopenias (affecting at least two of three lineages in the peripheral blood): hemoglobin level $< 9.0\text{ g/dL}$ ($< 10\text{ g/dL}$ in infants younger than 4 weeks), platelet count $< 100 \times 10^9/\text{L}$, neutrophil count $< 1.0 \times 10^9/\text{L}$
 - d. Hypertriglyceridemia and/or hypofibrinogenemia: fasting triglyceride level $\geq 3.0\text{ mmol/L}$ (i.e., $\geq 265\text{ mg/dL}$), fibrinogen level $\leq 1.5\text{ g/L}$
 - e. Hemophagocytosis in bone marrow or spleen or lymph nodes; no evidence of malignancy
 - f. Low or absent natural killer cell activity (according to local laboratory reference)
 - g. Ferritin level $\geq 500\text{ ug/L}$
 - h. Elevated soluble CD25 level (i.e., alpha chain of soluble IL-2 receptor) $\geq 2400\text{ IU/mL}$

Other findings consistent with the diagnosis include cerebromeningeal symptoms, lymph node enlargement, jaundice, edema, rash, hepatic enzyme abnormalities, hypoproteinemia, hyponatremia, elevated VLDL level, and decreased HDL level.

HDL, High-density lipoproteins; IL-2, interleukin-2; VLDL, very low-density lipoprotein.

From Henter J-I, Horne A, Arico M, et al. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer*. 2007;48:124–131.

immune activation (Stepp et al., 1999). Several additional mutations can also cause FHL, including mutations in *UNC13D* on chromosome band 17q25 and mutations in *STX11* on chromosome band 6q24 (Trizzino et al., 2008). Patients with FHL are usually well at birth, then develop clinical symptoms by age 2 to 6 months. Constant fever, cytopenias, marked hepatosplenomegaly, and

progressive cerebromeningeal symptoms characterize the disease course. Initial treatment is with steroids and etoposide. Progressive disease usually leads to death within 4 months of diagnosis, but hematopoietic cell transplant can increase the 3-year survival rate to 64% (Jordan and Filipovich, 2008).

Patients with IAHS, whose symptoms are similar to those of patients with FHL, experience a high recovery rate (59%) when appropriate antibiotic or antiviral treatment is promptly administered (Isaacs, 2006). Leukocytosis is common in IAHS, possibly reflecting the underlying infectious cause.

JXG, a disorder of dendritic-related cells, is less common in neonates than LCH. Two forms are recognized: cutaneous and extracutaneous. Patients have one or multiple cutaneous nodules, which are reddish to yellow-brown papules on the head, neck, and extremities. Three quarters of neonates with cutaneous JXG have a solitary nodule. The nodules typically resolve spontaneously within approximately 2 years and are present at birth in 34.5% of cases (Willard et al., 2013). Survival is excellent for neonates with JXG limited to the skin and subcutaneous tissue, with no deaths reported in one series (Isaacs, 2006). Treatment is not generally indicated. Extracutaneous or disseminated JXG can involve the subcutaneous and soft tissues, liver, lung, spleen, eye, lymph nodes, and brain. Immunohistochemical stains may be required to differentiate this from LCH. Twenty-five percent of neonates with disseminated JXG do not have cutaneous lesions. Jaundice, hepatosplenomegaly, and thrombocytopenia can be seen. Although most cutaneous and extracutaneous lesions resolve spontaneously without treatment, disseminated JXG has an 11% death rate (Isaacs, 2006). Ocular and CNS involvement can cause significant morbidity. Systemic JXG is usually treated with chemotherapy and/or corticosteroids.

Hepatoblastoma

Hepatoblastoma, the most common malignant tumor of the liver in children, is an embryonal neoplasm composed of malignant

epithelial tissue. The incidence in infants is 11.2 cases per million; premature infants with low birthweights have a particularly high incidence (Feusner and Plaschkes, 2002). A second peak in incidence occurs at 6 months to 3 years of age. In neonates the female-to-male ratio is 1:6 (Isaacs, 2007). Treatment and management of hepatoblastoma in neonates are similar to treatment in young children. Histologic subtype is associated with outcome, with pure fetal histology having the best prognosis. Hepatoblastoma is associated with a number of genetic abnormalities and malformation syndromes, including BWS and trisomy 18 (von Schweinitz, 2003). Patients with sporadic hepatoblastoma have tested positive 10% of the time for a germline mutation in the tumor suppressor *APC*, the gene associated with familial adenomatous polyposis (Aretz et al., 2006).

Chromosome abnormalities in tumor tissue include trisomy of chromosomes 2 and 20. Chromosomal gains at chromosome 8 and 20 may be associated with an adverse prognosis. Patients with BWS demonstrate loss of heterozygosity at 11p15.5 (a tumor suppressor gene associated with increased morbidity and mortality), the Beckwith–Wiedemann locus. Patients with BWS should be screened for hepatoblastoma by abdominal ultrasonography and measurement of AFP levels every 3 months (Aretz et al., 2006).

The most common presenting symptom in neonates is abdominal distention. Even in the absence of distention an abdominal mass can sometimes be palpated. Anemia, fetal hydrops, and respiratory distress are other initial findings. Serum AFP level is elevated above neonatal norms in half of patients. Hepatoblastoma is occasionally diagnosed prenatally by screening ultrasonography. Tumor rupture can occur during birth, resulting in massive hemorrhage, often necessitating birth by cesarean delivery (Aretz et al., 2006).

Ultrasonography is useful to distinguish cystic and solid masses from diffuse hepatic enlargement. CT scan of the abdomen demonstrates the extent of tumor involvement, anatomic landmarks, and operability; MRI most accurately shows tumor margins and vessel involvement.

The goal of therapy is complete surgical resection. Infants with pure fetal histology whose tumors are completely resected have a 92% 24-month survival rate, and the overall survival rate in a case series of 52 patients was 86% (Aretz et al., 2006). A lobectomy of the involved portion of the liver is performed if one lobe is free of malignancy and there is no evidence of distant metastases. For unresectable but nonmetastatic tumors, initial treatment consists of chemotherapy with cisplatin and vincristine. If adequate tumor shrinkage results, the tumor is then resected. Orthotopic liver transplant has been used in patients with unresectable, nonmetastatic hepatoblastomas in conjunction with chemotherapy.

Hepatic Hemangioendothelioma

Hepatic hemangioendothelioma, also referred to as *hepatic hemangioma*, is a benign vascular tumor of the liver; it is the most frequent liver tumor of infants (Warmann et al., 2003). The disease is classified into three subtypes: focal, multiple, and diffuse hepatic lesions. Some patients also have cutaneous hemangiomas. While some infants with small tumors are asymptomatic, infants with larger tumors can have multiple symptoms, including abdominal distention, respiratory distress, high output cardiac failure, and consumptive coagulopathy. The diagnosis is often suspected prenatally when a hepatic mass is detected by ultrasonography. The diagnostic evaluation usually includes abdominal ultrasonography and CT or MRI. If imaging studies fail to provide a diagnosis,

then a biopsy can be considered. Infants with focal or multifocal disease without evidence of cardiac failure can usually be carefully observed with periodic physical examinations and ultrasound examinations. Various modalities have been used for symptomatic infants, including use of corticosteroids, use of corticosteroids with vincristine, embolization or artery ligation, surgical excision, and use of antiangiogenic chemotherapy agents (van der Meijs, 2009; Qureshi et al., 2015). Propranolol, which is used for treatment of cutaneous hemangiomas, may also be effective for treatment of hepatic hemangiomas (Vergine et al., 2015). The overall survival rate for fetal and neonatal focal hemangioma is 86%; the overall survival rate for fetal and neonatal multifocal disease is 71% (Isaacs, 2007).

Treatment Considerations in Infants

Chemotherapy Dosing

Neonates and infants experience more frequent and more severe side effects and late effects from chemotherapy compared with older children and adults. This is likely due to age-related differences in body composition, drug bioavailability, and drug metabolism. Infants have an increased amount of total body water, decreased activity of drug-metabolizing enzymes, particularly cytochromes P450, and less efficient renal function. To compensate for these differences, specific chemotherapy dosing protocols have been developed for neonates and infants. The doses for systemic chemotherapy are reduced overall and are frequently calculated by weight instead of by body surface area. Paradoxically, the volume of the cerebrospinal fluid in relation to body surface area is much greater in young children than in adults; accordingly, the doses of intrathecal chemotherapy are adjusted for age to avoid the underdosing of young infants with leukemia.

Radiation Effects

The use of radiation therapy in newborns is reserved for acute life-threatening situations in which the benefits of radiation therapy clearly outweigh the risks of adverse late effects, which include growth impairment, cognitive impairment, and the risk of secondary malignancies. The goal is to minimize the use of radiation as much as possible, while not compromising disease-related outcomes, to spare the infant potentially morbid treatment-related side effects.

Pain Management

Infants experience pain and should be treated with adequate pain medication. Signs of pain in the neonate can be subtle and can include crying, grimacing, poor feeding, tachycardia, and high blood pressure. Acetaminophen can be an effective analgesic in infants, but it must be used judiciously to avoid the masking of a fever that could signify an infection, particularly if a central line is in place or in the setting of neutropenia. Use of nonsteroidal antiinflammatory medications is usually avoided in patients with cancer because of the risks of bleeding due to interference with platelet function in the setting of chemotherapy-induced thrombocytopenia. Narcotics can be used as needed provided that adequate monitoring of side effects such as respiratory depression is in place. Narcotics can produce physical dependence when used for more than 1 week, and the doses may need to be tapered to avoid withdrawal symptoms.

Nutrition

Adequate nutrition is particularly important for neonates and infants. Patients might require supplemental nutrition via a nasogastric tube or intravenous parenteral nutrition. A nutritionist should be consulted to help assess the infant's nutritional needs. Breastfeeding can usually be continued in neonates with malignancy.

Intravenous Access

Most neonates and infants with diagnosed cancer will require a central venous catheter to facilitate delivery of chemotherapy, blood product support, and parenteral nutrition. Some chemotherapy agents, such as vincristine and doxorubicin, are vesicants and can cause severe skin and subcutaneous burns if inadvertently infiltrated underneath the skin, which is a more common occurrence with peripheral intravenous access. The type of central line inserted depends on the size of the infant and the specific needs associated with the chemotherapy regimen.

Transfusions

Cancer patients frequently require blood product supports to correct life-threatening cytopenias and coagulopathies, which can be secondary to the underlying malignancy or treatments directed against the malignancy. To minimize the risk of transfusion-associated infections and complications, a number of guidelines exist. These include the use of CMV-negative blood to prevent transmission of CMV, which is potentially life threatening in an immunocompromised infant; the use of irradiated blood products to prevent the possibility of life-threatening graft-versus-host disease; and the use of leukocyte-depleted blood products to minimize nonhemolytic febrile or allergic reactions. Use of donor-designated blood is usually discouraged in infants with congenital leukemia, given the possibility of the need for future related donor hematopoietic stem cell transplant.

Immunizations

Immunizations are generally avoided until the patient has not been receiving chemotherapy for at least 6 months. In addition, close contacts of the patient should receive the inactivated polio vaccine rather than the live oral polio vaccine. There is no contraindication to immunization of first-degree relatives with the varicella vaccine. However, any person in whom a rash develops after the vaccination should be kept away from the patient.

Psychosocial Considerations

Social services and psychological support are essential for families. These services are usually best coordinated through the efforts of a multidisciplinary team composed of nurses, social workers, case managers, and the pediatric oncologist.

Late Effects

Infants who receive treatment for cancer during the first year of life are at risk of many late effects directly related to chemotherapy, surgery, and radiation therapy. Infants in the neonatal period will be particularly susceptible to therapies affecting normal

growth and development. Information about late effects of treatment and suggested screening after childhood cancer may be found on the Children's Oncology Group website (<http://www.childrensoncologygroup.org>). Infants require follow-up evaluations at routine intervals by a pediatric oncologist (or multidisciplinary cancer survivor team), who can help identify appropriate screening tests and support.

Conclusion

Cancer in the neonatal period is rare but must be diagnosed and treated promptly with careful attention to the epidemiologic and clinical features that differ from those of older children with similar malignancies. There are special challenges in treating the neonate with cancer, including the newborn's unique physiologic status, which results in marked susceptibility to immediate and late adverse effects of treatment. Careful teamwork is necessary among the neonatologist, pediatric oncologist, surgeon, radiation therapist, nurse, and social worker to support and treat the patient and the family.

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86

Renal Development

IRENE MCALEER

KEY POINTS

- Renal organogenesis is a complex interaction between gene stimulation of planned growth and complementary apoptosis that allows appropriate development of the functioning renal organ system, vascular bed, and intertwined genital system.
- The renin–angiotensin system (RAS) is critical to normal renal development through delicate interaction between the maternal–placental RAS and the developing fetal RAS that has central hormone-specific and sex-specific configurations that help the developing vascular and renal organogenesis.
- Fetal development, genetic factors, and environmental factors control ultimate nephron numbers. Lower than normal nephron numbers at birth may predispose the adult to renal and cardiac disease.

The human kidney, specifically the metanephric kidney, is an extremely complex organ that has more than 25 functionally and morphologically different distinct cell types situated to perform vitally important functions for the developing human. These actions include filtering waste products and maintaining electrolyte and fluid homeostasis, bone mineralization, blood pressure, and blood composition (O'Brien and McMahon, 2014; Little, 2015). The nephron is the portion of the kidney that performs these functions. The total complement of human nephrons averages 900,000–1,000,000, and their development is complete in the human by 34–36 weeks' gestation. Any further growth of the kidney is due to an increase in the size of the individual nephrons rather than an increase in nephron number.

Renal organogenesis is a complex and incompletely understood process. Factors affecting maternal and fetal health, including environmental factors in utero, can lead to failure of normal morphogenesis, resulting in congenital anomalies of the kidney and urinary tract. If the complement of nephrons is decreased by prematurity, low birth weight (LBW), or exposure to environmental agents causing damage to the developing renal system, adult renal disease and hypertension may occur subsequently (Little, 2015; Nuyt and Alexander, 2009; Maringhini et al., 2010; Dötsch et al., 2012). Many steps in the development of the human kidney are unknown but are extrapolated from the study of animal models. This chapter will review known (and postulated) molecular and cellular mechanisms of renal development, the impact of

environmental factors on the developing fetal kidney, and the long-term risks of renal disease and hypertension into adulthood (Andrew and Yelon, 2015).

Factors Influencing Organogenesis

Organogenesis, or organ formation, begins with early patterning of cell groups by the expression of genes and transcription factors that act to determine cell fates specific to a given organ (Andrew and Yelon, 2015).

Transcription factors, generally ribonucleic acids (RNAs) directed by DNA information, are incorporated into larger molecular networks and ultimately direct downstream signaling pathways. Transcription factors may have different purposes in each organ system that they are responsible for developing. Similarly, signaling pathways are used repeatedly in the development of different organs, and these same pathways may also be used in multiple stages of differentiation in the same organ.

Signaling pathways establishing epithelial morphogenesis by allowing cell polarity, bending, and folding to shape the developing tissues have been found to interact with the loose mesenchyme surrounding the epithelia through signaling between the two tissue types. This reciprocal induction between epithelia and mesenchyme is well illustrated in kidney development, where expression of the transcription factor Wilms tumor 1 (WT1) in the metanephric mesenchyme leads to glial cell–derived neurotrophic factor (GDNF) expression critical for the outgrowth of the developing epithelial ureteral bud. Reciprocally, the ureteral bud then signals back to the metanephric mesenchyme to induce formation of the renal nephron units. There is a delicate balance between the interplay between the ureteral bud and the developing mesenchymal metanephros to allow tissue differentiation into the final renal unit.

Enhanced imaging, ex vivo organ culture, and computational strategies have advanced our understanding of the physical forces inside and outside the cells in organ development (Andrew and Yelon, 2015). Study of different species has led to better understanding of the role of the extracellular matrix in regulating the mechanical properties of the developing tissues. The extracellular matrix also provides substrata for cell migration, rotation, and elongation necessary for organ development. Most of this research is in nonhuman tissue at present, but the basic understanding of generalized organogenesis is important in determining the steps critical in

human organ development, with potential implications for regenerative medicine as well. Unfortunately, the organisms most extensively studied are *Planaria* species, which have stem cell populations throughout their bodies able to replace almost everything that is damaged or missing, unlike in human and mammalian species.

Development of the Renal Vascular Bed

The vascular systems of all vertebrates are highly organized branched networks of arteries, veins, and capillaries. The circulatory system in combination with the hematologic system is the first functioning physiologic system to develop in embryogenesis (Crivellato, 2011). Development of the cardiovascular and hematologic systems must occur in tandem because simple diffusion of oxygen and nutrients would be insufficient for organ development as the embryo enlarges. Formation of the vascular system is therefore crucial for proper tissue growth and differentiation, delivery of oxygen and nutrients to the developing organism, and removal of waste.

Vasculogenesis and angiogenesis play a primary role in determining patterning of the developing embryo through the paracrine action of the endothelial cells. Blood vessels are critical for organ development, differentiation, and postnatal remodeling. There is a reciprocal relationship, with the developing organ providing signals to the endothelial cells of the developing vasculature, while the endothelial cells signal patterning instructions for organ formation. Vascular development (vasculogenesis) begins shortly after gastrulation when the blood islets form in the yolk sac and angioblast precursors form in the head mesenchyme and the posterior lateral plate mesoderm. The vascular precursor cells are present in the metanephric blastema. Local environmental cues initiate the differentiation of the endothelial layer in the developing kidney as evidenced by formation of the renal vascular tree after transplant of different extrarenal endothelial cells to the developing renal bed in mouse kidneys. Angiogenesis occurs mostly later in embryogenesis by increasing the previously laid vascular bed by sprouting, bridging, and intussusceptive growth. Ultimately, the primitive vessels branch, prune, and specialize to accommodate the probable function of each respective organ that they feed. Oxygen tension and hemodynamic forces are critical for developing the delicate patterns specific to the local vasculature. Angiogenic growth factors are necessary for development as well as organ patterning. The most important factors responsible for angioblast differentiation and tube formation are vascular endothelial growth factors (VEGFs). VEGFs are found early in blood vessel patterning and later help modulate endothelial maintenance in normal tissues before and after birth. The endothelial cells themselves are the source of these proteins. VEGF also helps mobilize blood elements from the bone marrow through interactions with hematopoietic stem cells there (Crivellato, 2011). The development of the area-specific branching pattern is likely due to local factors and different local progenitor cell populations. The podocyte epithelial cells in the developing glomerular region secrete large amounts of VEGF, stimulating increased branching of the vessels forming in this area (Fig. 86.1). Fibroblast growth factors (FGFs) are heparin-binding growth factors that interact with endothelial cell surface receptors. Their primary purpose is to help control branching morphogenesis of the vascular tree during organogenesis. The postglomerular vascular bed with its close alignment to the vasa recta in the renal tubule structures is likely regulated by angiogenic factors such as VEGF secreted by the tubules themselves. Postnatal development of the vasa recta has been found to be mediated by VEGF as well. Thus renal vasculature development occurs

through simultaneous vasculogenic and angiogenic means. VEGF is critical in the regulation of blood vessel formation in the kidney. VEGF is also responsible for the coordinated development of the glomerulus and renal tubular development at the same time it is regulating vasculogenesis. Current animal model studies suggest that postnatal branching may be mediated by the renin–angiotensin system (RAS).

The renal vascular bed is unique compared with other organ systems. The kidneys compose less than 0.8% of the human body weight but receive 20% of the cardiac output at any given time (Herzlinger and Hurtado, 2014). The volume of blood sent to the kidney is critical for its task of clearing the body of metabolic waste, as well as maintaining fluid and ion homeostasis in the bloodstream. The renal artery enters the kidney near the central portion of the kidney or hilum, which is close to the medulla of the kidney. Although the renal artery is proximal to the medulla of the kidney, 90% of the blood it delivers is sent directly to the glomerular capillary bed in the periphery of the kidney, and only 10% is delivered to the medulla.

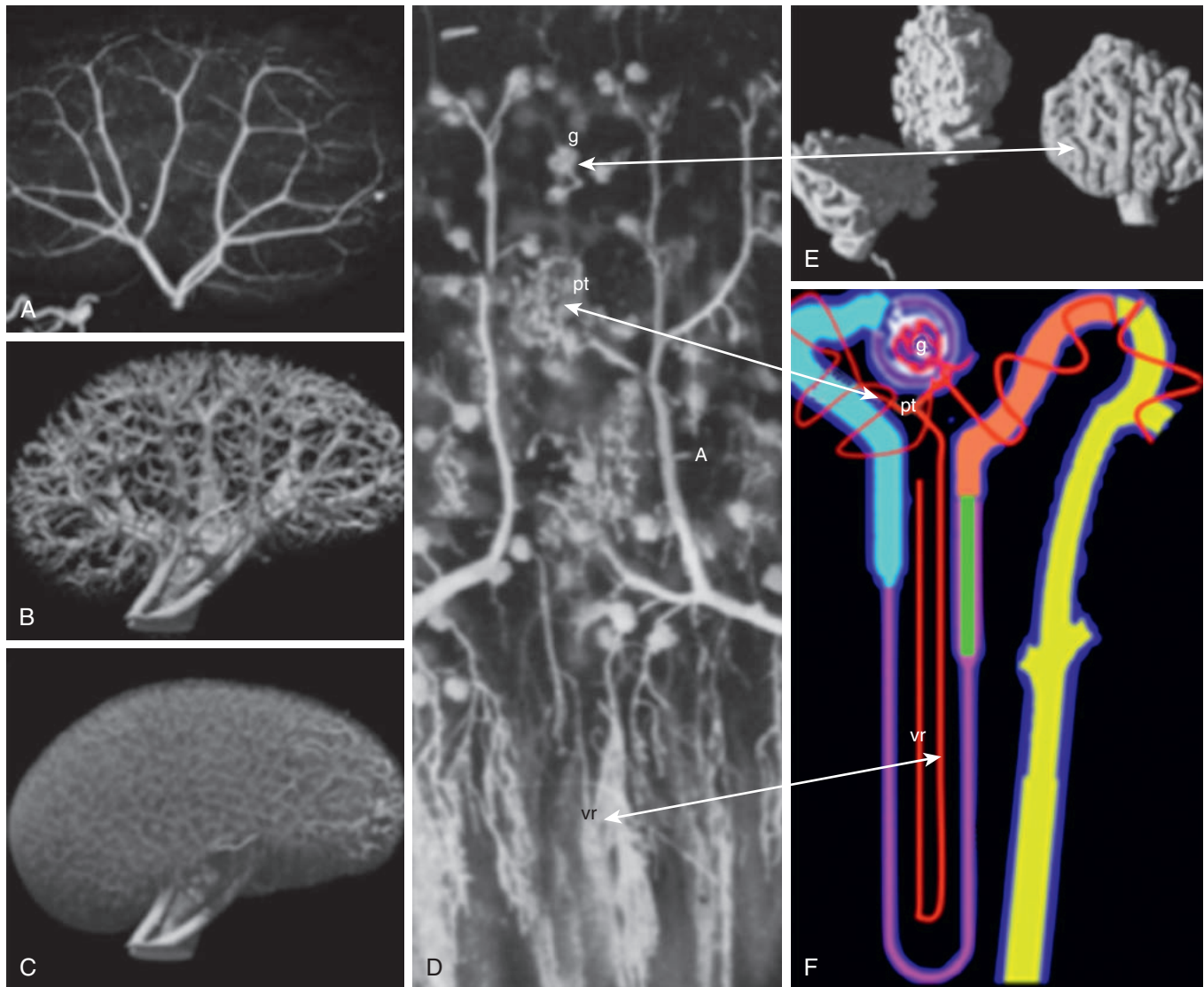
The arterial structure of the kidney is critical for directing blood primarily to the glomerulus. The mechanisms for this patterning during development are not well understood, but as development proceeds, the medullary portion of the arterial tree has very little branching, whereas the cortical arteries have extensive branching, especially the afferent arterioles surrounding the glomeruli.

For appropriate renal function the glomerulus endothelium is very permeable to fluids and low molecular weight solutes. Additionally, the resistance of the renal vasculature surrounding the glomerulus is very high to allow the ultrafiltrate produced to flow through the urinary space at 125 mL/min. These high-resistance arterioles allow the amount of fluid emanating from the glomerular capillaries to be about 50 times greater than the fluid outflow of other capillary systems in the body. In contrast, the peritubular capillaries have a fairly high oncotic pressure, allowing them to reabsorb solutes and fluids that may be lost by the high glomerular filtration rate (GFR). There is a complementary interplay between the renal tubules reabsorbing fluid, electrolytes, and solutes from the glomerular filtrate and the peritubular capillaries returning these substances to the systemic circulation because of their high oncotic pressure pull.

The vasa recta is an additional renal capillary bed adjacent to the renal tubules in the medulla. These vessels act by delivering oxygen and nutrients to the medulla and also return electrolytes and solutes reabsorbed by the medullary renal tubules. The long looped arrangement of the vasa recta close to the loop of Henle is critical for urine concentration by conservation of water through increased osmolarity in this region in the renal medulla (Herzlinger and Hurtado, 2014). The blood is ultimately collected in the venous system at the cortical medullary region of the kidney and flows out of the kidney through the renal vein.

Renal Morphogenesis

The mammalian urogenital system develops from the intermediate mesoderm. The paired epithelia nephric ducts arise dorsally and elongate caudally on the right and left sides of the embryo until they induce development of the pronephric and mesonephric duct formation from intermediate mesenchymal mesoderm. These ducts elongate until they reach and fuse with the cloaca, which is the bladder and urethral precursor. The primitive tubules that develop as the pronephros and mesonephros are transient and have minimal function, and only a portion remains of the mesonephric tubules



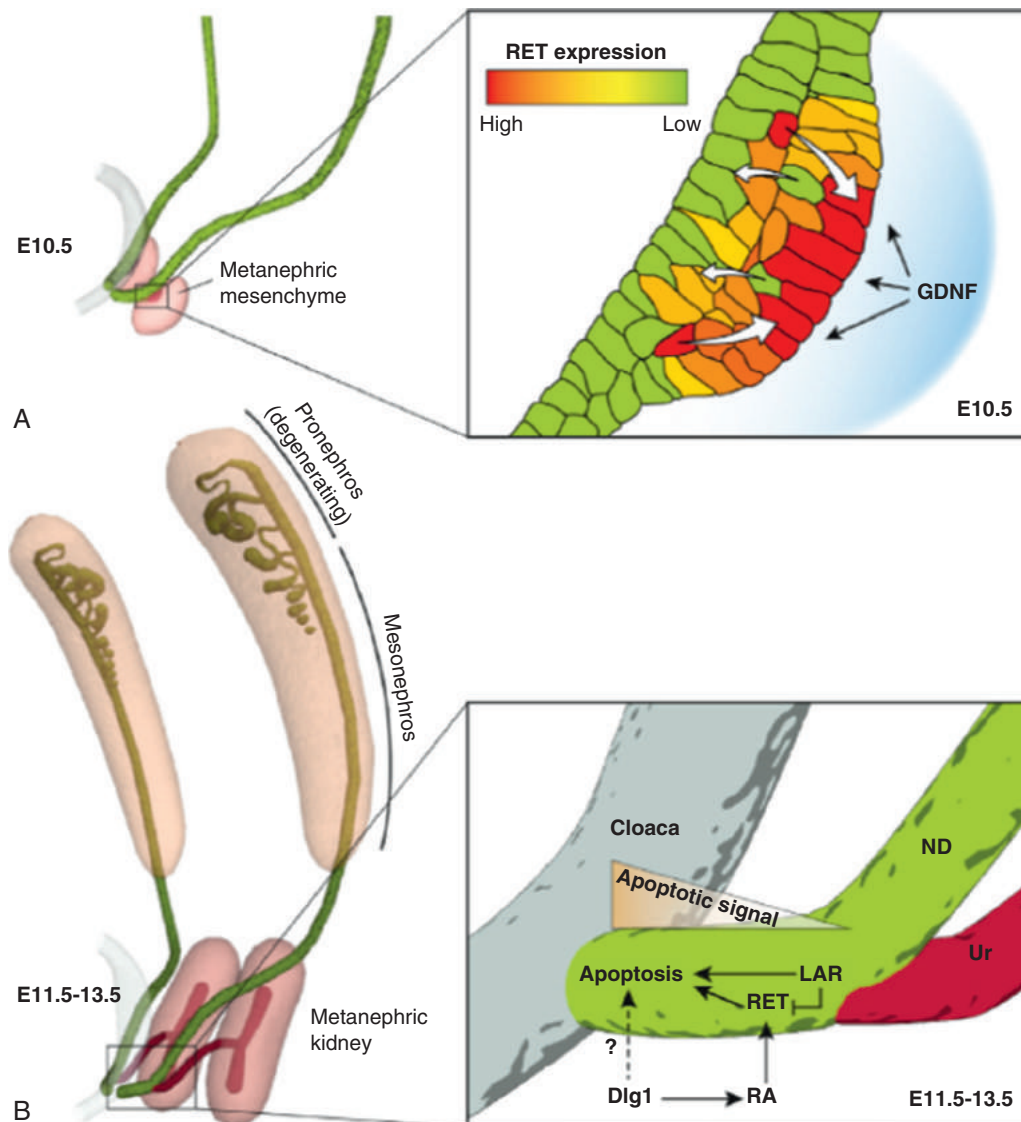
• **Fig. 86.1** Anatomy of the Renal Vascular Bed. Reconstructed scan of an entire mouse kidney corrosion cast by nano computed tomography (A–C). Thresholding for large vessels (A) illustrates the stereotypic architecture of the renal artery and its major branches. As thresholding is adjusted to visualize smaller vessels, the cortical arterial tree up to the level of the intralobular arteries is detected (B). Finer thresholding allows the visualization of the complex architecture of the cortical microvasculature (C). (D) Corrosion cast of rat kidney images by scanning electron microscopy. The glomeruli (*g*), peritubular capillaries (*pt*), and vasa recta (*vr*) are easily visualized. (E) A corrosion cast of an isolated glomerulus imaged by nano computed tomography illustrating the complexity of the capillary loops. (F) A single nephron and its associated glomerulus (*g*), peritubular capillaries (*pt*), and vasa recta (*vr*). ([D, E] Courtesy of Dr. Wilhelm Kriz; [F] From Herzlinger D, Hurtado R. Patterning the renal vascular bed. *Semin Cell Dev Biol.* 2014;36:50–56.)

as a portion of the male reproductive system (O'Brien and McMahon, 2014).

The metanephric kidney arises from the posterior nephric duct through reciprocal signaling induction between the metanephric mesenchyme and nephric duct causing the ureteral bud to form. Human metanephric differentiation starts at about 5 weeks' gestation, and the first functioning nephrons are formed at about week 8. The ureteral bud will then invade the metanephric mesenchyme to initiate the mesenchymal to epithelial differentiation giving rise to the glomerulus–nephron–collecting duct system of the mature kidney. The requirement for intermediate mesoderm appears to be regulated by bone morphogenetic protein (BMP) signaling, which is also required for maintenance of the pronephric duct

gene expression to allow survival and differentiation of the developing nephric duct.

Paired box genes (*PAX2* and *PAX8*) appear to be necessary to drive nephric duct development, elongation, and maintenance as well (Stewart and Bouchard, 2014). The final stage in early nephric development is the separation of the genital tract from the urinary tract. Once the ureteral bud extends from the nephric duct, the urinary tract separates the ureter from the common nephric duct through apoptosis to form the new ureterovesical junction into the cloacal structure that will be the urinary bladder. This apoptosis is most active at the caudal region and less so at the ureteral bud branching point. The apoptosis occurs through downregulation of RET (receptor tyrosine kinase) activity. RET is still needed for



• **Fig. 86.2** Interaction between mesenchymal and nephric duct epithelial tissues drives cell sorting to form the ureteric bud and programmed cell death during ureter maturation. (A) Ureteric bud evagination involves RET tyrosine kinase-dependent cell sorting (white arrows) to assemble the highest RET-responsive cells toward glial cell-derived neurotrophic factor (GDNF) secreted from the metanephric mesenchyme. (B) A graduated apoptotic signal drives elimination of the common nephric duct. Apoptotic cell death involves Dlg1 and retinoic acid (RA) signaling from the mesenchymal compartment, which appear to act through RET within the common nephric duct. Apoptosis additionally requires the expression of LAR-family receptor protein tyrosine phosphatases in the common nephric duct, which act partially by down-regulating RET pro-survival signaling. E, Embryonic day; ND, nephric duct; Ur, ureter. (From Stewart K, Bouchard M. Coordinated cell behaviours in early urogenital system morphogenesis. *Semin Cell Dev Biol.* 2014;36:13–20.)

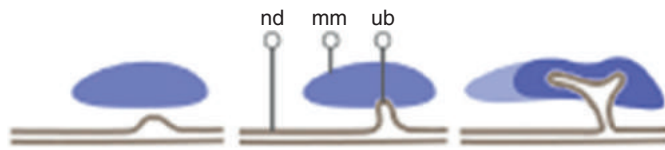
ureteral remodeling but at an expression level lower than that needed for generation of the ureteral bud branching off the nephric duct. Appropriate elimination of the common nephric duct is necessary for normal urinary tract function. Absence or delay in ureteral remodeling may result in ureteropelvic junction obstruction. Overactivation of apoptosis of the common nephric duct may result in vesicoureteral reflux (Stewart and Bouchard, 2014; Fig. 86.2).

Development of the ureteral bud requires an active RET–GDNF pathway. GDNF located in the metanephric mesenchyme adjacent to the nephric duct activates ureteral bud development by activation

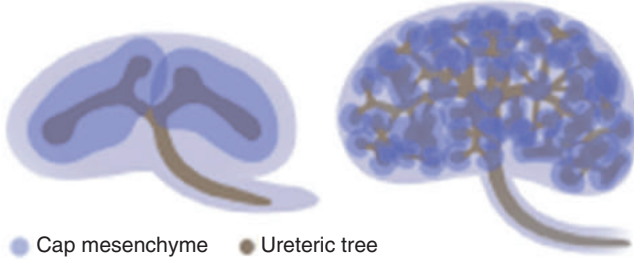
of RET located along the nephric duct. If RET is absent, ureteric induction fails, but the ureteral bud forms ectopically in studies where GDNF is cultured next to the nephric duct (Blake and Rosenblum, 2014). Wnt pathway factors, particularly factor 11, and FGFs upregulate GDNF to help with branching of the developing ureteral nephron tree.

Once the ureteral bud has been stimulated to extend out to the developing metanephric mesenchyme, it begins to branch, forming the ureteric tree, which occurs only at the tips of ureteric tips with RET–GDNF reciprocal signaling, which upregulates RET–Wnt11, further upregulating GDNF secretion. This ureteral

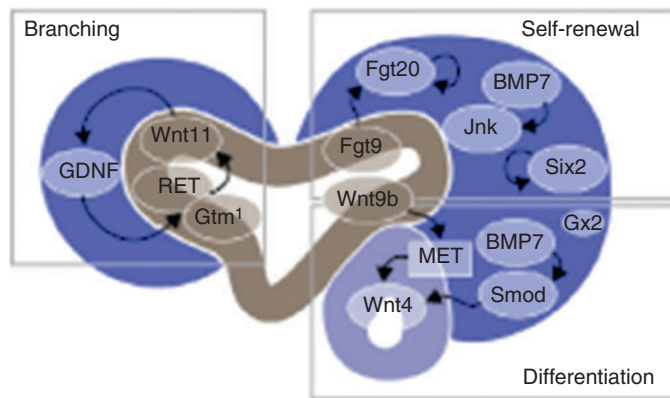
A. Ureteric budding



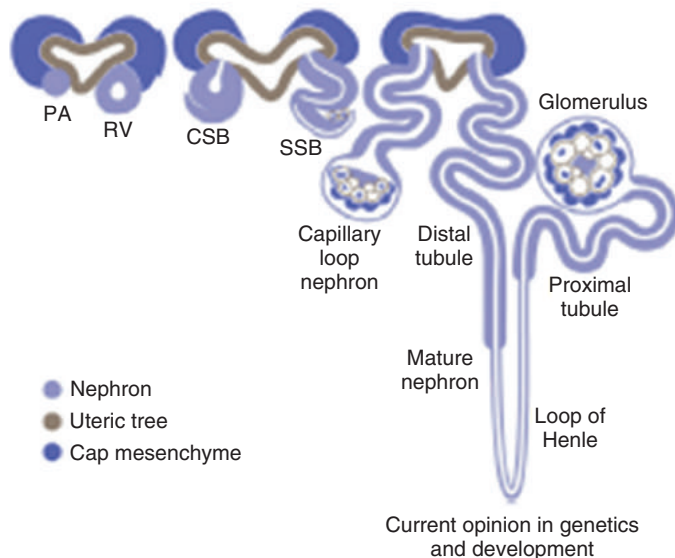
B. Ureteric branching



C. Key processes in the nephrogenic niche



D. Nephrogenesis and differentiation



signal to release RET is localized to the cap mesenchyme. With continued proliferation, elongation, patterning, and segmentation, the specific functioning regions of the nephron form the recognizable adult nephron (glomerulus, proximal tubule, loop of Henle, distal tubule), which all connect back to the collecting duct (Fig. 86.3).

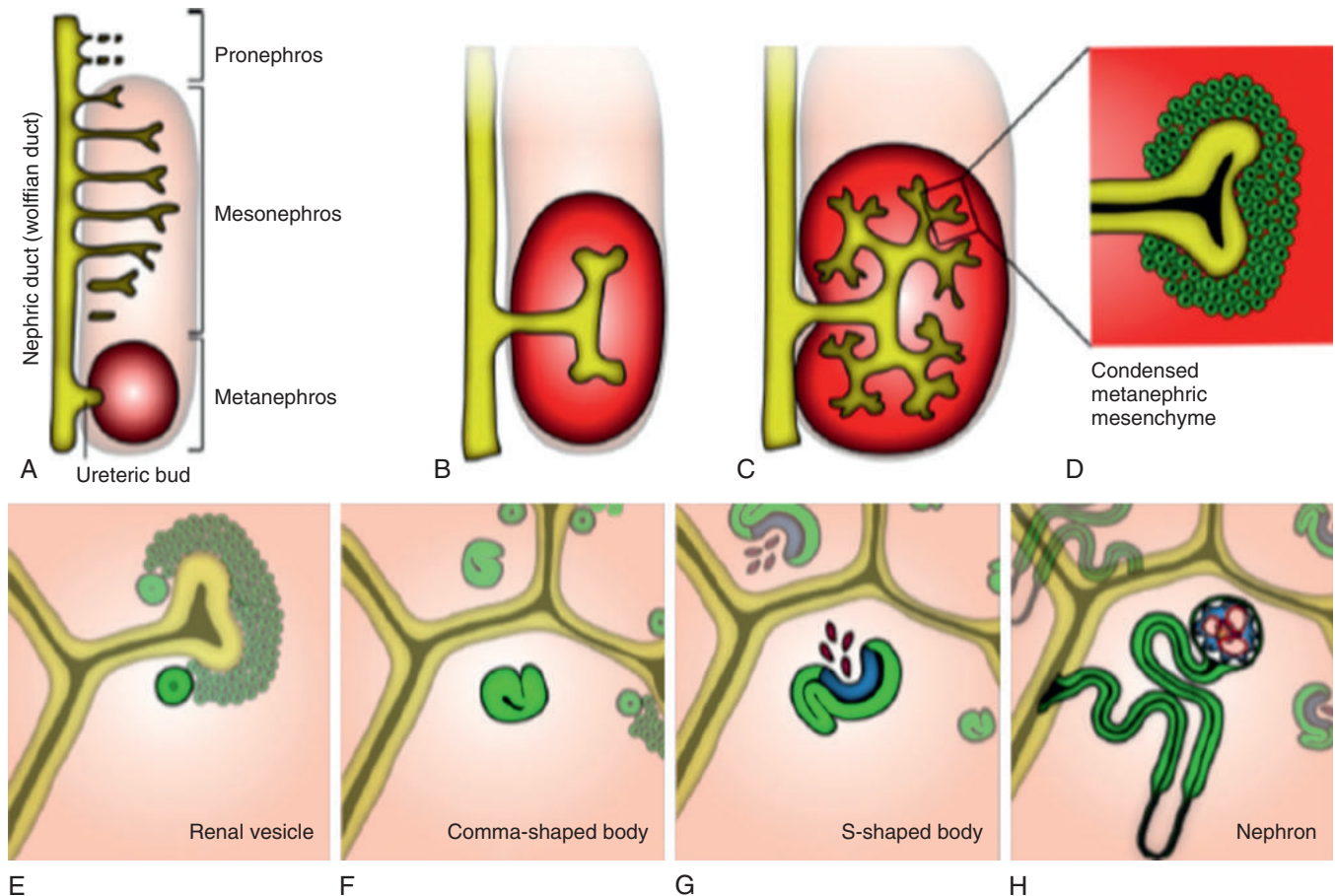
Once the ureteral bud epithelium invades the metanephric mesenchyme at the cap mesenchyme region, this mesenchyme

• **Fig. 86.3** Key inductive events in mammalian kidney morphogenesis. (A) Ureteric budding. The formation of the ureteric bud (*ub*) as a swelling of the nephric duct (*nd*), which grows toward the metanephric mesenchyme (*mm*) before undergoing initial bifurcation. (B) Ureteric branching. The branching ureteric epithelium of the developing mouse kidney from 11.5 days after conception (*left*) to 15.5 days after conception (*right*) showing the ureteric tree and surrounding cap mesenchyme. (C) Key processes in the nephrogenic niche. A nephrogenic niche illustrating the signaling pathways critical for branching (*left*) versus cap mesenchyme self-renewal (*top right*) and differentiation (*bottom right*). (D) Nephrogenesis and differentiation. The stages of nephron maturation from pretubular aggregate (*PA*) through renal vesicle (*RV*), comma-shaped body (*CSB*), S-shaped body (*SSB*), capillary loop nephron, and mature nephron. The *RV* represents the point of transition from mesenchyme to a polarized epithelial state. The formation of a connection between the forming nephron and the lumen of the adjacent ureteric epithelium occurs at the late *RV* stage and is shown here at the *CSB* stage. *BMP7*, Bone morphogenetic protein 7; *Fgt9*, *Fgt20*, *GDNF*, glial cell-derived neurotrophic factor; *Gtm*, *Gx2*, *Jnk*, *Six2*, *Smad*, . (From Little MH. Improving our resolution of kidney morphogenesis across time and space. *Curr Opin Genet Dev.* 2015;32:135–143.)

condenses around the ureteral bud tips under the influence of Wnt genes (Dressler, 2009). The renal vesicle, the first epithelial structure that will become the future nephron, is activated by the developing ureteral bud. The next phase is the development of the comma-shaped bodies as the first condensation of the renal vesicle metanephric mesenchyme. The formation of the cleft in the comma-shaped bodies denotes the development of the S-shaped bodies, which will ultimately initiate the development of the renal nephron (Fig. 86.4). The S-shaped bodies are derived from the cap mesenchyme and will become the glomerular tuft once the endothelial cells infiltrate this area of the developing nephron. The axis of the developing nephron is determined by the S-shaped body after it fuses to the ureteral tip. The proximal end of the S-shaped body will be the glomerulus, while the distal end will fuse to the ureteral bud branching system as the collecting duct. The S-shaped phase of development is when the nephrogenic and vasculogenic processes connect because of secretion of VEGF from the podocytes in the S-shaped body and attract migrating vascular endothelial cells to the S-shaped body cleft (Schell et al., 2014; Fig. 86.5).

With time and continued development, the ureteral tip and cap mesenchyme decrease in size. Ureteric branching ceases in the human at around week 14–15 of gestation. The human kidney is multipapillate, with about 8–15 lobes, each having about 15 generations of collecting duct branching. The extensive patterning and segmentation that occur in nephron development are driven by anchor genes that have absolute specificity for each cell compartment where they are located in the developing embryo. The kidney alone has more than 15 distinct anatomic compartments. To date, 37 anchor genes have been defined for only six subcompartments in the kidney. The actions of these genes are not yet well known, but the anchor genes may be responsible for initiating differentiation of the various compartments of the nephron by releasing promoters and tissue factors to the local mesenchyme to initiate formation of the functioning renal unit (Little, 2011).

Human nephron development is compared with mouse development to extrapolate similar or speculated steps in development that may be the same (see Fig. 86.5). The human nephron



• **Fig. 86.4 Nephron Development.** (A) In mammals the kidney develops from the metanephric mesenchyme on invasion of the ureteric bud from the nephric duct. (B, C) The ureteric bud starts branching within the growing metanephric mesenchyme. (D) The mesenchyme condenses around the ureteric bud tips, forming the Six2-positive cap mesenchyme. (E) Renal vesicles form from the condensed cap mesenchyme. (F) A cleft develops in the comma-shaped bodies. (G) Podocyte progenitors start to attract angioblasts in the S-shaped body. (H) The developing nephron connects with the collecting duct. (From Schell C, Wanner N, Huber TB. Glomerular development: shaping the multi-cellular filtration unit. *Semin Cell Dev Biol.* 2014;36:39–49.)

complement may differ by about 10-fold between individuals, with variability in number based on age, race, and gestation events such as birthweight, prematurity, and in utero exposure to maternal factors (smoking, malnutrition, diabetes, and other agents) (Little, 2015).

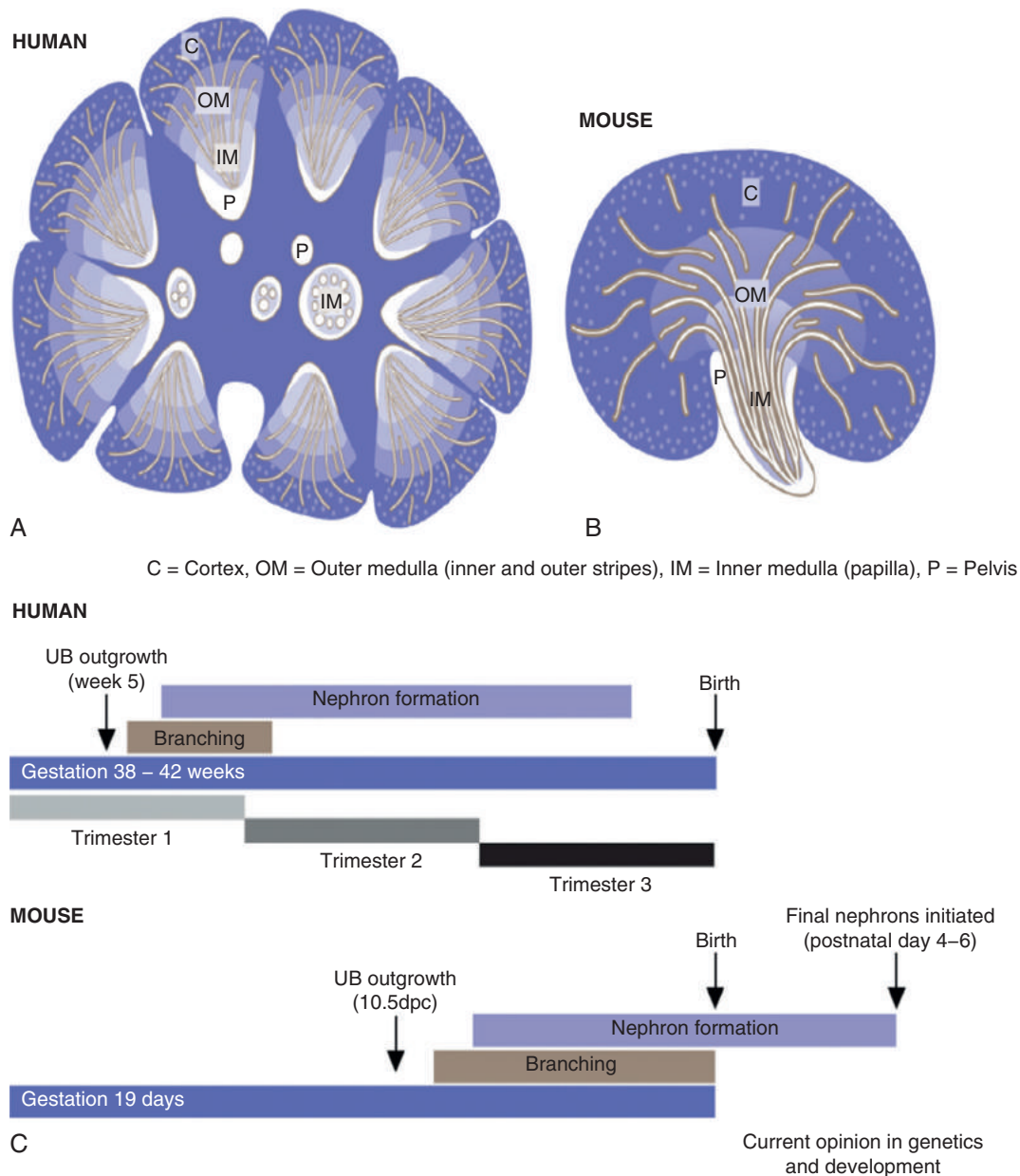
Glomerular Development

Glomerular formation occurs in stages and is dependent on specific cell type signaling within the glomerular capillary. Malformation of any component of glomerular development may result in decreased filtration ability of the nephron, typically characterized by proteinuria.

Development of the glomerulus is intricately linked with the development of the renal tubule. Glomerular formation begins at the S-shaped body stage of nephron development. Glomerular endothelial progenitors are found adjacent to the nephron developing podocytes at the cleft of the S-shaped structure during this stage. An initial capillary loop will ultimately mature into six to eight capillary loops representing the mature glomerular capillary tuft. This migration of glomerular endothelia progenitors is likely

due to VEGF secretion by the local podocytes. The amount of VEGF released by the podocytes controls capillary development by proangiogenic and antiangiogenic signaling (Herzlinger and Hurtado, 2014). It is critical that the podocyte foot processes connect tightly or the filtrate from the afferent arteriole will allow leakage of larger molecules such as protein and larger solutes, causing loss of important proteins, fluid, and solutes that need to be returned to the systemic vasculature. These foot processes are integral to the glomerular filtration barrier (Fig. 86.6).

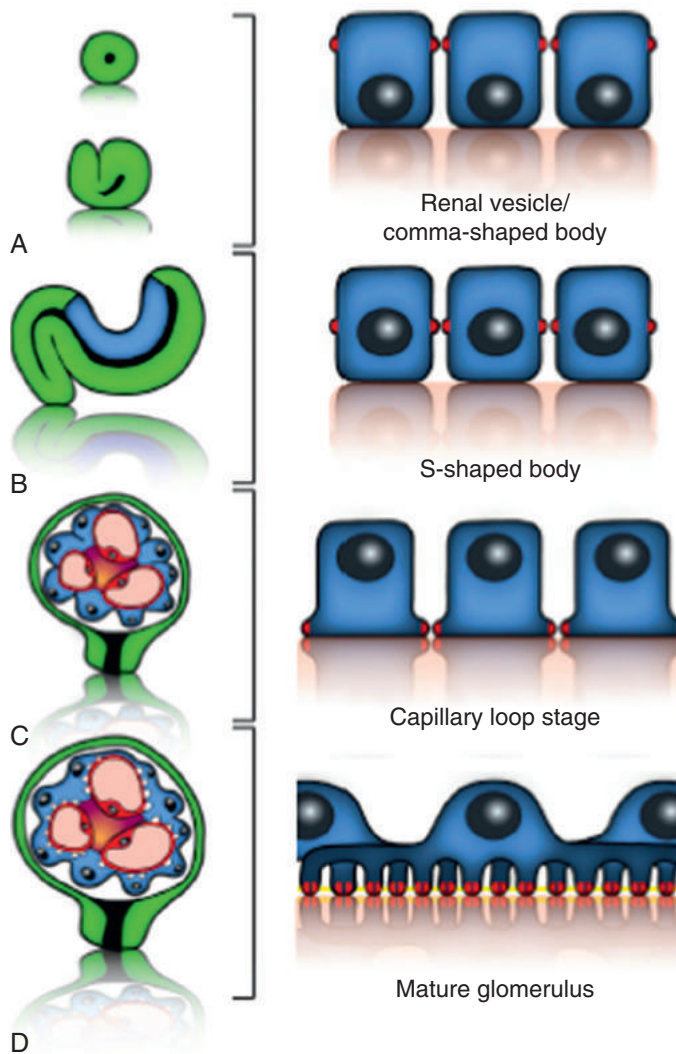
Vasculogenesis starts during the S-shaped body stage of glomerular development as mentioned earlier when expression of several VEGFs from the glomerular podocytes occurs, inducing migration of endothelial cells to the cleft of the S-shaped body. Blockage of these factors in mice causes renal disease resembling preeclampsia and end-stage renal failure by 9 weeks of age (Schell et al., 2014). VEGF-A secretion by the podocytes of the S-shaped body causes fenestration of the podocytes near the glomerular basement membrane in proximity to the glomerular endothelial cells, possibly allowing limited transmission of fluid from the vascular tuft into the glomerulus, but the function and mechanism of these fenestrations are not well understood at present.



• **Fig. 86.5** Comparative timeline of kidney development between human and mouse. The human (A) and mouse (B) kidney illustrating the anatomic differences. The human kidney consists of 8–15 lobes, each with a branching ureteric tree and inner medulla (IM; papilla), while the mouse kidney is unipapillate. (C) Comparative developmental timeline of human and mouse nephrogenesis identifying the duration of gestation, timing of initial ureteral bud (UB) outgrowth, period of ureteric branching, and period of nephron formation. Note the prolonged period of nephron formation in the human after the end of branching in comparison with the mouse. Note also that the final nephron formation in the mouse occurs in the immediate postnatal period. C, Cortex; dpc, date post conception; OM, outer medulla, P, pelvis. (From Little MH. Improving our resolution of kidney morphogenesis across time and space. *Curr Opin Genet Dev.* 2015;32:135–143.)

WT1 is a zinc finger transcription factor, with four different isoforms isolated at present. It is thought to be a transcription factor that binds to DNA as well as messenger RNA as either a repressor or an activator in different organs during development. Studies in mice showed that WT1 is important for genitourinary development. In humans, WAGR syndrome (Wilms tumor, aniridia, genitourinary abnormalities, and mental retardation) is caused by interstitial deletion of the 11p13 locus, where the *WT1* and *PAX6* genes are located. Similarly, *WT1* mutations are

thought to contribute to the abnormalities seen in Denys–Drash syndrome (mesangial sclerosis, Wilms tumor, and gonadal dysgenesis). Studies of *WT1* absence or mutations have been done extensively in mice, showing that *WT1* is essential for podocyte development and possibly for podocyte maintenance. Further study in humans is needed to better understand *WT1* interactions in normal genitourinary development as well as what aberration of *WT1* expression does in disease states (Schell et al., 2014).



• **Fig. 86.6** Specification of podocyte cell-cell contacts. (A) In the renal vesicle and comma-shaped body stage, the podocyte progenitors display a typical epithelial morphology with apical cell-cell contacts. (B) During the S-shaped body stage, apical membranes expand and cell contacts appear to shift to the basal sides of podocytes. (C) At the capillary loop stage of immature glomeruli, the podocyte cell-cell contacts are located at the basal side. (D) At mature glomeruli, the podocyte foot processes form a specialized cell-cell contact, the slit diaphragm. (From Schell C, Wanner N, Huber TB. Glomerular development: shaping the multi-cellular filtration unit. *Semin Cell Dev Biol.* 2014;36:39–49.)

Ureteral Growth and Development

The ureters are muscular tubes conveying urine from each renal pelvis to the urinary bladder. The upper ureter, leaving the kidney at the renal pelvis, is a thin-walled, funnel-shaped tube that thickens as it passes through the abdomen and enters the bladder obliquely to terminate at the bladder trigone. There are two tissue components to the ureter: the specialized endothelial-lined lumen and the outer mesenchymal coat consisting of the lamina propria, multilayered smooth muscle, and the outer tunica adventitia. This outer tunica adventitia also contains ascending and descending blood vessels, nerves, and lymphatics. The ureter does not passively allow the urine to drain from the kidney to the bladder but directs the urine to the bladder with unidirectional peristaltic contractions propelling

the urine to the bladder. The peristaltic waves, triggered by pacemaker cells, cause depolarizing contractions in the smooth muscle cells located in the ureteropelvic junction.

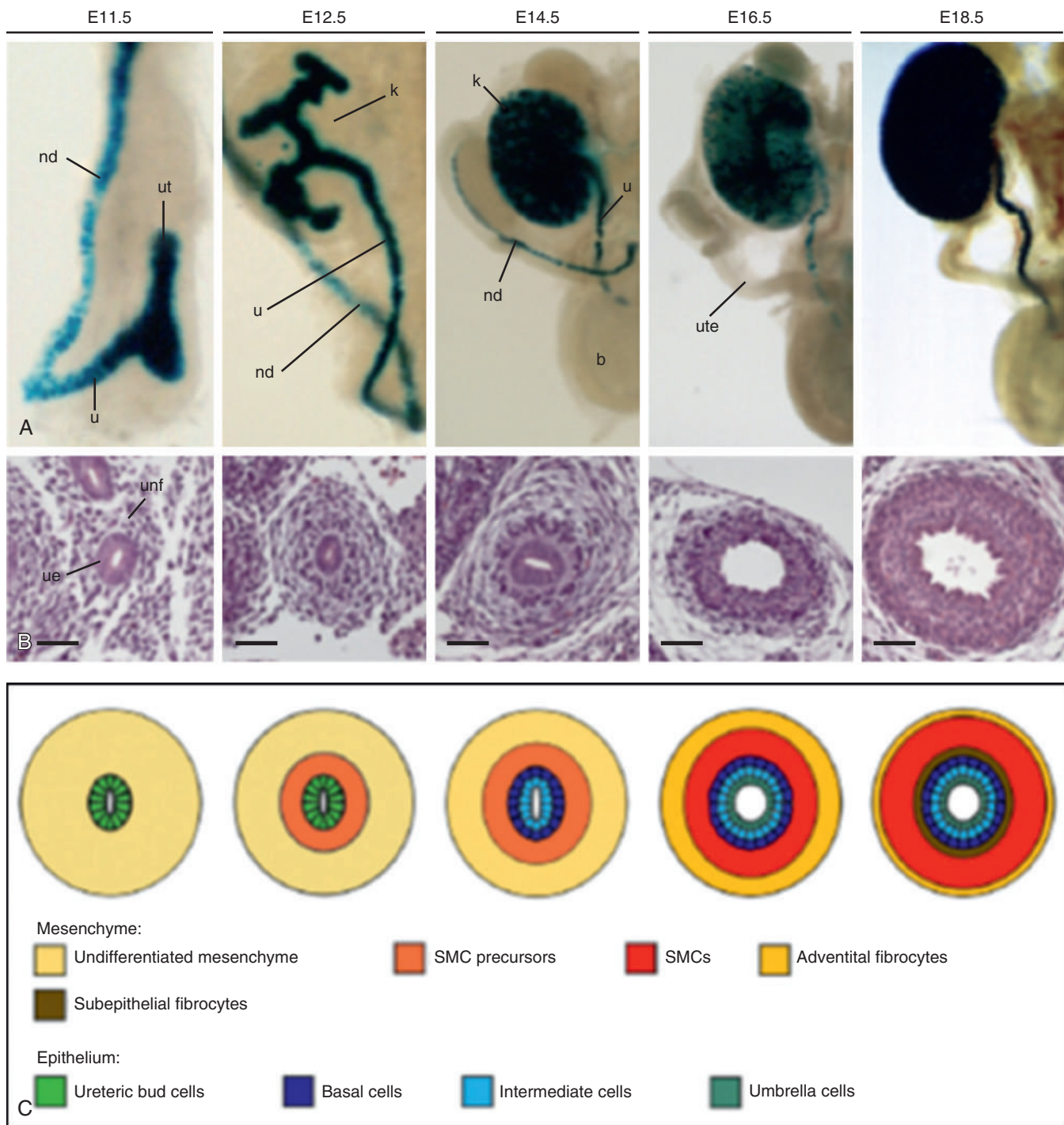
Abnormalities in the smooth muscle function, particularly in the ureteropelvic or ureterovesical junctions, prevent efficient drainage of urine from the renal pelvis to the bladder and can cause either urinary efflux or urinary reflux. Obstruction at either the upper ureter or the lower ureter, with dilation of the ureter (hydronephrosis) or renal pelvis (hydronephrosis), can cause damage to the renal parenchyma, with marked decrease or even absence of function if obstruction is particularly severe. These conditions comprise a large subset of congenital anomalies of the kidney and urinary tract and are frequent manifestations of underlying genetic defects. Diagnosis of such defects is now frequently made before birth with prenatal ultrasound imaging (Bohnenpoll and Kispert, 2014).

The ureters arise from the nephric duct as do the metanephric kidneys. The ureteral bud evaginates from the nephric duct and, ultimately through extensive branching and elongation, becomes the ducting system ending with the collecting ducts in the kidney, while the stalk portion of the ureteral bud becomes the epithelial portion of the ureter. In the human, starting on embryonic day E12.5, the distal end of the stalk separates from the nephric duct to integrate into the urinary bladder and separate from the genital tube structures. Urine production starts around E16.5, by which time the epithelium around the ureteral stalk differentiates into urothelium. The development of the smooth muscle layer starts around E13.5 to E15.5.

The development of the ureter depends on ureteral bud development and on GDNF binding to RET, which activates the formation of the endothelium from the tissue near the nephric duct. If the GDNF signaling is anterior or posterior to the normal placement of the ureteral bud, the ureters may develop ectopically in the bladder or urethra (Bohnenpoll and Kispert, 2014). The primordial ureters that arose from the nephric duct will differentiate into the vas deferens in the male, while they degenerate in the female.

To remain patent, the ureter must translocate from the nephric duct into the developing bladder wall in the cloaca. The first step occurs when the nephric duct fuses to the cloaca. This may occur from mutual signaling between the nephric duct to the cloaca; however, the precise mechanisms are poorly understood. In the second step, the distal part of the ureter is incorporated into the bladder wall, where it undergoes apoptosis until the ureter is placed in its final position on the bladder trigone. If there is aberrant distal ureteral maturation, the ureter will end blindly inside or outside the bladder as a ureterocele or an ectopically located ureter in the bladder, urethra, or other ectopic location along the genitourinary tract following the path of the involuting nephric duct. These ureters are hydronephrotic.

Further formation of the proximal, mid, and distal parts of the ureter depends on the local epithelia and mesenchyme tissue in the region of that portion of the developing ureter. The mesenchyme near the distal ureteral stalk will elongate, while the same mesenchyme transplanted to the proximal ureteral region will branch and induce nephrogenic aggregates that will lead to nephron-like structures. The signaling molecule for distal ureteral development through mesenchymal specialization is likely BMP4. Absence of or a decrease in the level of BMP4 leads to ectopic budding of the nephric duct and the distal ureteral stalk. In the human, by E12, experiments on explant metanephric cell cultures have shown that the ureteral mesenchyme is radially arrayed with connective

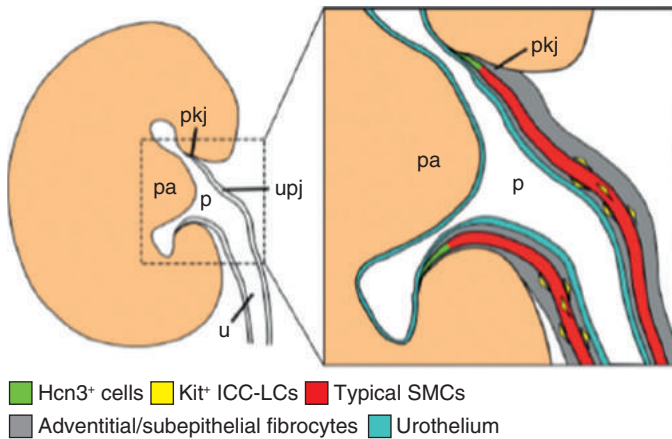


• **Fig. 86.7** Ureter morphogenesis and differentiation. (A) Visualization of the ureteric epithelium by β -galactosidase activity from a *Pax2(8.5)-lacZ* transgenic line. (B) Hematoxylin and eosin stainings on transverse 5- μ m proximal ureter sections. Scale bars represent 50 μ m. (C) Mesenchymal and epithelial differentiation in ureter development. Stages are as indicated. *b*, Bladder; *E*, embryonic day; *k*, kidney; *nd*, nephric duct; *SMC*, smooth muscle cell; *u*, ureter; *ue*, ureteric epithelium; *unf*, ureteric mesenchyme; *ut*, ureter tip; *ute*, uterus. (From Bohnenpoll T, Kispert A. Ureter growth and differentiation. *Semin Cell Dev Biol.* 2014;36:21–30.)

tissue and smooth muscle cells that depend on the adjacent epithelium to develop as the distal ureter. Signaling by sonic hedgehog (Shh) expressed by the ureteral epithelium at E11.5 to E18.5 also stimulates development of the ureter by increasing mesenchymal growth of the ureter. BMP4 appears to stimulate Shh to help

differentiate the smooth muscle cells into the developing ureter (Fig. 86.7).

At E16.5, terminally differentiated smooth muscle cells with abundant contractile filaments are located all along the developing ureter. These muscle fibers are tightly packed and interconnected



• **Fig. 86.8** Pacemaker cells in the upper urinary tract. Localization of Hcn3⁺ and Kit⁺ pacemaker cells in the upper urinary tract. (Left) A sagittal view of the kidney, pelvis, and the upper ureter. (Right) The localization of pacemaker cell populations in the magnified pelvis region of the kidney. ICC-LC, interstitial cells of cajal-like cells; p, pelvis; pa, papilla; pkj, pelvic-kidney junction; SMC, smooth muscle cell; u, ureter; upj, ureteropelvic junction. (From Bohnenpoll T, Kispert A. Ureter growth and differentiation. *Semin Cell Dev Biol.* 2014;36:21–30.)

with gap junctions, causing smooth muscle contractions to be propagated in proximal to distal waves. The waves are coordinated and are preceded by spontaneous electrical activity in the renal pelvis. This is independent of nerve function or stimulation (Bohnenpoll and Kispert, 2014). The smooth muscle cells in this region show spontaneous oscillations of a membrane potential that spreads to the other smooth muscle cells and generates the wave down the ureter to the distal part of the ureter (Fig. 86.8).

The urothelium itself is highly specialized stratified epithelium, which is the inner lining of the urinary drainage system from the renal pelvis to the proximal part of the urethra. The derivation of the epithelium in the bladder is endoderm in nature, while the ureteral epithelium is mesodermal in origin. It is uncertain if the development of this urothelium from the different origins is the same for the bladder as the ureter, but ureteral development lags that of bladder development.

Renin–Angiotensin System Interaction for Programing Fetal Development

The RAS is a systemic hormonal process mediating sodium reabsorption, vasoconstriction, aldosterone production, and vasopressin secretion. Angiotensinogen is produced by the liver and activated by renin (from the kidney) to form angiotensin I. Angiotensin II is cleaved by angiotensin-converting enzyme (ACE) to create angiotensin II, which is the most biologically active peptide.

Angiotensin II acts on the angiotensin type 1 (AT1) and angiotensin type 2 (AT2) receptors. Beyond the circulating RAS, there is also a local RAS in many other organs, including the kidney and the placenta, which will act as an autocrine or a paracrine agent (Moritz et al., 2010). The RAS plays a critical role in the growth, development, and functioning of many organs. These include the kidney and the placenta. During renal angiogenesis, angiotensin II acting on AT1 receptors mediates renal tubular growth and branching. AT2 receptors in the fetal kidney act as a growth mediator to prevent uncontrolled renal tubule growth through apoptosis. The uteroplacental circulation has a local RAS

that aids in placental angiogenesis and modulates placental cytokine, growth factor, and vasoactive substance production, which all affect fetal growth and development. Angiotensin II–stimulated AT1 receptor activity promotes trophoblast invasion, angiogenesis, growth, and branching of the placenta, while also regulating vasoconstriction in the uterine spiral arteries. Additionally, AT1 receptors have been found in the chorionic villi, showing regulation of function of fetal blood flow within the placenta.

Studies have shown that sex hormones differentially regulate the RAS pathway: testosterone increases the expression of renin and AT1 receptor with upregulation of the vasopressor arm, and estrogens increase the upregulation of the vasodepressor arm (ACE2 and AT2 receptor) (Moritz et al., 2010). The renal RAS and the placental RAS may be differentially expressed in male and female tissues, may respond differently to adverse stimuli, and may result in differences in adult males and females.

Prenatal exposure of the fetus to excessive glucocorticoids may have adverse effects. Glucocorticoids are potent regulators of fetal growth and development. Maternal glucocorticoid exposure can reduce nephron endowment and alter expression of the RAS in fetal kidneys. Studies in sheep, rats, and spiny mice have shown that even a short-term, early-gestation exposure of the fetus to dexamethasone infusions can decrease the number of nephrons.

Fetal Programing of Renal Function and Perinatal Environmental Factors Influence Development of Renal Function and Adult Renal Disease

Genetic and environmental factors are important determinants of the development and function of the major organ systems of the body. It is becoming more and more evident that prenatal programing can affect subsequent organ function and some adult diseases. Brenner has studied the effects of fetal programming on the kidney and its subsequent risks for development of hypertension and renal disease in adulthood. LBW, as defined as birthweight less than 2500 g, has been associated with high incidence of cardiovascular disease, diabetes, hypertension, and renal disease. The risk of intrauterine growth restriction (IUGR) and LBW is twice as high in African Americans as in white populations. Risk factors for IUGR and LBW are consistently found more often in certain populations. For example, fetuses with higher risk of IUGR and LBW include those of African-American or Asian origin as well as those exposed to maternal hypertension, maternal smoking, inadequate maternal weight gain or malnutrition, shorter maternal height, poor prenatal care, advanced maternal age, and lower socioeconomic status.

Animal models have shown that LBW is associated with later development of hypertension. The hypertension may be due to lower nephron numbers frequently seen with IUGR. Additionally, LBW adversely affects RAS activity. Brenner hypothesized that low nephron numbers at birth result in decreased filtration surface area, limiting renal sodium excretion, causing an increased risk of development of essential hypertension.

Human studies have also found an inverse relationship between LBW and higher blood pressures in infancy through adulthood. Although not all children with LBW have hypertension, the blood pressure measurements in these infants, children, and young adults tend to be higher than those of normal birthweight individuals. High birthweight (>4000 g) has also been associated with adverse

renal outcomes in later life. It is uncertain in humans if there is any relationship with abnormal nephron number (Luyckx et al., 2011). LBW infants also have an increased adult risk of coronary heart disease, insulin sensitivity, and hypertension. Females have approximately 12% fewer glomeruli than males. After 18 years of age, the kidneys lose approximately 3676 nephrons per kidney per year. Adult height also has a positive correlation with the number of nephrons an individual may have: for each centimeter increase in height, there are about 28,000 more glomeruli. Height was also found to contribute about two-thirds of the variance in glomerular number.

The numbers of nephrons are determined before birth, but the kidney can increase filtration capacity by hypertrophy of the glomerulus (Luyckx and Brenner, 2005; Luyckx et al., 2011). Mean glomerular volumes correlate inversely with glomerular numbers but directly with current body size. Hypertensive individuals have a 133% higher mean glomerular volume but a 46.6% reduction in glomerular numbers compared with nonhypertensive controls.

Perinatal programing controls nephrogenesis from early fetal development until 34–36 weeks' gestation, at which time the full nephron development is complete. If an infant is born before 36 weeks' gestation, nephrogenesis is still ongoing, but the number of nephrons produced may be reduced because of intrauterine stress or prenatal/perinatal stressors on development. Potential perinatal programming for hypertension and diabetes may be associated with the development of chronic kidney disease because of total nephron number reduction (Puddu et al., 2009).

Barker speculated that maternal intrauterine factors may affect prenatal development of the various fetal organ systems that will ultimately affect the function of those systems into adulthood. "Barker's hypothesis" is best demonstrated by the prenatal programming of ultimate adult development of hypertension and cardiovascular disease found in LBW infants who have a lower nephron number because of adverse maternal factors transmitted to the fetus during nephrogenesis. Barker further suggested that there is variance in gene expression during development that may be caused by different in utero conditions to which the fetus is exposed. The most critical times for fetal programming with either normal or abnormal expression are during periods of rapid cell migration, division, and differentiation. The critical programing time for human renal development is between weeks 31 and 36, as nephrogenesis is completed by week 36 (Koleganova et al., 2009).

Diabetes is the world's leading cause for end-stage renal disease. Unfortunately, LBW also has an association with development of pancreatic insufficiency and development of diabetes. In studies in monozygotic twins, the lower birthweight infant had a higher risk of developing diabetes later in life than the normal birthweight infant. The risk is highest in those LBW infants who caught up the fastest in weight. The proposed explanation for this finding is that the LBW or undernourished infant resets its insulin hormone axis to adapt to increased growth when needed for development in utero, and this upregulated metabolism may not correct itself once the infant has caught up in growth (Luyckx and Brenner, 2005).

Kidney volume is dependent on nephron number and can be measured by ultrasound testing. Young adults with a history of prematurity, regardless of their birth size, have smaller kidneys than term-matched controls, and even the effect of IUGR on kidney size as a young adult was not significant (Luyckx et al., 2011). Genetic and environmental factors can cause a reduction in total nephron number. This interaction between genetic

programing and environmental factors influences renal function potential from fetal life to adulthood.

Small for gestational age (SGA) infants have a shorter renal length than normal-sized infants, and, with aging, the kidneys of the SGA infant demonstrate either accelerated renal maturation or early compensatory kidney hypertrophy. Under the hyperfiltration hypothesis, hypertrophy of glomeruli may predispose the individual to development of hyperperfusion injury of the tubules, which then leads to deterioration of renal function and development of proteinuria, glomerulosclerosis, and tubular and interstitial inflammation and fibrosis (Puddu et al., 2009).

The kidney is central in regulating blood pressure and is implicit in causing hypertension. The kidney's regulation of sodium homeostasis, intravascular fluid regulation, and regulation of solute filtering is part of renal regulation of blood pressure and is why the kidney is implicated in development of hypertension when these functions do not occur appropriately. Other risk factors for the development of hypertension and renal disease are a family history of the same renal problems, hypertension, diabetes, obesity, gestational diabetes, dietary factors, insulin resistance, and being a member of certain otherwise at-risk ethnic groups.

Malnutrition of the mother through low protein intake during intrauterine and neonatal life may have an adverse effect on renal function; ongoing renal damage is caused by continued high salt intake and an unbalanced fat and protein diet later in life. The developing kidney may also be adversely affected by vitamin A deficiency, urinary tract malformation with obstruction or infection, and exposure to nephrotoxic drugs (antibiotics, nonsteroidal antiinflammatory drugs [NSAIDs]). Other fetoplacental factors causing a decrease in the necessary nutrients by restricting their transfer include maternal smoking, hypertension, poor nutrition, and socioeconomic factors affecting nutrition. Additionally, IUGR may be caused by decreasing available intrauterine space through primiparity, low maternal height, and being a mother who was also an SGA baby (Puddu et al., 2009).

Perinatal obstructive uropathy may cause reduced nephron numbers in the affected individual. The diminution of nephrons with obstruction may be due to mechanical stretching of the tubules, which in turn activates ion channels that increase intracellular calcium levels and, subsequently, cellular apoptosis (Puddu et al., 2009). In animal models with unilateral ureteral obstruction with subsequent relief of the obstruction, the major damage found was due to tubular apoptosis and atrophy causing subsequent impaired growth of the postobstructed kidney, reduced glomerular numbers, and decreased GFR. The uninvolved kidney will show compensatory growth, but both kidneys will show glomerular sclerosis, tubular atrophy, macrophage infiltration, and interstitial fibrosis. Urinary obstruction in adults does not appear to cause reduction in the nephron number that is seen in congenital or perinatal obstruction. There may possibly be an increased vulnerability of the nephrons to damage with obstruction occurring during or just after completion of nephrogenesis. High-grade vesicoureteral reflux diagnosed shortly after birth may also be associated with poor renal outcome. Unfortunately, early postnatal diagnosis and treatment did not improve the renal outcomes in this group of patients either (Puddu et al., 2009).

Vitamin A has been found to determine fetal renal programming in rats through modulation of nephron number and vascular supply to the developing kidney. Vitamin A and other retinoids help regulate cell proliferation, differentiation, immune function, and apoptosis. These animal findings provide indirect evidence of the importance of vitamin A in human kidney development. Low

circulating levels of vitamin A are common in women who are smokers, abuse alcohol, or have poor perinatal nutrition, which may cause low or absent vitamin A levels in the developing fetus and is associated with IUGR in infants.

Maternal intake of nephrotoxic drugs during pregnancy has also been associated with neonatal development of acute renal failure, especially in preterm or LBW infants. It is postulated that maternal exposure to NSAIDs may cause hypoperfusion of the kidneys and fetal nephrotoxicity from vasomotor nephropathy due to decreased levels of prostaglandin E_2 . There appears to be less damage to the newborn kidney with NSAID exposure suggesting that the risk of damage is highest during nephrogenesis itself.

Treatment of children with low renal reserve who have hypertension or hyperalbuminuria with ACE inhibitors may have a renal protective role through reducing angiotensin II–modulated cellular apoptosis and decreased renal tubular and interstitial fibrosis. However, use of ACE inhibitors is not without risks to kidney function as they have been found to cause hypotension, oliguria, hyperkalemia, acute renal failure, and reduced glomerular filtration, especially in patients with bilateral renal artery stenosis.

Although low nephron number is associated with renal disease and hypertension, not all renal programming models show this; some studies have found associated hypotension. Some ethnic groups, for example, African Americans, with decreased nephron numbers have not exhibited associated hypertension. Some other as yet undetermined factors may be required to induce hypertension and renal disease. Some factors that may or may not have an association with low nephron number and hypertension/renal disease may include individual genetics, including the sex of the individual, with less hypertension generally seen in premenopausal females with decreased nephron numbers. Sex differences in arterial pressure may be due to sex hormones as well as sex chromosomal complement. Female fetuses also appear to be less vulnerable to adverse in utero environmental factors than male fetuses (Kett and Denton, 2011).

Some modifiable risk factors such as diet and stress may improve or worsen ultimate renal outcome. Limited human studies have shown a higher sensitivity to dietary salt intake with respect to high blood pressure development in adults with a history of LBW as compared with normal birthweight adults. This salt sensitivity for blood pressure has also been found in studies of children. Animal studies have shown that salt sensitivity can be modulated early in life if a low-salt diet is instituted (Kett and Denton, 2011).

Early renal programming studies in animals demonstrated an increase in systemic blood pressure when there was in utero exposure to a low-protein maternal diet, malnutrition, or dexamethasone. More recent studies have found that the resting blood pressure in these animals is not different from the same resting pressures in controls. Blood pressure elevations appeared to be related to secondary stressors being present. Historic human studies using the Dutch Famine cohort found that the baseline blood pressures were similar between those exposed to famine and those who were not. Blood pressure elevations were higher in famine-exposed groups when they had additional stress, especially if they were exposed to famine early in gestation (Kett and Denton, 2011).

Although fetal renal programming may put the LBW, IUGR, and individuals with in utero environmental exposure to renal damaging factors at risk of hypertension and poor renal function as an adult, there appears to be a significant ability of the kidney

to compensate for these prenatal insults to normal renal development. By control of secondary risk factors such as diet and stress in the adult, there is the possibility of reducing adverse outcomes (hypertension and renal insufficiency) because of abnormal renal developmental (Kett and Denton, 2011).

Conclusion

Renal organogenesis is a complex process that is not yet fully understood but has complementary interactions stimulated by anchor genes, activator molecules, growth factors, and hormone effects. Environmental factors in the maternal–placental–fetal interface are being further elucidated, with implications in adverse adult health outcomes, but some of these may be offset by modification of secondary factors.

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Developmental Abnormalities of the Kidneys

RACHEL ENGEN AND SANGEETA HINGORANI

KEY POINTS

- Renal malformations account for 20%–30% of all prenatally diagnosed developmental anomalies and are responsible for 31% of all childhood end-stage renal disease.
- Patients with unilateral renal agenesis are at risk for hypertension in childhood and chronic kidney disease in adulthood.
- Multicystic dysplastic kidney typically presents as a collection of large renal cysts on ultrasound, and patients generally do well. Autosomal recessive polycystic kidney disease presents as bilaterally enlarged hyperechoic kidneys, often without cysts, and patients may have significant pulmonary and renal complications.
- The outcome for patients with renal dysplasia depends significantly on the amount of functioning renal tissue and the associated congenital anomalies.
- Infant dialysis has improved rapidly over recent years, and 2-year survival outcomes as high as 80% are now reported by multinational dialysis databases.

Normal renal development begins with two precursor organs, the pronephros and mesonephros, followed by the definitive metanephros at approximately 5 weeks' gestation. The metanephros develops from an interaction between a branch of the mesonephric (Wolffian) duct, called the ureteric bud, and the surrounding metanephric mesenchyme. The repeatedly branching ureteric bud will become the collecting system, including the collecting ducts, renal pelvis, ureter, and bladder trigone. The metanephric mesenchyme will form the glomerular podocytes, proximal tubule, loop of Henle, and distal convoluted tubule. Nephrogenesis begins at 9 weeks' gestation and is complete by 32–36 weeks' gestation. A complex and carefully timed web of signaling “crosstalk” between the mesonephric duct, metanephric mesenchyme, and surrounding tissue is necessary to coordinate proper renal development. Any interruption of this process can lead to abnormalities in kidney structure and function (Reidy and Rosenblum, 2009).

Developmental abnormalities of the kidney and the urinary tract affect 3–6 per 1000 births (Sanna-Cherchi et al., 2007) and account for 31% of all children with end-stage renal disease (ESRD) in the United States (Reidy and Rosenblum, 2009). Renal malformations represent 20%–30% of all prenatally diagnosed developmental

anomalies; prenatal ultrasound allows malformations that are asymptomatic at birth to be detected. Long-term outcomes for children with development anomalies of the kidney depend on the amount of functional renal tissue at birth, associated urinary tract anomalies, degree of prematurity, urine output, and presence of additional congenital anomalies.

With advances in neonatal critical care, nutritional therapy, and peritoneal dialysis techniques, many children who previously would have died in early infancy are surviving, growing, and receiving a renal transplant. Reviews of multinational dialysis databases have reported 2-year survival rates as high as 80% in infants who start dialysis (van Stralen et al., 2014). Successful provision of care requires a multidisciplinary team approach that includes the neonatologist or pediatrician, nephrologist, surgeon, urologist, nutritionist, nurse, and social worker. Above all, it requires close communication with and strong support of the child's family, who may bear enormous emotional and financial burdens in caring for their child.

Abnormalities of Kidney Number

Unilateral Renal Agenesis

Unilateral renal agenesis, or a congenital solitary kidney, results from a unilateral early and complete failure in the signaling interaction between the ureteric bud and metanephric mesenchyme. The estimated incidence is 1 in 500 to 1 in 1000 children (Shapiro et al., 2003). Approximately 30%–40% of children with unilateral renal agenesis will have other developmental anomalies; roughly 25% will have vesicoureteral reflux with the remainder having ureter–pelvic junction obstruction and gastrointestinal, cardiac, and musculoskeletal anomalies (Westland et al., 2013). The same embryologic insult that led to failure of kidney development can also cause abnormalities of other mesonephric duct derivatives, including the seminal vesicles, vas deferens, and epididymis (Shapiro et al., 2003) and Müllerian duct organs. Approximately 50% of girls with unilateral renal agenesis will have genital tract abnormalities, including unicornate or didelphic uterus and vaginal obstruction (Hollander et al., 2008).

Renal agenesis was once thought to be a benign condition if the contralateral kidney appeared healthy. However, there is now increasing concern that unilateral renal agenesis increases the risk

TABLE 87.1 Syndromes With Renal Agenesis and Ectopia

| Syndrome | Inheritance | Genes | Percent With Renal Disease | Type of Renal Involvement | Other Key Features |
|--------------------------------|----------------------|---|----------------------------|---|--|
| Fraser | AR | <i>FRAS1</i> , <i>FREM1</i> , <i>FREM2</i> , <i>GRIP1</i> | 67 | Bilateral or unilateral renal agenesis | <ul style="list-style-type: none"> Failure of eyelid formation Syndactyly Genital anomalies Laryngeal stenosis |
| Kallman | XR | <i>KAL-1</i> | 30 | Unilateral or bilateral renal agenesis Horseshoe kidney Vesicoureteral reflux | <ul style="list-style-type: none"> Hypogonadism Olfactory defects Mirror movements of arms Sensorineural hearing loss |
| Mayer–Rokitansky–Küster–Hauser | Sporadic or familial | <i>WNT4</i> | 20–40 | Unilateral renal agenesis | <ul style="list-style-type: none"> Congenital absence of uterus and upper two-thirds of vagina Vertebral, cardiac, skeletal, ocular, and ear involvement |
| OEIS complex | Sporadic | Unknown | 36 | Renal agenesis Vesicoureteral reflux | <ul style="list-style-type: none"> Omphalocele Cloacal extrophy Imperforate anus Spinal defects |
| Turner syndrome | Sporadic | XO karyotype | 33 | Renal agenesis Horseshoe kidney Ectopic kidney | <ul style="list-style-type: none"> Coarctation of aorta Bicuspid aortic valve Short webbed neck Ovarian failure |
| Thrombocytopenia-absent radius | AR | <i>RBM8A</i> | 23% | Horseshoe kidney | <ul style="list-style-type: none"> Bilateral absent radii Bilateral thumbs present Thrombocytopenia Cardiac malformations |

AR, Autosomal recessive; OEIS, omphalocele-exstrophy-imperforate anus-spinal defects; XR, X-linked recessive.

Data from Zenteno et al., 1999; Keppler-Noreuil, 2001; Greenhalgh et al., 2002; Massin et al., 2003; Oppelt et al., 2012; Barisic et al., 2013; Bernardo et al., 2015; Rall et al., 2015.

for chronic kidney disease in adulthood (Chevalier, 2009; Sanna-Cherchi et al., 2009). The “hyperfiltration” hypothesis suggests that compensatory hyperfiltration in the healthy kidney leads to single-nephron hypertension, glomerulosclerosis, and nephron loss over time (Brenner et al., 1996). Recent studies of outcomes for children with congenital solitary kidney identified proteinuria and hypertension in 5%–25% of patients during childhood (Vu et al., 2008; Westland et al., 2011), while chronic kidney disease affects approximately 13%–30% of adults with a solitary kidney (Shapiro et al., 2003; Vu et al., 2008). The presence of other urinary tract abnormalities or any dysplasia in the remaining kidney worsens the prognosis.

Unilateral renal agenesis is asymptomatic in infants with a healthy contralateral kidney. The routine use of voiding cystourethrogram to screen for vesicoureteral reflux in infants without evidence of a urinary tract infection is now controversial. Children with unilateral renal agenesis should be monitored for appropriate compensatory hypertrophy of the healthy kidney during infancy and should have annual screening for proteinuria and hypertension during childhood (Vu et al., 2008). Girls with a solitary kidney should be screened for uterine abnormalities before puberty (Hollander et al., 2008; Table 87.1).

Bilateral Renal Agenesis

Bilateral renal agenesis occurs in 1 in 3000 births. It may be an isolated finding or part of a syndrome, such as the brachio-oto-renal

dysplasia syndrome or a hereditary renal adysplasia. It is typically diagnosed prenatally in a pregnancy complicated by severe oligohydramnios or anhydramnios and nonvisualization of the fetal kidneys and bladder. Bilateral renal agenesis is responsible for approximately 20% of cases of the oligohydramnios sequence (Potter syndrome) (Elder, 2016), in which decreased amniotic fluid causes compression of the fetus. Patients have the classic low-set ears, wide-set eyes with epicanthal folds, flat nose, and receding chin (Fig. 87.1). Infants are typically born alive but die within the first hours to days of life because of pulmonary hypoplasia. There is one case report of treatment with serial amniotransfusion that resulted in the birth of an infant who survived with minimal respiratory support (Bienstock et al., 2014).

Abnormalities of Renal Position

Ectopic Kidney

Renal ectopia occurs when the kidney fails to ascend from its embryologic position in the fetal pelvis to its final position in the renal fossa. Ectopia can be simple, with the kidney located ipsilateral to its uterine insertion, or crossed, with the kidney located contralaterally. Crossed ectopic kidneys typically fuse to the orthotopic kidney (van den Bosch et al., 2010). Approximately 20%–30% of patients will have vesicoureteral reflux usually into the orthotopic kidney (Guarino et al., 2004; van den Bosch et al., 2010). It is found in 1 in 1000 individuals on autopsy and is typically

asymptomatic; approximately 90% of patients are never diagnosed. Ectopia is not associated with hypertension, proteinuria, or chronic kidney disease. Patients are typically not followed closely if there is no evidence of reflux or obstruction.

Horseshoe Kidney

A horseshoe kidney occurs when the two kidneys are fused, typically at the lower poles, by a parenchymal or fibrous isthmus. This fusion impedes the embryologic ascent of the horseshoe kidney



• **Fig. 87.1** Potter Syndrome. Potter syndrome facies with low-set ears, wide-spaced eyes with epicanthal folds, flattened nose, and receding chin. (Courtesy of Dr. Laura Finn, Seattle Children's Hospital, Department of Pathology, Seattle, Washington.)

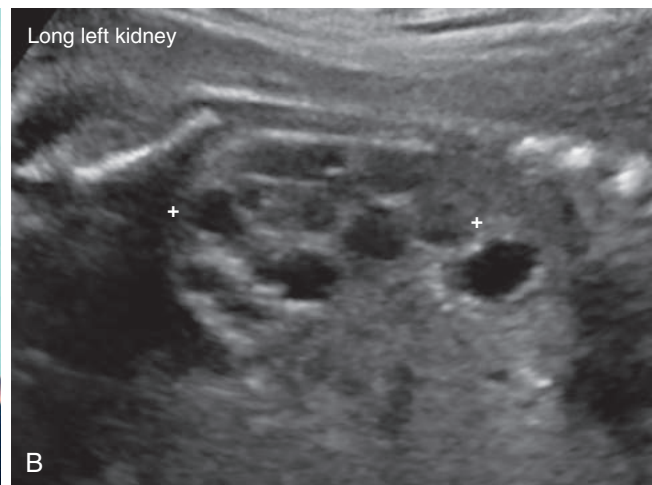
past the origin of the inferior mesenteric artery. It occurs in 1 in 400 children. Twenty-two percent of cases are associated with other systemic abnormalities, including vertebral anomalies, anorectal malformations, and Turner syndrome, and 20%–30% of children will have vesicoureteral reflux. Urinary tract infections and renal stones are common. The risk of progression to chronic kidney disease is primarily dependent on the amount of kidney damage suggested by proteinuria, hypertension, and renal scarring (Yavuz et al., 2015).

Abnormalities of Renal Organization

Multicystic Dysplastic Kidney

A multicystic dysplastic kidney (MCDK) develops when there is an impairment of nephrogenesis, resulting in branched ducts surrounded by connective tissue and undifferentiated cells. Occasionally there will be areas of recognizable renal tissue, but the ureter is not patent and the kidney is nonfunctional (Schreuder et al., 2009). MCDK is typically a sporadic and unilateral finding that occurs in 1 in 4300 births. It is the second most common cause of a flank mass in newborns. MCDK can be accurately diagnosed on ultrasound as a collection of large cysts that do not communicate with the renal pelvis (Kerecuk et al., 2008); a nuclear medicine scan is not necessary for diagnosis (Sarhan et al., 2014; Whittam et al., 2014) (Fig. 87.2).

Approximately 20% of patients with MCDK have vesicoureteral reflux in the contralateral kidney; 40% of these children will have severe (grade III–V) reflux (Schreuder et al., 2009). However, given the low rate of clinical intervention in some recent cohorts, the need for routine screening voiding cystourethrogram (VCUG) is controversial. If VCUG is not performed, the parents should be given instructions on monitoring for urinary tract infections (Calaway et al., 2014). Five percent of MCDKs are associated with a contralateral ureteropelvic or ureterovesical junction obstruction; approximately 15% will have other developmental abnormalities (Schreuder et al., 2009).



• **Fig. 87.2** Multicystic Dysplastic Kidney. (A) Gross pathology specimen of a multicystic dysplastic kidney. (B) Prenatal ultrasound image of a multicystic dysplastic kidney. Note the many large cysts. (A, Courtesy of Dr. Laura Finn, Seattle Children's Hospital, Department of Pathology, Seattle, Washington; B, Courtesy of Jennifer McBroom, Seattle Children's Hospital, Department of Radiology, Seattle, Washington.)

Multicystic dysplastic kidneys undergo spontaneous involution over time, with 5% of prenatally detected MCDKs no longer visible at birth and 40%–55% involuted by age 5 years. If the contralateral kidney is normal, MCDK is usually asymptomatic, with a prognosis similar to unilateral renal agenesis (see earlier). In rare cases MCDK may be a bilateral finding; these infants generally die soon after birth because of pulmonary hypoplasia. Patients should have routine ultrasound monitoring in infancy to ensure involution of the MCDK and appropriate compensatory hypertrophy of the contralateral kidney, with annual screening throughout childhood for hypertension and proteinuria. Nephrectomy is generally only performed if there is a clear indication. Older studies associated MCDK with an increased incidence of Wilms tumor, but more recent cohorts have not found an association (Eickmeyer et al., 2014; Moralioglu et al., 2014). Currently, there is no routine tumor monitoring recommended for MCDK.

Isolated Renal Dysplasia

Renal dysplasia occurs when either failure of ureteric bud-metanephric mesenchyme signaling or early urinary flow obstruction disrupts the normal development and differentiation of the fetal kidney (Chen and Chang, 2015). The tissue is made up of primitive ducts, branches of the ureteric bud, surrounded by a ring of fibromuscular tissue and disorganized lobar development (Kakkar et al., 2006). Renal dysplasia may be unilateral or bilateral, isolated or syndromic, and sporadic or genetic. Mutations in *ITGA8*, an integrin important to cell structure and signaling, and *FGF20*, a fibroblast growth factor with a variety of functions in growth and development, have been associated with dysplasia and/or agenesis. Renal dysplasia occurs in 0.1%–3% of births and is the most common cause of childhood ESRD (Chen and Chang, 2015).

Renal dysplasia is typically diagnosed prenatally or postnatally with the appearance of large, bright kidneys on ultrasound. Cysts may or may not be present. Treatment and prognosis depend on the degree of dysplasia and associated findings. Children with unilateral renal dysplasia and a normal contralateral kidney may have outcomes similar to children with unilateral renal agenesis. Children with bilateral renal dysplasia have variable outcomes depending on the degree of residual renal function. Mild bilateral dysplasia may result in adequate amniotic fluid production for lung development; however, generally, there is a progressive decline in renal function in infancy or childhood. Severe bilateral dysplasia has significantly worse postnatal outcomes, particularly if children develop the oligohydramnios sequence (Potter syndrome) with pulmonary hypoplasia (Winyard and Chitty, 2008). Treatment may include dialysis, but the appropriateness of dialysis is typically determined on a case-by-case basis after discussion of the multidisciplinary care team and parents.

Prognosis for children with renal dysplasia associated with genetic syndromes often depends on the patient's other developmental abnormalities. Some of the more common syndromes are presented below and in Table 87.2.

Renal Coloboma Syndrome

Renal coloboma syndrome (aka papillorrenal syndrome) is an autosomal dominant disorder caused by mutations in *PAX2*, a transcription factor involved in development. Affected children have optic nerve coloboma (dysplasia) and small dysplastic kidneys (Schimmenti, 2011). Vesicoureteral reflux, high-frequency hearing

loss, and central nervous system anomalies may also be present. Most patients have progressive renal dysfunction, though the timing is highly variable, even in families with the same *PAX2* mutation (Cheong et al., 2007; Schimmenti, 2011).

Brachio–Oto–Renal Syndrome

Branchio–oto–renal syndrome is an autosomal dominant condition affecting 1 in 40,000 newborns. It is caused by mutations in *EYA1*, *SIX1*, or *SIX5*, which interact in the development of the branchial arches, inner ear, and kidney (Bertucci et al., 2015). Clinical manifestations include branchial arch anomalies (clefts, fistula, cysts), preauricular pits, hearing impairment (conductive or sensorineural), and renal anomalies ranging from unilateral dysplasia to bilateral agenesis (Kochhar et al., 2007).

Hypothyroidism–Deafness–Renal Dysplasia Syndrome

Hypoparathyroidism–deafness–renal dysplasia syndrome (aka Barakat syndrome) is an autosomal dominant disorder with variable penetrance caused by mutations in *GATA3*, a transcription factor involved in embryologic development (Gaynor et al., 2009). Patients can present at any age with symptomatic hypocalcemia secondary to hypoparathyroidism or early-onset bilateral sensorineural hearing loss that worsens with age. The associated renal abnormalities include unilateral or bilateral renal dysplasia or agenesis, though vesicoureteral reflux, proteinuria, and progressive chronic kidney disease have been described (Maleki et al., 2013; Shim et al., 2015).

VACTERL

The VACTERL association consists of vertebral defects, anal atresia, cardiac anomalies, trachea–esophageal fistula, renal malformations, and limb abnormalities; diagnosis is made by the presence of three of the component features. The genetics of VACTERL are heterogeneous, and many of the currently identified mutations are related to the Sonic hedgehog signaling cascade (Reutter et al., 2016). Renal anomalies are one of the most common component features of VACTERL, found in approximately 65%–80% of affected children, and typically consist of unilateral renal agenesis or dysplasia. There is an increased risk of chronic kidney disease in childhood. Twenty-seven percent of those with renal anomalies and 12% of children without visible renal anomalies will have vesicoureteral reflux (Cunningham et al., 2014).

Eagle–Barrett Syndrome

Eagle–Barrett (prune-belly) syndrome is the triad of deficient abdominal wall musculature, bilateral undescended testes, and urinary tract abnormalities including renal dysplasia and an enlarged, hypotonic bladder (Fig. 87.3). It affects 3.7 per 100,000 live male births; females comprise less than 5% of cases. Cardiac, gastrointestinal, and musculoskeletal abnormalities are common; 10% of children have respiratory insufficiency related to oligohydramnios and pulmonary hypoplasia. Ten to 25% of children die in the neonatal period of respiratory failure or prematurity. Because of poor bladder tone, most patients have vesicoureteral reflux and develop urinary tract infections. Urologic management with intermittent catheterization or surgery is important to allow adequate bladder drainage and preserve renal function. Forty to 50% of children develop chronic kidney disease, and 15% will ultimately

TABLE 87.2 Syndromes With Renal Dysplasia

| Syndrome | Inheritance | Genes | Percent With Renal Disease | Type of Renal Involvement | Other Key Features |
|---|-------------|---|----------------------------|--|---|
| Alagille | AD | <i>JAG1</i> , <i>NOTCH2</i> | 40 with <i>JAG1</i> | Renal dysplasia Renal tubular acidosis Vesicoureteral reflux | <ul style="list-style-type: none"> • Paucity of intrahepatic bile ducts • “Butterfly” vertebrae |
| Brachio–oto–renal | AD | <i>EYA-1</i> , <i>SIX1</i> , <i>SIX5</i> | 67 | Unilateral dysplasia Bilateral renal agenesis | <ul style="list-style-type: none"> • Branchial arch anomalies • Hearing impairment |
| Cornelia de Lange | AD, XD | <i>NIPBL</i> , <i>SMC1A</i> , <i>SMC3</i> , <i>RAD21</i> , <i>HDAC8</i> | 36 | Renal dysplasia Renal ectopia Vesicoureteral reflux | <ul style="list-style-type: none"> • Short stature • Hirsutism, low hairline • Limb abnormalities • Hearing loss |
| DiGeorge | AD | 22q11.2 deletion | 30 | Renal dysplasia Renal agenesis MCDK Vesicoureteral reflux | <ul style="list-style-type: none"> • Facial dysmorphism • Cardiac malformations • Congenital hypoparathyroidism • Absent thymus |
| Ectrodactyly, ectodermal dysplasia, and cleft lip/palate (EEC1) | AD | 7q11.2–q21.3 | 20 | Renal dysplasia Ureterocele Vesicoureteral reflux | <ul style="list-style-type: none"> • Absent digits, “split” hand/foot • Fair hair • Hyperkeratotic skin • Cleft palate, cleft lip |
| Fanconi anemia | XR | Heterogeneous | 5 | Renal dysplasia Renal agenesis | <ul style="list-style-type: none"> • Microcephaly • Café au lait spots • Absent radii • Thumb malformations |
| Fryns | AR | Unknown | 35 | Renal dysplasia | <ul style="list-style-type: none"> • Congenital diaphragmatic hernia • Pulmonary hypoplasia • Distal finger hypoplasia • Craniofacial anomalies |
| Hypoparathyroidism–deafness–renal dysplasia | AD | <i>GATA3</i> | >60 | Renal dysplasia Renal agenesis Nephrotic syndrome Vesicoureteral reflux | <ul style="list-style-type: none"> • Hypoparathyroidism • Bilateral hearing loss |
| Pallister–Hall | AD | <i>GLI3</i> | 21–36 | Renal agenesis Renal dysplasia Vesicoureteral reflux | <ul style="list-style-type: none"> • Hypothalamic hamartoma • Polydactyly • Imperforate anus |
| Prune-belly | AR | Unknown | >97 | Hydronephrosis Renal dysplasia | <ul style="list-style-type: none"> • Absent abdominal wall muscle • Bilateral cryptorchidism |
| Renal coloboma | AD | <i>PAX2</i> | >90 | Renal dysplasia Vesicoureteral reflux | <ul style="list-style-type: none"> • Optic nerve coloboma • Hearing impairment • Central nervous system anomalies |
| Townes–Brocks | AD sporadic | <i>SALL1</i> | 27–42 | Renal dysplasia Chronic kidney disease | <ul style="list-style-type: none"> • Imperforate anus • Dysplastic ears • Hearing loss • Thumb anomalies |
| VACTERL | Sporadic | Unknown | 65–80 | Renal dysplasia Renal agenesis Vesicoureteral reflux | <ul style="list-style-type: none"> • Vertebral defects • Anal atresia • Tracheoesophageal fistula • Limb abnormalities |
| Wolf–Hirschhorn | Sporadic | 4p16 deletion | 40 | Renal dysplasia Renal hypoplasia | <ul style="list-style-type: none"> • Seizures • Cognitive disabilities • Growth delay |

AD, Autosomal dominant; AR, autosomal recessive; MCDK, multicystic dysplastic kidney; XR, X-linked recessive.

Data from Kohlhasse, 2007; Slavotinek, 2004; Goodship et al., 1997; Selicorni et al., 2005; Kochhar et al., 2007; Allen and Maestri, 2008; Narumi et al., 2010; Schimmenti, 2011; Alter and Rosenberg, 2013; Debost-Legrand et al., 2013; Kamath et al., 2013; Maleki et al., 2013; Cunningham et al., 2014; Seidel et al., 2015.



• Fig. 87.3 Eagle–Barrett Syndrome.

require dialysis or renal transplantation, often before school age (Seidel et al., 2015).

Abnormalities With Renal Overgrowth

Beckwith–Wiedemann syndrome is an overgrowth disorder caused by abnormal methylation in two gene regulation regions, *IC1* and *IC2*. Most cases are sporadic; in rare cases there is autosomal dominant inheritance. Patients are large at birth with hemihyperplasia, abdominal wall defects, enlarged tongues and internal organs, hyperinsulinemic hypoglycemia, *nevus flammeus* capillary malformations, and dysmorphic facial features. Nephromegaly is the most common kidney finding, though renal cysts, nephrolithiasis, and urinary tract malformations have also been reported (Mussa et al., 2012). The kidneys contain nephrogenic rests, areas of embryonic kidney tissue that are at high risk for malignant transformation. Ten percent of patients with Beckwith–Wiedemann syndrome develop embryologic cancers before 10 years of age, and 40%–60% of these cases are Wilms tumor (nephroblastoma). Tumor screening by abdominal ultrasound is recommended for all patients every 3–4 months until age 8 years (Mussa et al., 2015).

Simpson–Golabi–Behmel syndrome is an X-linked condition associated with mutations in *glypican3*, which may be involved in regulating embryologic growth. Affected children have many of the same findings as in Beckwith–Wiedemann syndrome, though hemihypertrophy and *nevus flammeus* are typically absent. There is a similar risk for Wilms tumor, and a similar screening schedule is typically recommended (Knopp et al., 2015).

Perlman syndrome is an autosomal recessive congenital overgrowth disorder caused by mutations in *DIS3L2*, a gene involved in ribonucleic acid processing. Patients are large at birth with poor muscle tone and characteristic facial features. There is often polyhydramnios in utero and nephromegaly. In one case series, 29% of children with Perlman syndrome developed Wilms tumor, half of those tumors were bilateral, and the average age at diagnosis was less than 2 years (Morris et al., 2013). Over half of patients

with Perlman syndrome die of respiratory or renal failure within the first month of life (Alessandri et al., 2008).

Abnormalities Predominated by Renal Cysts

Ciliopathies

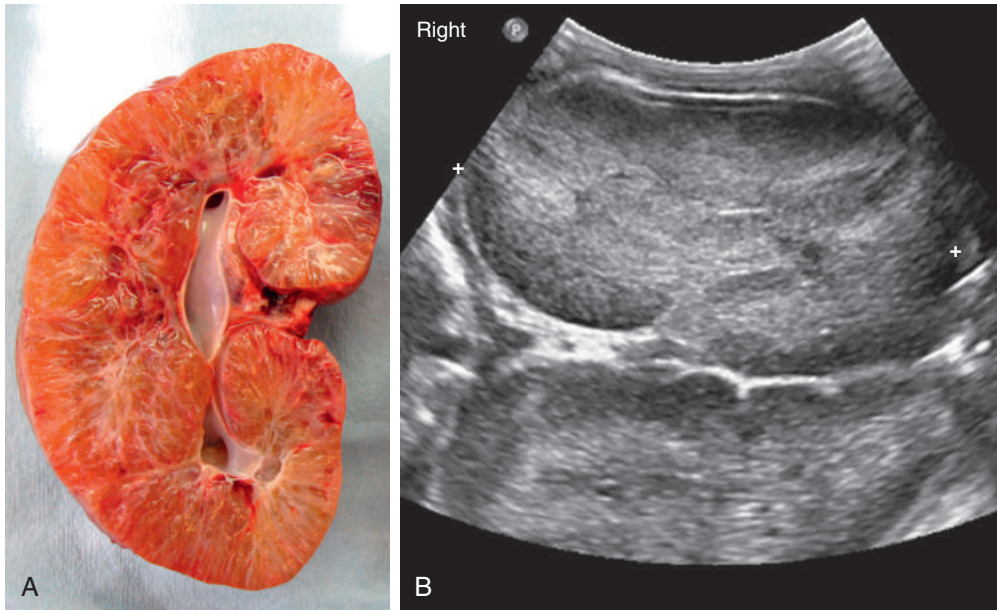
Primary cilia are protrusions of the cell membrane overlying a microtubule-based axon core. At the base of the cilium is a basal body, which is one of the two centrioles of the centrosome. Primary cilia sense the extracellular environment and transmit signals to the center of the cell, and they are critical to maintaining coordinated cell polarity (Schwartz et al., 2011). Failure to maintain cell polarity in renal tubular cells results in the development of renal cysts, which is why diverse renal cystic syndromes are now being grouped as ciliopathies. With the exception of a few diseases, including autosomal recessive and autosomal dominant polycystic kidney disease, the ciliopathies are highly heterogeneous genetically. Many syndromes are associated with a dozen or more genes, and single genes are often associated with multiple syndromes depending on the exact mutation and the presence of other cilia gene abnormalities. This genetic diversity can also lead to a high degree of clinical variability within any one syndrome and significant overlap between genetically related syndromes.

Autosomal Recessive Polycystic Kidney Disease

Autosomal recessive polycystic kidney disease (ARPKD) is an autosomal recessive hepatorenal fibrocystic syndrome associated with mutations in *PKHD1*, which encodes a protein of unknown function that localizes to the primary cilia, mitotic spindle, and apical membranes of renal tubule and biliary duct cells. *PKHD1* is expressed in a number of tissues during fetal development, including the ureteric bud, mesonephric tubules, and immature hepatocytes, and probably has a role in organogenesis and tubule formation. In the kidney the abnormality causes dilation of collecting ducts, forming cysts.

ARPKD affects 1 in 20,000 live births, and carrier rates as high as 1:70 have been suggested (Telega et al., 2013). Most patients present at or before birth with enlarged echogenic kidneys with poor corticomedullary differentiation; macrocysts are generally not visible on ultrasound at birth but do develop later in life (Fig. 87.4). Severely affected patients may have the oligohydramnios sequence with pulmonary hypoplasia, but oligohydramnios alone is not predictive of a poor neonatal outcome (Mallett et al., 2015). A small subset of patients present in childhood or adolescence with liver disease.

The kidneys may be so enlarged as to compromise respiratory function after birth, and 20%–30% of affected infants will die due to respiratory insufficiency. The kidneys are often palpable, and the abdomen may be grossly distended. Most patients have abnormal renal function from birth. Hypertension can be severe. As blood flow to the kidney increases after birth, polyuria may develop due to a urinary concentrating defect. All patients with ARPKD have liver disease due to bile duct malformation, and some will develop congenital hepatic fibrosis. Portal hypertension and ascending cholangitis are common complications, and some infants will have fat malabsorption due to abnormal bile flow. The enlarged kidneys may also compress other abdominal organs, further complicating enteral nutrition (Mallett et al., 2015). Hepatic synthetic and metabolic functions generally remain normal until late in the disease course (Telega et al., 2013).



• **Fig. 87.4** Autosomal Recessive Polycystic Kidney Disease. (A) Autosomal recessive polycystic kidney disease. Many microcysts without large cysts. (B) Ultrasound image of an autosomal recessive polycystic kidney measuring 9.1 cm in length, twice the normal length for age. Note the significant increased echogenicity. (A, Courtesy of Dr. Laura Finn, Seattle Children's Hospital, Department of Pathology, Seattle, Washington.)

Initial management is focused on respiratory support and the initiation of peritoneal dialysis, if necessary (Sweeney and Avner, 2011). Polyuric patients require high levels of daily fluid intake to match their excessive urine output, and some may require sodium supplementation (Sweeney and Avner, 2011). Hypertension is often treated with angiotensin-converting enzyme inhibitors (Guay-Woodford, 2014). Infants with fat malabsorption may require supplementation of their fat-soluble vitamins (A, D, E, and K). If the enlarged kidneys are causing respiratory failure, intractable hypertension, or feeding intolerance, bilateral nephrectomy may be performed; however, this necessitates dialysis and may cause hypotension due to the absence of renin production (Mallett et al., 2015).

Approximately 70%–75% of patients with ARPKD survive the newborn period; among those survivors the 15-year survival rate is 67%–79% (Sweeney and Avner, 2011). Over 50% of affected patients require renal replacement therapy, either dialysis or a renal transplant, during childhood, and 7%–10% require a liver transplant (Hoyer, 2015).

Autosomal Dominant Polycystic Kidney Disease

Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited renal disease, affecting 1 in 500 to 1 in 1000 live births. It is associated with mutations in *PKD1* and *PKD2*, with *PKD1* mutations being more frequent and associated with earlier onset of symptoms. *PKD1* encodes polycystin-1, a membrane protein involved in cell–matrix interactions and possibly calcium homeostasis. *PKD2* encodes polycystin-2, a nonselective cation channel that increases membrane permeability to calcium and interacts physically with polycystin-1 at the primary cilia. ADPKD has an autosomal dominant inheritance pattern but is recessive at the molecular level; it is now generally understood that cells must develop a second, somatic mutation to begin forming a cyst (Sweeney and Avner, 2011).

ADPKD generally presents with bilateral renal cysts in early adulthood followed by progressive cyst development, renal enlargement, hypertension, and renal function decline. Patients may also have cysts in the liver, pancreas, spleen, and seminal vesicles and are at increased risk for vascular abnormalities, including cerebral aneurysms, aortic dilatation, and aortic dissection. Two to five percent of patients with ADPKD present prenatally or in early childhood with symptoms ranging from asymptomatic renal cysts on ultrasound to the oligohydramnios sequence. A history of early-onset ADPKD in a family member portends a high risk of early-onset disease in the patient (Sweeney and Avner, 2011). The majority of infants diagnosed with ADPKD are asymptomatic at birth and remain so throughout childhood. Chronic renal insufficiency has been reported in 8% of children and hypertension in 19% of children by adolescence (Boyer et al., 2007).

It may be difficult to differentiate severe early ADPKD from ARPKD by ultrasound. There are no diagnostic criteria for ADPKD in children less than 15 years old; the kidneys of affected infants may be enlarged, hyperechoic with or without visible cysts, and have increased corticomedullary differentiation. Children rarely have liver or pancreatic cysts, but 10% will have inguinal hernias. Screening the patient's parents for renal cysts may be helpful in determining the correct diagnosis, though normal parental ultrasound does not rule out a diagnosis of ADPKD, as 8%–10% of cases are associated with new mutations.

Tuberous Sclerosis Complex

Tuberous sclerosis complex (TSC) is an autosomal dominant condition associated with mutations in either *TSC1*, encoding hamartin, or *TSC2*, encoding tubulin. *TSC1* and *TSC2* are important regulators of the mechanistic target of rapamycin (mTOR) kinase, a key signaling molecule involved in cell proliferation. Patients develop uncontrolled cellular proliferation, leading to hamartomas of the brain, skin, heart, lungs, and kidneys.

Hypomelanotic skin macules (ash leaf marks) may be the only skin manifestation in infants, and the presence of 3 or more ash leaf marks at birth is suggestive of TSC. Shagreen patches, angiofibromas, and nail fibromas tend to develop later in childhood (DiMario et al., 2015). Seizures and renal angiomyolipomas are common; simple renal cysts are a minor feature of TSC but can be seen. *TSC2* is physically located near *PKD1* on chromosome 16, and 2% of patients with TS will also have ADPKD secondary to a contiguous gene deletion (Rijal et al., 2014). The cysts in ADPKD-TSC appear earlier, are larger, and are more numerous than the simple renal cysts of isolated TSC. Enlarged kidneys also raise the concern for ADPKD-TSC (Back et al., 2015). ESRD is rare in patients with isolated TSC but may occur before age 30 years old in patients with ADPKD-TSC.

Bardet–Biedl Syndrome

Bardet–Biedl syndrome is an autosomal recessive ciliopathy caused by mutations in any of at least 17 genes whose products are involved in the “BBsome,” a complex involved in signaling receptor trafficking to the primary cilia. It affects 1 in 125,000 to 1 in 175,000 children. The abnormal primary cilia signal trafficking that leads to nonspecific glomerular and tubulointerstitial changes in the kidney, with or without renal cysts, and reduced nephron mass. Retinitis pigmentosa, obesity, renal anomalies, learning disabilities, polydactyly, and hypogonadism are the cardinal features (Scheidecker et al., 2014). Hearing loss, developmental or behavioral problems, diabetes, hypertension, cardiac defects, and limb anomalies have also been reported.

Renal anomalies may be both structural and functional. Fetal ultrasound may show enlarged hyperechoic kidneys without corticomedullary differentiation. Cysts and abnormalities of the renal pyramids may be seen. By 3 months of age there is inversion of the corticomedullary differentiation with a hyperechoic medulla. Renal size normalizes over time, and renal cysts may disappear. In childhood, one-third of patients will have polyuria and polydipsia; renal tubular acidosis and other signs of tubular dysfunction are less commonly seen. Hypertension is also common. Approximately 10% of children will develop ESRD, and 25% will require dialysis or transplant by 40 years of age (Putoux et al., 2012).

Jeune Syndrome

Jeune syndrome, also called asphyxiating thoracic dystrophy, is an autosomal recessive skeletal ciliopathy affecting 1 in 126,000 live births and is associated with mutations in a large number of genes, including *IFT80*, *DYNC2H1*, *WDR19*, and *TTC21B*, that are involved in transport along the axon of primary cilia. Since 2006 it has been considered one of the short-rib thoracic dysplasias. Patients have characteristic skeletal abnormalities, including a small narrow chest with short ribs, short squared iliac wings, and short digits and extremities that can typically be diagnosed on prenatal ultrasound. Liver and eye abnormalities may be present. Approximately 40% of patients will have renal anomalies, including cystic dysplasia, renal hypoplasia, or hydronephrosis causing a tubulointerstitial nephropathy and tubular dysfunction.

The abnormal chest development in Jeune syndrome prevents the intercostal muscles from contributing to respiration, and half of children with Jeune syndrome die before 6 months of age secondary to respiratory failure. Surgical techniques for chest wall reconstruction are currently being explored. In limited case series, outcomes appear good if surgery is performed after 1 year of age

but demonstrate 50% mortality before 1 year of age (Betz et al., 2008). If affected children have mild respiratory involvement and live past infancy, they develop a urinary concentrating defect, polyuria, and polydipsia. After 3 years of age renal failure is the leading cause of death among children with Jeune syndrome (Poyner and Bradshaw, 2013).

Nephronophthisis

Nephronophthisis is an autosomal recessive ciliopathy associated with at least 14 different genes involved in primary cilia structure and function, the most common being *NPHP1*. Nephronophthisis is a tubulointerstitial nephropathy with tubular atrophy, corticomedullary cysts, and interstitial fibrosis that presents as polydipsia and polyuria around age 6 years, progressing to renal failure in early adolescence. Infantile nephronophthisis progresses to ESRD around 1 year of age. It has similar pathologic findings as polycystic kidney disease, including cysts seen throughout enlarged kidneys (Wolf and Hildebrandt, 2011), and is typically associated with mutations in *NPHP2* and *NPHP3* (Sun et al., 2016). It has been reported in isolation but is more common as a component of a number of genetic syndromes, including Meckel–Gruber syndrome, Joubert syndrome, and the Joubert-related disorders.

Meckel–Gruber Syndrome

Meckel–Gruber syndrome is an autosomal recessive disorder associated with mutations in any of at least 11 genes, all of which are associated with proper functioning of the primary cilia. It occurs in 2.6 per 100,000 live births and is more common if there is parental consanguinity. Patients have the characteristic triad of bilateral renal cystic dysplasia, occipital encephalocele, and polydactyly. Hepatic fibrosis may also be present. Over 90% of cases are diagnosed prenatally by ultrasound, and 80% of cases result in fetal death or termination of pregnancy. Outcomes are poor; only about one-third of patients survive the first week of life, and the longest recorded survival is 28 months (Barisic et al., 2015).

Joubert Syndrome and Joubert-Related Disorders

Joubert syndrome is an autosomal recessive disorder associated with mutations in any of 25 genes that are associated with the primary cilia and basal body. Patients have developmental delay, hypotonia, an irregular neonatal breathing pattern (episodic apnea and/or tachypnea), and abnormal eye movements including nystagmus and oculomotor apraxia. Facial features may be dysmorphic with a broad forehead, arched eyebrows, ptosis, wide-spaced eyes, and polydactyly. Magnetic resonance imaging shows the characteristic “molar tooth sign,” an unusual combination of cerebellar vermis hypoplasia/dysplasia, elongated superior cerebellar peduncles, and a deep interpeduncular fossa. The molar tooth sign has now been associated with seven other conditions, including some cases of Senior–Løken syndrome, COACH syndrome, Dekaban–Arima syndrome, orofaciocigital syndrome type VI (Varadi–Papp) syndrome, and Malta syndrome, all of which have variable levels of ocular and renal involvement.

Renal disease in Joubert syndrome and Joubert-related disorders may present as cystic dysplasia or nephronophthisis, with corticomedullary cysts and tubulointerstitial nephritis. Ultrasound shows increased renal echogenicity in normal-sized kidneys with poor corticomedullary differentiation. Affected infants may be

asymptomatic at birth but develop urinary concentrating defects, polyuria, and progressive renal dysfunction during childhood. ESRD is common in the teenage years. Rarely, patients will present with a clinical picture similar to ARPKD; this is more common in patients with COACH syndrome and *MKS3* mutations.

Orofaciodigital Syndrome

Orofaciodigital (OFD) syndrome is a heterogeneous ciliopathy of 14 subtypes that are grouped together by the common finding of malformations of the face, oral cavity, and digits. OFD1 is an X-linked dominant condition that is lethal in males; the other 13 OFD subtypes are autosomal recessive and linked to a variety of cilia-associated genes. Tooth and tongue abnormalities, a buccal frenulum, agenesis of the corpus colosseum, and brachydactyly are common findings. Cleft lip and/or cleft palate occur in approximately one-third of patients. Renal cysts have been reported in 6% of children with orofacioidigital syndrome and in up to 70% of adults, and patients develop progressive renal insufficiency that results in ESRD in early adulthood (Saal et al., 2010).

Cranioectodermal Dysplasia

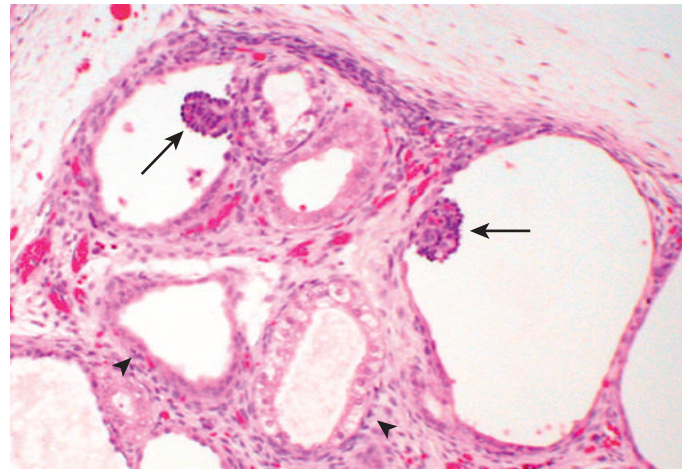
Cranioectodermal dysplasia, also called Sensenbrenner syndrome, is an autosomal recessive or sporadic ciliopathy associated with mutations in *IFT122*, *WDR35*, *IFT43*, and *WDR19*, all of which encode proteins that localize to the cilia; *IFT43* and *WDR35* are involved in axonal transport within the primary cilia. Patients have frontal bossing, dolichocephaly, low-set ears, wide-spaced eyes with epicanthal folds, small and widely spaced teeth, sparse hair, abnormal nails, short stature, a narrow thorax, short humeri, and brachydactyly. The liver and eyes may also be affected. Over half of patients will develop nephronophthisis that progresses to ESRD.

Renal–Hepatic–Pancreatic Dysplasia

Renal–hepatic–pancreatic dysplasia (RHPD), also called Ivemark syndrome, is an autosomal recessive disorder associated with mutations in *NPHP3*, which encodes nephrocystin 3, a protein that localizes to primary cilia (Fiskerstrand et al., 2010). Patients often present in utero with oligohydramnios caused by renal dysplasia with peripheral cortical cysts. Hepatic and pancreatic fibrosis is also present. Most patients develop the oligohydramnios sequence and die of respiratory failure soon after birth.

Glomerulocystic Kidney Disease

Glomerulocystic kidney disease is defined as dilation of the Bowman capsule around the glomeruli with or without tubular dilatation and renal cysts (Fig. 87.5). It is an autosomal dominant disorder associated with mutations in *UMOD* or *HNF1β* but may also be seen in ciliopathies, renal dysplasia, or associated with severe fetal renal damage. *HNF1β* encodes a transcription factor critical to embryologic development of the kidney, pancreas, liver, and Müllerian duct. Mutations in *HNF1β* are also associated with maturity-onset diabetes of the young, as in the “renal cysts and diabetes syndrome.” *UMOD* encodes uromodulin, which is linked to urinary tract renal defense but probably has other, currently unknown, functions in the kidney. Glomerulocystic kidney disease often presents in young children with renal hyperechogenicity on ultrasound, but presentation may be delayed into adulthood. Patients may be hyperuricemic. The kidneys generally progress to ESRD,



• **Fig. 87.5** Glomerulocystic Kidney Disease. Arrows show glomeruli inside dilated Bowman capsule; arrowheads indicate normal tubules. (Courtesy of Dr. Laura Finn, Seattle Children's Hospital, Department of Pathology, Seattle, Washington.)

but the rate of progression is highly variable (Bissler et al., 2010; Lennerz et al., 2010; Iorembere and Vehaskari, 2014).

Renal Tubular Dysgenesis

Renal tubular dysgenesis is an autosomal recessive disorder associated with mutations in genes for the renin–angiotensin system (*AGT*, *REN*, *ACE*, and *AGTR1*) that result in failure of renal proximal tubule development. Similar pathology may develop if renal blood flow is interrupted in utero, such as in a major cardiac anomaly, renal artery stenosis, twin–twin transfusion syndrome, or use of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers during the second or third trimesters.

Patients present with severe oligohydramnios by 20–22 weeks' gestation, leading to the oligohydramnios sequence. The kidneys may appear normal on ultrasound, or there may be hyperechogenicity or decreased corticomedullary differentiation. The skull may have large fontanelles and sutures. Diagnosis is confirmed by renal biopsy showing the absence or dramatic reduction in the number of differentiated proximal tubules.

Most patients die in utero or in the neonatal period because of pulmonary hypoplasia and hypotension that is not responsive to standard medical therapy. In cases of refractory hypotension, fludrocortisone may improve the blood pressure; fresh frozen plasma may also be helpful in patients with an angiotensinogen mutation. Of the 150 patients reported with renal tubular dysgenesis, there are at least 10 reported cases of survivors past 18 months of age, 4 of whom have ESRD and 5 of whom have chronic kidney disease (Gubler, 2014).

Renal Teratogens

A number of commonly used medications have been associated with abnormal renal development. Angiotensin-converting enzyme inhibitors and angiotensin receptor blockers inhibit nephrogenesis when taken in the second and third trimesters, and they have been associated with renal tubular dysgenesis, ureteropelvic junction obstruction, and renal agenesis. Nonsteroidal antiinflammatory drugs, especially indomethacin, are linked to fetal and neonatal renal failure, probably because of their effects on the ductus

arteriosus. Mycophenolate mofetil is associated with a variety of organ anomalies, including renal anomalies. Tacrolimus may cause a mild and transient decline in renal function, but the placenta prevents an estimated 60% of the dose from reaching the fetus (Morgan et al., 2014). The breast cancer drug trastuzumab has been associated with oligohydramnios when used in the second or third trimester and is an Federal Drug Administration pregnancy category D medication. Gentamicin has been associated with nephrotoxicity in animal studies and two human cases of cystic dysplasia; however, these findings were not confirmed by a large Swedish study (Morgan et al., 2014). Maternal diabetes is associated with renal and urinary tract abnormalities, especially if it is present in the first trimester (Dart et al., 2015).

Inborn Errors of Metabolism

Multiple Acyl-CoA Dehydrogenase Deficiency

Multiple acyl-CoA dehydrogenase deficiency (MADD), also called glutaric acidemia type II or glutaric aciduria type II, is an autosomal recessive defect in the mitochondrial electron transfer chain caused by mutations in *ETFDH*, *ETFA*, or *ETFB*. This causes degeneration of cells that use fatty acids as a primary source of energy, including renal tubular epithelial cells. Affected children present in the neonatal period with lethargy, vomiting, a “sweaty feet” odor, nonketotic hypoglycemia, metabolic acidosis, and organic aciduria. Some will have other congenital anomalies, including a high forehead, low-set ears, wide-spaced eyes, a small midface, renal dysplasia, and renal cysts. Most patients who present near birth die within weeks to months despite attempts at dietary interventions. There is a milder, late-onset form of the disease, not associated with renal anomalies, which may be treated with riboflavin.

Smith–Lemli–Opitz Syndrome

Smith–Lemli–Opitz syndrome is an autosomal recessive disorder of cholesterol synthesis associated with mutations in *DHCR7*. The clinical spectrum is broad, ranging from asymptomatic cases to perinatal lethality. Affected children may have delayed growth, microcephaly with bitemporal narrowing, ptosis, a short nose with anteverted nares, micrognathia, epicanthal folds, and low-set ears. Mental retardation, cleft palate, heart defects, genital anomalies, and syndactyly of the second and third toes are common. One-fourth of patients have renal anomalies, including renal hypoplasia or agenesis, renal cortical cysts, or urinary tract anomalies.

Zellweger Syndrome

Zellweger syndrome, also called cerebrohepatorenal syndrome, is an autosomal recessive condition associated with mutation in any of 12 pexin genes, usually *PEX1*, leading to peroxisome dysfunction. Affected infants have severe hypotonia, absent reflexes, a high forehead and large anterior fontanelle, small supraorbital ridges, a triangular mouth, and low-set ears with abnormal lobes. There are usually small renal cysts and calcific stippling of the epiphyses. Severe liver dysfunction typically appears after 3 months of age. Some patients present with seizures caused by abnormal neuronal

migration. Death before age 1 year is common, though some patients survive longer with varying developmental outcomes (Baumgartner and Saudubray, 2002; Poll-The et al., 2004).

Congenital Disorders of Glycosylation

The congenital disorders of glycosylation (CDG) are a family of autosomal recessive defects in the synthesis of the glycans of glycoproteins (Hertz-Pannier et al., 2006). Patients can present with a variety of symptoms, including developmental delay, seizures, hypotonia, liver disease, protein-losing enteropathy, and dysmorphic facial features. Patients may have enlarged hyperechoic kidneys on ultrasound and renal tubular microcysts. Congenital nephrotic syndrome, associated with diffuse mesangial sclerosis pathology, has also been reported (Sinha et al., 2009).

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Developmental Abnormalities of the Genitourinary System

PAUL A. MERGUERIAN AND COURTNEY K. ROWE

KEY POINTS

- Current American Urological Association guidelines do not recommend ultrasound for the evaluation of undescended testicles.
- Children with an undescended testicle should be referred to a pediatric urologist at approximately 6 months of age.
- Infants with bilateral nonpalpable testicles should be evaluated for possible congenital adrenal hyperplasia.
- Infants with hypospadias and unilateral or bilateral undescended testicles should be evaluated for disorder of sexual development.
- Repair of hypospadias is recommended at between 6 months and 1 year of age.
- Bladder exstrophy repairs are currently delayed to 3 months of age.
- A voiding cystourethrogram should be performed in infants with high-grade hydronephrosis and bilateral hydronephrosis.
- Most hydroceles in infants are noncommunicating, and infants should be referred to a urologist if they fluctuate in size suggesting a communicating hydrocele.
- Vesicoureteral reflux is more common in male infants and has a high rate of resolution.

Congenital anomalies of the kidney and urinary tract (CAKUTs) are found in around 0.5% of pregnancies and account for 20%–30% of all prenatally diagnosed congenital anomalies. In spite of prenatal diagnosis and aggressive treatment, they remain one of the main causes of renal failure in children. They are responsible for 30%–50% of all children with chronic renal failure worldwide (Pope et al., 1999).

Prenatal ultrasonography has dramatically changed the management of these conditions. Close to 90% of these conditions are detected prenatally. Most of these are asymptomatic and can be managed conservatively. It is imperative for pediatric providers caring for these patients to identify patients at risk of developing renal impairment.

The phenotypic spectrum of these disorders is shown in [Box 88.1](#). Ureteropelvic junction obstruction (UPJO) and vesicoureteral reflux (VUR) are the most common CAKUTs. Other forms of CAKUTs include multicystic dysplastic kidneys (MCDK), primary megaureter, duplicated collecting systems, ureterocele, renal

dysplasia, and bladder outlet obstruction such as posterior urethral valves (PUVs) (Ichikawa et al., 2002).

There are well-recognized features related to these anomalies in infants, and they include higher incidence of UPJ obstruction and VUR in males; UPJO and MCDK are often unilateral; most of these anomalies are associated with VUR; most infants with high-grade reflux have renal dysplasia; and primary megaureter is overwhelmingly found in males.

On the other hand, in older children there is a female preponderance of several of these CAKUTs.

It is postulated that these anomalies are based on defects in the same molecular pathways involved in kidney development. According to the Genitourinary Molecular Anatomy Project, there may be several hundred genes involved in the process of kidney development (Renkena et al., 2011; Schedl et al., 2007; Song and Yosypic, 2011).

Before we discuss the evaluation and management of these anomalies, it is important to discuss embryogenesis.

Early Kidney and Urinary Tract Embryologic Development

The development of the kidney and urinary tract begins when the nephric duct (ND) is formed from the intermediate mesoderm ([Fig. 88.1](#)). The ND extends caudally and induces the adjacent mesoderm to form two transient kidneys, the pronephros and the mesonephros (Mitchell and Sharma, 2009).

The pronephros contains rudimentary tubules that open into the pronephric duct and disappears at the end of the fourth week of gestation (Mitchell and Sharma, 2009).

The mesonephros then begins to develop and contains well-developed nephrons with vascularized glomeruli connected to proximal and distal tubules draining into the mesonephric duct. The mesonephric duct then fuses with the cloaca and contributes to the formation of the bladder trigone. In the male it also forms part of the genital system, including the vas deferens, seminal vesicles, epididymis, ejaculatory ducts, and the efferent ductules of the testis. In females the mesonephros forms vestigial structures, the epoophoron and the paroophoron (Renkena et al., 2011; Duke University School of Medicine, 2015).

The metanephros is the final stage of renal development and is identified at around 5–6 weeks' gestation. This structure consists of two components: the ureteric bud and the metanephric mesenchyme. The ureteric bud forms from the nearby caudal mesonephric (wolffian) duct and grows to penetrate the metanephric blastema. The reciprocal interaction between the ureteric bud and the metanephric mesenchyme results in branching of the ureteric bud to form the collecting system of the kidney. A mesenchymal-to-epithelial transition of the metanephric mesenchyme at each of the newly formed ureteric bud tips results in the development of the nephrons. With each division of the ureteric bud, a new layer of nephrons is induced from stem cells in the periphery of the organ. As development proceeds, the metanephros is located at progressively higher levels, reaching the lumbar position by 8 weeks' gestation (Duke University School of Medicine, 2015).

• BOX 88.1 Spectrum of Congenital Anomalies of the Kidney and Urinary Tract (CAKUT)

Renal agenesis
Renal dysplasia
Renal hypoplasia
Duplex kidney and ureter
Horseshoe kidney and other fusion anomalies (crossed fused ectopia)
Ureteropelvic junction obstruction
Megaureter (obstructing, refluxing, nonobstructing, nonrefluxing)
Ectopic ureter
Ureterocele

It has also been found that the main ureteric duct (future ureter) undergoes a process of temporary obliteration followed by recanalization of the lumen as the embryo grows. This process begins in the middle zone of the ureter and progresses proximally and distally. In embryos of approximately 17 mm in length the primary ureter forms a solid cord, and in a 23-mm embryo it is totally patent. These observations have given rise to the theory that UPJO and ureterovesical junction obstruction arise from incomplete recanalization of the ureter at its most proximal and distal ends (Baker and Gomez, 1998; Duke University School of Medicine, 2015).

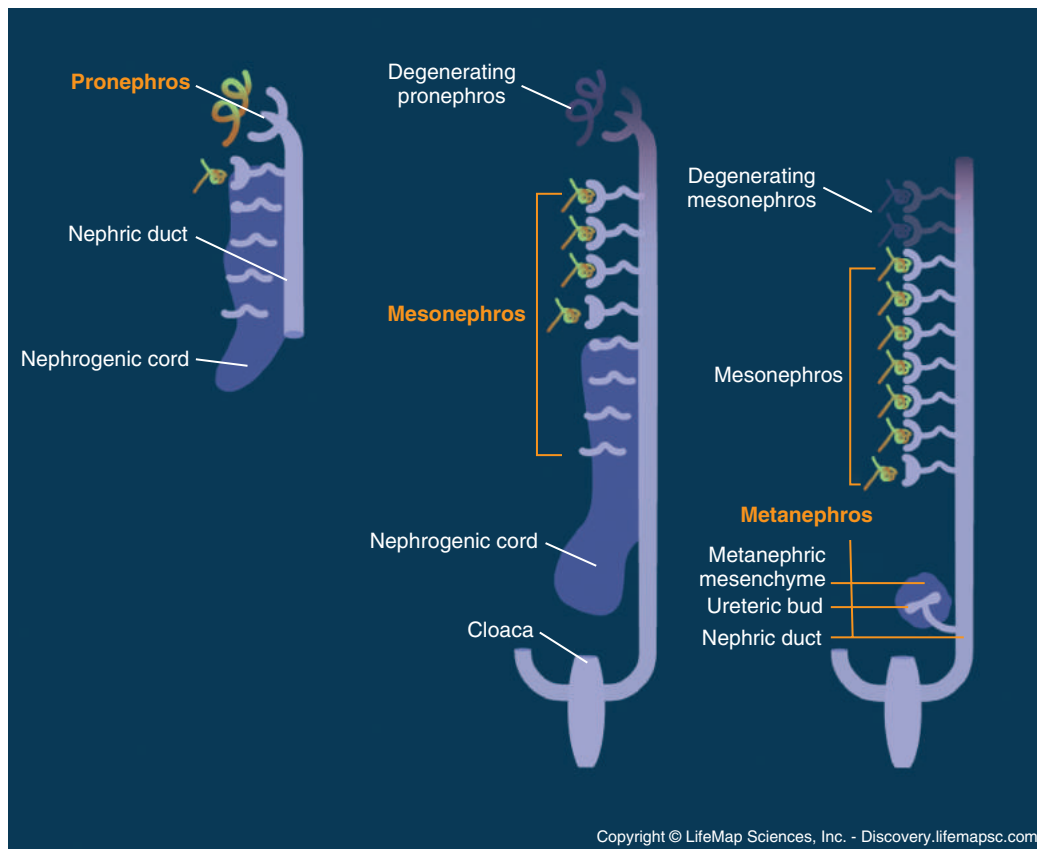
The ureteral orifice is also transposed from its original budding site on the wolffian duct into the bladder. This transposition occurs with expansion of the terminal part of the duct and its incorporation into the base of the bladder as the hemitrigone. If, for example, the bud arises caudally on the duct, the orifice becomes incorporated onto a long cornu of the hemitrigone and is therefore laterally displaced. This lateral displacement causes the submucosal tunnel to be short, leading to VUR (Baker and Gomez, 1998).

All the branches of the ureteric bud and the nephrons have been formed by 32–36 weeks' gestation. However, these structures will continue to mature after birth. Once matured, humans have an estimated 300,000 to 1 million nephrons per kidney (Bertram et al., 2011).

Anomalies of the Kidney

Renal Agenesis

Renal agenesis is the congenital absence of the kidneys and can be bilateral or unilateral. Bilateral renal agenesis occurs in roughly



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• Fig. 88.1 Development of the human kidney.

1 in 4000 births, with male predominance (Potter, 1965). Prenatally it is discovered in the second or third trimester because of severe oligohydramnios on ultrasound. Findings of absent kidneys, no bladder filling, and lack of renal arteries on color Doppler ultrasonography support the diagnosis (Dias et al., 2014). Postnatally, infants are noted to have the characteristic Potter facies with a prominent skin fold from the eye to the cheek, blunted nose, low-set ears, and orthopedic abnormalities, including clubbed legs or fused lower extremities. Lack of adequate amniotic fluid results in pulmonary hypoplasia and respiratory distress (Peters et al., 1991). These findings or lack of urine output within 24 hours should prompt renal ultrasound. Bilateral renal agenesis is almost universally fatal, with 40% demise in utero, and those born alive surviving for less than 48 hours because of pulmonary compromise, although there have been reports of survival of monoamniotic twins discordant for bilateral renal agenesis (Perez-Brayfield et al., 2004) and a report of bilateral renal agenesis managed with serial amnioinfusion with survival of the infant to 9 months (Bienstock et al., 2014). There is an increased prevalence of congenital renal anomalies in relatives, and screening ultrasound has been recommended for parents and siblings (Roodhooft et al., 1984; Schwaderer et al., 2007).

Unilateral renal agenesis occurs in roughly 1 in 500 to 1 in 1000 births. The true incidence can be difficult to measure because of the frequent involution of multicystic dysplastic kidneys. In fetal ultrasounds with a unilateral empty renal fossa, 47% have an absent kidney at birth, while most of the rest have renal ectopia (Chow et al., 2005). Unilateral renal agenesis is associated with abnormalities of the paramesonephric and mesonephric ducts, including absence of the ipsilateral uterine horn or vas deferens. It is also associated with a number of multisystem syndromes, including DiGeorge syndrome, Kallmann syndrome, Klinefelter syndrome, Mayer-Rokitansky-Küster-Hauser syndrome, Townes-Brocks syndrome, and Williams-Beuren syndrome (Woolf and Hillman, 2007). There is an increased risk of contralateral renal anomaly, including VUR in a third of patients; infants with unilateral renal agenesis should undergo a screening VCUG (Kaneyama et al., 2004).

Despite reassuring studies indicating that patients with solitary kidneys after donor nephrectomy have a low risk of developing end-stage renal disease (Reese et al., 2015), new evidence shows increased risk of renal failure in patients with a congenital cause of their solitary kidney, with 50% requiring dialysis by 30 years of age (Sanna-Cherchi et al., 2009). The American Academy of Pediatrics does not recommend sports restrictions because of the low risk of renal injury (Grinsell et al., 2012), but children with unilateral renal agenesis should be followed up regularly to monitor blood pressure, for urinalysis, and to monitor glomerular filtration rate (Westland et al., 2013).

Renal Ectopia and Fusion

Renal ectopia describes a kidney that fails to reach its standard location; instead it can be pelvic, iliac, abdominal, thoracic, and contralateral or crossed. This occurs in about 1 in 700 on ultrasound screenings (Caiulo et al., 2012). Abnormalities of renal ascent during fetal development are thought to be the cause of ectopia. Most patients are asymptomatic.

Half of ectopic kidneys are hydronephrotic, of which 50% are due to obstruction, 25% are due to vesicoureteral reflux, and 25% are due to abnormal rotation without obstruction (Gleason et al., 1994). There is a strong association with genital anomalies, which

are seen in almost half of patients (Rivard et al., 1978). This is pronounced in females, and a large number of girls with müllerian anomalies, including cloacal anomalies, are also noted to have ectopic kidneys (Leduc et al., 1968; Warne et al., 2002). Because of this, the finding of an ectopic kidney in a female should prompt further investigation. Prognosis for the ectopic kidney is good, with no evidence of adverse effects on blood pressure or renal function (van den Bosch et al., 2010).

When the ectopic kidney is located on the opposite side from where its ureter enters the bladder, this is called *crossed renal ectopia*. This is found in about 1 in 1000 on autopsy, and 90% of these kidneys are also fused to the adjacent kidney (Abeshouse and Bhisitkul, 1959). The cause is unknown. This condition is found more commonly in males, and left-to-right crossover is most frequent (Gu and Alton, 1991; Bhatt and Herts, 2014). About 20% of patients with crossed renal ectopia also have VUR (Guarino et al., 2004), and there is a high incidence of associated skeletal and genital anomalies with solitary crossed renal ectopia, although it is not known if this is due to the ectopia or the agenesis (Gu and Alton, 1991; Gleason et al., 1994).

The most common fusion abnormality is the horseshoe kidney, which consists of two renal masses on either side of the midline connected by an isthmus of tissue. It is found in approximately 1 in 600 on radiographic review (Weizer et al., 2003). There is an increase in associated genitourinary anomalies, including hypospadias, undescended testes, bicornuate uterus, septate vagina, and duplication of the ureter (Boatman et al., 1972). Up to half of children with horseshoe kidney have associated VUR (Taghavi et al., 2016).

Most patients with horseshoe kidney are asymptomatic, although the incidence of Wilms tumor is higher in patients with horseshoe kidney than in the average population (Huang et al., 2004), and there is a high prevalence of stones in adulthood (Taghavi et al., 2016). Neither risk is significant enough to justify routine screening of these patients.

Supernumerary Kidney

A supernumerary kidney is a separate or loosely attached renal mass with its own blood supply and collecting system. It is exceedingly rare, with only about 100 cases reported (Sureka et al., 2014). This condition is usually diagnosed when the supernumerary kidney becomes infected or obstructed, as about 50% have a structural abnormality such as a duplicated system or hydronephrosis (N'Guessan and Stephens, 1983).

Cystic Disease of the Kidney

Cystic diseases of the kidney are classified as having inheritable or noninheritable causes (Box 88.2). Many will present during the neonatal period.

Autosomal recessive polycystic kidney disease presents as symmetric enlargement of the kidneys bilaterally due to collecting duct cysts and is associated with biliary dysgenesis and portal fibrosis. This disease used to be referred to as *infantile*; however, mild cases can present later in life. The incidence is estimated at 1 in 20,000 live births, with a mutation of *PKHD1* located on chromosome 6 responsible for this disease (Zerres et al., 1994). Ultrasound findings of bilateral hyperechoic kidneys with poor corticomedullary differentiation are suggestive, and infants often have Potter facies and respiratory issues due to oligohydramnios. Thirty percent to 40% of patients die shortly after birth of

• BOX 88.2 Cystic Diseases of the Kidney

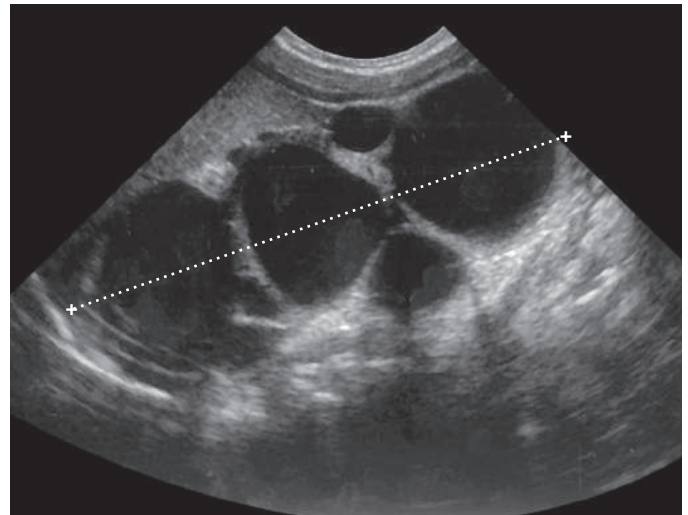
Inheritable

- Autosomal recessive (infantile) polycystic kidney disease
- Autosomal dominant (adult) polycystic kidney disease
 - Juvenile nephronophthisis and medullary cystic disease complex
 - Juvenile nephronophthisis (autosomal recessive)
- Medullary cystic disease (autosomal dominant)
- Congenital nephrosis (familial nephrotic syndrome) (autosomal recessive)
- Familial hypoplastic glomerulocystic disease (autosomal dominant)
- Multiple malformation syndromes with renal cysts (e.g., tuberous sclerosis, von Hippel–Lindau disease)

Noninheritable

- Multicystic kidney (multicystic dysplastic kidney)
- Benign multilocular cyst (cystic nephroma)
- Simple cysts
- Medullary sponge kidney
- Sporadic glomerulocystic kidney disease
- Acquired renal cystic disease
- Calyceal diverticulum (pyelogenic cyst)

Modified from the American Academy of Pediatrics, Section on Urology (Glassberg et al., 1987).



• **Fig. 88.2** Sonographic view of a multicystic dysplastic kidney in which multiple cysts that do not communicate with one another are grouped together, giving the typical “bunch of grapes” appearance. No function was noted on the patient’s renal scan, confirming this diagnosis.

pulmonary complications or sepsis (Guay-Woodford et al., 2014). Those who survive have a high risk of developing childhood renal insufficiency, systemic hypertension, and portal hypertension, with many requiring renal or liver transplants (Guay-Woodford and Desmond, 2003).

Autosomal dominant polycystic kidney disease involves progressive cystic enlargement of bilateral kidneys due to a mutation in at least three different genes (*PKD1*, *PKD2*, and *PKD3*) that leads to an abnormal form of the protein polycystin (Grantham, 1996). It is the most commonly inherited renal disease, occurring in 1 in 500 to 1 in 1000 live births. While it used to be diagnosed late in life, advances in radiographic imaging have allowed early detection in fetuses and infants (Boyer et al., 2007). Renal failure is variable and is usually preceded by hypertension and hematuria between 30 and 50 years of age (Johnson and Gabow, 1997). The condition is associated with intracranial aneurysms, abdominal wall hernias, and cysts in the liver, seminal vesicles, and other areas (Luciano and Dahl, 2014). Screening of asymptomatic family members is recommended for adults but not children (Chapman et al., 2015).

Tuberous sclerosis complex is a neurocutaneous disease that presents with benign hypopigmented skin lesions and is associated with epilepsy or autism. Renal manifestations can mimic other cystic diseases, and long-term urologic sequelae include renal angiomyolipomas, which can be treated with surgery or the mammalian target of rapamycin complex 1 (mTORC1) inhibitor everolimus (DiMario et al., 2015).

Multicystic dysplastic kidney disease is the most common type of renal cystic disease (Fig. 88.2), occurring in 1 in 4000 live births, with increasing incidence (Schreuder et al., 2009). It is diagnosed on the basis of ultrasound findings of a collection of cysts of various sizes without any evidence of renal parenchyma. There is a male and a left-sided predominance. The cause is unknown. Since half of MCDK will involute spontaneously by age 10 years, nephrectomy is generally reserved for symptoms such as urinary tract infection or hypertension (Eickmeyer et al., 2014). One-third of patients will have an anomaly in the contralateral

kidney, and most of these will have vesicoureteral reflux; a screening voiding cystourethrogram (VCUG) can be considered (Schreuder et al., 2009). Less than 10% of patients will go on to develop hypertension, while up to 17% will develop proteinuria and chronic kidney disease; both appear to be most common in patients with contralateral renal anomalies (Mansoor et al., 2011).

Renal Tumors

Malignancies are rare in neonates, and renal tumors make up only approximately 7% of neonatal tumors (Powis, 2010). About 15% are discovered on prenatal ultrasound, with half noted on physical examination as a palpable abdominal or flank mass (Isaacs, 2008). The first line of treatment is radical nephrectomy for all unilateral renal tumors. Biopsy before surgery is generally reserved for complex cases such as those with bilateral disease or metastases at presentation.

The most common renal tumor in neonates is congenital mesoblastic nephroma, generally diagnosed before 3 months of age. There is a male predominance of 1.5:1. The mainstay of treatment is a radical nephrectomy, and surgery is generally curative in stage I/II tumors, with rare need for chemotherapy. The overall survival rate is excellent, around 95% (Gooskens et al., 2017).

Wilms tumor is the second most common neonatal renal tumor and affects males and females equally. Treatment generally begins with radical nephrectomy, although partial nephrectomy for bilateral tumors or patients with a syndrome that predisposes them to reoccurrence is becoming more common (Wilcox Vanden Berg et al., 2016). After surgery, treatment is based on risk stratification and may include chemotherapy and radiation therapy based on protocols from two international oncologic groups, the Children’s Oncology Group and the International Society of Paediatric Oncology (Dome et al., 2015). The overall survival rate is high at 90%, but 60% of long-term survivors report chronic health problems, with 25% of those experiencing severe issues, including congestive heart failure, hypertension, and renal failure (Termuhlen et al., 2011).

Malignant rhabdoid tumor of the kidney is a rare and aggressive cancer that generally presents at advanced stages. The overall survival rate in neonates is less than 10%. Other renal tumors in neonates include clear cell carcinoma of the kidney, which presents at advanced stage but for which there is an overall survival rate of 50%, ossifying renal tumor of infancy, which is generally benign, nephroblastomatosis, which is a premalignant condition requiring observation, and cystic nephroma, which is benign but indistinguishable from rare malignancies and therefore generally surgically removed (Powis, 2010).

Renal Vein Thrombosis

The risk factors for renal vein thrombosis include perinatal asphyxia, maternal diabetes, and dehydration as well as the presence of prothrombotic factors such as protein C and protein S deficiency and factor V Leiden (Lai et al., 2012). The classic presentation is gross hematuria, a palpable flank mass, and thrombocytopenia. Contrast angiography is the gold standard for diagnosis, but because of concerns regarding radiation, Doppler ultrasound is often used as an alternative. This will show enlarged and echogenic kidneys with either absent flow in the renal vein or increased resistance in the renal artery (Resontoc and Yap, 2016). The mainstay of treatment is supportive care, but heparin and low-molecular-weight heparin are being used more often for bilateral disease or inferior vena cava involvement. Regardless of the treatment chosen, about 70% of the affected kidneys will become atrophic (Lau et al., 2007; Marks et al., 2005).

Adrenal Hemorrhage

Adrenal hemorrhage occurs after birth in approximately 2 in 1000 live births and presents with anemia, hyperbilirubinemia, or scrotal hematoma (Lai et al., 2012). While palpable abdominal masses have been reported, this is a less common finding in the era of ultrasound diagnosis. Predisposing factors include vaginal birth, macrosomia, perinatal hypoxia, and sepsis (Gyurkovits et al., 2015). Treatment is generally supportive, with spontaneous resolution on subsequent imaging (Postek et al., 2011).

Anomalies of the Ureters

Duplication of the Ureters

Ureteral duplication can develop if there are duplicate ureteral buds or early division of these buds. It occurs in between 0.8% and 1.8% of the population (Privett et al., 1976; Wein et al., 2016). Ureteral triplication and even quadruple ureters have also been reported in the literature but are much rarer. Complete duplication results in two separate ureters, while partial duplication results in a bifid renal pelvis with distal confluence into a single ureter. There is a strong genetic link to duplication of the ureter, which occurs in 12% of screened siblings and parents of affected patients (Carter, 1984). Diagnosis is generally by ultrasound, and some clues can indicate a duplex kidney prenatally, including renal length greater than the 95th percentile with a cyst-like structure in the upper pole surrounded by a rim of parenchyma, two noncommunicating renal pelvises, or a cystic structure in the bladder consistent with a ureterocele (Dias et al., 2014). The location of the ureters in the bladder of patients with complete ureteral duplication generally follows the Weigert–Meyer law, with the upper pole ureter found caudal to the lower pole ureter.

About half of patients with duplex kidneys are otherwise asymptomatic, and the anomaly by itself is not thought to have any clinical significance. It is, however, associated with other conditions; a quarter of patients have upper pole obstruction, usually associated with a ureterocele, 10% have lower pole scarring, and 4% have lower pole VUR (Doery et al., 2015). Both lower pole UPJO and rarely upper pole UPJO have been reported, as has upper pole ectopia. Long-term renal outcome is generally related to the presence of these associated conditions, although ureteral duplication itself is a risk factor for a slower rate of spontaneous reflux resolution (Estrada et al., 2009).

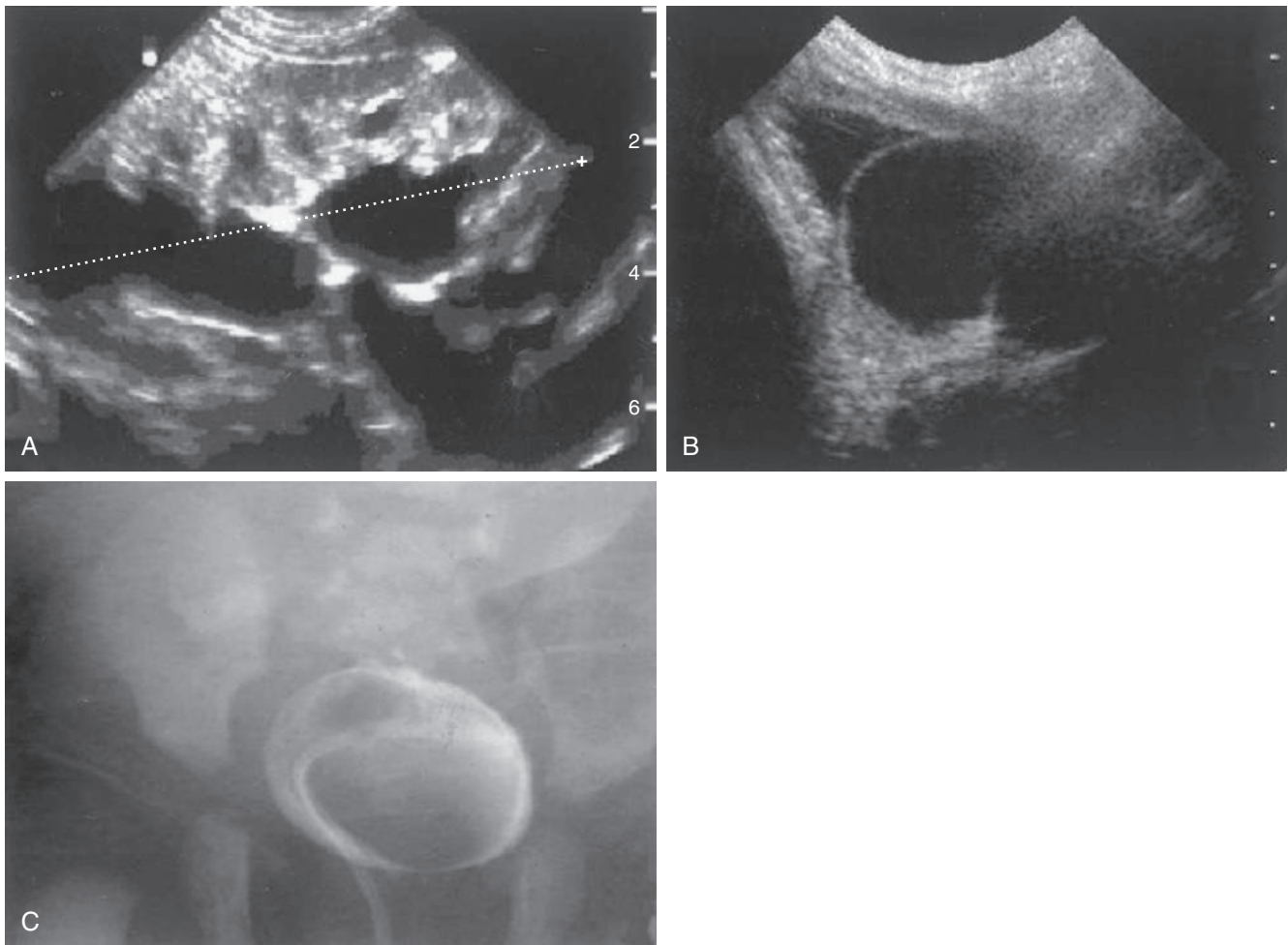
Ureteral Ectopia

Ureteral ectopia occurs whenever the opening of the ureter is in a position other than the expected location in the bladder trigone. The incidence is 1 in 1900 on autopsy study (Mathiot et al., 2004). The cause is thought to be from ectopic location of the initial ureteric bud, and because of the predictable pathway of the bud during embryologic development, these ureters are generally found in predictable locations. The most common form is lateral migration, which is thought to cause vesicoureteral reflux. Medially located ureters are generally found along the path of the wolffian duct. In males an ectopic ureter can be found in the bladder neck, the posterior urethra, seminal vesicles, the vas deferens, or the epididymis. In females an ectopic ureter can be found in the bladder neck, the urethra, the uterus, the proximal part of the vagina, or the Gartner duct (Wein et al., 2016).

In males the ectopic ureter can present with dilatation or infection of the structure into which it inserts (El-Ghar and El-Diasty, 2013). In females the ectopic position is more likely to be distal to the urethral sphincter, resulting in continuous incontinence, which is often discovered after failed attempts at toileting train. Ectopic ureters are generally from the upper pole of a duplex kidney. While single-system ureteral ectopia occurs, it is difficult to diagnose because of the association with poor renal function on that side but should be considered in females who present with continuous urinary incontinence and what appears to be a solitary kidney on conventional imaging (Borer et al., 1998). Dimercapto-succinic acid scanning and computed tomography are the most sensitive techniques for diagnosis, although ultrasound, MRI, and intravenous pyelogram can also detect a single-system ectopic ureter (Lee et al., 2016).

Ureterocele

A ureterocele is a cystic dilatation of the distal submucosal or intravesical ureter that results in obstruction of urine flow. It can occur within a single system or a duplex system; if the system is duplex, the ureterocele is associated with the ectopic upper pole. Ureteroceles occur in between 1 in 500 and 1 in 4000 in autopsy series (Sözübir et al., 2005). The cause is thought to be failure of apoptosis of a distal membrane at the ureteral orifice known as the *Chwalle membrane*. Because of familial occurrence, this is thought to have a genetic cause (Mendelsohn, 2009). Ultrasound of ureteroceles shows hydronephrosis associated with a cystic structure within the bladder (Fig. 88.3). Most are now diagnosed prenatally and have been detected as early as 16 weeks (Godinho et al., 2013). Prenatal symptoms include infection, retention, or prolapse through the urethra in a female, which generally presents as a bulging vulvar mass (Abdelgadir et al., 2010).



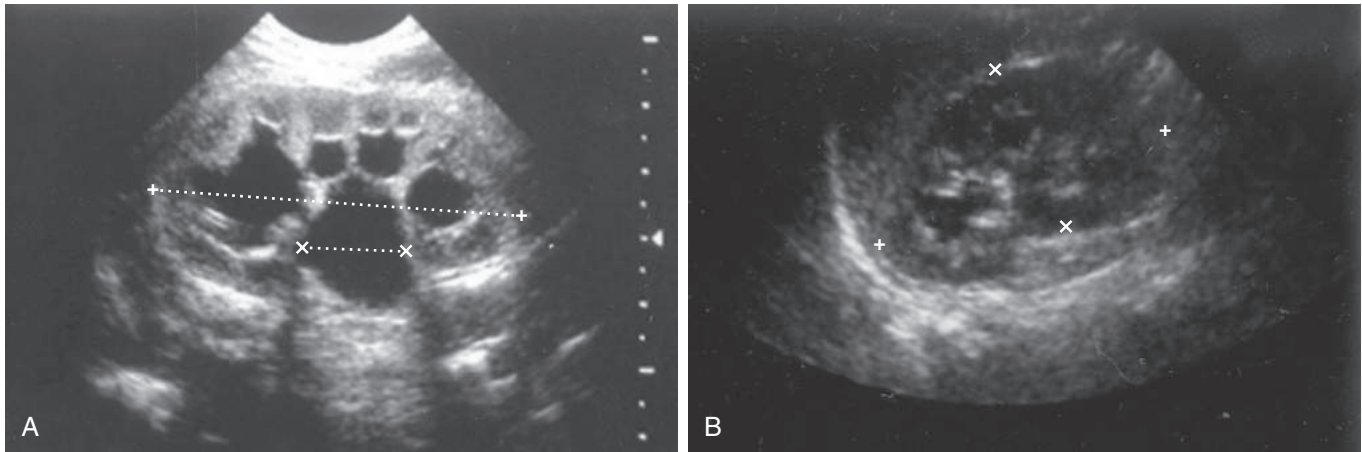
• **Fig. 88.3** Images demonstrating upper pole hydronephrosis that is secondary to a dilated ureter (A), which empties into a larger ureterocele within the bladder (evident in [B]). There is also secondary dilatation of the lower pole renal pelvis. The ureterocele is demonstrated as the cystic filling defect on bladder views from the ultrasound study (B) as well as the voiding cystourethrogram (C).

Multiple options have been described for management. Ureteroceles can be managed with observation if the child has no infections and minimal associated vesicoureteral reflux, and a diuretic renogram shows no obstruction or an already nonfunctioning portion of the kidney. Appropriately selected patients have needed no further intervention (Pohl, 2011). For patients who require treatment, transurethral incision or puncture provides decompression in a minimally invasive fashion, although it can result in secondary vesicoureteral reflux. It is most successful as a definitive operation in patients with intravesical single-system ureteroceles and can also be used as an urgent temporizing measure in septic, obstructed children (Byun and Merguerian, 2006). Fetoscopic incision and puncture have also been described (Godinho et al., 2013). More definitive surgical procedures include ureteroureterostomy, ureteropyelostomy, and ureterocele excision with reimplantation into the bladder for functioning renal units or nephrectomy or heminephrectomy for nonfunctioning renal units. Regardless of the management selected, most children do well in the long term, with low risk of major bladder dysfunction (Paye-Jaouen et al., 2015).

Ureteropelvic Junction Obstruction

Ureteropelvic junction obstruction refers to a blockage where the renal pelvis meets the ureter. It occurs in an estimated 1 in 1500 live births and is the most common cause of prenatal hydronephrosis (Fig. 88.4), accounting for 41% of cases (Brown et al., 1987; Morin et al., 1996). UPJO in newborns and infants is generally caused by intrinsic narrowing of the area, while in childhood and adolescence it is generally caused by extrinsic compression by an accessory vessel to the lower pole of the kidney. Because of increased prenatal ultrasound screening, many cases are diagnosed prenatally, although delayed presentations can occur with flank pain, nausea, and emesis later in life. Diagnosis of functional obstruction is confirmed with a diuretic renogram.

Up to half of patients will require intervention because of worsening hydronephrosis or decreasing renal function (Chertin et al., 2006). Surgery is still primarily performed in an open fashion, but laparoscopic or robotic surgery is equally successful (Liu et al., 2014). While surgical success rates are between 90% and 100%, patients are still at risk of later development of hypertension and



• **Fig. 88.4** An example of prenatal hydronephrosis consistent with a partial ureteropelvic junction obstruction (A) that spontaneously resolved over a 6-month period (B).

proteinuria (Lee et al., 2014). Half of patients with UPJO will have other urologic anomalies, including contralateral obstruction and VUR (Lebowitz and Blickman, 1983; Karnak et al., 2008). A routine VCUG is generally recommended if UPJO is present, although some pediatric urologists are questioning the benefit of this invasive test because of the high prevalence of clinically insignificant reflux (ElSheemy et al., 2017; Weitz and Schmidt, 2017).

Uterovesical Obstruction

Uterovesical obstruction, also sometimes called an *obstructed megaureter*, refers to a blockage where the ureter meets the bladder. The cause is thought to be an adynamic segment of the distal ureter with insufficient peristalsis (Wein et al., 2016). Megaureter accounts for 5%–10% of prenatal hydronephrosis, and up to 80% of cases resolve without the need for intervention (Nguyen et al., 2010; Di Renzo et al., 2013). The true incidence of obstructed megaureter is not known. As with UPJO, uterovesical obstruction is confirmed with the functional study of a diuretic renogram since not all megaureters are obstructed. Surgical management can be with endoscopic treatment or open reconstruction (Farrugia et al., 2014).

Vesicoureteral Reflux

Vesicoureteral reflux describes retrograde flow of urine from the bladder to the ureters. The normal ureterovesical junction is designed with a long intramural tunnel through which the ureter travels before reaching the bladder that prevents retrograde flow of urine. Primary VUR is thought to be due to an abnormal or immature formation of this area, allowing urine to reflux from the bladder into the ureters and kidneys.

As many as 17% of all children may have VUR, including 30% of those who develop a urinary tract infection (Sargent, 2000). The first sign of VUR is often hydronephrosis on the prenatal ultrasound, with 20% of these infants eventually found to have reflux (Passerotti et al., 2011). Neonates with a history of unilateral prenatal hydronephrosis should undergo ultrasound again within the first week of life; if the hydronephrosis was bilateral or in a solitary kidney, this should be done before discharge from the hospital (Nguyen et al., 2010). Patients with high-grade

hydronephrosis on late prenatal or postnatal imaging should be screened with a VCUG (Peters et al., 2010).

VUR is graded after a VCUG from I to V. Lower-grade reflux is more likely to resolve spontaneously without the need for intervention, with spontaneous resolution seen in about 50% of patients with grade III reflux (Estrada et al., 2009). Continuous antibiotic prophylaxis is recommended for infants with grade III–V VUR or any grade if the infant has a history of febrile urinary tract infection. There is evidence that prophylaxis prevents recurrent infections, but its benefit in terms of renal scarring is less clear (RIVUR Trial Investigators, 2014). Another option for reducing the risk of urinary tract infection is circumcision (Morris and Wiswell, 2013). Breakthrough infections despite continuous administration of prophylactic antibiotics generally trigger a change in the antibiotic or surgical correction via an open or endoscopic approach.

About 27% of siblings and 36% of children of patients with VUR will have reflux. A screening ultrasound can be considered, with a VCUG pursued if there is evidence of renal scarring or a history of urinary tract infection (Skoog et al., 2010).

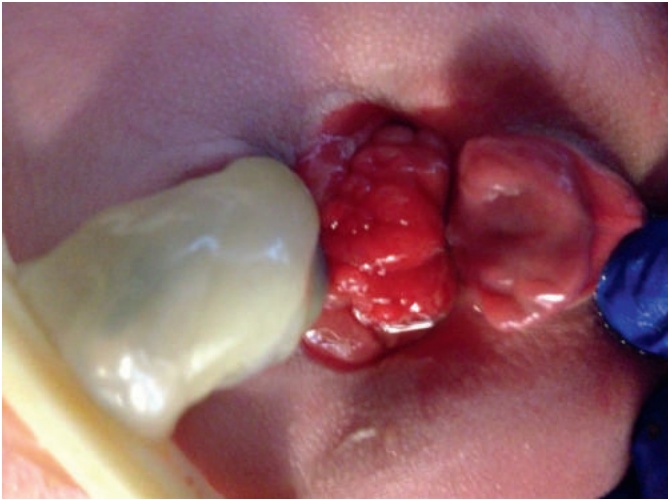
Anomalies of the Bladder

Bladder Exstrophy

Bladder exstrophy and epispadias are congenital anomalies that have characteristic external physical manifestations. The anterior portion of the bladder and/or urethra and abdominal wall structures are deficient, and the symphysis pubis is widely separated from the midline (Figs. 88.5–88.6).

This deformity has been described as “if one blade of a pair of scissors were passed through the urethra of a normal person; the other blade were used to cut through the skin, abdominal wall, anterior wall of the bladder and urethra, and the symphysis pubis; and the cut edges were then folded laterally as if the pages of a book were being opened” (Brock and O’Neill, 1998). This defect is usually found in isolation, with other organ systems, including the kidneys, rarely affected. Bladder exstrophy occurs at a rate of 1 per 10,000 to 1 per 50,000 live births. It occurs more commonly in males than females, with a ratio of 2.3:1 (Engel, 1974).

The defect may be diagnosed prenatally as ultrasonography can reliably detect exstrophy with the following findings: (1) an absent



• **Fig. 88.5** Male patient with bladder exstrophy showing exstrophied bladder, low umbilicus, and penile and urethral defect.



• **Fig. 88.6** Female patient with bladder exstrophy showing exstrophied bladder and bifid clitoris.

or nonfilling bladder, (2) a mass protruding from the abdominal wall, (3) normal kidneys with a low-set umbilicus, (4) an abnormal iliac crest widening, and (5) an anteriorly displaced scrotum (Mirk et al., 1986; Gearhart et al., 1995; Wilcox and Chitty, 2001). Prenatal diagnosis also allows better counseling of parents as the overall prognosis of these children is excellent if they are treated in specialized centers. Counseling of families by healthcare providers unfamiliar with the long-term outcomes has led to an increase in the abortion rate for these fetuses (Cacciari et al., 1999).

The pathogenesis of bladder exstrophy is thought to be caused by persistence of the cloacal membrane during development. This, in return, prevents the mesoderm from fusing in the midline. The persistent cloacal membrane then ruptures to produce an exstrophic condition (Muecke, 1964).

Management after delivery includes ligation of the umbilical cord with silk suture rather than plastic or metal clamp to prevent trauma to the exposed bladder. A hydrated gel dressing or even

plastic wrap placed on the exposed bladder protects it from superficial trauma. The dressing should be replaced daily and the bladder irrigated with saline with each diaper change. A renal ultrasound and a pelvic radiograph should be performed to evaluate the kidneys and the pubic diathesis. If a sacral dimple is found, a spinal ultrasound should be performed to rule out spinal cord tethering.

Surgical management has evolved, with most centers today performing a complete primary repair of exstrophy, which includes closure of the bladder exstrophy, reconstruction of the bladder neck, repair of the dorsal chordee, repair of epispadias with urethroplasty, and bilateral iliac osteotomies. Some still advocate a staged repair with closure of the bladder exstrophy and bladder neck as the first stage, repair of the epispadias as a second stage, and bladder neck reconstruction at around age 4–5 years (Grady and Mitchell, 1999; Inouye et al., 2013).

The timing of repair has also undergone some recent changes. In the past these repairs were primarily performed at birth. Recently many have advocated delayed closure at around 3 months of age, and this was based on studies showing that delayed closure does not compromise the rate of bladder growth (Baradaran et al., 2012; Ferrara et al., 2014; Borer et al., 2015). Delayed closure allows better coordination of care with the best team of urologists, orthopedic surgeons, anesthesiologists, and operating room staff present at the time of closure. Moreover, it allows the child to bond with the parents and allows penile growth to occur because of the physiologic surge of testosterone.

All children will develop VUR after surgery and should be given antibiotic prophylaxis for at least the first 18 months of life.

The goal of reconstruction includes preservation of renal function, urinary continence with volitional voiding, and functional and cosmetically acceptable external genitalia. With the primary repair, volitional voiding can be achieved with a single operation in around 40% of children, with around 40% requiring further bladder neck reconstruction at age 4–5 years to achieve continence. A small percentage of patients with small bladders will require reconstruction, including bladder augmentation and use of intermittent catheterization (Ellison et al., 2016).

Cloacal Exstrophy

Patients with cloacal exstrophy have multiple organ systems affected, and as a result it is also referred to the *OIES complex* (omphalocele, exstrophy, imperforate anus, and spinal defects). Other organ systems affected may include the extremities, the upper urinary tract, and the cardiovascular, pulmonary, and craniofacial systems. In this condition the hindgut is also open to the abdominal wall and separates the hemibladder plates.

Before the 1960s cloacal exstrophy was considered incurable, with a very high mortality rate. In the past 2 decades, survival rates have increased to close to 100% (Fullerton et al., 2016). The principles of management include neurologic evaluation for assessment of myelomeningocele, neonatal closure of omphalocele and intestinal diversion using the exstrophied hindgut, approximation of bladder halves, delayed bladder exstrophy closure with placement of the bladder deep in the pelvis, and iliac osteotomies (Shah et al., 2014).

Patent Urachus

The urachus is a tubular structure that connects the urogenital sinus and the allantois. It begins to narrow at between 4 and 5

months' gestation, generally being obliterated before birth. A patent urachus is often diagnosed because of drainage of clear fluid from the umbilicus. While ultrasound will often show the anomaly, a VCUG or sinogram can be used to confirm the diagnosis (Mesrobian et al., 1997). Because there is evidence that a patent urachus will be obliterated with time, surgery is generally avoided in patients younger than 1 year (Lipskar et al., 2010). If excision is required, it can be performed via the open or laparoscopic approach.

Posterior Urethral Valves

PUVs are a congenital obstruction of the posterior urethra. They are the most common cause of bladder outlet obstruction in newborn males, affecting approximately 2 in 10,000 live births, and occur only in males (Malin et al., 2012). They have been classified into three types since the 1920s. Type 1 valves account for the majority of PUVs, with leaflets of tissue arising from the verumontanum and fusing in the midline. Type 2 valves have not been described since the initial reports and likely do not exist. Type 3 valves are an annular ring thought to be due to persistence of the urogenital membrane (Young et al., 1919).

Prenatal ultrasound is often able to detect PUVs, with classic findings of a thickened, dilated bladder with bilateral hydroureter, especially in the presence of oligohydramnios and a dilated posterior urethra with the "keyhole sign" although some of these anomalies may be present in urethral atresia as well (Robyr et al., 2005). A VCUG is used to confirm the diagnosis after birth and will clearly show the obstructing membranes in the urethra (Fig. 88.7).

PUVs have consequences for multiple organ systems. Pulmonary hypoplasia and Potter facies can be seen if the obstruction was severe enough to cause oligohydramnios. Renal dysplasia is common

and can lead to progressive renal failure later in life, often associated with polyuria and nephrogenic diabetes insipidus. The bladder is generally small, with poor compliance that progresses to myogenic failure over time. The ureters often remain chronically dilated (Wein et al., 2016).

Initial management of PUVs involves support in the neonatal intensive care unit and passage of a urinary catheter for drainage. There is some evidence that inflation of a catheter balloon can cause ureteral obstruction in the valve bladder, and so this should be avoided (Jordan and Hoover, 1985). Once the infant is stable to undergo the procedure, the valves are ablated endoscopically. Vesicostomy is reserved for infants with urethras that are too small to accommodate the scope, while upper tract diversion is not felt to offer any significant benefit in terms of drainage or future renal function (Farhat et al., 2000).

Long-term renal outcomes are poor, with 20%–60% of boys progressing to end-stage renal disease. Nadir creatinine level is predictive of eventual renal outcome, with initial creatinine nadir level greater than 1.0 mg/dL generally used in modern series as the cutoff to predict a high risk of future renal damage (Sarhan et al., 2011; Bilgutay et al., 2016). The bladder decompensates to an overly large bladder with poor sensation and elevated postvoid residuals that often require overnight catheter drainage (Koff et al., 2002).

In the past decade a number of fetal interventions have been trialed for PUVs. These include vesicoamniotic shunting and fetoscopic valve ablation. While oligohydramnios abates and perinatal mortality is reduced, there is no evidence of a decrease in the percentage of patients who progress to end-stage renal disease (Farrugia, 2016).

Genital Abnormalities in Males

Cryptorchidism

Cryptorchidism, also known as *undescended testicle*, is one of the most common congenital anomalies. The incidence is variable and dependent on gestational age. The incidence ranges from 1% to 4.6% in full-term infants and from 1.1% to 45.3% in premature infants (Kolon et al., 2004). During the first 3–6 months of life the testicle may spontaneously descend such that the incidence at 1 year of age is around 0.7%–1%.

The main reasons for treating this condition are impaired fertility potential, testicular cancer, and testicular torsion. The number of spermatogonia per tubule is adversely affected in boys with undescended testicles beyond 1 year of age, and therefore it is recommended that orchiopexy be performed before 1 year of age to minimize germ cell loss (Hadziselimovic and Herzog, 2001).

This condition is commonly seen in healthy infants but can occur in conjunction with more than 400 syndromes (Visser, 1982). The cause is thought to be multifactorial and includes familial predisposition and environmental exposure (phthalates, pesticides, flame retardants). It has also been included in "testicular dysgenesis syndrome," a mechanism that interferes with normal fetal testicular development and includes undescended testicles and genitourinary disorders such as hypospadias, semen production abnormalities, and infertility (Barthold, 2008). A newborn "male" with bilateral nonpalpable gonads should be evaluated for possible disorder of sexual differentiation (DSD) such as congenital adrenal hyperplasia (CAH). Patients with a unilateral nonpalpable testicle and hypospadias should also be evaluated for a DSD; namely, mixed gonadal dysgenesis or ovotesticular DSD (Kolon et al., 2014).



• **Fig. 88.7** This voiding cystourethrogram shows a classic posterior urethral valve, with narrowing of the urethra at the most distal end of the prostate. This area corresponds to a flap of tissue that serves as an obstructing valve leaflet.

The most recent American Urological Association guidelines on the management of cryptorchidism state: “Providers should refer infants with a history of cryptorchidism (detected at birth) who do not have spontaneous testicular descent by six months (corrected for gestational age) to an appropriate surgical specialist for timely evaluation (Evidence Strength: Grade B).” The consensus on surgical management is that orchiopexy should be performed at around 6 months of age and before the age of 1 year. Surgically bringing the testicles into the scrotum may reduce but does not prevent the long-term sequelae such as infertility and cancer (Kolon et al., 2014).

Hypospadias and Chordee

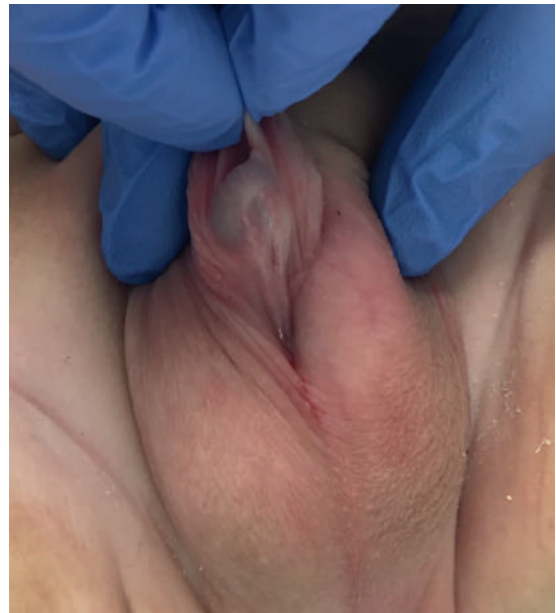
This is one of the most common abnormalities of the penis and occurs in 1 in every 250–300 male births, or around 0.5% of all male births (Stoll et al., 1990; Schnack et al., 2008). From an analysis of all boys born in Denmark between 1973 and 2005, it was found that the relative recurrence risk for a brother to have hypospadias was 13.4 (95% confidence interval [CI] 11–16.4) and 10.4 (95% CI 7.54–14.4) for offspring of an affected male. The risk in same-sex twins was 50.8 (95% CI 34.2–75.5) (Schnack et al., 2008).

The term *hypospadias* means an abnormally located urethra. The defect is a spectrum that includes an abnormal ventral curvature (chordee) of varied degree, a dorsally hooded foreskin, and penoscrotal transposition. The spectrum ranges mild chordee without hypospadias to severe chordee and hypospadias with the meatus in a perineal location. A small group of patients will have a normally formed foreskin such that the hypospadias is discovered at the time of circumcision. These children also have a wide urethral plate and thus are described as having megameatus hypospadias (Centers for Disease Control and Prevention, 2017).

In most cases hypospadias is the only abnormal finding. In about 10% of children, hypospadias may be a part of a syndrome with multiple anomalies (Stoll et al., 1990; Schnack et al., 2008). The most common defect is cryptorchidism, reported in around 3% of infants with distal hypospadias and in up to 10% of infants with proximal hypospadias (Wu et al., 2002). In one study chromosomal anomalies were found in 22.2% of patients with hypospadias and cryptorchidism (Yamaguchi et al., 1991). DSD such as CAH and mixed gonadal dysgenesis should be considered when hypospadias is found with either a unilateral or bilateral undescended testicles (McAleer and Kaplan, 2001; Cox et al., 2008).

Hypospadias is usually classified on the basis of the location of the meatus (distal portion of the shaft, midshaft, proximal portion of the shaft; penoscrotal, scrotal, perineal). This classification, however, is not sufficient to explain the complexity of the defect. Because of this, a new classification scoring system called the *GMS score* (glans, meatus, shaft) has been used that includes a scale of 1–4 for each component (glans size and urethral plate quality; meatal location; and degree of shaft curvature), with the more unfavorable characteristics assigned higher scores. This new scoring system provides a means by which hypospadias severity can be standardized and surgical outcomes better classified (Merriman et al., 2013; Arlen et al., 2015).

Hypospadias is usually repaired at 6–18 months of age, with the more severe repairs postponed until after the age of 1 year (American Academy of Pediatrics, 1996). A two-stage repair is advocated for those with severe hypospadias and chordee (Fig. 88.8). If the phallus is small, testosterone injections may be given to increase phallic size, but some have found that adjuvant therapy is an independent risk



• **Fig. 88.8** Severe scrotal hypospadias with chordee. The opening is in between the scrotal folds.

factor, with higher complication rates (Wong and Braga, 2015). Poor response to testosterone injections may reveal children with androgen insensitivity (Snodgrass et al., 2014). Complication rates are reported as being 2%–5% for distal hypospadias and in excess of 30% for proximal hypospadias (Snodgrass et al., 2010; Long and Canning, 2016; Pippi Salle et al., 2016).

Other Penile Anomalies

Webbed Penis

Webbed penis, also called *penis palmatus* or *penoscrotal fusion*, is a congenital condition where the scrotal skin extends to the ventral penile shaft with deficient ventral penile shaft skin. The penile shaft may be buried in the scrotum. Usually the urethral meatus and the penile shaft are normal (Perlmutter and Chamberlain, 1972).

These infants should not be circumcised and should be referred to a specialist for repair at around 6 months of age. Repair will include use of the foreskin to cover the ventral penile shaft and recreation of the penoscrotal and penopubic junction (El-Koutby and El Gohary, 2010).

Buried Penis

Buried penis, also known as *hidden penis*, is a congenital condition where the penis is partially or completely hidden below the surface of the skin. It can sometimes lead to obstruction with ballooning of the foreskin during voiding.

These children should not be circumcised at birth and should be referred to a specialist at around 6 months of age for possible repair (Maizels et al., 1986; Wollin et al., 1990).

Micropenis

This is a congenital condition defined as a male with normal internal and external genitalia with a stretched penile length 2.5 standard deviations below the mean (Table 88.1). The reported incidence of this condition is 1.5 in 10,000 male births (Hatiboğlu and Kurtoglu, 2013).

TABLE 88.1 Stretched Penile Length by Age

| Age | Mean (cm) | SD (cm) | Mean – 2.5 SD (cm) |
|----------------------------|-----------|---------|--------------------|
| Preterm newborns, 30 weeks | 2.5 | 0.4 | 1.5 |
| Preterm newborns, 34 weeks | 3.0 | 0.4 | 2.0 |
| Term newborn | 3.5 | 0.4 | 2.5 |

SD, Standard deviation.

Aphallia

Agenesis of the phallus is very rare and occurs in 1 in 10 million to 1 in 30 million live births. This suggests an early embryologic failure in development of the genital tubercle. The urethra is located in the perineum (Skoog and Belman, 1989). The karyotype is mostly 46XY, but females have been described with absence of corporal bodies (Friedman et al., 2016a). These conditions have been reported with other anomalies, such as renal agenesis, horseshoe kidney, skeletal and neural disorders, and imperforate anus. Male children were previously raised as females, but this practice has been abandoned. These children have normal testicles, with normal testosterone production and normal spermatogenesis, and should be raised as males. With improvements in phallic reconstruction and in vitro fertilization, these patients could father children and have the potential of having a functional phallus (Macedo et al., 2015).

Epispadias

This condition is usually associated with bladder exstrophy but can appear as an isolated defect. The incidence is around 1 in 117,000 males and 1 in 150,00 to 1 in 300,000 females (Frimberger, 2011). Epispadias is characterized by failure of the urethral plate to tubularize on the dorsum, with a defect ranging from the glandular to the phallopubic location. Male patients also have dorsal curvature or chordee where females have a bifid clitoris (Grady and Mitchell, 2002).

Those with the more proximal defects (penopubic) are incontinent because of incompetence of the bladder neck, but urinary incontinence has also been reported in the more distal defects (Canon et al., 2008; Surer et al., 2000).

There are different techniques for repairing this defect, with the proximal defects also requiring a bladder neck reconstruction for continence (Mitchell and Bagli, 1996; Surer et al., 2000). The current recommendation is to delay repair of these defects until 3–6 months of age. The more proximal defects (penopubic) will also require a bladder neck reconstruction including pelvic osteotomies to place the bladder neck area in an anatomically correct location, deep in the pelvis.

Urethral Duplication

This is an uncommon anomaly and can present as a complete or partial duplication. It is more common in males than in females. The two urethras are oriented in an anteroposterior plane, with the ventral urethral meatus being the functional urethra and the dorsal urethra being stenotic (Pippi Salle et al., 2000).

In females, urethral duplication may accompany cloacal anomalies and is usually associated with duplication of the bladder, with the urethral openings being on either side of the common urogenital sinus (Pippi Salle et al., 2000).

Differences in Sex Development

This is discussed in a separate chapter. These patients pose a difficult diagnostic and therapeutic challenge and require a multidisciplinary approach to evaluation and management, including genetics, urology, gynecology, endocrinology, social work, and pediatric psychiatry.

Any phenotypically looking male, even if completely masculinized, with bilateral nonpalpable gonads should be evaluated for CAH. Failure to do so may result in an Addisonian crisis due to salt-losing adrenogenital syndrome.

Phimosis

The prepuce forms as a roll of epithelium that fuses ventrally at the frenulum. Incomplete formation of the prepuce is associated with other penile anomalies such as hypospadias, chordee, and epispadias. Once formed, the inner preputial surface fuses with the glans epithelium and may not separate from it until later in childhood. In the process of separation, cystic spaces between the two layers may form and fill with desquamated epithelium that form white beads or infantile smegma. These may be quite large and present as a mass under the foreskin. They eventually drain spontaneously.

Current recommendations are to allow the foreskin to separate from the glans and to consider management if the phimosis does not resolve during childhood or by 5–7 years of age. Forceful retraction of the foreskin is contraindicated as this produces tears in the foreskin that will result in scarring that leads to pathologic phimosis and also to inflammation and infection (posthitis).

The benefits of circumcision at birth have been the subject of debate and controversy. An American Academy of Pediatric statement states that circumcision may have benefits in reducing the risk of urinary tract infections (UTIs) and sexually transmitted diseases (American Academy of Pediatrics Task Force on Circumcision, 2012). On the other hand, the Canadian Pediatric Society concluded that since the benefits do not exceed the risks, circumcision should be performed only in boys in high-risk populations or circumstances (Brian et al., 2016).

The most common complications of newborn circumcision include bleeding, infection, formation of penile adhesions between the skin and the glans, and incomplete circumcision. Other less common complications include iatrogenic amputation of the glans, loss of penile skin, and injury to the urethra (Friedman et al., 2016b).

When newborn circumcision is being discussed with the parents, it is important to discuss the pros and cons of circumcision, including the possible benefits, risks, and complications.

Testicular Tumors

There is a bimodal age distribution for the incidence of testis tumors, with one peak occurring during the first 2 years of life and a second, larger peak occurring in young adulthood. The incidence of pediatric testis tumors is 0.5–2.0 per 100,000 children, accounting for only 1%–2% of all pediatric tumors (Coppes et al., 1994).

Teratomas are the most common benign tumors in prepubertal patients. The median age of presentation is 13 months, with several reported cases in the neonatal period (Levy et al., 1994; Grady et al., 1997). Testis-sparing surgery is a consideration for prepubertal children, but frozen sections should be obtained to confirm the diagnosis. Orchiectomy is necessary if the entire testicle is involved.

Testicular Torsion

This is a rare event, and its management is controversial. The newborn presents with swelling of the hemiscrotum with or without discomfort. In this condition the testicle, epididymis, and tunica vaginalis twist around the spermatic cord (extravaginal torsion). This condition may be unilateral or bilateral, and the bilateral torsion can either be synchronous or asynchronous (Riaz-UI-haq et al., 2013).

The cause is unknown, but it is theorized that complicated pregnancies and vaginal delivery are predisposing factors (Riaz-UI-haq et al., 2013).

The presentation depends on when the torsion occurred. If it occurred several days before birth, the newborn will have a firm painless scrotal mass. If it occurred hours before birth, the newborn usually has a painful, enlarged, and high-riding testicle (Riaz-UI-haq et al., 2013).

Diagnosis is usually made by physical examination and Doppler testicular ultrasonography showing no blood flow to the testicle.

Management of neonatal testicular torsion is controversial. Because of the low salvage rate, some advocate conservative management (Kaye et al., 2008). Most pediatric urologists today advocate immediate surgical exploration. The rationale behind this recommendation is that viability of the testicle can be assessed only surgically. Moreover, there is an increased risk of asynchronous torsion in the first month of life, and fixing the normal testicle in place will prevent it from experiencing torsion (Yerkes et al., 2005).

Urinary Tract Infections

UTIs are more common in male infants, with a male-to-female ratio of around 3:1. Uncircumcised male infants appear to be at increased risk of UTIs in the first few months of life. Uncircumcised males have a 4.1% incidence of UTIs during the first year of life, while females have a 0.5% incidence and circumcised males a 0.2% incidence. The periurethral area was found to be more frequently and more heavily colonized with uropathogens, especially *Escherichia coli*, in uncircumcised infants compared with circumcised infants (Schoen et al., 2000; Singh-Grewal et al., 2005).

Newborns with culture-documented urinary tract infection should be evaluated with at least a renal ultrasound. Some advocate a VCUG. If the ultrasound findings are abnormal, a VCUG should be performed.

Myelodysplasia

The birth prevalence of spina bifida in the United States is approximately 30 cases per 100,000 live births. The urinary tract is involved in most children with myelomeningocele. Increased bladder pressures and impaired bladder function due to the neurologic deficit eventually result in hydronephrosis and ultimately renal damage. The goal of treating children with myelomeningocele is preventing upper tract deterioration.

In utero closure of myelomeningocele has resulted in reduced need for shunting and improved motor outcomes (Adzick et al., 2011). Prenatal surgery, however, did not significantly reduce the need for clean intermittent catheterization but was associated with reduced bladder changes (Brock et al., 2015).

All children should be evaluated with a renal ultrasound.

Urodynamic studies are currently recommended early in life to risk stratify children into those at high risk of renal damage (those with high pressure bladder, high leak point pressures,

dyssynergic voiding) and those at low risk (normal bladder compliance, low leak point pressure) (Bauer et al., 2015).

An indwelling catheter is placed in the immediate postclosure period, and clean intermittent catheterization is implemented every 3–4 hours. If the catheterization volumes are low, clean intermittent catheterization can be stopped. Parents should be taught to perform clean intermittent catheterization (Wu et al., 1997). Although controversial, early initiation of clean intermittent catheterization may decrease the need for future reconstruction such as bladder augmentation (Snow-Lisy et al., 2015).

These children should be treated by a multidisciplinary team that includes neurologists, neurosurgeons, urologists, and orthopedic surgeons.

Prune-Belly Syndrome

Prune-belly syndrome, also known as *Eagle-Barrett syndrome*, is a rare defect occurring in about 1 in 40,000 births (Fig. 88.9). This abnormality is usually characterized by the triad of abdominal wall deficiency, cryptorchidism, and dilatation of the urinary tract, including severe hydronephrosis, bladder distension, and urethral dilatation (Woods and Brandon, 2007).

Prognosis ranges from death in utero to near normal life expectancy. Management of the disorder depends on the severity of the symptoms. Patients with incomplete bladder emptying and recurrent UTIs may require a vesicostomy. All male children will have to undergo bilateral orchiopexy before the age of 1 year. Some children will require urinary tract reconstruction and reconstruction of the abdominal wall (Wheatley et al., 1996).

Anorectal Malformation

Children with anorectal malformations have a higher incidence of other organ system anomalies known as *VACTERL association* (vertebral, anorectal, cardiac, tracheal, esophageal, renal and/or radial, and limb). Urologic anomalies are frequently seen and can result in severe renal impairment if treated inadequately. The most common renal anomalies seen are hydronephrosis and VUR (Wiener and Keisewetter, 1973; Goossens et al., 2011).

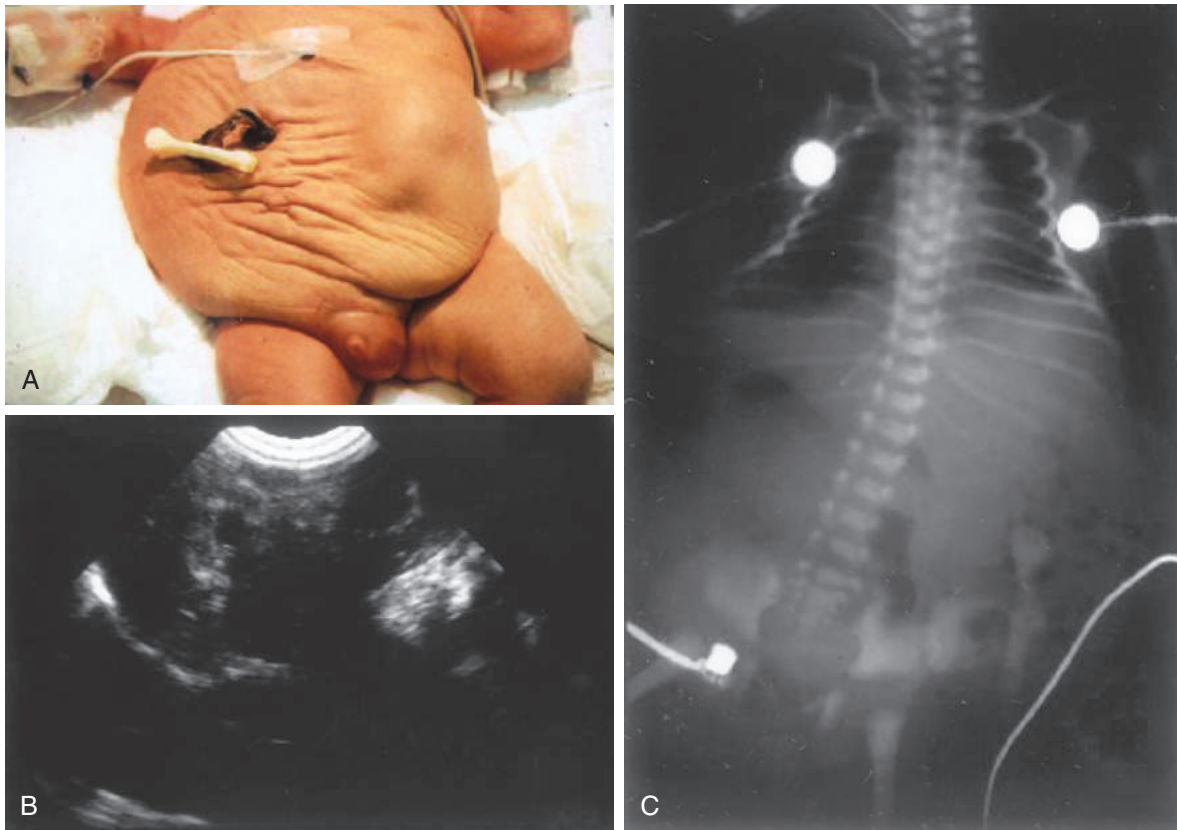
All children should undergo a renal and bladder ultrasound and, if the findings are abnormal, a VCUG. Sacral X-rays and ultrasound of the spine will further detect defects of the spinal cord that may also affect bladder function and cause renal impairment (Wiener and Keisewetter, 1973; Goossens et al., 2011).

Female Genital Anomalies

Female Genital Tract Development

The female reproductive system is formed by the müllerian or paramesonephric ducts. The process is complex and involves many genes. Mostly because of the absence of müllerian inhibiting substance (produced by the testicle), there is involution of the wolffian ducts. The müllerian ducts form lateral to the wolffian duct at around 6–8 weeks' gestation. The müllerian ducts then migrate medially, fuse in the midline, and are incorporated into the urogenital sinus to form the uterovaginal canal by the 10th week of gestation. The vagina forms from the fused müllerian ducts and the urogenital sinus. The upper four-fifths of the vagina is müllerian derived, and the lower fifth is of urogenital sinus in origin.

The external genitalia differentiate during the 12th to 16th weeks. The genital tubercle forms the clitoris, the urethral folds



• **Fig. 88.9** The classic wrinkled abdominal wall seen in prune-belly syndrome is accompanied by bilateral undescended testes (A). Affected patients will have marked hydronephrosis. In severe cases as illustrated here, these small kidneys may have a markedly dysmorphic sonographic appearance (B), and renal insufficiency may be present from the beginning. In cases with severe renal insufficiency, pulmonary development may be compromised, as evident on the radiograph (C); the patient required prolonged mechanical ventilation in the neonatal period.

become the labia minora, and the genital swellings become the labia majora (Masse et al., 2009).

Hydrocolpos and Hydrometrocolpos

Congenital hydrocolpos is an uncommon disorder characterized by vaginal distension with fluid. Hydrometrocolpos is associated with accumulation of fluid in both the vagina and the uterus. It is believed to be due to increased secretion by cervical mucous glands secondary to maternal hormone stimulation that expands and builds up into a pelvic mass because of vaginal outlet obstruction (Nazir et al., 2006).

Hydrocolpos can be associated with genitourinary anomalies such as persistent urogenital sinus and cloacal anomalies (Bischoff et al., 2010; Levitt and Peña, 2010; Fig. 88.10). Vaginal atresia can be associated with several syndromes (e.g., McKusick–Kaufman syndrome and Bardet–Biedl syndrome).

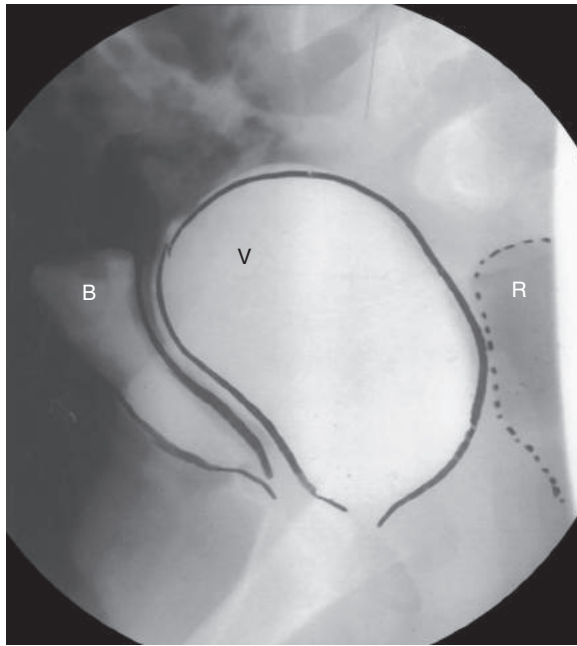
The most common complication of hydrocolpos is compression of the bladder, leading to urinary retention and hydronephrosis, which can ultimately cause kidney damage (Nazir et al., 2006). This can be prevented by drainage of the accumulated fluid.

If the hydrocolpos is secondary to an imperforate hymen, incision with drainage is performed. If, on the other hand, the hydrocolpos is due to vaginal atresia or cloacal anomaly, drainage of the vagina

can be accomplished either by clean intermittent catheterization or a transabdominal vaginostomy performed by an interventional radiologist under ultrasound guidance, which enables real-time evaluation without radiation exposure. The transabdominal drainage of hydrocolpos with an indwelling tube is more preferred than transvaginal drainage to prevent reaccumulation. In general, infants with hydrocolpos and urogenital sinus have increased risk of sepsis due to collection of urine in the vaginal vault. There have been reported deaths due to sepsis associated with hydrocolpos.

Vaginal Agenesis

Vaginal agenesis (Mayer–Rokitansky–Küster–Hauser syndrome) occurs in 1 in 4000 to 1 in 5000 live female births and is due to failure in canalization of the vaginal plate. Most of these patients present later in life with amenorrhea with normal secondary female sex features. The ovaries and external genitalia are normal, and the uterus may be rudimentary. Associated genitourinary abnormalities are common, such as renal agenesis and renal ectopia. Skeletal malformations are also common, particularly spine and rib anomalies. These have been termed *MURCS* (müllerian duct aplasia, renal aplasia, and cervicothoracic somite malformations). Surgical correction and timing are individualized on the basis of the extent of atresia and development of the uterine and vaginal remnants.



• **Fig. 88.10** This genitogram, performed by retrograde injection of contrast medium into a single sinus anterior to the patient's rectum, demonstrates the anteriorly placed bladder (B) and posteriorly placed vagina (V). The vagina and urethra merged into a common sinus, which then traveled a distance of 2 cm before emerging on the perineal body. The rectum (R) was normally placed. The vagina distended as a result of urinary entrapment, and the patient presented with a lower abdominal mass.

Cloacal Anomalies and Urogenital Sinus

A common urogenital sinus is a normal part of development of the fetus. If müllerian duct development stops during the first trimester, a common urogenital sinus will persist at birth. The confluence of the vagina into the common channel varies and depends on when the development arrest occurred. The earlier the arrest, the higher the connection of the vagina will be. The anus will be normally located but sometimes is anteriorly displaced. On examination, only one opening is found in the introitus, and there is a second, anal opening.

Sometimes the vagina is distended, with urine causing compression of the ureters and hydronephrosis. This can be easily managed

with initiation of intermittent catheterization, which will decompress the distended vagina (Rink and Cain, 2008).

Evaluation includes an examination of the patient under anesthesia and determination of the length of the common channel and the length of the urethra from the bladder neck to the common channel. A cloacogram with three-dimensional CT reconstruction will also provide details of the anatomy, which aids in surgical planning. Surgical repair is usually undertaken during the first year of life, and the reconstruction depends on the confluence of the vagina into the urogenital sinus (Patel et al., 2012; Valentini et al., 2016).

A cloacal anomaly is defined when the urogenital sinus is combined with an anorectal malformation. These infants have only one opening into the perineum and confluence of the vagina, rectum, and urethra into a common channel (Bischoff et al., 2010; Levitt and Peña, 2010; Peña, 2016). These children require a colostomy for stool divergence, and at the same time an examination under anesthesia can be performed to determine the extent of the common channel and the length of the urethra and vaginal channels. The reconstruction is usually performed at around 6–8 months of age or when the child is nutritionally stable. Multiple teams are involved in the reconstruction, including pediatric general surgery, pediatric urology, and pediatric gynecology teams.

Müllerian Duplication Anomalies

If fusion of the müllerian duct is incomplete, duplication anomalies occur. This can range from a septate vagina to vaginal duplication, where one or both vaginas are open to the perineum. These children may have a uterus that is partially fused (bicornuate uterus) to complete duplication with two cervixes (uterus didelphys) (Acien and Acien, 2016).

Introital Masses in Children

These are seen on physical examination of the infant, and the differential diagnosis includes:

- Imperforate hymen
- Prolapsed ureterocele
- Urethra prolapse
- Skene or Gartner duct cysts
- Rhabdomyosarcoma (Nussbaum and Lebowitz, 1983)

Complete references used in this text can be found online at www.expertconsult.com

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Clinical Evaluation of Renal and Urinary Tract Disease

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KEY POINTS

- Renal anomalies are identified in 0.5% of pregnancies and account for 20% of all identified prenatal anomalies.
- Hydronephrosis (abnormal increase in the fetal renal pelvic diameter) is the most common identified renal anomaly.
- Oligohydramnios should prompt an evaluation for severe, bilateral, poorly functioning, or absent kidneys.
- Fetal magnetic resonance imaging may be useful as a complementary imaging modality when ultrasound results are inconclusive.
- Prenatal intervention strategies that improve long-term kidney and urinary tract outcomes remain in need of ongoing research.
- Monogenic gene disorders account for a significant number of structural and/or glomerular kidney disorders identified in infants.
- Palpable abdominal masses in neonates most often originate from the urinary tract and are commonly cystic or obstructive.
- Absolute/corrected glomerular filtration rate is much lower in newborns than in older children and adults.

Prenatal Evaluation of Renal and Urinary Tract Disease

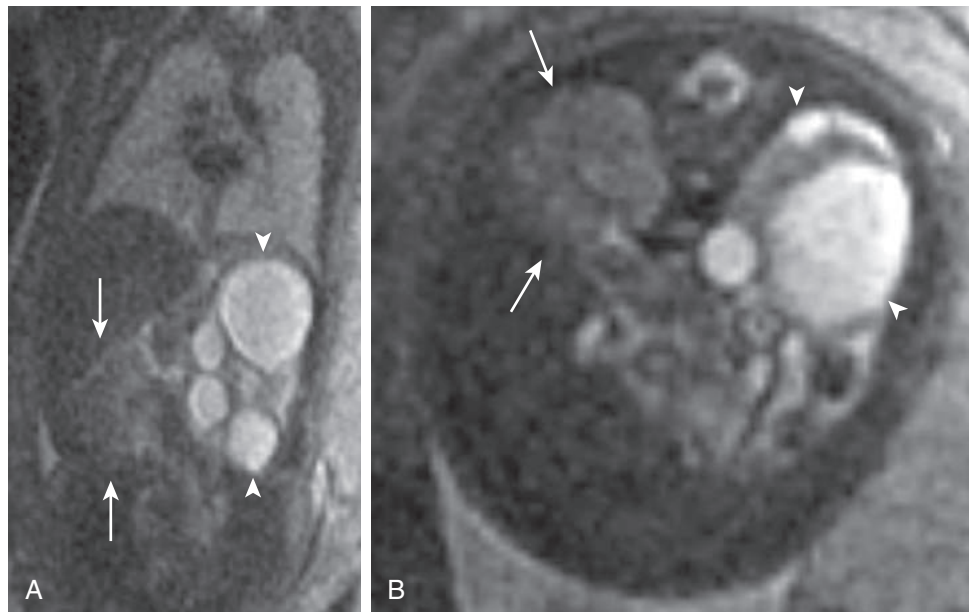
Prenatal Diagnosis

Renal anomalies will be frequently encountered in neonatal practice as they are identified in 0.5%–5% of pregnancies and account for 20% of all anomalies diagnosed with prenatal imaging (Scott and Renwick, 1988; Scott, 2002). Disorders of the renal and urinary tract include a broad spectrum of clinical entities with a wide variation of severity. This variation may result in challenging clinical diagnosis and management scenarios. Thus an understanding of the clinical evaluation in the prenatal and neonatal periods is an essential component of neonatal clinical practice.

Prenatal Renal Ultrasound

The primary tool utilized in the diagnosis of disorders of the renal and urinary tract is the prenatal ultrasound. Although the kidneys may be visualized at 10 weeks' gestation, accurate determination of renal anatomy is usually not possible until 16 weeks' gestation (Stamilio and Morgan, 1998). Findings on a prenatal ultrasound

that suggest renal or urinary tract disease include hydronephrosis, renal cysts, hyperechoic kidneys, renal mass, oligohydramnios, and polyhydramnios. Hydronephrosis (abnormal increase in the fetal renal pelvic diameter) is the most common identified renal anomaly, affecting 1%–5% of all pregnancies (Dicke et al., 2006; Nguyen et al., 2010). The presence of prenatal hydronephrosis may indicate a variety of renal disorders including vesicoureteral reflux, upper urinary tract obstruction (ureteropelvic junction obstruction, ureterovesical junction obstruction, and obstructive megaureter), and lower urinary tract obstruction (posterior urethral valves and prune belly syndrome) (Stamilio and Morgan, 1998; Yee and Wilcox, 2008). While mild hydronephrosis is often transitory and not associated with sequelae, higher degrees of hydronephrosis, early onset of hydronephrosis (first trimester), hydronephrosis that worsens with time, and bilateral hydronephrosis are usually associated with more severe disease, such as posterior urethral valves in male fetuses (Yee and Wilcox, 2008; Nguyen et al., 2010). Prenatal renal cysts can be seen with autosomal dominant polycystic kidney disease (ADPKD), multicystic dysplastic kidneys, cystic renal dysplasia, or obstructive dysplasia (Stamilio and Morgan, 1998; Avni et al., 2012). Hyperechoic kidneys are often associated with overt cysts as in ADPKD, with microscopic cysts (autosomal recessive polycystic kidney disease [ARPKD], Bardet–Biedl syndrome, Meckel–Gruber syndrome, hepatocyte nuclear factor 1 β [HNF1 β] mutations, or nephrotic syndrome), or renal dysplasia (Chaumoitre et al., 2006; Avni et al., 2012). The cause of oligohydramnios is multifactorial; however, its presence can indicate decreased fetal urine output and renal function (Kemper and Mueller-Wiefel, 2007). Oligohydramnios should prompt an evaluation for severe, bilateral, poorly functioning or absent kidneys as can be seen with renal agenesis, renal dysplasia, ARPKD, and lower urinary tract obstruction (Kemper and Mueller-Wiefel, 2007; Deshpande and Hennekam, 2008). Polyhydramnios is caused by renal anomalies in a small percentage of cases but can indicate the presence of a renal concentrating defect associated with renal dysplasia, nephrotic syndrome, or inherited renal tubular defects (Cole and Quamme, 2000; van Eijk et al., 2002; Deshpande and Hennekam, 2008). Congenital renal tumors are rare, and most are benign. The most common tumor is the mesoblastic nephroma, which is a benign tumor appearing as a unilateral single solid mass on prenatal ultrasound in the third trimester (Giulian, 1984; Cho and Lee, 2014; Do et al., 2015). Neuroblastomas are the most



• **Fig. 89.1** Coronal (A) and axial (B) images of fetal kidneys on T2-weighted magnetic resonance imaging. Note the normal right kidney (arrows) and large multicystic dysplastic left kidney (arrowheads) with the hyperintense peripheral cyst.

common fetal malignant tumors, appearing in the third trimester most often on the right side and suprarenal in location (Cho and Lee, 2014).

Fetal Magnetic Resonance Imaging

An emerging adjunct therapy to ultrasound is fetal magnetic resonance imaging (MRI). The typical indication for fetal urinary tract MRI evaluation is a second or third trimester pregnancy with oligohydramnios and/or an inconclusive prenatal renal ultrasound (Poutamo et al., 2000; Abdelazim and Belal, 2013). Fetal MRI lends additional insight into the evaluation of renal cysts and renal agenesis and can clarify diagnoses that would have been missed by ultrasound alone (Abdelazim and Belal, 2013). Differentiating renal cysts from a dilated collecting system is often problematic; the detailed anatomy provided by MRI can help determine if the apparent cysts are limited to the periphery (consistent with cyst) (Fig. 89.1) or central in location (consistent with a dilated collecting system) (Hormann et al., 2006). An increase in signal intensity in the renal cortex can differentiate between ARPKD and other polycystic diseases, which can have a similar hyperechoic appearance on ultrasound (Liu et al., 2006). When oligohydramnios is present and the kidneys cannot be conclusively identified on ultrasound, an MRI is effective at identifying an empty renal pouch, underlying changes (renal dysplasia), or ectopic kidneys (Hormann et al., 2006). Comparison of kidney and urinary tract fetal MRIs and ultrasounds reveals that MRI is able to identify abnormalities not identified by ultrasound in 26%–31% of patients (Cassart et al., 2004; Gupta et al., 2010; Behairy et al., 2015). Therefore fetal MRI may be very useful as a complementary imaging modality when ultrasound results are inconclusive. In addition to providing more detail of the renal and urinary tract anatomy, MRIs are also useful in measuring the amniotic fluid, evaluating bladder filling, and assessing lung maturation (Hormann et al., 2006). The aforementioned findings are indirect measures of kidney function that can help define the degree of renal impairment. While not yet available clinically, MRI has been shown in animals and in

postmortem human kidneys to accurately assess glomerular number (Puelles and Bertram, 2015).

Amniotic Fluid and Chorionic Villus Sampling

The diagnosis of most genetic disease with renal and urinary tract manifestations is based on the characteristic multiorgan findings on imaging. With an increasing number of conditions, analysis of DNA or tissue from amniocentesis or chorionic villus biopsy can be utilized to confirm a suspected disorder or determine prognosis. Examples include:

- Molecular testing for the mutations in genes for suspected congenital forms of nephritic syndrome (in fetuses with polyhydramnios and hyperechoic kidneys) by chorionic villus sampling in fetuses. Studies have shown that up to 84% of congenital forms of nephrotic syndrome are caused by mutations in *NPHS1*, *NPHS2*, *WT1*, or *LAMB2A* genes (see later) (Hinkes et al., 2007; Deshpande and Hennekam, 2008).
- Fetal karyotyping by amniocentesis or chorionic villus sampling performed when kidney defects occur with anomalies of other organ systems can be used to identify the precise chromosome anomaly and the likely genetic causes for the disorders (Deshpande and Hennekam, 2008; Chen et al., 2013; Li et al., 2014).
- Beckwith–Wiedemann syndrome, which can be associated with Wilms tumor and other renal anomalies, can be diagnosed by specific 11p methylation studies in amniotic fluid (Deshpande and Hennekam, 2008).
- Historically, most cases of prenatally enlarged echogenic kidneys were thought to be due to ADPKD, ARPKD, and cystic dysplasia. Microarray comparative genomic hybridization (CGH) arrays have demonstrated that other conditions, including deletions of 17q12, which includes *HNF1B*, are also responsible for some prenatally enlarged echogenic kidneys (Jones et al., 2015). Subsequently, prenatal CGH arrays have been proposed for echogenic kidneys with an unknown cause (Jones et al., 2015).
- While not yet available clinically, panels of peptides, extracellular vesicles, and exosomes containing CD24 in amniotic fluid have

been shown to be more predictive of more severe forms of congenital urinary tract obstruction than imaging alone (Chevalier, 2015).

- Hypotonic fetal urine, characterized by a sodium level of less than 90 milliequivalent (mEq)/L, chloride less than 80 mEq/L, and osmolality less than 180 mOsm/L in patients with bilateral hydronephrosis and no evidence of renal dysplasia, is associated with a good prognosis, particularly if present on sequential samples taken 48–72 hours apart (Johnson et al., 1995; Wu and Johnson, 2009).

Prenatal Management of Renal and Urinary Tract Disease

Goals of Management and Prognostic Factors

Identification of renal and urinary tract disease on prenatal screening provides the basis for formulating a differential diagnosis, determining prenatal and postnatal risks, and screening for other anomalies. The goals of prenatal intervention include the preservation of renal function and the promotion of lung maturation (Yiee and Wilcox, 2008). Potential management options include parental counseling, follow-up or serial prenatal imaging, postnatal imaging (ultrasound, voiding cystourethrogram [VCUG] etc.), fetal interventions, and early multispecialty collaboration (Pates and Dashe, 2006; Yiee and Wilcox, 2008; Swords and Peters, 2015). Factors associated with a poor outcome include bilateral involvement of the kidneys and oligohydramnios. If oligohydramnios is severe the oligohydramnios sequence can occur. This is characterized by pulmonary hypoplasia, “flattened” face, “squashed” nose, and positional limb abnormalities (Deshpande and Hennekam, 2008). When oligohydramnios is present the prognosis may vary based upon the underlying renal diagnosis. The prognosis for bilateral renal aplasia is usually much less favorable than if the oligohydramnios is secondary to obstruction (Cendron et al., 1994; Kemper and Mueller-Wiefel, 2007; Schwaderer et al., 2007).

Interventions

The effectiveness of fetal intervention for hydronephrosis is controversial (Hubert and Palmer, 2007). The primary procedure is placement of a vesicoamniotic shunt with the goals of relieving the obstruction (potentially preventing further renal damage) and decreasing oligohydramnios (Wu and Johnson, 2009). This procedure may be considered when bladder outlet obstruction is present, the life of the neonate is at risk, the fetus would likely benefit from bladder decompression, the pregnancy is singleton, and the fetus has a normal karyotype (Hubert and Palmer, 2007). A multicenter prospective study aimed at determining therapeutic and cost effectiveness of vesicoamniotic shunting in fetuses with suspected obstruction was terminated early due to poor enrollment but did show some modest increases in survival at 28 days and 1 year of age over conservative management; unfortunately, survival and renal function were relatively poor in both groups, and costs were higher in the shunted group than in the conservative treatment group (Morris et al., 2013). It is a matter of debate as to whether amniocentesis prevents or decreases pulmonary hypoplasia. Some reports have suggested a benefit for oligohydramnios secondary to premature rupture of membrane (De Carolis et al., 2004; Klaassen et al., 2007), and others have reported long-term success with serial amniocentesis in patients with bilateral renal aplasia (Bienstock et al., 2014). Rarely, fetal surgery is indicated for removal of tumors,

such as aggressive subsets of neuroblastomas (Bruny and Crombleholme, 2013). In utero repair of myelomeningocele has been proposed as a strategy to improve bladder function. Compared with postnatal myelomeningocele repair, prenatal repair results in less bladder trabeculation but does not reduce the need for clean intermittent catheterization at 30 months of age (Brock et al., 2015). Prenatal intervention strategies that improve long-term kidney and urinary tract outcomes remain an area in need of ongoing research.

Postnatal Evaluation of Renal and Urinary Tract Disease

Prenatal and Perinatal History

As noted earlier, a history of oligohydramnios is often associated with poor urine flow and poor kidney function. Forms that occur in the first trimester (e.g., bilateral renal aplasia or severe dysgenesis) are often associated with poor prognoses, in part from poor pulmonary development. Other causes such as obstructive nephropathy may have a more favorable outcome (Kemper and Mueller-Wiefel, 2007). As noted earlier, polyhydramnios is often idiopathic or secondary to nonrenal problems such as gastrointestinal or central nervous system abnormalities (Deshpande and Hennekam, 2008) but is associated with renal anomalies such as infantile Bartter syndrome and nephrogenic diabetes insipidus. A large placenta (>25% of the birthweight) can be a sign of congenital nephrotic syndrome (Holmberg et al., 1996). Hypoxia–ischemia (e.g., perinatal anoxia, placental abruption), nephrotoxin exposure (e.g., pre/perinatal use of nonsteroidal antiinflammatory agents, aminoglycosides, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers), and shock (e.g., blood loss, sepsis) increase the risk of acute kidney injury (AKI), which has an incidence of over 20/100,000 neonatal patients (Andreoli, 2004; Askenazi et al., 2009; Selewski et al., 2015). Studies in very low birth weight infants have reported 12%–40% rates of AKI that then confer much higher risks of mortality above those patients without AKI (Selewski et al., 2015).

Family History

Renal structural diseases such as aplasia, dysplasia, and multicystic dysplastic kidneys in parents or siblings increase the risks of congenital kidney disease in newborn patients (Roodhooft et al., 1984; Belk et al., 2002; Schwaderer et al., 2007). Mutations in many genes (e.g., *HNF1B*, *PAX2*, or *EYA1*) account for a large number of autosomal dominant inherited forms of either isolated structural kidney disease or as part of syndromes with extrarenal manifestations (Madariaga et al., 2013; Hwang et al., 2014). Some forms of PKD, vesicoureteral reflux, and medullary cystic kidney disease follow autosomal dominant inheritance patterns; thus screening should be considered in neonates whose parents have these disorders. Appropriate screening would be indicated in neonates whose siblings have autosomal recessive diseases (e.g., autosomal recessive PKD, cystinosis) or X-linked diseases (e.g., Alport disease or Lowe syndrome).

Physical Examination

Hypertension

As discussed in Chapter 93, increased blood pressure in the neonatal period is frequently secondary to renovascular and/or parenchymal disorders and should receive appropriate evaluation.

Micturition

Frequently, healthy neonates will not void until 12 hours or later after birth, although nearly all will produce some urine by 24 hours (Clark, 1977). Once voiding commences, it almost never occurs during sleep in term infants, whereas 60% of preterm infants will void when asleep; furthermore, both term and preterm neonates tend to void frequently (often hourly) with quite variable volumes (Sillen, 2001; Sillen and Hjalmas, 2004). Male preterm and full-term neonates have greater postvoid residual volumes and decreased bladder emptying rates than their female counterparts, likely because of the need for higher voiding pressures due to the longer male urethra (Wen et al., 2014). Delayed voiding (beyond 24 hours after birth) is often seen after stressful deliveries and has been correlated with enhanced arginine vasopressin and aldosterone secretion (Vuohelainen et al., 2008). Prolonged absence of urine formation should prompt evaluation for AKI or structural kidney/urinary tract disease.

Abdominal Masses

Palpation of the abdomen for masses is best performed within the first few days after birth, during which time there is relative hypotonia of the abdominal musculature (Perlman and Williams, 1976). Palpable abdominal masses, particularly flank masses, in neonates most often originate from the urinary tract and are commonly cystic or obstructive (Chandler and Gauderer, 2004; Ranganath et al., 2012). Among three studies that each enrolled over 10,000 newborns, the incidence of renal/urinary tract anomalies detected by deep palpation ranged from 0.2%–0.6% (Chandler and Gauderer, 2004). The most common urinary tract anomalies detected as abdominal masses are hydronephrotic kidneys, followed by multicystic dysplastic kidneys (Chandler and Gauderer, 2004). Bilateral flank masses may be palpated in patients with autosomal recessive PKD. Although less common, neonatal tumors of renal origin are usually not detected prenatally (Shapiro, 2014). The most common neonatal renal tumors are congenital mesoblastic nephromas (typically benign), followed by Wilms tumor (which is usually diagnosed after 6 months of age), rhabdoid tumors, and clear cell sarcomas (Chandler and Gauderer, 2004; Glick et al., 2004; Shapiro, 2014). While rarely originating in the kidney or lower urinary tract, neuroblastomas are the most common neonatal malignancy, accounting for greater than 20% of neonatal tumors (Shapiro, 2014). An abdominal mass should be evaluated by appropriate laboratory and imaging studies (see later).

Edema

Edema occurs when there is an imbalance between capillary hydrostatic and interstitial oncotic forces. Neonatal edema is more commonly seen in preterm infants than term infants and is often transient, resolving a few days after birth (Griffiths, 1959; Cartledge and Rutter, 1986; Hahn et al., 1997). Most cases of persistent pathologic edema, including cases of nonimmune fetal hydrops (which includes edema and ascites) are extrarenal in origin (Bellini and Hennekam, 2012). The major renal causes include total body fluid overload secondary to a decrease in glomerular filtration rate (GFR) (from acute or chronic renal injury) and low intravascular oncotic pressure from urinary protein losses (from congenital nephrotic syndrome, see later).

Ascites

As with edema, ascites can arise as an imbalance between hydrostatic and oncotic pressures, but it can also occur secondary to decreased

lymphatic drainage. Although neonatal ascites is somewhat rare, urinary tract abnormalities account for a significant number of cases (Aslam et al., 2007; Griscom et al., 1977). The most common urinary tract cause is urinary ascites, which can result from perforation of the ureter, renal pelvis, or bladder from obstruction (e.g., posterior urethral valves) or, less commonly, from a persistent urogenital sinus (Loganathan et al., 2014). Less common conditions leading to ascites are congenital nephrotic syndrome and renal vein thrombosis.

Laboratory Tests

Serum Estimates of Glomerular Filtration Rate

Absolute and relative (corrected for body surface area) GFR is much lower in newborns than older children and adults; at day 1 of life, GFR may be as low as 30 mL/min per 1.73 m² in term infants (and even lower in preterm infants) rising to about 50 mL/min per 1.73 m² by 1 month of life and 75 mL/min per 1.73 m² by 2 months of life (Arant, 1987; Chevalier, 1996). Because of technical difficulties with 24-hour urine collections for clearance measurements and/or the use of exogenous agents that are freely filtered at the glomerulus, most often assays of endogenous serum molecules are used to estimate GFR in neonates. As in adults, serum creatinine is both filtered and secreted in the kidney; however, creatinine also appears to be reabsorbed within the tubules of immature kidneys (with more reabsorption in preterm infants) (Guignard and Drukker, 1999). This likely accounts for the delay in creatinine drop that occurs from the time of birth (at which time serum creatinine reflects the mother's level) until about 7–10 days after birth in term newborns, which may extend to 3 weeks in preterm newborns (tubular reabsorption may also explain creatinine levels slightly greater than the mother's in many preterm newborns just after birth). In one study, term newborns had a mean serum creatinine of 0.85 ± 0.43 mg/dL at 2 days of age, 0.57 ± 0.40 mg/dL at 7 days of age, and 0.42 ± 0.23 mg/dL at 14 days of age (Rudd et al., 1983). In 28 weeks' gestation preterm newborns, the same study reported serum creatinine measurements of 1.31 ± 0.45 mg/dL at 2 days of age, 0.95 ± 0.36 mg/dL at 7 days of age, and 0.81 ± 0.36 mg/dL at 14 days of age. Serum cystatin C levels have some potential advantages to serum creatinine levels. For instance, unlike creatinine, cystatin C is not influenced by muscle mass and does not cross the placenta (Cataldi et al., 1999). Serum cystatin C reference values for neonates have been determined longitudinally up to 1 year of age (Nakashima et al., 2016). However, the difficulty of comparing a "gold standard" inulin or iothexol clearance in preterm infants makes determining the most accurate GFR measurement difficult. Blood urea nitrogen concentration is also often used as another indirect measure of kidney function but is less reliable than creatinine due to alterations based on protein intake and hydration status.

Other Serum Chemistries

In addition to GFR, renal tubular function often differs in the neonatal kidney from that of adults. Acidification differs with normal serum bicarbonate levels in the term neonate, ranging from 19–21 mEq/L, and in the preterm infant ranging from 16–20 mEq/L (Shaw, 2008). The lower serum bicarbonate is due to a lower threshold at the proximal tubule; the lowered threshold is likely due to the presence of different acid transporter isoforms in maturing kidneys versus adult kidneys as well as due to hormonal influences on abundance and activity of these transporters (Baum and Quigley, 1995; Baum, 2008). Serum potassium is less well

excreted in the collecting ducts of neonates compared with older children and adults, with normal newborn levels up to 6.7 mmol/L (Lorenz, 1997). This physiologic increase in potassium (to accommodate vigorous growth over the first year of life) is due to a paucity of aldosterone-sensitive secretory potassium channels and an abundance of potassium reabsorbing transporters on the luminal surface of collecting ducts (Gurkan et al., 2007). Sodium must remain in positive balance for rapidly growing neonates (despite a diet that is typically low in sodium). The ability to excrete less sodium chloride appears to be due to differences in expression of sodium/proton antiporter isoforms and tight junction proteins, called claudins, in neonates versus adults and older children (Baum and Quigley, 2004; Baum, 2008). Serum phosphate levels are also typically higher in newborns with serum levels ranging from 5.8–9.3 mg/dL on the first day of life (with the higher levels typically in preterm infants (Spitzer and Barac-Nieto, 2001; Hellstern et al., 2003)). This physiologic increase in serum phosphate levels compared with older humans (again, to accommodate rapid growth) is due to the enhanced capacity of sodium phosphate transport at the proximal tubule in neonates (Spitzer and Barac-Nieto, 2001).

Urine

Urinary Tract Infection

Most frequently, urine collections in newborn infants are performed to evaluate for suspected urinary tract infection. Evidence-based guidelines were published recently regarding evaluation for urinary tract infection (UTI) in patients from 2 months to 2 years of age (Roberts, 2011). Some of these guidelines concluded that UTI should be considered in all patients with unexplained fever and is best assessed with a urine culture. For patients under 2 months of age, suprapubic aspiration or transurethral catheterization is highly recommended when evaluating for urinary tract infections. According to the most recent American Academy of Pediatrics guidelines, a renal ultrasound should be performed in patients from 2 months to 2 years after their first confirmed UTI and a VCUG performed after the second UTI (Roberts, 2011).

Concentrating/Diluting

Compared with adults, newborns have a diminished capacity to concentrate their urine, because of their relative insensitivity to antidiuretic hormone and the presence of a less hypertonic medulla due to less sodium chloride and urea transport (Quigley et al., 2001; Bonilla-Felix, 2004). Neonates have less ability to dilute their urine compared with adults and older children, although they can handle the typically hypotonic fluids they receive for nutrition.

Proteinuria

Physiologic and pathologic proteinuria can be seen in newborns, particularly in preterm and/or low birth weight infants. Normal preterm and term infants have an average of 182 mg/m² per day and 145 mg/m² per day of total urine protein, respectively, compared with 91 mg/m² per day in children 2–4 years of age (Loghman-Adham, 1998). A recent study also found that very low birth weight infants had a higher risk of pathologic albuminuria (albumin/creatinine ratio >20 mg/g) than normal infants, particularly those that developed hypotension after birth (Iacobelli et al., 2007). Another study showed that preterm infants were more likely to develop pathologic tubular proteinuria (α 1 microglobulin/creatinine ratio >10 mg/mmol) than term infants (Ojala et al., 2006). Low gestational age enhanced the risk of early tubular proteinuria, the

highest α 1 microglobulin/creatinine ratios, and delayed normalization of proteinuria.

A more rare but serious pathologic finding is congenital nephrotic syndrome, defined as high-grade proteinuria, low serum albumin, and edema within the first year of life. The differential diagnosis includes primary/genetic causes (e.g., Finnish-type nephrotic syndrome and diffuse mesangial sclerosis) and secondary causes such as infections (e.g., cytomegalovirus, syphilis, hepatitis), genetic syndromes (e.g., Denys-Drash and Frasier syndromes), toxins/drugs, hemolytic uremic syndrome, systemic lupus erythematosus, and nephroblastoma (Papez and Smoyer, 2004). Unlike nephrotic syndrome in later childhood, congenital nephrotic syndrome usually portends a poor prognosis, including end-stage renal failure (with the exception of some infectious forms that respond to appropriate therapy). Interestingly, a classic study found that 2/3 of all cases of primary/genetic congenital nephrotic syndrome were caused by mutations in 1 of 4 genes, encoding for nephrin (major cause of Finnish type), podocin, Wilms tumor suppressor gene 1 (found in Denys-Drash and Frasier syndromes), and laminin β 2 (a cause of diffuse mesangial sclerosis) (Hinkes et al., 2007).

Other Urinary Findings (Hematuria, Hemoglobinuria, Myoglobinuria, Uricosuria)

Hematuria is a rare finding in neonates and when present has a wide differential diagnosis including renal vein thrombosis, PKD, obstructive nephropathy, tumor, congenital malformations, urinary tract infection, and AKI (Emanuel and Aronson, 1974). Hemoglobinuria, another rare finding in newborn infants, occurs secondary to intravascular hemolysis, of which the most common cause is ABO blood group incompatibility (Murray and Roberts, 2007). Myoglobinuria is even more rarely detected in neonates but has been reported secondary to rhabdomyolysis from asphyxia and shock (Sirota et al., 1988). Finally, pink or red uric acid crystals are often seen in diapers of otherwise healthy newborns; while this has been reported to be a consequence of high urine uric acid levels in normal newborns, one study challenges that contention, arguing that there must be other reasons for uric acid crystallization in these neonates (Kupeli et al., 2005). Urinary neutrophil gelatinase-associated lipocalin (NGAL) levels correlate with creatinine-based AKI (Goldstein, 2015). Additionally, urine NGAL levels above a certain threshold are associated with clinical outcomes even when measured at day 1 when newborn serum creatinine levels are representative of maternal levels (Essajee et al., 2015; Goldstein, 2015).

Imaging

Renal Ultrasound

The primary indications for renal ultrasound in the neonatal period include a palpable abdominal mass, hypertension, renal failure, or suspected malformations of the renal and urinary tract (McInnis et al., 1982). The neonatal presentation of a renal mass or renal failure is often suggested by the prenatal evaluation (Riccabona, 2006). An abdominal mass of renal etiology might appear as large kidneys, hydronephrotic kidneys, or a renal tumor. Renal ultrasound can be useful to distinguish between intrinsic, prerenal, and postrenal failure. Regarding intrinsic causes of renal failure, neonatal nephrotic syndrome and glomerulonephritis will present as large echogenic kidneys; renal vein thrombosis will present as large echogenic kidneys with renal vein color signal missing on veins with thrombosis and increased resistive indices on a duplex examination; and congenital dysplasia will present as small echogenic kidneys

(Riccabona, 2006). Postrenal failure is characterized by hydronephrosis, and prerenal failure is characterized by echogenic kidneys with decreased flow velocities with elevated resistive index values on duplex examination (Riccabona, 2006). Ultrasound imaging has been recommended as a noninvasive study that should be obtained in all hypertensive infants as it may identify correctable causes of the hypertension including renal vein thrombosis, aortic/renal artery thrombi, or anatomic renal abnormalities (Flynn, 2000). If a renal ultrasound is planned to follow-up prenatal hydronephrosis, it is advantageous to wait until day 3 of life as the initial period of neonatal oliguria might mask renal collecting system dilation (Kennedy, 2002).

Computed Tomography Scan

The risk of ionizing radiation from computed tomography (CT) is higher in neonates secondary to more radiosensitive tissues and longer life expectancies (Brenner et al., 2001). Further, the CT evaluation of the kidney is limited by reduced contrast uptake by the renal parenchyma (Olsen and Gunny, 2006). Because of the aforementioned factors, CT has limited use in the evaluation of the renal and urinary tract during the neonatal period. Nonetheless, CT does have a role when ultrasound results are inconclusive for complex anomalies of the kidney, abdominal masses, and suspected renal vascular anomalies and when MRI would be problematic due to sedation risk or a contraindication to MRI contrast (Gnanasambandam and Olsen, 2006; Olsen and Gunny, 2006; Grobner and Prischl, 2007).

Magnetic Resonance Imaging

MRI has several advantages in imaging the neonatal kidney and urinary tract. The absence of radiation and detailed anatomy of soft tissue with MRI make it an attractive tool to clearly define normal and abnormal renal anatomy when ultrasound is insufficient. However, MRI has disadvantages as sedation is sometimes necessary for infants (Michael, 2008). There are risks for MRI contrast (gadolinium)-associated nephrogenic systemic fibrosis in the immature neonatal kidney and gadolinium depositions in the bone marrow (Michael, 2008). Applications in which MRI are particularly useful include the identification of a suspected ectopic or dysplastic kidney and the identification of a renovascular cause of hypertension in neonates unable to tolerate angiography (Mustafa et al., 2006; Michael, 2008).

Voiding Cystourethrography

VCUG is utilized to evaluate for lower urinary tract obstruction and vesicoureteral reflux. In males a fluoroscopic as opposed to a nuclear VCUG should be obtained as the anatomic details of the male urethra warrant a complete evaluation (Kennedy, 2002).

Nuclear Medicine

The use of nuclear medicine is limited in neonatal practice. Two studies that might be occasionally used include DMSA cortical scintigraphy and the DTPA or MAG3 diuretic renogram. Cortical DMSA cortical scintigraphy can be used to evaluate left and right relative renal function and evaluate for acute pyelonephritis (Piepsz

and Ham, 2006). The diuretic renogram is indicated to evaluate for upper urinary tract obstruction when fetal hydronephrosis has persisted and vesicoureteral reflux has been excluded (Kennedy, 2002; Piepsz and Ham, 2006). It may also be used to identify ectopic renal tissue (Piepsz and Ham, 2006). In the future, MRI may supplant nuclear medicine studies. A 2014 study demonstrated that magnetic resonance urography was superior to DMSA scan for identification of renal parenchymal defects in a population of children with a mean age of 9 months (Cerwinka et al., 2014).

In conclusion, structural abnormalities are common causes of kidney and urinary tract disorders in the perinatal period of life. Thus imaging modalities such as renal ultrasound remain very useful tools in evaluating patients with suspected kidney and/or urinary tract disease. A careful history and physical examination will also offer insights into the underlying causes of kidney and urinary tract disorders. Finally, compared with that of older children and adults, the neonatal kidney is less efficient at clearance and has reduced tubular function (acidification, potassium secretion, sodium transport), except for phosphate, which is reabsorbed more efficiently in the very young.

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Acute Kidney Injury and Chronic Kidney Disease

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KEY POINTS

- Acute kidney injury (AKI) is common in critically ill neonates. AKI affects survival, hospital expenditures, and long-term outcomes, independent of severity of illness and comorbidities.
- Renal development continues until 34 weeks' gestation. Neonatal intensive care unit graduates, especially those with AKI, premature infants, and those with intrauterine growth retardation, are at risk for long-term chronic kidney disease (CKD).
- Clinical sequelae of CKD include anemia, acidosis, electrolyte abnormality, growth restriction, renal osteodystrophy, fluid overload, hypertension, and uremia. Attention to these complications is critical to optimizing long-term outcomes.
- Long-term survival of neonates with end-stage renal disease (ESRD) appears to be approaching that of older infants and young children, but they continue to have higher morbidity and mortality due to infectious and cardiovascular complications.
- Renal replacement therapy can be performed in neonatal patients and is likely to improve outcomes in children with AKI and those with ESRD.

Acute Kidney Injury

Acute kidney injury (AKI) is characterized by a sudden impairment in kidney function, which may result in dysregulation of fluid balance, acid–base, electrolytes, and nitrogenous waste products. AKI has supplanted the term *acute renal failure* as the accepted terminology to describe acute changes in renal function across medicine, including neonatology (Mehta et al., 2007; Selewski et al., 2015). The term *injury* highlights the spectrum of organ injury and differentiates a damaged organ from an organ that has dysfunction. The main impetus to change the terminology was to highlight early detection, as even small changes in kidney function (rise of serum creatinine [SCr] by 0.3 mg/dL) can be associated with adverse outcomes. AKI is now staged into mild, moderate, and severe, based on either the most severe oliguria or rise in SCr.

In 2005, the introduction of an empiric, categorical staged AKI definition for adults dramatically changed the field of AKI as it brought consensus and enabled comparison between studies (Hoste and Kellum, 2006). In 2007, modifications were made based on improved understanding from observational data (Mehta et al., 2007). Additional modifications occurred in 2012, and currently Kidney Diseases: Improving Global Outcomes (KDIGO) defines

AKI when there is a rise in SCr of 0.3 mg/dL over 48 hours or a decrease in urine output (UOP) occurs. The development and utilization of standardized definitions of AKI have created commonality in defining AKI and clearly show that incremental degrees of AKI independently impact survival after correcting for comorbidities, complications, and severity of illness in neonatal (Selewski et al., 2015), pediatric (Akcan-Arikan et al., 2007), and adult studies (Hoste and Kellum, 2006).

A neonatal classification of AKI used to define AKI in critically ill neonates (Table 90.1) parallels the KDIGO adult definition and uses the lowest SCr as a baseline for subsequent SCr values. In April 2013, neonatologists and pediatric nephrologists participating in the NIDDK workshop carefully scrutinized this definition. They concluded that, at that time, this definition offered a reasonable starting point and would allow for consistency throughout studies yet warned that rigorous evaluation of the definition was necessary.

Importantly, SCr-based AKI definitions do not detect kidney damage; instead they document changes in kidney function. Although SCr is the most common method of documenting changes in kidney function, it has significant shortcomings, including:

- SCr does not change until 25%–50% of the kidney function has been lost, and thus it may take 48–72 hours for SCr levels to rise after an insult (Brion et al., 1986).
- At a lower glomerular filtration rate (GFR), SCr will overestimate renal function due to tubular secretion (Brion et al., 1986).
- SCr varies by muscle mass, hydration status, sex, age, and gender.
- Different measurement methods (Jaffee reaction vs enzymatic) produce different values, and medications and bilirubin can affect SCr measured by the Jaffee method (Rajs and Mayer, 1992; Lolekha et al., 2001).
- Once a patient receives dialysis, SCr can no longer be used to assess kidney function, since SCr is easily dialyzed.

Additional problems specific to neonates with using SCr as a measure of AKI include:

- SCr measurements in the first few days of life reflect the mother's levels; thereafter, the distribution of normal SCr values varies greatly, dependent on level of prematurity and age (Gallini et al., 2000) (Fig. 90.1).
- Normal nephrogenesis in the healthy fetus continues until 34 weeks of gestation when the number of nephrons, 1.6 to 2.4 million, approximates that of an adult (Abrahamson, 1991).

Dependent on degree of prematurity, GFR steadily improves from 10–20 mL/min per 1.73 m² during the first week of life to 30–40 mL/min per 1.73 m² by 2 weeks after birth, concomitant with alterations in renal blood flow. GFR improves steadily over the first few months of life (Brion et al., 1986) (Table 90.2).

An alternative to SCr is the measurement of serum cystatin C levels. Cystatin C is a low-molecular-weight protein that is freely filtered in the glomerulus and not resorbed. Cystatin C is a member of the cystatin superfamily of cysteine protease inhibitors, and is made by all nucleated cells within the body at a relatively constant rate. Cystatin C does not cross the placental barrier and, as a result of this, does not reflect maternal values. Serum cystatin C remains a functional biomarker that has shown promise in neonates but warrants further study. As a functional biomarker serum, cystatin C has been shown to provide more accurate assessments of neonatal renal function than SCr. Studies evaluating the potential role of cystatin C in neonates have been recently extensively reviewed (Filler and Lepage, 2013).

As mentioned previously, SCr, UOP, and cystatin C are markers of kidney function, not damage. Over the last decade, there has been a significant amount of work to identify urine and serum biomarkers of AKI. Such biomarkers include urine and serum neutrophil gelatinase-associated lipocalin (NGAL), urine interleukin-18, kidney injury marker-1, and liver fatty acid-binding protein, originally identified and studied in neonates undergoing cardiopulmonary bypass (Mishra et al., 2005; Parikh et al., 2006) (Fig. 90.2). These markers will ideally show acute damage hours after an insult, distinguish between different causes and locations of tissue injury, and prognosticate clinical outcomes.

In neonates, it is important to note these urine biomarkers will vary based on gestational age (GA), day of life, and gender (Saeidi et al., 2015). Urine biomarkers of AKI have been tested and show promise in the ability to predict AKI in very low birth weight (VLBW) (Askenazi et al., 2011) and near-term/term neonates (Tanigasalam et al., 2016). Future work is needed before these biomarkers can be used at the bedside. Once we can reliably identify AKI early in the disease process, preventive/therapeutic interventions can be studied to improve outcomes in neonates with AKI.

TABLE 90.1 Definition of Neonatal Acute Kidney Injury

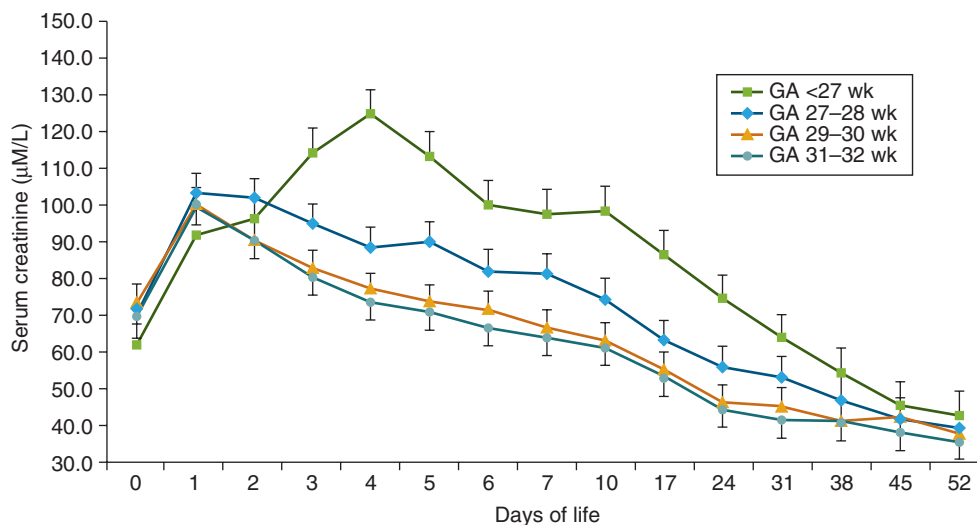
| Stage | Serum Creatinine | Urine Output/24 Hours |
|-------|---|------------------------------|
| 0 | No change in serum creatinine or rise <0.3 mg/dL | >1 mL/kg per hour |
| 1 | SCr rise ≥0.3 mg/dL rise from baseline or SCr rise ≥1.5–1.9 mg/dL × baseline SCr ^a | >0.5 and ≤1 mL/kg per hour |
| 2 | SCr rise ≥2.0–2.9 mg/dL × baseline SCr ^a | >0.3 and ≤0.5 mL/kg per hour |
| 3 | SCr rise ≥3 × baseline SCr ^a or SCr ≥2.5 mg/dL ^a or Receipt of dialysis | ≤0.3 mL/kg per hour |

^aBaseline SCr = lowest SCr prior to measurement.
SCr, Serum creatinine.

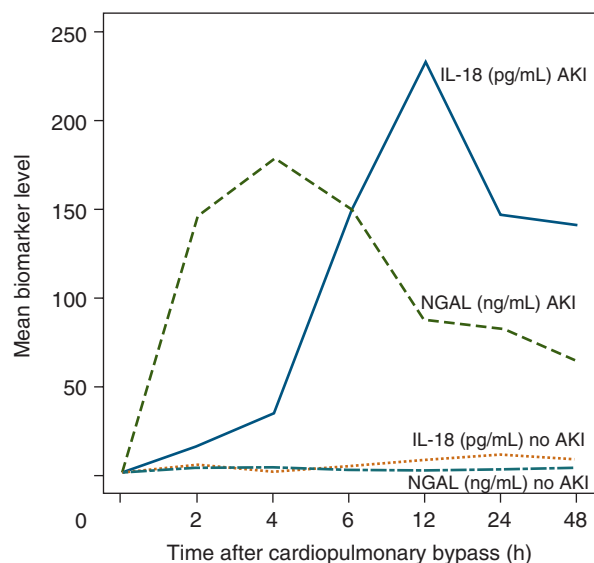
TABLE 90.2 Insulin Clearance Glomerular Filtration Rate in Healthy Premature Infants

| Age | mL/min per 1.73 m ² |
|--------------|------------------------------------|
| 1–3 days | 14.0 ± 5.0 (Brion et al., 1986) |
| 1–7 days | 18.7 ± 5.5 (Guignard et al., 1975) |
| 4–8 days | 44.3 ± 9.3 (Barnett et al., 1948) |
| 3–13 days | 47.8 ± 10.7 (Barnett et al., 1948) |
| 1.5–4 months | 67.4 ± 16.6 (Barnett et al., 1948) |
| 8 years | 103 ± 12 (Vanpee et al., 1992) |

Adapted from Schwartz GJ, Furth SL. Glomerular filtration rate measurement and estimation in chronic kidney disease. *Pediatr Nephrol*. 2007;22:1839–1848.



• **Fig. 90.1** Serum creatinine concentrations (μM/L) during the first days of life, with values given as means and standard error for infants born at different gestational ages. GA, Gestational age.



• **Fig. 90.2** Mean values of urine interleukin-18 (pg/mL) and neutrophil gelatinase-associated lipocalin (ng/mL) over the first hours after cardiopulmonary bypass in infants who develop acute kidney injury (50% increase in serum creatinine) compared with those who did not develop acute kidney injury. *AKI*, Acute kidney injury; *IL-18*, interleukin-18; *NGAL*, neutrophil gelatinase-associated lipocalin.

Epidemiology

Critically ill neonates experience multiple risks for the development of AKI during their hospitalization, including infections, nephrotoxic medications, and hypotension. The exact incidence of neonatal AKI is difficult to quantify because infants commonly have nonoliguric renal failure and may therefore may not be screened with SCr for AKI. Utilizing legacy definitions of AKI, such as SCr greater than 1.5 mg/dL or initiation of renal replacement therapy (RRT), previous studies had estimated the incidence of neonatal AKI to be 8%–24% with associated mortality rates between 10%–61% (Andreoli, 2004). Over the past decade there has been a significant amount of research utilizing modern staged definitions of AKI to evaluate the incidence and impact of AKI in a number of high-risk patient populations, including neonates with perinatal asphyxia or necrotizing enterocolitis (NEC), those undergoing cardiac surgery or receiving extracorporeal membrane oxygen (ECMO), sick term/premature infants, low birth weight babies, and general neonatal populations (Table 90.3).

Infants With Perinatal Asphyxia

Infants with perinatal asphyxia represent a population known to be at high risk for the development of AKI. Recently there have been several single-center studies evaluating the incidence of AKI, utilizing modern AKI definitions in neonates with perinatal asphyxia. Selewski et al. (2013) evaluated 96 newborns undergoing therapeutic hypothermia for perinatal asphyxia and found that 38% had AKI. In this cohort, AKI was associated with adverse outcomes, with

TABLE 90.3 Neonatal Acute Kidney Injury Studies

| Study | Population | Definition | Incidence of AKI | Findings |
|-----------------------|---|----------------------------------|----------------------------|--|
| Askenazi et al. 2009 | Very low birth weight infants (<i>n</i> = 195) | AKIN criteria | Matched case–control study | AKI is associated with increased mortality after adjustment for confounders. |
| Gadepalli et al. 2011 | Congenital diaphragmatic hernia on extracorporeal membrane oxygenation (<i>n</i> = 68) | RIFLE criteria | 71% | Increased risk of mortality at highest level of AKI (failure) |
| Kaur et al. 2011 | Perinatal asphyxia (<i>n</i> = 36) | AKIN criteria | 41.7% | Modern staging systems (AKIN) capture AKI previously missed by previous standard of SCr >1.5 mg/dl. |
| Koralkar et al. 2011 | Very low birth weight infants (<i>n</i> = 229) | Neonatal modified KDIGO criteria | 18% | Adjusting for severity of illness AKI was associated with increased mortality. |
| Askenazi et al. 2013 | Sick near-term neonates (<i>n</i> = 58) | Neonatal modified KDIGO criteria | 15.6% | AKI associated with increased mortality and positive fluid balance |
| Alabbas et al. 2013 | Cardiac surgery <28 days (<i>n</i> = 122) | AKIN criteria | 62% | Severe AKI (stage III) was associated with increased mortality and length of stay after adjusting for severity of illness. |
| Selewski et al. 2013 | Perinatal asphyxia (<i>n</i> = 96) | Neonatal modified KDIGO criteria | 38% | AKI predicted prolonged mechanical ventilation, length of stay, and abnormal brain MRI findings at 7–10 days of life. |
| Zwiers et al. 2013 | Extracorporeal membrane oxygenation <28 days (<i>n</i> = 242) | RIFLE criteria | 64% | Increased risk of mortality at highest level of AKI (failure) |
| Rhone et al. 2013 | Very low birth weight infants (<i>n</i> = 107) | Neonatal modified KDIGO criteria | 26.2% | AKI is associated with nephrotoxic medication exposure. |

AKI, Acute kidney injury; *AKIN*, acute kidney injury network; *KDIGO*, kidney disease improving global outcomes; *MRI*, magnetic resonance imaging; *RIFLE*, risk, injury, failure, loss, end-stage; *SCr*, serum creatinine.

prolonged mechanical ventilation by a mean of 4 days ($P < .001$) and prolonged hospitalization by 3.4 days ($P < .03$). In the same cohort, those with AKI were more likely to have abnormal brain magnetic resonance imaging (MRI) findings at 7 to 10 days of life, implicating AKI as a potential marker and/or mediator of poor neurologic outcomes in infants with perinatal asphyxia (Sarkar et al., 2014). Recently, in a randomized controlled trial of 120 term neonates with perinatal asphyxia, those randomized to therapeutic hypothermia had lower rates of AKI compared with those who received standard care only (32% vs 60%, $P < .05$), suggesting therapeutic hypothermia may protect against the development of AKI (Tanigassalam et al., 2016).

Infants Undergoing Cardiac Pulmonary Bypass Surgery

Several factors contribute to the risk of postoperative AKI in neonates undergoing cardiac pulmonary bypass (CPB) surgery, including prematurity, cardiopulmonary bypass characteristics and duration, surgical complexity, perioperative morbidities, hypotension, deep hypothermic circulatory arrest, and hypoxia (Blinder et al., 2011). The renal outcomes associated with cardiac surgery in pediatric patients have been well studied, and the association of AKI with adverse outcomes is clear. Alabbas et al. (2013) reported an incidence of AKI in 62% of 122 neonates (<28 days) following CPB. Stage 3 AKI was associated with increased mortality and prolonged length of stay. In a multicenter cohort of 264 babies younger than or 6 weeks of age undergoing CPB, Morgan et al. (2013) reported an incidence of AKI of 64%; those with AKI had longer duration of intubation and length of stay, after adjusting for covariates. In a study of 430 infants (≤ 90 days old) Blinder et al. (2011) reported an incidence of CPB-AKI of 52%. After correcting for severity of illness, AKI stage II and III were associated with increased hospital mortality (stage II odds ratio [OR] 5.1, 95% confidence interval [CI] 1.7–15.2; $P = .004$; stage III OR 9.46, 95% CI 2.9–30.7; $P = .0002$).

Infants Requiring Extracorporeal Membrane Oxygenation

Neonates on ECMO are predisposed to AKI for a number of reasons, including those inherent to their underlying critical illness (sepsis, ischemia, respiratory failure, cardiac failure, hypotension, nephrotoxic medications) and elements associated with ECMO (hemodynamic fluctuations, hemolysis, systemic inflammation). Several early studies of infants and children (Sell et al., 1987; Weber et al., 1990; Meyer et al., 2001; Cavagnaro et al., 2007; Shaheen et al., 2007) who receive ECMO suggest both AKI and RRT are associated with mortality. In a retrospective cohort study of 7941 neonates in the extracorporeal life support organization registry, where AKI was defined as infants in the registry who had an SCr greater than 1.5 mg/dL or an ICD-9 code for acute renal failure, neonatal mortality was 2175/7941 (27.4%). Nonsurvivors experienced more AKI than survivors (413/2175 [19.0%] vs 225/5766 [3.9%]; $P < .0001$), and more received RRT (863/2175 [39.7%] vs. 923/5766 [16.0%]; $P < .0001$). After adjusting for confounding variables, the adjusted OR for mortality was 3.2 ($P < .0001$) following AKI and 1.9 ($P < .0001$) for those given RRT. Zwiers et al. (2013) evaluated AKI in 242 neonates on ECMO, reporting an AKI incidence of 64% and a mortality of 65% when AKI progressed to the highest stage. These findings are similar to those reported by Gadepalli et al. (2011) in their evaluation of 68 neonates with congenital diaphragmatic hernia on ECMO, where AKI occurred in 71% of neonates, and those with the highest stage of AKI had a significantly increased mortality of 73%.

Very Low Birth Weight and Extremely Low Birth Weight Neonates

There are now several single-center studies describing the epidemiology of AKI in premature infants. Askenazi et al. performed a case-control study matching premature infants by GA and birthweight and found that for every 1 mg/dL increase in SCr, the odds of death doubled (OR 1.94, 95% CI 1.13–3.32). The odds of death increased when confounding variables were adjusted (adjusted OR 3.44, 95% CI 1.23–9.61). In 2011, Koralkar et al. (2011) reported an 18% incidence of AKI in 229 VLBW infants and showed that infants with AKI had significantly higher mortality than those without AKI (42% vs 5%; $P < .001$), which persisted after adjusting for confounding variables (hazard ratio [HR] 2.4, 95% CI 0.95–6.00; $P = .06$). Viswanathan et al. (2012) reported an incidence of AKI of 12.5% in 472 extremely low birth weight (ELBW) infants in a single-center retrospective study. In this case-control study, those with AKI had significantly increased mortality (70% vs 22%; $P < .001$). Carmody et al. (2014) reported a higher incidence of AKI of 39.8% in a retrospective study of 455 VLBW infants. In this study, AKI was independently associated with increased mortality (OR 4.0, 95% CI 1.4–11.5) and length of stay (11.7 hospital days, 95% CI 5.1–18.4). Large prospective multicenter cohort studies with contemporary definitions of AKI are currently under way.

One of the most common morbidities of prematurity is bronchopulmonary dysplasia (BPD), affecting 10% and 40% of surviving VLBW and ELBW infants, respectively (Eichenwald and Stark, 2008). The pathophysiology of this chronic lung condition involves elevated levels of proinflammatory interleukins, tumor necrosis factor- α (TNF- α), leukotrienes, and increased pulmonary vasculature permeability, which culminate in abnormal lung development and fibrosis. Not only does AKI cause pulmonary edema secondary to volume overload, but evidence in ischemic, nephrotoxic, and bilaterally nephrectomized animal models shows that AKI induces a proinflammatory process highlighted by increased levels of neutrophils, TNF- α , interleukins, free radicals, endothelial growth factors, and granulocyte colony-stimulating factor (G-CSF) (Kim et al., 2006; Hoke et al., 2007; Faubel, 2008). In 2015, Askenazi et al. showed an association between AKI and BPD in premature infants. Those with AKI had a 70% higher risk of oxygen requirement/death at 28 days old (risk ratio [RR] 1.71, 95% CI 1.22–2.39; $P < .002$). This association remained after controlling for GA, preeclampsia, 5-minute Apgar score, and maximum percentage weight change in the first 4 days (RR 1.45, 95% CI 1.07–1.97; $P < .02$). Those without AKI were 2.5 times more likely to come off oxygen (HR 1.3–5.0; $P < .02$) than those with AKI, even when controlling for GA, preeclampsia, 5-minute Apgar, and maximum percentage weight change (multivariate HR 2.0, 95% CI 0.9–4.0; $P < .06$) (Askenazi et al., 2015).

Pathophysiology of Neonatal Acute Kidney Injury

Prerenal

Prerenal azotemia occurs in response to decreased renal blood flow (RBF). Causes of prerenal azotemia in neonates include loss of effective circulating blood volume (perinatal blood loss, hemorrhage), dehydration (diarrhea, transepidermal free water losses, poor intake, gastric or chest tube losses), capillary leak (hydrops, infection, or hypoalbuminemia), increased abdominal pressures (NEC, repair or reduction of gastroschisis, omphalocele,

TABLE 90.4 Causes of Acute Kidney Injury in the Newborn

| Prenatal Azotemia | Intrinsic Acute Kidney Injury | Obstructive Renal Failure |
|---------------------------------|-------------------------------|---------------------------|
| Loss of effective blood volume | Acute tubular necrosis | Congenital malformations |
| Absolute loss | Severe renal ischemia | Imperforate prepuce |
| Hemorrhage | Nephrotoxins | Urethral stricture |
| Dehydration | Infections | PUV |
| Relative loss: ↑ capillary leak | Congenital infections | Urethral diverticulum |
| Sepsis | Pyelonephritis | Ureterocele |
| NEC | Bacterial endocarditis | Megaureter |
| RDS | Renal vascular causes | UPJ obstruction |
| ECMO | Renal artery thrombosis | Extrinsic compression |
| Hypoalbuminemia | Renal vein thrombosis | Sacroccygeal teratoma |
| Renal hypoperfusion | DIC | Hematocolpos |
| Congestive heart failure | Nephrotoxins | Intrinsic obstruction |
| Pharmacologic agents | Aminoglycosides | Renal calculi |
| Indomethacin | Indomethacin | Fungus balls |
| Tolazoline | Amphotericin B | Neurogenic bladder |
| ACE inhibitors | Radiocontrast dyes | |
| | Acyclovir | |
| | Intrarenal obstruction | |
| | Uric acid nephropathy | |
| | Myoglobinuria | |
| | Hemoglobinuria | |
| | Congenital malformations | |
| | Bilateral renal agenesis | |
| | Renal dysplasia | |
| | Polycystic kidneys | |

DIC, Disseminated intravascular coagulation; ECMO, extracorporeal membrane oxygenation; NEC, necrotizing enterocolitis; PUV, posterior urethral valve; RDS, respiratory distress syndrome; UPJ, ureteropelvic junction.

congenital diaphragmatic hernia, ascites), and decreased cardiac output (cardiac surgery, heart failure, or use of ECMO), which results in a lack of pulsatile flow (Liem et al., 1995). Nonsteroidal antiinflammatory drugs (NSAIDs) such as indomethacin and angiotensin-converting enzyme inhibitors (ACE-Is) can also decrease RBF (Table 90.4).

When low RBF occurs, renal autoregulation preserves GFR by increasing renal sympathetic tone, activation of the renin-angiotensin-aldosterone system, and increased activation of hormones such as vasopressin and endothelin. Increase in filtration fraction ($GFR/RBF \times 100$) increases peritubular oncotic pressure, resulting in enhanced proximal tubular sodium and water reabsorption (Feld et al., 1986) in those with intact tubular function. These renal hemodynamic changes lead to a decrease in water and sodium losses, to maintain systemic volume expansion and blood pressure. In some newborns, oliguria does not develop because of poor vasopressin secretion, weak renal responsiveness to vasopressin (Dixon and Anderson, 1985), poor tubular function in underdeveloped tubular cells, or prolonged/severe hypoperfusion. In the context of renal hypoperfusion, correction of the underlying condition restores normal renal function unless renal hypoperfusion has been so severe or prolonged that renal parenchymal damage has already developed. Once parenchymal damage occurs, renal tubular cell damage (acute tubular necrosis) occurs even if renal perfusion is restored.

Intrinsic Acute Kidney Injury

Prolonged or severe hypoperfusion is the most common cause of intrinsic AKI. Other causes of intrinsic AKI include nephrotoxic

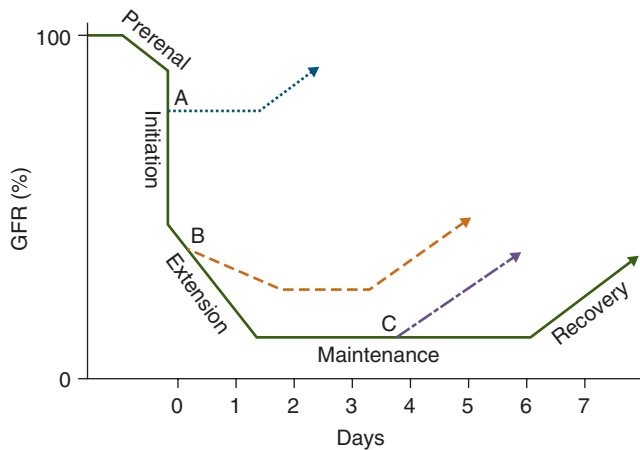
medications and sepsis, which can cause AKI with either hypodynamic or hyperdynamic blood flow. Other rare causes of AKI include renal vein thrombosis, renal artery thrombosis, uric acid nephropathy, hemoglobinuria, and myoglobinuria (Table 90.4). Congenital abnormalities of kidneys and urinary tract are discussed further in the section under chronic kidney disease (CKD) of the newborn.

Ischemic Acute Kidney Injury

The presentation and course of the renal damage after hypoxic ischemic injury depends on the severity and duration of the insult. In contrast to prerenal azotemia, renal function abnormalities in intrinsic AKI are not immediately reversible. The severity of intrinsic AKI ranges from mild tubular dysfunction, to acute tubular necrosis, to renal infarction and corticomedullary necrosis with irreversible renal damage (Feld et al., 1986).

Prerenal azotemia and ischemic AKI are a continuum of physiologic responses. The main difference between prerenal AKI and ischemic AKI is that in the latter, hypoperfusion induces renal parenchymal cell injury, particularly to the tubular epithelium of the terminal medullary portion of the proximal tubule (S3 segment) and of the medullary portion of the thick ascending limb of the loop of Henle.

The course of ischemic AKI may be subdivided into the prerenal, initiation, extension, maintenance, and recovery phases (Sutton et al., 2002) (Fig. 90.3). If, during prerenal azotemia, restoration of RBF occurs, GFR can return promptly to normal. The initiation phase includes the original insult and the associated events resulting in a drop in GFR. Tubular dysfunction with low GFR represents



• **Fig. 90.3** Schematic representation of stages of the progression in acute kidney injury. *GFR*, Glomerular filtration rate.

the maintenance phase. The duration of the maintenance phase depends, at least in part, on the severity and duration of the initial insult. The recovery phase is characterized by the gradual restoration of *GFR* and tubular functions, which can take months to occur. During the maintenance and recovery phase of AKI, the kidney is susceptible to further damage from additional insults. Recognition of the different phases of intrinsic AKI is helpful in the diagnosis, clinical management, and prognostication of the disorder.

The histologic hallmark of severe ischemic AKI is damage to epithelial tubular cells with characteristic bleb formation and loss of brush border in the apical portion of the cell cytoskeleton disruption and loss of tight junctions between cells. If injury is severe enough, apoptosis and necrosis will occur with resultant desquamation of cells, which lead to tubular obstruction. Not only are tubular epithelial cells critical in the pathophysiology of ischemic AKI, but damage to the innermost lining of the renal vascular system, the endothelial cells, has a critical role in the initiation, extension, maintenance, and recovery phases of ischemic AKI (Basile, 2007).

When endothelial cell damage occurs, activation of vasoconstriction, impaired vasodilation, and impaired leukocyte adhesion result in capillary obstruction and distorted peritubular capillary morphology. Capillary obstruction and impaired morphology lead to a cycle of increasing ischemia and vascular inflammation. The loss of endothelial cell function may represent an important therapeutic target in which vascular support and/or endothelial regeneration by progenitor cell may impact the short- and long-term consequences of AKI (Liu and Brakeman, 2008).

Damaged endothelial and tubular cells not only lead to dysfunction within the kidney, but they produce a systemic inflammatory response, which leads to significant distant organ dysfunction. The inflammatory dysregulation is due (at least in part) to dysfunctional immune, inflammatory, and soluble mediator metabolism. AKI also has been shown to directly affect brain, lung, heart, liver, bone marrow, and gastrointestinal tract (Awad and Okusa, 2007). Mice with AKI (induced by bilateral renal ischemia for 60 minutes) had increased levels of the proinflammatory chemokines keratinocyte-derived chemoattractant and G-CSF in the cerebral cortex and hippocampus, which resulted in increased neuronal pyknosis and microgliosis in the brain (Liu et al., 2008; Liu and Brakeman, 2008). In the lung, mechanistic studies demonstrate that AKI induces increased pulmonary vascular permeability, soluble and cellular inflammation, and dysregulated salt and water channels.

TABLE 90.5 Nephrotoxic Medications

| Drug | Mechanism |
|--|--|
| Acyclovir | Urinary precipitation, especially with low flow and hypovolemia, with renal tubular obstruction and damage and decreased <i>GFR</i> . May cause direct tubular toxicity (metabolites) |
| Angiotensin-converting enzyme inhibitors | Decreased angiotensin II production inhibiting compensatory constriction of the efferent arteriole to maintain <i>GFR</i> |
| Aminoglycosides | Toxic to the proximal tubules (transport in the tubule, accumulate in lysosome, intracellular rise in reactive oxygen species and phospholipidosis, cell death); intrarenal vasoconstriction and local glomerular/mesangial cell contraction |
| Amphotericin B | Distal tubular toxicity, vasoconstriction, and decreased <i>GFR</i> |
| Nonsteroidal antiinflammatory drugs | Decreased afferent arteriole dilatation as a result of inhibiting prostaglandin production resulting in reduced <i>GFR</i> |
| Radiocontrast agents | Renal tubular toxicity secondary to increase in reactive oxygen species; intrarenal vasoconstriction may play a role |
| Vancomycin | Mechanism of AKI unclear, possible mechanism includes proximal tubular injury with generation of reactive oxygen species |

AKI, Acute kidney injury; *GFR*, glomerular filtration rate.

Because neurologic and pulmonary morbidity are very high in the critically ill neonatal population, the potential deleterious effects of AKI on these organs need to be explored.

In many cases, a combination of several causative factors contributes to the development of acute renal failure. For instance, absolute hypovolemia, increased capillary leak-induced loss of effective circulating blood volume, and reflex renal vasoconstriction all may contribute to renal hypoperfusion and ensuing renal injury in newborns with severe forms of shock.

Nephrotoxic Acute Kidney Injury

Exposure to nephrotoxic medications is a potentially modifiable risk factor for intrinsic AKI in critically ill children and neonates. Table 90.5 lists commonly used nephrotoxic medications in the neonatal intensive care unit (NICU) and their mechanism of nephrotoxicity. Until recently the epidemiology and burden of nephrotoxic medication exposure in neonates were unknown. Rhone et al. (2014) recently evaluated the cumulative nephrotoxic medication exposure of a cohort of 107 VLBW neonates. In this study, 87% of the cohort was exposed to at least one nephrotoxic medication, and on average these neonates were exposed to over 14 days of nephrotoxic medications. In a number of pediatric patient populations, nephrotoxin-associated AKI has been linked to adverse outcomes including increased length of stay and cost (Zappitelli et al., 2011).

In the NICU, nephrotoxins can cause AKI by decreasing renal perfusion (NSAIDs, diuretics, ACE-Is), direct tubular injury (aminoglycosides, cephalosporins, amphotericin B, rifampin, vancomycin, NSAIDs, contrast media, myoglobin/hemoglobin [Hgb]), interstitial nephritis, and tubular obstruction (acyclovir). Although not a comprehensive review, some of the most common nephrotoxic medications in neonates are described.

Indomethacin, a prostaglandin inhibitor used to treat patent ductus arteriosus in premature infants, is one of the most commonly used medications in the NICU. Severe, although usually transient, nephrotoxicity can occur with indomethacin administration. The potentiation of the vasoconstrictive and sodium- and water-retaining effects of angiotensin II, norepinephrine, and vasopressin by the indomethacin-induced inhibition of renal prostaglandin production is the primary mechanism of the renal actions of the drug. Because neonatal renal function is more dependent on local prostaglandin production (especially when intravascular volume is decreased as a result of fluid restriction, increased capillary leak, and transepidermal water losses in the preterm infant with patent ductus arteriosus), indomethacin administration is commonly associated with elevated SCr concentrations, decreased urine output, and hyponatremia (Cifuentes et al., 1979).

Amphotericin B alters renal function by directly affecting tubular function, resulting in renal tubular acidosis and increased urinary potassium excretion. Although these nephrotoxic effects are most often reversible, cases of fatal neonatal renal failure caused by amphotericin B toxicity have been reported (Baley et al., 1984). Amphotericin B lipid complex (ABLC), liposomal amphotericin, and other lipid formulations of this drug consist of nonliposomal lipid bilayers complexed with amphotericin B. This lipid bilayer results in a higher affinity to fungal rather than mammalian cellular membranes and therefore is less nephrotoxic (Wurthwein et al., 2005). Auron et al. (2007) recently showed no differences in blood urea nitrogen or SCr, serum sodium, or serum potassium in 35 premature infants (average birthweight 764 ± 196 g) treated with ABLC compared with similar infants (controlling for GA and birthweight). Thus a 2-week course of ABLC is likely safe in premature infants, although studies to explore longer-term use of ABLC are needed.

Aminoglycosides are one of the most commonly used medications used in the treatment of suspected or proven neonatal sepsis. Aminoglycosides inhibit lysosomal phospholipases, leading to primary proximal tubule cell damage (Giuliano et al., 1984), although changes in the ultrastructure of the glomerulus also occur (Ojala et al., 2001). A metaanalysis of 11 studies in septic neonates showed that both once-a-day and multiple-dose regimens showed adequate clearance of sepsis. Even though rates of ototoxicity or nephrotoxicity were not different among the two groups, pharmacokinetic studies revealed that once-a-day dosing caused less drug accumulation in the kidney's proximal cells and more commonly achieved adequate peak concentrations (>5.0 mcg/dL) while avoiding toxic trough levels (<2 mcg/dL) (Rao et al., 2006).

Aminoglycosides should be used with caution in any person with renal dysfunction, concomitant nephrotoxic medication use or poor renal perfusion (due to volume, hypoalbuminuria, heart failure). In those with renal dysfunction, serial monitoring to assure proper clearance of the medication is needed to prevent AKI. Because aminoglycoside toxicity is usually nonoliguric, serial monitoring of SCr values is necessary, especially during prolonged administration of these antibiotics, to detect their potential nephrotoxicity in the newborn.

Acyclovir has replaced vidarabine for treatment of infection with herpes simplex virus (HSV) because of its ease of use and

more favorable side effect profile. High-dose acyclovir (60 mg/kg per day for 21 days) decreased the mortality of neonatal HSV sepsis and central nervous system disease to 29% and 4%, respectively (Kimberlin et al., 2001). Acyclovir is an antiviral agent that is eliminated rapidly in the urine through glomerular filtration and tubular secretion. It is nearly insoluble in the urine and may precipitate, particularly in the distal tubular lumen. Intravenous high-dose acyclovir treatment may lead to intratubular crystal precipitation and renal failure. Acyclovir-related nephrotoxicity can be limited by avoiding its use in those with renal insufficiency or intravascular volume depletion, infusing the drug slowly (over several hours), and by assuring adequate hydration to maintain high urinary flow rate, which will reduce the likelihood of crystal deposition in the tubules (Izzedine et al., 2005).

Finally, medications given to pregnant women may cause combined ischemic and nephrotoxic renal injury in the fetus, resulting in the clinical presentation of AKI after birth. A frequently used class of medications that falls into this category is the NSAIDs prescribed for tocolysis—both nonselective and selective cyclooxygenase inhibitors such as indomethacin or ketoprofen (Bannwarth et al., 1999; Peruzzi et al., 1999), which can lead to severe AKI in the newborn.

Postrenal Acute Kidney Injury

The most common causes of obstructive kidney dysfunction in the newborn are congenital malformations, including imperforate prepuce, urethral stricture, prune belly syndrome, and posterior urethral valves. Other causes of acute obstruction include neurogenic bladder, extrinsic compression (e.g., hematocolpos, sacrococcygeal teratoma), and intrinsic obstruction from renal calculi or fungal balls. Depending on the cause and associated damage to the kidneys, relief of the obstruction will markedly improve renal function.

Evaluation and Management of Neonatal Acute Kidney Injury

There are three main goals in the care of neonates with AKI. First is to understand the cause of the problem. Second is to take steps to intervene to prevent further deterioration in kidney function. Third is to provide renal support to the patient by helping achieve proper homeostasis and blood pressure control, provide the synthetic substance that the failed kidney lacks, and assist in toxic clearance. Similar steps parallel proper care for the newborn with CKD.

Step 1: Understand the Cause of Acute Kidney Injury

The pregnancy history, findings on prenatal tests, vital signs, changes in weight, physical examination, interventions, and medications prescribed provide important clues about the cause of neonatal AKI. SCr often does not rise for days after an injury, thus monitoring these values for several days after the inciting event is necessary to determine if AKI occurred. If urine is available, a urinalysis, urine culture, and a spot urine sample for sodium, creatinine, and osmolality can help differentiate the cause. Serum laboratory values can help understand the cause of AKI and should be monitored sequentially. These include serum sodium, potassium, chloride, bicarbonate, calcium, phosphorus, magnesium, urea, creatinine, uric acid, glucose, blood gases, Hgb, and platelets.

One of the major goals in the initial evaluation of neonatal AKI is to determine if the kidney is hypoperfused. Several laboratory, clinical, and therapeutic interventions can help delineate prerenal azotemia from intrinsic AKI (Table 90.6). Decrease in body weight, tachycardia, dry mucous membranes, poor skin turgor, flattened

TABLE 90.6 Diagnostic Indices Suggestive of Prerenal Azotemia Versus Intrinsic Acute Kidney Injury in the Newborn

| | Prerenal Azotemia | Intrinsic Acute Kidney Injury |
|------------------------------------|---------------------------------------|------------------------------------|
| Urine flow rate (mL/kg per hour) | Variable | Variable |
| Urine osmolality (mOsm/L) | >400 | ≤400 |
| Urine-to-plasma osmolar ratio | >1.3 | ≤1.0 |
| Urine-to-plasma creatinine ratio | 29.2 ± 1.6 ^c | 9.7 ± 3.6 ^c |
| Urine [Na ⁺] (mEq/L) | 10–50 | 30–90 |
| FENa ^a (%) | <0.3 (0.9 ± 0.6) ^c | >3.0 (4.3 ± 2.2) ^c |
| Renal failure index ^{a,b} | <3.0 (1.3 ± 0.8) ^c | >3.0 (11.6 ± 9.5) ^c |
| Response to fluid challenge | Improved tachycardia Increased UOP | No effect on tachycardia or UOP |

^aFractional excretion of sodium (FENa) = (urine [Na⁺]/serum [Na⁺])/(urine [Cr]/serum [Cr]) × 100.

^bRenal failure index = urine [Na⁺]/(urine [Cr]/serum [Cr]).

^cMean ± standard deviation.

UOP, Urine output.

Data from Feld et al., 1986; Karłowicz and Adelman, 1992; and Mathew et al., 1980. See text for details.

anterior fontanel, and elevation of serum sodium can be seen in those with low intravascular volumes.

When the kidney is hypoperfused, the kidney will avidly retain sodium and water to preserve overall intravascular volume. Laboratory markers of prerenal azotemia include low urinary sodium excretion, low fractional excretion of sodium (FENa), low renal failure index, and high blood urea nitrogen:SCr ratio. However, it is important to recognize that preservation of urine sodium and water is dependent on intact tubular function; therefore disturbances of tubular function (from diuretic use, tubular injury, or primary tubular diseases) can affect the tests. As premature infants have poor tubular function, these studies have important limitations. Normal FENa in preterm infants born at less than 32 weeks of gestation is usually higher than 3% (Ellis and Arnold, 1982). Additionally, because of the developmentally regulated limitation of their concentrating capacity and the effects of low protein intake and urea excretion on urine osmolality, the urine-to-plasma creatinine ratio instead of the urine-to-plasma osmolar ratio should be used in newborns to evaluate their renal tubular reabsorptive capacity (Feld et al., 1986).

If the suspicion of renal hypoperfusion is high, an appropriate fluid challenge with 10–20 mL/kg of isotonic fluids (usually normal saline) over 30 minutes should be given. Close observation of vital signs and UOP may serve to delineate if intravascular hypoperfusion is present. Several boluses may be necessary with careful prescription of fluid volume for the next 24 hours. Care to avoid fluid challenges is advised in those with suspected urinary outlet obstruction, lung pathology such as BPD, or congestive heart failure.

Another important part of the evaluation of AKI is to assure that there is no obstruction to urine flow. A renal and bladder

ultrasound should be performed without delay if an obstructive process is suspected and to determine if congenital renal abnormalities are present. If hematuria or hypertension (or both) are present, the possibility of renal vascular disease should also be considered. Doppler ultrasound of the renal vessels can be performed if renal vascular thrombosis is suspected.

Step 2: Intervene to Preserve or Prevent Further Acute Kidney Injury

The approach using provision of fluid boluses (if appropriate) as part of the evaluation of prerenal azotemia also serves as the initial management of AKI. Careful evaluation of any potentially nephrotoxic medications to determine if they are necessary, and/or if alternatives are available, is crucial. Clearly the clinician can reverse or prevent further damage by maintaining adequate renal perfusion, relieving abdominal compartment syndrome if present, assuring adequate oncotic pressure (keeping a serum albumin of 2.5 mg/dL or higher) and, if obstruction of the urinary outflow is discovered, provide interventions to eliminate the obstruction. If systemic hypotension develops despite adequate volume administration, early initiation of blood pressure support often establishes appropriate renal perfusion (Seri et al., 1993; Seri et al., 1998). In cases of pressor/inotrope-resistant hypotension and shock, a brief course of low-dose hydrocortisone can be effective in restoring systemic perfusion and renal function in preterm neonates (Seri, 2001). Other management goals include the maintenance of blood oxygen content, provision of blood products for specific indexes, limiting severe acidosis, and maintenance of normal serum albuminemia (at least 2.5 mg/dL preferably). If abdominal compartment syndrome is noted, the kidney will need higher blood pressure or relief of abdominal pressure with a drain to maintain perfusion.

Several therapies are commonly employed in AKI; however, little, if any, data are available to support the use of low-dose dopamine, fenoldopam (a selective dopamine-1 receptor agonist), or diuretics for the treatment or prevention of AKI.

Low-dose dopamine can increase renal perfusion in the sick preterm and term infant with prerenal azotemia caused by hypoxemia, acidosis, or indomethacin administration (Seri, 1995; Seri et al., 1998; Seri et al., 2002). Although low-dose dopamine increases renal perfusion, well-powered randomized controlled studies (Bellomo et al., 2000) and several metaanalyses in adults with AKI have come to the same conclusion: compared with placebo, low-dose dopamine does not improve survival, shorten hospital stay, or limit dialysis use (Marik, 2002; Friedrich et al., 2005; Hoste et al., 2006). Similar studies have not been performed in children or neonates.

Fenoldopam is a selective dopamine-1-receptor agonist the effects of which include vasodilation of renal and splanchnic vasculature, increased RBF, and increased GFR. It is approved in adults for treatment of severe hypertension but is not approved for the treatment of AKI. Nonetheless, its use in neonates with AKI has been explored in several single-center analyses, with conflicting results. Two retrospective single-center analyses (Moffett et al., 2008; Yoder and Yoder, 2009) found increased urine output in a select group of neonates with oliguria. In contrast, in a prospective placebo-controlled trial of low-dose fenoldopam (0.1 mcg/kg per min) in infants undergoing cardiac surgery with cardiopulmonary bypass (Ricci et al., 2008), low-dose fenoldopam did not show beneficial effects on AKI incidence, fluid balance control, time to sternal closure, time to extubation, or time to intensive care discharge.

Diuretics are commonly used to induce diuresis in critically ill neonates; however, no studies in neonates, children, or adults have

shown that diuretics are effective in preventing AKI or improving outcomes once AKI occurs (Bellomo et al., 2000). The mannitol test (used to test if a patient has renal hypoperfusion) is contraindicated in newborns with a predisposition to the development of intraventricular hemorrhage or periventricular leukomalacia, because of the drug-induced sudden increase in serum osmolality.

Step 3: Provide Renal Support

The clinician has an important role in helping to achieve homeostasis, and careful attention to what fluid/electrolytes are being delivered to the patient is critical. Managing fluids in the critically ill neonate with AKI can be very difficult. These infants may require large volumes of fluid to maintain adequate nutrition and hematologic indices and to provide appropriate medications. However, in an oliguric/anuric child these fluids can be detrimental as they can cause congestive heart failure, chest wall edema, and pulmonary failure. Therefore once adequate intravascular volume has been restored, prevention of severe fluid overload (by limiting crystalloid infusions) and the maximization of nutritional supplement concentration should be undertaken. Severe fluid restriction, limiting intake to insensible, gastrointestinal, and renal losses, is sometimes required but is performed at the heavy price of inadequate nutrition. Decisions on placement of dialysis access should be considered early in the course of AKI before severe fluid overload has occurred, because once severe fluid overload occurs, placement of a peritoneal dialysis (PD) or a hemodialysis (HD) catheter can be significantly more difficult, as is support of the infant with severe pulmonary edema.

Although diuretics do not impact the course of AKI, they can be used to assist in maintaining fluid homeostasis. If an adequate dose of a loop diuretic (i.e., 1 mg/kg of furosemide intravenously) does not improve UOP, it is unlikely that higher doses, changing diuretic, and/or changing to continuous dose will benefit the infant, and their use could have important side effects. In cardiac surgery patients, continuous doses were shown to be as effective with smaller total quantities of medication required and may provide less risk for nephrotoxicity or ototoxicity than larger intermittent dosing (Luciani et al., 1997). The potential toxicity of long-term and aggressive furosemide therapy, including ototoxicity, interstitial nephritis, osteopenia, nephrocalcinosis, hypotension, and persistence of patent ductus arteriosus, should be taken into consideration, especially in the preterm newborn (Karlowicz and Adelman, 1992).

Hypertension is common in neonates with AKI. It can be due to increased renin release in malformed/damaged kidneys or secondary to increased intravascular volume from a lack of free water clearance. If hypertension is due to fluid overload, inducing free water clearance with diuretics or fluid removal with dialysis will address its cause. Calcium-channel blockers work by selectively causing vasodilation of the venous system. Short-acting calcium-channel blockers (isradipine for example) are reliable, have a quick onset of response, and are well-tolerated medications. Long-acting calcium-channel blockers (amlodipine for example) take longer to take effect but provide less lability with longer dosing intervals. β -Blockers (propranolol or labetalol) are also commonly used to treat hypertension in neonates. Use of ACE-Is in children with ischemic AKI should be avoided as they can produce further renal hypoperfusion and alter intrarenal hemodynamics in an already injured kidney. See Chapter 93 for more information on neonatal hypertension.

Electrolyte abnormalities can vary depending on the cause of AKI. For example, aminoglycoside toxicity is commonly nonoliguric

with ongoing potassium and magnesium losses. Alternatively, ischemic AKI causes oliguria/anuria hyponatremia, hyperkalemia, hyperphosphatemia, and hypocalcemia. Polyuria with electrolyte (especially bicarbonate) losses may occur following the relief of a urinary obstruction. Management of electrolyte disorders can usually be managed by attention to electrolyte intake during initial course of AKI, frequent evaluation, and specific therapies.

Most cases of hyponatremia are due to water overload and, less commonly, low total sodium body composition. Attention to fluid status is critical to determine the cause and proper therapy of hyponatremia. In cases of nonsymptomatic hypervolemic hyponatremia (serum sodium concentrations usually between 120 and 130 milliequivalent [mEq]/L), restriction of free water intake is recommended. If hyponatremia at this level results in clinical signs and symptoms (lethargy, seizures) or serum sodium concentration falls below 120 mEq/L, use of 3% sodium chloride over 2 hours according to the following formula should be considered, but caution should be used to assure that the sodium (Na) is not corrected too quickly.

Possible complications of hypertonic saline administration include congestive heart failure, pulmonary edema, hypertension, intraventricular hemorrhage, and periventricular leukomalacia. Care should be taken not to increase serum sodium concentration more rapidly than 0.5 mEq/hour.

Severe hyperkalemia is a life-threatening medical emergency. Signs of progressive hyperkalemia on the electrocardiogram, in order of severity, consist of tall peaked T waves, heart block with widened QRS complexes, U wave formation, the development of sine waves, and finally cardiac arrest. Medications used to manage hyperkalemia, with their dose, onset, and duration of action, are listed in Table 90.7. Measures to remove potassium from the body

TABLE 90.7 Medical Management of Hyperkalemia in the Newborn

| Drug | Dose | Onset of Action | Duration of Action |
|-------------------------------------|---|---------------------|---------------------|
| Calcium gluconate (10%) | 0.5–1 mL/kg (IV over 10 min) | 1–5 min | 15–60 min |
| Sodium bicarbonate (3.75% solution) | 1–2 mEq/kg (IV over 10 min) | 5–10 min | 2–6 hr |
| Insulin | 1 IU/5 g glucose (IV bolus or continuous infusion) | 15–30 min | 4–6 hr |
| Glucose | ≤14 mg/kg per min (IV bolus or continuous infusion) | 15–30 min | 4–6 hr |
| Furosemide | 1 mg/kg dose or as continuous infusion | 5–10 min | 2–3 hr |
| Sodium polystyrene sulfonate | 1 g/kg dose q6h as needed (orally/rectally) | 1–2 hr ^a | 4–6 hr |
| Dialysis | As per nephrology | Immediate | Duration of therapy |

^aOnset of action may take up to 6 hours, and the drug may be ineffective in preterm infants born at less than 29 weeks of gestation. See text for details.

IU, International unit; IV, intravenous.

include oral or rectal sodium polystyrene powder (Kayexalate), loop diuretics to enhance potassium excretion (if not anuric), and dialysis. Several methods to move potassium from the extracellular to the intracellular compartment are available including albuterol inhalation, sodium bicarbonate, and insulin/glucose. Adequate ionized calcium levels for cardioprotection should be sought in context of hyperkalemia. In 2007, Vemgal and Olhson performed a metaanalysis of studies on the management of hyperkalemia in premature infants. Given the limited data available, no firm clinical practice recommendations on which treatment modality was best for the treatment of infants with hyperkalemia were able to be made except that insulin/glucose may be better in premature infants (Vemgal and Ohlsson, 2007).

Hyperphosphatemia is common in AKI and should be treated with low phosphorus intake. Breast milk and Similac 60/40 both contain low phosphorous and low potassium in comparison with other neonatal infant formulas. Significant elevations in serum phosphate represent a risk of development of extraskeletal calcifications of the heart, blood vessels, and kidneys in the newborn, especially when the calcium–phosphorus product exceeds 70 (Sell et al., 1987). Calcium carbonate may be used as a phosphate-binding agent in those whose phosphorous intake exceeds excretion. Although rarely an indication for dialysis without fluid overload or hyperkalemia, severe hyperphosphatemia is best treated with dialysis.

Hypocalcemia is low in neonates with severe and prolonged AKI, especially those who develop an inability to convert 25-hydroxy-vitamin D to 1-25-hydroxy vitamin D. Ionized calcium should be measured in those with low total calcium levels as concurrent hypoalbuminemia can affect total calcium levels. If ionized calcium is decreased and the newborn is symptomatic, 100–200 mg/kg of calcium gluconate should be infused over 10 to 20 minutes and repeated every 4 to 8 hours as necessary. Oral or intravenous calcitriol may be administered to increase intestinal reabsorption of calcium.

Normal acid–base homeostasis depends on the kidneys' ability to reabsorb bicarbonate. Thus infants with AKI commonly have a nonanion gap metabolic acidosis. Replacement with bicarbonate or acetate as a base is indicated in those with AKI to avoid or treat metabolic acidosis. In infants with severe respiratory failure, large doses of bicarbonate should be avoided as this can result in respiratory acidosis with increased carbon dioxide retention.

Nutritional goals in infants with AKI are similar to those of infants without AKI. Commonly, parental nutrition and/or feeds will need to be concentrated to avoid excessive fluid gains. If nutritional goals are unable to be achieved due to oliguria/ongoing fluid overload, the potential risks of dialysis therapy versus the potential risks associated with inadequate caloric and protein needs should be discussed. If a neonate is on continuous peritoneal or HD, an additional 1 g/kg per day of protein is needed to supplement the protein losses that occur with this form of dialysis (Zappitelli et al., 2008, 2009).

In a neonate with AKI, careful assessment of medication dosing is imperative. Because many drugs are excreted in the urine, impaired metabolism or clearance from the kidneys can cause drug accumulation and adverse side effects. In those on dialysis, pharmacokinetic properties (volume of distribution, protein binding, size, charge) of drugs, dialysis modality (peritoneal vs HD), and interval of dialysis (intermittent vs continuous) will affect drug availability (Churchwell and Mueller, 2009). Consultation with pharmacists and nephrologists familiar with drug dosing in renal failure is invaluable for neonates with AKI.

Renal Support Therapy With Dialysis

Renal support therapy, either through the use of the peritoneal membrane or with the extracorporeal blood system, does not prevent or treat AKI or CKD; it is used solely to support the infant who lacks adequate kidney function. The decision to initiate dialysis (especially in those infants with severe congenital malformations of the kidney and urinary tract) is complex and requires a multidisciplinary approach to guide the family as they consider very difficult decisions (see later on decisions to initiate RRT). Access placement and some technical challenges make neonatal dialysis more difficult than in older children, but this therapy is feasible in experienced programs with dedicated pediatric nephrologists, neonatologists, dialysis nurses, and surgeons.

Indications for Dialysis Initiation

Absolute indications to initiate dialysis include severe electrolyte abnormalities that are not correctable with medical interventions, life-threatening intoxications of medications that can be cleared with dialysis, inborn errors of metabolism, fluid overload that leads to pulmonary edema or other organ dysfunction, inability to provide adequate nutritional requirements because of renal compromise, and uremia. If renal dysfunction and/or fluid overload occurs, discussions about dialysis initiation should occur early because prolonged fluid overload/uremia can worsen pulmonary edema and cardiopulmonary instability and make placement of access for dialysis very difficult.

The timing of initiation of dialysis for those with AKI is controversial. Several observational studies show a clear advantage in adults who are dialyzed early versus late (Ronco et al., 1986; Liu et al., 2006), and pediatric studies have begun to show similar findings. The impact of fluid overload on outcomes in critically ill patients has been a hot topic across medicine. Pediatricians have been at the forefront of identifying the degree of fluid overload at the initiation of renal replacement as an independent risk factor for survival in critically ill children (Goldstein et al., 2001; Gillespie et al., 2004; Symons et al., 2007). Further studies evaluating the impact of fluid overload on outcomes in neonates need to be performed. Advocates for early initiation of renal support argue that critically ill patients benefit from early dialysis because they can remove excess fluid sooner, gain metabolic control faster, and provide renal support to allow for provision of maximal nutrition without progressive fluid overload. As technical access and machine advances have made neonatal dialysis safer and technically possible, early initiation of dialysis may improve outcomes in critically ill neonates with AKI. Further studies are needed before recommendations on timing of dialysis can be made.

Access

The limiting factor in performing dialysis in the smallest of babies is access to the peritoneal space or the vascular space for dialysis. The ideal acute PD access is a noncuff or single-cuff coiled catheter specifically designed for neonates undergoing PD. If this is not available, the use of a catheter that is used for chest tube drainage, or other catheters that may be available, can be lifesaving. The advantage to the straight uncuffed catheter is that it can be placed at the bedside and can be used soon after insertion. However, these catheters are more likely to become infected and/or develop leakage of fluids around the insertion site. For the patient who requires chronic dialysis, a catheter with two subcutaneous cuffs and the use of a downfacing exit site away from the diaper area and away from a gastrostomy tube are recommended (Auron et al.,

2007). As with all pediatric surgery procedures, the exact type of catheter and the timing and location of catheter insertion need to be tailored to the individual patient (Shahen et al., 2007). Repair of hernia (if present) should be performed at the time of catheter insertion.

Vascular access for HD requires a large (at least 7 French [F] but preferably an 8F) double lumen catheter that can be placed in the femoral or internal jugular vein. Double lumen catheters that are smaller than 7F are much more likely to develop problems during the dialysis procedure (Symons et al., 2007). Standard intravenous catheters are too flexible and too small to maintain patency with high blood flows. The use of 7F or 8F catheters is ideal; two 5F catheters in different sites can be lifesaving. If the need for dialysis is likely to last more than 1 week, a cuffed catheter is preferred to decrease the likelihood of infection. The length of the catheter should be chosen so that the tip of the catheter resides in the right atrium for internal jugular catheters and in the inferior vena cava for femoral catheters. Unless no other choices are available, the subclavian artery should not be used in infants who are likely to require long-term RRT because future forearm fistula of the ipsilateral arm can fail with “mild stenosis” of the subclavian vein.

Peritoneal Dialysis

Once PD access is placed and the decision to start dialysis has occurred, small volume continuous cycles (10 cc/kg) are performed. The dialysate solution is left dwelling in the peritoneal cavity, during which time solute and fluid removal takes place and is then drained. Continuous cycles are performed with each cycle lasting about an hour. The dextrose concentration in the fluid will determine the amount of net water loss (ultrafiltration). Complications associated with PD include peritonitis, leakage around catheter exit site, tunnel infection, catheter malfunction, and obstruction by omentum (Coulthard and Vernon, 1995). Leakage of fluid into other compartments (including the chest in patients without an intact diaphragm) can occur, and, if suspected, the fluid composition will reveal high glucose levels if a leak is indeed present. Absolute or relative contraindications to PD include NEC, abdominal wall defects, and the presence of an intra-abdominal foreign body, such as a ventriculoperitoneal shunt or diaphragmatic patch.

Hemodialysis

Once reliable access to the vascular space is achieved, the HD procedure can be performed. The two types of HD, intermittent HD and continuous RRT (CRRT), differ mainly by the duration of the procedure. Intermittent HD is significantly more efficient than CRRT. The blood flow and time on therapy are the limiting factors for solute clearance on HD. Even with the smallest dialyzers and neonatal tubing, most infants need blood priming of the extracorporeal circuit for the therapy. Skilled pediatric HD nurses are required at the bedside during the entire procedure, which typically lasts 3–4 hours. Achieving the adequate fluid removal necessary for the entire day can be difficult to achieve in the few hours on dialysis, especially in hemodynamically unstable infants. This technique usually requires systemic heparinization, with activated clotting time usually kept at 180 to 200 seconds, rendering the technique risky in preterm newborns and others at high risk for intracranial bleeding.

The main advantage to a continuous modality is that lower blood flow and fluid removal rates can be used to accomplish the desired ultrafiltration and clearance goals. Ronco et al. (1986) described

the use of CRRT in a critically ill newborn. CRRT requires a double lumen venous catheter whereby the CRRT pump pulls blood from one port of the catheter and returns the blood via the other side of the catheter. This is now a relatively common procedure in level IV NICUs.

Anticoagulation with CRRT is achieved with either systemic heparin or regional citrate anticoagulation. The advantage of regional citrate anticoagulation is that the patient is not systemically anticoagulated; however, this approach has the added risk of hypocalcemia caused from citrate excess (especially in those with impaired liver metabolism) and metabolic alkalosis (Tolwani and Wille, 2009). Because citrate is metabolized by the liver, caution must be exercised when dialyzing premature infants or newborns with multiorgan failure who may have impaired liver function.

Outcome data in neonates who require CRRT are scarce. Symons et al. (2003) reported a survival rate of 32/85 (37.6%) in neonates who received CRRT in five large children's hospitals in the United States. Between 2001 and 2007, the prospective pediatric CRRT group, a multicenter registry of 14 pediatric CRRT programs, reported outcomes for neonates in whom CRRT was initiated before 1 month of age (Symons et al., 2007). About 8% (35 neonates) in the registry were dialyzed in the first month of life. In this group, the median age was 8 days old; the median weight was 3.2 kg with the smallest infant weighing 1.3 kg. Of the 35 infants in the registry, 24 were dialyzed for either fluid overload, electrolyte imbalance, or both, and 11/35 were dialyzed for inborn errors of metabolism. Overall survival was 43%. Infants dialyzed for inborn errors had a better survival rate (73%) than the others (30%).

Several technical issues specific to infants arise when using CRRT for dialysis. The extracorporeal volume can incorporate greater than 50% of the infants' blood volume. For example, a 2.5-kg baby has a blood volume of 80 mL/kg (200 mL); the smallest circuit volume available on the most commonly used machine in the United States has an extracorporeal volume of around 100 mL, about 50% of infants' blood volume. Priming the blood circuit with blood will minimize the risks of hypotension during circuit initiation, but additional complications (acidosis, hypocalcemia, thrombocytopenia, and dilution of coagulation factors) make initiation of CRRT challenging. The risk for the bradykinin reaction that can occur at the initiation of CRRT with AN69 dialyzer (Baxter, Chicago, IL) membranes can be reduced using several techniques that minimize exposure of acidotic blood to the membrane (Brophy et al., 2001; Hackbarth et al., 2005).

Acute Kidney Injury as a Cause of Long-Term Chronic Kidney Disease

Total GFR is determined by the filtration rate of single nephrons and the number of nephrons present. When the number of nephrons is diminished, single nephron GFR increases as the kidney works to compensate for low nephron numbers. This compensatory hypertrophy causes glomeruli to function under increased intracapillary hydraulic pressure, which, over time, causes damage to capillary walls. This abnormal process leads to progressive glomerulosclerosis, proteinuria, hypertension, and CKD (Brenner et al., 1996). The hyperfiltration hypothesis has been applied and confirmed in autopsy data of hypertensive patients (Ohishi et al., 1995; Keller et al., 2003) and has been written about at length regarding infants with intrauterine growth restriction (Wadsworth et al., 1985; Barker and Osmond, 1988; Barker et al., 1989; Manalich et al., 2000;

White et al., 2009). A systematic review and metaanalysis in 2009 concluded that low birth weight babies (≤ 5.5 lbs) were 70% more likely to develop CKD later in life compared with individuals with normal birthweight (White et al., 2009).

Nephrogenesis continues through 34 weeks' gestation. Premature infants (even those born appropriate for GA) are therefore born with low nephron numbers compared with term infants. Using computer-assisted morphometry, Rodriguez et al. (2004) showed that premature infants who survived to at least 36 weeks' postconception had nephron numbers similar to premature infants with short survival, suggesting that the extrauterine environment does not support normal neoglomerulogenesis. In addition, preterm infants with AKI had fewer nephrons than similar infants without AKI.

Recent animal and epidemiology data suggest that AKI leads to CKD. As discussed in the section on the pathophysiology of ischemic AKI, tubular and vascular endothelial cellular damage occur with prolonged hypoperfusion. Animal models suggest that although tubular recovery occurs, damage to the vascular endothelial cells remains and can lead to interstitial fibrosis and progressive kidney dysfunction (Basile et al., 2001).

Studies of children with AKI show that over 50% have at least one sign of CKD 3–5 years after the inciting event. Large adult studies suggest that after AKI, rates of CKD (low GFR) are 5%–10%, with about 3%–5% developing ESRD.

The exact prevalence of CKD after neonatal AKI is unknown. Stapleton et al. (1987) reviewed the published single-center data and reported a 40%–88% prevalence of long-term CKD after oliguric renal failure. Since then, other retrospective small single-center studies describe similar tubular/glomerular dysfunction and hypertension in survivors of neonatal AKI (Chevalier et al., 1984; Polito et al., 1998; Abitbol et al., 2003). Outcome data on premature infants after AKI are scarce. Rodriguez et al. performed a cross-sectional study on premature infants during childhood born weighing less than 1000 grams and found that estimated GFR and tubular function were lower than in term-born children. Despite the limitations of these single-center studies, these data suggest that prematurity, intrauterine growth restriction, and AKI lead to a lower number of nephrons and/or endothelium dysfunction and an increased risk of long-term renal dysfunction. To further delineate the likelihood and extent that AKI causes CKD, and to provide guidelines for long-term follow-up, a prospective study is greatly needed. Future studies of interventions (such as ACE-Is) to decrease the rate of CKD progression in this growing population should be explored.

Renal Vascular Disease in the Newborn

Thromboembolic events in neonates usually result from an imbalance of the delicate homeostasis between bleeding and thrombosis. Some may be of genetic origin, some may relate to underlying stresses during pathologic processes, and some may relate to treatments for the pathologic processes.

Renal Arterial Thrombus

Incidence and Etiology

Renal artery thrombosis in the neonate is far less common than renal vein thrombosis. A major risk factor for renal arterial obstruction is umbilical artery catheterization. Other significant risk factors are shock, coagulopathy, and congestive heart failure. The reported incidence of umbilical artery-related thromboembolism reflects, in large part, the diagnostic test chosen. Doppler ultrasonography

estimates the incidence of umbilical artery-related thromboembolism from 14%–35%, whereas studies using angiography document incidences up to 64%. Autopsy studies have shown an incidence of umbilical artery-related thromboembolism between 9% and 28%, although major clinical symptoms of umbilical artery-related thromboembolism occur in 1%–3% of infants (Andrew et al., 2001). Trauma (endothelial injury) at the time of insertion of an umbilical artery catheter is postulated to be the cause of aortic thrombus formation, which then leads to thrombosis of one or both renal arteries.

High umbilical artery catheters, placed at the T6 to T10 vertebral level, have been associated with a decreased incidence of clinical vascular complications without a statistically significant increase in any adverse effects (Barrington, 2000b). The chances of umbilical artery catheter occlusion can be decreased by adding heparin to the infusing fluid at a concentration as low as 0.25 unit/mL (Barrington, 2000a).

Clinical Presentation

Clinical presentation varies with the extent and severity of thrombosis. Thrombosis of the abdominal aorta or renal arteries can manifest in any of the following ways: signs of congestive heart failure, hypertension, oliguria, renal failure, decreased femoral pulses with lower limb ischemia, or bowel ischemia/frank NEC secondary to superior or inferior mesenteric artery thrombosis. Symptoms of renal arterial thrombosis manifest within the first few postnatal days in a term neonate, compared with a median age of 8 days in a preterm neonate. The symptoms can be classified based on clinical severity; minor thrombosis with mildly decreased limb perfusion, hypertension, and hematuria; moderate thrombosis with decreased limb perfusion, hypertension, oliguria, and congestive heart failure; and major thrombosis with hypertension and multiorgan failure.

Laboratory findings associated with renal arterial thrombosis are thrombocytopenia, hypofibrinogenemia, elevated fibrin split products, variable prothrombin and thromboplastin times, conjugated hyperbilirubinemia, elevated blood urea nitrogen and creatinine, hyperreninemia, and hematuria.

Diagnosis

Doppler ultrasonography is used as the first line of imaging for diagnosing neonatal thrombosis although it usually fails to detect smaller intra-arterial thrombi and some larger asymptomatic venous thrombi (Roy et al., 2002). If ultrasonography is inconclusive, radionuclide imaging can be used. Angiography is the standard diagnostic modality and should be performed through the umbilical artery line if surgical intervention or fibrinolytic therapy is being considered.

Treatment

For asymptomatic or minimally symptomatic newborns, only supportive care is recommended, such as removal of the umbilical artery catheter and close ultrasonographic monitoring. Most of these thrombi resolve spontaneously. In newborns with mild signs of organ dysfunction and stable aortic and renal arterial thrombosis, management of hypertension, transient renal insufficiency, and mild congestive heart failure is recommended. Systemic heparin is given for anticoagulation. Close laboratory monitoring is done to avoid excessive heparinization, and clinical response is monitored by Doppler ultrasonography. The best method for monitoring heparinization remains controversial. (McDonald et al., 1981; Ignjatovic et al., 2006; Monagle et al., 2008; Newall et al., 2009).

Low molecular weight heparins (LMWHs) have some advantages over unfractionated heparin, thereby making them safe and efficacious alternatives to unfractionated heparin therapy. LMWHs have superior bioavailability, a longer half-life, and dose-independent clearance, which give a more predictable anticoagulant response. The incidences of heparin-induced thrombocytopenia and osteoporosis are rare with LMWHs; they can be used in neonates with poor venous access because they are administered subcutaneously. LMWHs also do not need frequent laboratory monitoring and dose adjustment (Albisetti and Andrew, 2002).

In case of potential life-threatening complications of aortic or renal thrombosis, fibrinolytic therapy (systemic or intrathrombotic) along with supportive care is indicated. There are limited data on efficacy, dose, and safety of fibrinolytic agents in infants (Manco-Johnson et al., 2002; Monagle et al., 2008). The intrathrombotic infusion of fibrinolytic agent reduces the cumulative dose and possible systemic adverse effects. Close monitoring by ultrasonography or angiography should be done to evaluate the response to this therapy. Fibrinolytic agents act by catalyzing the conversion of endogenous plasminogen to plasmin. The most commonly used agent is recombinant tissue plasminogen activator (tPA).

The major complication of tPA therapy is bleeding. Thrombocytopenia and vitamin K deficiency, if present, should be corrected before the start of treatment. Development of intraventricular hemorrhage or cerebral edema should be monitored closely. Mild bleeding secondary to fibrinolytic therapy can be stopped with local pressure. In the event of major bleeding, tPA should be stopped and intravenous fresh frozen plasma or cryoprecipitate should be given. The antifibrinolytic agent aminocaproic acid (Amicar) should be considered if the bleeding is life threatening.

Prognosis

The overall mortality rate with aortic and renal arterial thrombosis is between 9% and 20%, with mortality being higher with major aortic and renal arterial thrombosis (Nowak-Gottl et al., 1997). Renovascular hypertension is the most common long-term complication of renal arterial thrombosis. In most cases, these infants eventually are weaned from antihypertensive medications and remain normotensive. Another consequence of renal arterial thrombosis is chronic renal insufficiency caused by irreversible renal parenchymal damage; this is seen less frequently but always in cases with severe aortic and bilateral renal arterial thrombosis.

Renal Vein Thrombosis

Incidence and Etiology

Renal vein thrombosis (RVT) is the most common thrombosis in infancy and occurs primarily in the newborn period. It has an incidence of 2.2 cases per 100,000 live births (Bokenkamp et al., 2000). RVT has a male predominance of approximately 67%; it is unilateral in more than 70% of patients and more prevalent on the left side (approximately 63%). The thrombus also involved the inferior vena cava in approximately 43% of the cases, and it was associated with adrenal hemorrhage in approximately 15% (Dauger et al., 2009; Lau et al., 2007).

The cause of RVT is unknown, although a number of factors are associated with this disorder. Prothrombotic factors—including lupus anticoagulant, protein C, protein S, plasma antithrombin III activity, lipoprotein (a), factor V Leiden mutation, prothrombin gene mutation, and methylenetetrahydrofolate (*MTHFR*) thermolabile mutation—have a significant role in the pathogenesis of neonatal RVT (Kosch et al., 2004; Marks et al., 2005; Lau et al.,

2007). Other associated factors are maternal diabetes, traumatic delivery, prematurity, hyperviscosity, hypovolemia, hemoconcentration, sepsis, birth asphyxia, cyanotic congenital cardiac disease, congenital renal vein defects, and an indwelling umbilical venous catheter (Nowak-Gottl et al., 1997; Bokenkamp et al., 2000; Proesmans et al., 2005; Lau et al., 2007).

Clinical Presentation

There are three cardinal signs of RVT: macroscopic hematuria, palpable abdominal mass, and thrombocytopenia; these signs have been found in approximately 56%, 45%, and 47% of cases, respectively (Lau et al., 2007). Other signs and laboratory findings associated with RVT are oliguria or anuria, hemolytic anemia, metabolic acidosis, azotemia, and variable prothrombin and partial thromboplastin times.

Diagnosis

Renal ultrasonography is a useful and convenient way of diagnosing RVT. It shows unilaterally or bilaterally enlarged and echogenic kidneys with attenuation or loss of corticomedullary differentiation and little blood flow. In many cases, calcification and thrombus may be seen extending into the inferior vena cava (Proesmans et al., 2005). Doppler studies are useful for detecting resistance or absence of flow in renal venous branches and collateral vessels. Thrombosis in small intrarenal veins can cause increased resistance in renal arteries, even when blood flow in the main renal vein and its branches is normal (Lau et al., 2007). Length of the kidney has been reported to correlate negatively with renal outcomes (Winyard et al., 2006). Ultrasonography may also be used as a prognostic tool.

Although renal ultrasonography is the most commonly used imaging modality for diagnosing RVT, contrast angiography is considered the gold standard. Angiography, however, is invasive and requires exposure to ionizing radiation and can be performed only in a neonate in a stable condition. MRI has also been reported to give excellent diagnostic findings in RVT, although it should be reserved for those cases in which Doppler findings are inconclusive (Basterrechea Iriarte et al., 2008).

Treatment

Treatment of neonatal RVT remains controversial because there is not enough literature to compare supportive therapy with anticoagulation, fibrinolysis, or both. Supportive therapy should be provided to all affected infants in an attempt to correct any abnormalities in fluid, electrolyte, and acid–base balance. Hypertonic solutions, nephrotoxic medications, hyperosmotic radiographic contrast agents, and unnecessary use of diuretics should be avoided. Prophylactic heparin therapy has been recommended in a majority of cases to prevent thrombus extension by some authors (Dauger et al., 2009), whereas others report similar renal outcomes between supportive treatment and heparin therapies, including a similar proportion of atrophic kidneys secondary to RVT in neonates whether they were managed supportively or with heparin (Lau et al., 2007). LMWH is being used more frequently than unfractionated heparin for anticoagulation. Fibrinolysis is usually reserved for more severe cases, such as bilateral thrombosis and systemic effects (Dauger et al., 2009). Whichever the treatment approach, affected neonates must be followed closely for renal complications such as hypertension, chronic renal insufficiency, and renal atrophy.

Surgical interventions such as thrombectomy or nephrectomy have not shown any benefit. Thrombectomy prevents the main

thrombus from extending into the inferior vena cava or contralateral kidney, but it does not prevent renal infarction because smaller intrarenal veins are almost always involved.

Prognosis

Renal scarring and atrophy are well-recognized complications of RVT in the affected kidney, which can be assessed with a radio-nuclide scan. Approximately 19% of patients have persistent elevation of blood pressure, which has been shown to be slightly higher—at 21% for those with bilateral RVT. The mortality rate for neonates with RVT is approximately 3% (Lau et al., 2007). Most of the deaths are due to underlying disease and not RVT or secondary renal dysfunction. Because more than 80% of neonates with RVT have shown persistent abnormalities on renal imaging and there are not enough data on long-term outcome of such neonates, continued follow-up is strongly recommended.

Renal Cortical and Medullary Necrosis

Incidence and Etiology

Renal cortical and medullary necrosis is uncommon in newborns and usually encountered in critically ill newborns as a manifestation of perinatal and postnatal stress leading to end-organ injury. It is usually diagnosed on autopsy or manifested as elevated and persistent kidney dysfunction. The incidence is 5% in infants who die at less than 3 months of age (Lerner et al., 1992). Risk factors associated with renal cortical and medullary necrosis are congenital heart disease, perinatal anoxia, placenta abruption, twin–twin or twin–maternal transfusions, sepsis, infectious myocarditis, vascular malformations, dehydration, prematurity, respiratory distress syndrome, bleeding diathesis, cardiac catheterization, and intravenous contrast agents (Nygren et al., 1988; Lerner et al., 1992).

Pathophysiology

Medication administration, blood loss, and ischemia can interfere with compensatory mechanisms to maintain renal perfusion and can lead to acute tubular necrosis that, depending on the severity of the insult, then may lead to vasculature injury and microthrombi formation with subsequent renal cortical and medullary necrosis. Administration of ACE-Is in the context of hypoperfusion can decrease the perfusion pressure in the glomerulus and can precipitate acute renal failure and, eventually, renal cortical necrosis.

Clinical Presentation

The clinical manifestations include hematuria, oliguria, rising SCr, and renal enlargement, which are nondiagnostic and associated with many other common neonatal renal abnormalities. Because renal cortical and medullary necrosis usually develops in critically ill newborns in the setting of shock, this needs to be explored in all critically ill neonates with abnormal renal function.

Diagnosis

In renal cortical and medullary necrosis, laboratory features may be present, such as hematuria, elevated blood urea nitrogen and creatinine, and thrombocytopenia. Renal ultrasound examination results are normal initially but may show small kidneys that are hyperechoic for age, loss of corticomedullary differentiation, and progressive decreased kidney size. A radionuclide renal scan shows decreased to no perfusion with delayed or no function (Andreoli., 2004).

Management and Prognosis

Infants with cortical necrosis may have partial recovery or no recovery at all. Typically they need RRT, short-term or long-term, but those who recover enough renal function to be managed without dialysis are at risk for late development of chronic renal failure.

Chronic Kidney Disease

Neonatal CKD is diagnosed when sustained derangements of glomerular filtration or tubular function occur with minimal to no resolution over time. In many cases, CKD follows AKI, and in others the acute phase of the renal compromise has not been detected or has occurred in utero, often as a result of anatomic abnormalities (e.g., hypoplasia, dysplasia, malformations). In that setting, the diagnosis of CKD is established without documented evidence of preexisting AKI. According to guidelines published by the Kidney Disease Outcomes Quality Initiative (KDOQI), CKD is present if there is evidence of kidney damage for more than 3 months, as defined by structural or functional abnormalities, with or without decreased GFR, or a GFR less than 60 mL/min per 1.73 m² for more than 3 months in children older than 2 years with or without kidney damage. These guidelines do not apply to infants less than 2 years of age, as a result of ongoing maturation of the kidney and improvement in GFR over the first 2 years of life. In turn, the KDIGO guidelines from 2012 recommend that for the classification of CKD in neonates and infants, available normative values and conventionally accepted equations should be used to classify neonatal CKD into one of three categories: normal (GFR <1 standard deviation [SD] below the mean); moderately reduced (GFR >1 SD to ≤2 SD below the mean); or severely reduced (GFR >2 SD below the mean) (Zaritsky and Warady, 2014).

Currently, the updated Schwartz formula derived using iohexol clearance and enzymatically measured creatinine is the most commonly used equation to estimate GFR using SCr; however, that equation is based on data derived from children greater than 1 year of age (Schwartz et al., 2009). Recent studies suggest that equations incorporating the use of cystatin C, renal mass, and body surface area may provide a more accurate assessment of GFR, at least for neonates (Treiber et al., 2015). ESRD, the point at which dialysis or kidney transplantation is necessary to ameliorate the physiologic complications of uremia owing to kidney failure, represents the most severe stage of CKD. The pathophysiologic mechanisms leading to the progression of AKI to CKD and ESRD are discussed earlier in this chapter.

Epidemiology

There is little information on the incidence and prevalence of CKD in neonates and infants, due to the lack of a uniform definition. In one small study, the estimated incidence of CKD was 1:10,000 live births with a male to female ratio of 2.8:1. The most common causes of CKD in neonates are renal dysplasia and obstructive uropathy (Wedekin et al., 2008; Mekahli et al., 2010; Harambat et al., 2012; Carey et al., 2015). The male predominance results from the finding that posterior urethral valves are the most frequent congenital obstructive disorder. In a small German study, 53% of children with CKD were premature, a figure significantly higher than the rate experienced by the total infant population of Germany (Wedekin et al., 2008). A publication from the Chronic

Kidney Disease in Children (CKiD) cohort also revealed a high prevalence of children with CKD who had an abnormal birth history as defined by low birth weight (17%), small for GA (14%), or prematurity defined as GA less than 36 weeks (12%) (Greenbaum et al., 2011). In a study of more severe CKD, 35% of affected patients were born prematurely, and approximately 50% had a comorbidity, such as cardiopulmonary and/or neurologic involvement (Mekahli et al., 2010).

Most epidemiologic reports focus on the development of ESRD in this age group. These studies demonstrate a varying regional incidence of ESRD, and the worldwide incidence is unknown. In one study, the estimated incidence of neonatal ESRD in the United States and Canada was 0.32 in 100,000 live births (Carey et al., 2007). The European Registry for Children on Renal Replacement Therapy collects data from many countries across Europe and recently reported the incidence of ESRD in children aged 0–4 years to be around 5.2 per million children (Chesnaye et al., 2015). The incidence in the 0–4 years age group as reported by the United States Renal Data System was 10.3 per million US population in this age group. This age group makes up between 10%–20% of all children who receive RRT (dialysis and transplantation) (Assadi, 2013; Borzych-Duzalka et al., 2013). Studies show that wealthier countries, those that spend more on health care, and countries where patients pay less out of pocket expenses have higher rates of RRT initiation. Thus much of the variability is likely explained by socioeconomic factors and less by genetic susceptibility to renal disease (Chesnaye et al., 2015).

Clinical Sequelae

Pathophysiology of Anemia

Anemia is a frequent complication of CKD in infants and children, and the prevalence of anemia increases with worsening stages of CKD. Recently, a study by Atkinson et al. (2010) revealed that 73% of pediatric patients with CKD stage 3 (GFR 30–59 mL/min per 1.73 m²) were anemic. This percentage increased to 93% of those with CKD stage 5 (GFR <15 mL/min per 1.73 m² or dialysis). The anemia of CKD is an important predictor of patient morbidity and mortality. It is associated with a number of physiologic abnormalities, including decreased tissue oxygen delivery, increased cardiac output, cardiac enlargement, ventricular hypertrophy, congestive heart failure, and impaired immune responsiveness (Gafer et al., 1994; Mitsnefes et al., 2000; Borzych-Duzalka et al., 2013). There is also evidence to suggest that the presence of anemia is associated with an increased risk of hospitalization in children with CKD (Staples et al., 2009).

The pathophysiology of anemia in infants and young children with CKD is primarily the result of a decrease in the renal production of erythropoietin, iron deficiency, or both (Koshy and Geary, 2008). Other potential contributing factors include a shortened red blood cell life span, secondary hyperparathyroidism, hypothyroidism, folate and vitamin B₁₂ deficiency, chronic inflammation, and hemoglobinopathies. In the early stages of CKD, iron deficiency tends to be common, and in one study of pediatric CKD patients, Baracco et al. (2011) found that 25% of patients with CKD stage 2 and 55% of patients with stage 3 CKD had iron deficiency. The cause of absolute iron deficiency is multifactorial and can be related to poor intake, gastrointestinal blood loss, and repeated phlebotomies for laboratory tests.

Erythropoietin deficiency becomes more prevalent in the later stages of CKD. Children may also be particularly prone to factors that contribute to relative erythropoietin resistance including

hyperparathyroidism, aluminum toxicity, and hemolysis (Bamgbola 2011). The decreased erythropoietin production from the kidney ultimately leads to anemia through increased apoptosis of red blood cell precursors in the bone marrow, often accompanied by iron deficiency related to the factors listed above, as well as an increased production and decreased excretion of hepcidin (Atkinson and Furth, 2011; Ganz and Nemeth, 2016).

Management of Anemia

According to the KDOQI *Clinical Practice Guidelines and Clinical Practice Recommendations for Anemia in Chronic Kidney Disease* produced by the National Kidney Foundation, anemia is defined as an Hgb concentration less than the fifth percentile of normal for age and sex (Table 90.8) (KDOQI, 2007). The normative values used to define anemia in children older than 1 year are taken from the third National Health and Nutrition Examination Survey (Astor et al., 2002) database, whereas the norms for infants younger than 1 year are derived from other reference sources (Nathan, Orkin, 2003). Recently, KDIGO released recommendations for the diagnosis of anemia in CKD that defined anemia as Hgb less than 11.0 g/dL in children 0.5–5 years (KDIGO Group, 2012).

The KDOQI guidelines recommend checking Hgb in all patients with CKD at least annually. In those children found to be anemic, the initial work-up should include red blood cell indices, reticulocyte count, white blood cell count with differential and platelet count, and iron parameters (serum iron, total iron binding capacity, and serum ferritin). These guidelines also recommend targeting an Hgb level between 11.0 and 13.0 g/dL as part of anemia management in pediatric patients with CKD (KDOQI and National Kidney Foundation, 2006; KDOQI, 2007). Erythropoiesis-stimulating agents (ESAs) such as recombinant erythropoietin- α (EPO) and darbepoetin, an analogue of erythropoietin with a longer half-life, along with iron supplements, are the key elements of anemia management in CKD. Both ESAs appear to have equal efficacy and similar safety profile (Hattori et al., 2014; Schaefer et al., 2016) and have been studied in premature infants (Ohls et al., 2013). When treated with an ESA, infants and young children

TABLE 90.8 Hemoglobin Levels in Children Between Birth and 24 Months for Initiation of Anemia Work-Up

| Age | Mean Hb (gm/dL) | –2 SD ^a |
|-------------------|-----------------|--------------------|
| Term (cord blood) | 16.5 | 13.5 |
| 1–3 days | 18.5 | 14.5 |
| 1 week | 17.5 | 13.5 |
| 2 weeks | 16.5 | 12.5 |
| 1 month | 14.0 | 10.0 |
| 2 months | 11.5 | 9.0 |
| 3–6 months | 11.5 | 9.5 |
| 6–24 months | 12.0 | 10.5 |

^aValues two standard deviations below the mean are equivalent to less than 2.5th percentile.
Data taken from normal reference values.
From National Kidney Foundation. KDOQI Clinical Practice Guidelines and Clinical Practice Recommendations for Anemia in Chronic Kidney Disease. *Am J Kidney Dis.* 2006;47:S88.

generally require larger doses than older children and adults, despite having a higher capacity for hematopoiesis. Whereas the average dose of EPO for children with CKD is 200–250 units/kg per week given by the subcutaneous route, younger children (<1 year) often require doses as high as 400 units/kg three times per week. This dose discrepancy holds true for patients receiving dialysis as well. Infants on dialysis have required doses averaging from 300–350 units/kg per week compared with those patients greater than 12 years whose dose averages around 200 units/kg per week. Children receiving HD typically require more EPO than patients receiving PD, as a result of the blood loss that routinely occurs with HD.

Iron therapy typically consists of the provision of oral elemental iron in doses ranging from 2 to 3 mg/kg per day up to 6 mg/kg per day in two to three divided doses (KDOQI and National Kidney Foundation, 2006). Iron should be taken 2 hours before or 1 hour after all calcium-containing phosphate binders in patients with CKD and a history of hyperphosphatemia to maximize gastrointestinal absorption. In HD patients, intravenous iron administration is often recommended because of inadequate iron absorption after oral administration coupled with increased losses of blood and iron during the HD treatments. Levels of serum ferritin greater than 100 ng/mL and transferrin saturation values greater than 20% are believed to reflect adequate iron stores in patients with CKD (KDOQI and National Kidney Foundation, 2006).

Growth and Development

Children with CKD often experience some degree of growth failure, which may start as early as CKD stage 3 (Hamasaki et al., 2015). The cause of the disordered growth in patients with CKD is a multifactorial process. Protein-calorie malnutrition, metabolic acidosis, electrolyte disarray, renal osteodystrophy, changes in the gonadotropic hormone axis in the face of uremia, and corticosteroid treatment are all factors that contribute to this challenging problem (Haffner, 2008).

The growth failure that exists is especially concerning when CKD occurs in infancy, a time that is normally characterized by rapid growth. Many studies have demonstrated already delayed growth at the time of dialysis initiation that has persisted through at least the first year following dialysis initiation (van Stralen, 2011; Borzych-Duzalka et al., 2014). Young children on dialysis have historically often failed to grow normally, despite meeting 100% of the recommended daily allowance of caloric and protein intake (Shroff et al., 2003; Stańczuk et al., 2016). Thankfully, growth outcomes of infants with ESRD have improved over time, possibly because of advances in medical, nutritional, and surgical therapies (Ledermann et al., 1999; Hijazi et al., 2009). Recent reports have also described improved longitudinal growth and sustained catch-up growth in infants with CKD in whom recombinant growth hormone treatment was initiated in the first year of life. Similar results have also been obtained with intensive nutritional regimens (Fine et al., 1995; Maxwell, 1996; Mencarelli et al., 2009; Santos et al., 2010; Rees, 2015). Finally, growth outcomes regularly improve after transplantation in young patients in whom accelerated growth may occur (Fine et al., 2010; NAPRTCS, 2014).

Renal impairment in infancy, a crucial time of neural development, raises concerns regarding the neurodevelopmental outcomes in children with ESRD. Advanced CKD has been linked to poor neurocognitive function in the areas of attention, memory, and inhibitory control (Hooper et al., 2011; Ruebner et al., 2015). Recently, small studies examining the effect of ESRD during infancy on neurocognitive development have suggested that there may be minor delays in intellectual and metacognitive function but that

most children without other comorbidities do not experience significant developmental delay (Johnson and Warady, 2013). Other comorbidities of CKD (e.g., anemia, iron deficiency, hypertension, cerebral vascular accidents, adverse effects of therapy) have been implicated in the neurodevelopmental impairments in many patients, and these comorbidities may explain the association between duration of CKD and impaired executive function (Geary, 1998; Lande et al., 2011; Johnson and Warady, 2013; Mendley et al., 2015). Genetic syndromes involving the central nervous system may also influence neurocognitive outcomes for these patients (Verbitsky et al., 2015). While larger studies are needed to provide additional data, it is clear that neurocognitive outcomes have improved for infants with ESRD over the past two decades, likely related to improved dialysis techniques, better nutrition, and avoidance of exposure to aluminum (Andreoli et al., 1984; Freundlich et al., 1985).

Nutrition

Nutritional Assessment

The origin of malnutrition in children with CKD is multifactorial; however, an inadequate voluntary intake is considered a major contributing factor, especially in infants. Nausea and vomiting are common in infants and children with CKD, with delayed gastric emptying and gastroesophageal reflux detected in as many as 75% of patients with these problems (Ruley et al., 1989). Protein energy wasting (PEW) is a common problem in patients with CKD and may be another major contributor to poor growth in the first few years of life. The International Society of Renal Nutrition and Management has identified specific biochemical evidence (i.e., low albumin, cholesterol, or transthyretin), reduced body mass index, and reduced muscle mass as the primary characteristics of PEW in adults (Fouque et al., 2008; Ingulli and Mak, 2014). Additional studies specific to the pediatric CKD and dialysis population have confirmed the importance of these factors, along with short stature as features characterizing PEW. Whereas few body composition studies of very young CKD patients have been performed, weight-for-length is often below average for infants with CKD who have not received calorie supplements, and small studies suggest that infants with CKD may have lean muscle mass deficits (Foster et al., 2012). However, the most prominent feature of inadequate nutrition in this population is linear growth restriction (KDIGO, 2009). Modified PEW scores as a reflection of poor nutrition in pediatric patients have also been associated with an increased risk for hospitalization, disease progression, and neurocognitive complications (Abraham et al., 2014).

In view of the importance of nutritional status to the outcome of the infant and young child with CKD, frequent monitoring of the patient is mandatory. Collaboration with a pediatric renal dietician is beneficial to assist in the nutritional evaluation and treatment strategy. An age-related schema for parameters and frequency of nutritional assessment for patients with CKD has recently been published (Table 90.9) (KDIGO, 2009). The World Health Organization Growth Standards of length-for-age, weight-for-age, weight-for-length, body mass index-for-age, and head circumference-for-age should be used as the reference for children from birth to 2 years (WHO, 2006). Nutritional intervention is indicated in children with CKD when there are findings that include an impaired ability to ingest or tolerate oral feedings, a body mass index value less than the fifth percentile of height-for-age, an acute weight loss of 10% or more, or a length/height ratio more than two SDs less than the mean. However, neonates with

TABLE 90.9 Recommended Parameters and Frequency of Nutritional Assessments for Children With Chronic Kidney Disease Stages 2 to 5 and 5d

| Measure | MINIMUM INTERVAL (MONTHS) | | | | | |
|---|---------------------------|---------|--------|------------------|---------|--------|
| | AGE 0 TO <1 YEAR | | | AGE 1 TO 3 YEARS | | |
| | CKD 2–3 | CKD 4–5 | CKD 5D | CKD 2–3 | CKD 4–5 | CKD 5D |
| Dietary intake | 0.5–3 | 0.5–3 | 0.5–2 | 1–3 | 1–3 | 1–3 |
| Height or length-for-age percentile or SDS | 0.5–1.5 | 0.5–1.5 | 0.5–1 | 1–3 | 1–2 | 1 |
| Height or length velocity—for-age percentile or SDS | 0.5–2 | 0.5–2 | 0.5–1 | 1–6 | 1–3 | 1–2 |
| Estimated dry weight and weight-for-age percentile or SDS | 0.5–1.5 | 0.5–1.5 | 0.25–1 | 1–3 | 1–2 | 0.5–1 |
| BMI-for-height-age percentile or SDS | 0.5–1.5 | 0.5–1.5 | 0.5–1 | 1–3 | 1–2 | 1 |
| Head circumference—for-age percentile or SDS | 0.5–1.5 | 0.5–1.5 | 0.5–1 | 1–3 | 1–2 | 1–2 |

BMI, Body mass index; CKD, chronic kidney disease; SDS, standard deviation score.
Modified from National Kidney Foundation. KDOQI Clinical Practice Guideline for Nutrition in Children with CKD: 2008 Update. *Am J Kidney Dis.* 2009;53:S16.

TABLE 90.10 Equations to Estimate Energy Requirements for Children at Healthy Weights

| Age (months) | EER (kcal/d) = Total Energy Expenditure + Energy Deposition |
|--------------|---|
| 0–3 | EER = $[89 \times \text{weight (kg)} - 100] + 175$ |
| 4–6 | EER = $[89 \times \text{weight (kg)} - 100] + 56$ |
| 7–12 | EER = $[89 \times \text{weight (kg)} - 100] + 22$ |
| 13–35 | EER = $[89 \times \text{weight (kg)} - 100] + 20$ |

EER, Estimated energy requirement.
Modified from National Kidney Foundation. KDOQI Clinical Practice Guideline for Nutrition in Children with CKD: 2008 Update. *Am J Kidney Dis.* 2009;53:S36.

CKD should be considered to be at nutritional risk if they are preterm or are characterized by any of the following:

- Low birth weight (<2500 g)
- A birthweight *z* score less than –2 SD for GA
- Polyuria and associated renal salt wasting

Nutritional Management

In children with CKD, the spontaneous energy intake decreases with deteriorating kidney function. Energy intake is the principal determinate of growth during infancy. Energy requirements should, in turn, be considered to be 100% of the estimated energy requirement for chronologic age (Table 90.10) (Ruley et al., 1989; National Academies Press, 2002; KDOQI, 2009; Foster et al., 2012). Malnutrition has the most marked negative effect on the growth of children with congenital disorders leading to CKD. Supplemental nutritional support is indicated when the voluntary intake by the child fails to meet energy requirements and the child is not achieving expected rates of weight gain or growth for age. In infants requiring fluid restriction because of their impaired kidney function, oral intake of an energy-dense diet with a milk formula that has a caloric density greater than 20 kcal/oz and the appropriate phosphorus content for CKD stage is preferred. If poor appetite or vomiting preclude an adequate oral intake, tube feedings (e.g., nasogastric, gastrostomy, gastrojejunostomy) should be considered

and provided by either bolus or continuous infusion. The development of repeated emesis in children fed per nasogastric tube has prompted the use of gastrostomy as the preferred route of therapy (Warady et al., 1996). In infants younger than 1 year, an initial infusion rate of 10 to 20 mL/hour or 1 to 2 mL/kg per hour is generally well tolerated, to be followed by a daily increase of 5 to 10 mL per 8 hours or 1 mL/kg per hour toward achieving the treatment goal. It is imperative that tube-fed infants be encouraged to continue some oral intake or have oral stimulation (e.g., pacifier) if persistent feeding dysfunction is to be prevented.

Whereas the spontaneous dietary protein intake (DPI) is reduced in progressive CKD in a manner similar to that of energy intake, the DPI of the patient with CKD is typically far in excess of the average requirements. At the same time, there is no evidence that strict dietary protein restriction has any nephroprotective effect (Chaturvedi and Jones, 2007), whereas aggressive restriction has been noted to compromise the growth of infants with CKD (Uauy et al., 1994). Because moderate dietary protein restriction reduces the accumulation of nitrogenous waste products, decreases acid load, and helps to lower dietary phosphorus intake (which helps preclude bone-mineral and cardiovascular complications), it is appropriate to gradually lower the DPI toward 100% of the dietary reference intake (DRI) as CKD progresses toward the need for dialysis (Ruley et al., 1989). More specifically, a DPI of 100%–140% DRI for CKD stage 3, 100%–120% DRI for CKD stage 4 to 5, and 100% DRI for CKD stage 5D (dialysis) have been proposed (Table 90.11) (KDOQI, 2009). In patients receiving dialysis, the dietary protein requirements are increased to account for dialysis-related protein losses.

Acid–Base and Electrolytes

Fluid and electrolyte requirements of children with CKD vary according to their primary kidney disorder and the degree of residual kidney function. Infants and children normally have a relatively larger endogenous hydrogen ion load (2–3 mEq/kg) than adults, resulting in metabolic acidosis as a common manifestation of CKD in children and an important negative influence on growth. Metabolic acidosis leads to changes in bone composition and decreases in 1,25-(OH)₂D synthesis, in addition to endogenous growth hormone and recombinant growth hormone resistance (de-Brito Ashurst et al., 2015). Based on the experience of successfully

TABLE 90.11**Recommended Dietary Protein Intake for Children with Chronic Kidney Disease Stages 3 to 5 and 5d**

| Age | DRI (g/kg per day) | DRI | | | |
|-------------|--------------------|--|---|--|--|
| | | Recommended for CKD Stage 3 (g/kg per day) (100%–140% DRI) | Recommended for CKD Stages 4–5 (g/kg per day) (100%–120% DRI) | Recommended for HD (g/kg per day) ^a | Recommended for PD (g/kg per day) ^b |
| 0–6 months | 1.5 | 1.5–2.1 | 1.5–1.8 | 1.6 | 1.8 |
| 7–12 months | 1.2 | 1.2–1.7 | 1.2–1.5 | 1.3 | 1.5 |
| 1–3 years | 1.05 | 1.05–1.5 | 1.05–1.25 | 1.15 | 1.3 |

^aDRI + 0.1 g/kg per day to compensate for dialytic losses.
^bDRI + 0.15–0.3 g/kg per day depending on patient age, to compensate for peritoneal losses.
 CKD, Chronic kidney disease; DRI, dietary reference intake; HD, hemodialysis; PD, peritoneal dialysis.
 Modified from National Kidney Foundation. KDOQI Clinical Practice Guideline for Nutrition in Children with CKD: 2008 Update. *Am J Kidney Dis.* 2009;53:S49.

enhancing the growth of infants and children with isolated renal tubular acidosis with alkali therapy (McSherry and Morris, 1978), it is recommended that children with CKD be treated to achieve a serum bicarbonate level of at least 22 mmol/L (KDOQI, 2009). A recent publication from the CKiD study revealed that a serum bicarbonate less than 18 mmol/L was a risk factor for poor growth. The fact that a minority of the children with a low bicarbonate level were receiving supplemental bicarbonate therapy emphasizes the importance of being vigilant with respect to evaluation and treatment (Rodig et al., 2014). In recent studies in adults with CKD, correction of metabolic acidosis led to a decreased rate of CKD progression (de Brito-Ashurst et al., 2009; Mahajan et al., 2010; Phisitkul et al., 2010). This finding may translate to the pediatric population as well, but further studies are required.

Whereas restriction of sodium and water is often indicated in children with CKD complicated by sodium and fluid retention and systemic hypertension, infants and children with CKD secondary to obstructive uropathy or renal dysplasia are often polyuric and may experience substantial urinary sodium and water losses despite experiencing advanced stages of CKD. Infants and children with salt-wasting forms of CKD who do not receive salt supplementation may in turn experience extracellular volume contraction, vomiting, constipation, and significant growth retardation (Parekh et al., 2001). The same holds true for infants receiving PD, with or without polyuria, as most patients lose significant quantities of sodium in the dialysate. Sodium depletion in the infant PD population has resulted in hypotension, cerebral edema, and blindness (Lapeyraque et al., 2003). Individualized therapy can, in turn, be achieved by first prescribing at least the age-related DRI of sodium and chloride (Table 90.12), with subsequent modification of therapy based on regular assessment of clinical and laboratory data. In PD patients, a dialysis sodium balance study may be performed to formally assess sodium losses through dialysis to help guide sodium supplementation (Foster et al., 2012). Close monitoring of blood pressure is imperative because, as noted previously, episodes of severe hypotension in young infants on PD have been associated with tragic neurologic complications.

Potassium homeostasis in children with CKD is usually unaffected until the GFR falls to less than 10% of normal. However, infants and children with disorders such as renal dysplasia and reflux nephropathy often demonstrate renal tubular resistance to aldosterone and may experience hyperkalemia, even when the GFR

TABLE 90.12**Dietary Reference Intake for Healthy Children for Sodium, Chloride, and Potassium**

| Age | SODIUM (mg/d) | | CHLORIDE (mg/d) | | POTASSIUM (mg/d) | |
|-------------|---------------|-------------|-----------------|-------------|------------------|-------------|
| | Upper Limit | | Upper Limit | | Upper Limit | |
| | AI | Upper Limit | AI | Upper Limit | AI | Upper Limit |
| 0–6 months | 120 | ND | 180 | ND | 400 | ND |
| 7–12 months | 370 | ND | 570 | ND | 700 | ND |
| 1–3 years | 1000 | 1500 | 1500 | 2300 | 3000 | ND |

AI, Adequate intake.
 Modified from National Kidney Foundation. KDOQI Clinical Practice Guideline for Nutrition in Children with CKD: 2008 Update. *Am J Kidney Dis.* 2009;53:S49.

is preserved. The hyperkalemia can be exacerbated by volume contraction, as can be seen in patients with salt-wasting forms of CKD. In patients who remain hyperkalemic despite repletion of salt and water, restriction of dietary potassium intake is critical. For infants and young children, the provision of 40 to 120 mg (1–3 mmol/kg per day) of potassium may be a reasonable place to start. Breast milk has a lower potassium content (546 mg/L; 14 mmol/L) than commercial milk-based infant formula (700–740 mg/L; 18–19 mmol/L) and may be preferred (KPOQI, 2009). Pretreatment of infant formula with a potassium binder such as sodium polystyrene sulfonate may also help address hyperkalemia in infants. Typically, 0.5 to 1.5 g of sodium polystyrene sulfonate is added to every 100 mL of formula or expressed breast milk. Recent studies suggest that this therapy significantly alters the composition of other electrolytes in a formula-dependent manner and mandates close monitoring. Calcium supplementation and sodium restriction may be required (Thompson et al., 2013). The powdered form of sodium polystyrene sulfonate is also recommended due to the presence of a high aluminum concentration in liquid preparations (Taylor et al., 2015). Constipation and certain medications (e.g., potassium-sparing diuretics, ACE-Is, angiotensin-receptor blockers) may exacerbate hyperkalemia in infants with CKD and should be addressed. Finally, the use of potassium-wasting diuretics

(e.g., furosemide) may also be utilized for the treatment of hyperkalemia in those patients who have urine output (Bunchman et al., 1991; Fassinger et al., 1998).

Renal Osteodystrophy

Infants with CKD and secondary hyperparathyroidism may experience improvement subsequent to the initiation of RRT. One study followed 17 patients initiating HD between birth and 2 years of age and found that the percentage of patients with intact parathyroid hormone (iPTH) concentrations less than twice the upper limit of normal increased after 3 months of HD (41% at initiation vs 69% after 3 months) (Shroff et al., 2003). Another study of 20 infants on long-term PD revealed similar results (58% after 6 months of PD vs. 100% after 1 year of PD) (Ledermann, 2000). Further study regarding the prevalence of renal osteodystrophy in this population is needed.

Renal Replacement Therapy

ESRD is an uncommon disorder in children less than 4 years of age, with an incidence of 5.2–10.3 per million age-related population (Chesnaye et al., 2015). Neonatal ESRD is even less common with an incidence of approximately 0.32 per 100,000 live births (Carey et al., 2007). A large international registry study of neonates on RRT showed that they made up between 6.8% and 18.3% of all infants less than 2 years of age on dialysis. Major causes of ESRD in the 0 to 2-year age group include congenital anomalies of the kidney and urinary tract and cystic kidney disease (van Stralen et al., 2014). Although kidney transplantation is the nearly universal goal for children who develop ESRD, data from the most recent North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) annual report revealed that 80% of children under 2 years old who received a renal transplant also received chronic dialysis before transplantation (NAPRTCS, 2014).

Peritoneal Dialysis

PD is the preferred chronic dialysis modality for infants with ESRD. A recent publication from NAPRTCS has shown that greater than 90% of patients 0 to 1-year-old receiving dialysis were receiving PD at dialysis initiation (NAPRTCS 2011; Carey et al., 2015). The mechanics of chronic PD (CPD) are similar to those discussed above for acute PD. While the CPD prescription initially mirrors that of acute PD, the fill volume is subsequently titrated upwards during CPD to a goal of 600 to 800 mL/m² body surface area in infants during each exchange; the duration of the dwell is adjusted to reach predefined adequacy metrics in terms of solute and fluid removal (National Kidney Foundation, 2006).

The complications of PD are similar for acute and chronic PD. The single most serious complication is peritonitis, which occurs more frequently in infants than older children (NAPRTCS, 2011; Sethna et al., 2016). Some data suggest that peritonitis is increased in infants with oligoanuria (Vidal et al., 2012). Whereas gram-positive organisms account for the majority of infections, gram-negative episodes of peritonitis are common in infants and young children (Zurowska et al., 2008), and the percentage of gram-negative and polymicrobial peritonitis episodes may be on the rise (Hijazi et al., 2009). Empiric therapy for peritonitis should, in turn, always provide coverage for gram-positive and gram-negative organisms (Warady et al., 2012). Other CPD-related complications that occur most frequently during infancy include anterior ischemic

optic neuropathy and sudden blindness secondary to hypovolemia, excessive loss of protein across the peritoneal membrane, and hernia formation (Quan and Baum, 1996; Lapeyraque et al., 2003; Hijazi et al., 2009).

Hemodialysis

The use of HD during infancy is most often dictated by the presence of a medical condition (e.g., omphalocele, gastroschisis, diaphragmatic hernia, bladder exstrophy) that compromises the ability to use the peritoneal membrane as a dialyzing membrane. The HD procedure during infancy is complicated, and limited clinical experience has revealed a high incidence of patient morbidity (Al-Hermi et al., 1999; Shroff et al., 2003; Kovalski et al., 2007). As noted above, the complicated nature of the HD procedure mandates that it is performed only in highly qualified centers with access to pediatric dialysis expertise. Recent studies of complications for infants on chronic HD reveal improvement in central venous catheter life in recent years but continued high rates of hypertension, psychomotor retardation, and hospitalization (Feinstein et al., 2008; Quinlan et al., 2013; Pollack et al., 2016).

Transplantation

The topic of kidney transplantation in patients who develop ESRD as neonates or young infants is complicated, and a lengthy discussion is beyond the scope of this chapter. In short, however, transplantation is a viable alternative for these young patients and is their best hope for long-term survival. In a review of NAPRTCS data, 20% of 0 to 1-year-olds and 24% of 2 to 5-year-olds with ESRD received a preemptive (e.g., no prior dialysis) transplant. Only eight infant transplants (<1 year of age) have been performed and entered into the NAPRTCS registry since 2008 (NAPRTCS, 2014). The overall transplant rates and graft survival rates for some of the largest and most recent studies of outcomes in neonates or young children with ESRD are listed in Table 90.13. Graft survival in patients who developed ESRD as neonates or infants has improved (Carey et al., 2015), and the rates of graft survival for the two groups are similar. These improvements in transplant rates and graft survival likely contribute to the improved overall survival of these patients and likely will improve further as outcomes in regards to other comorbidities, such as cardiovascular disease and growth, also improve.

What is often most important for the neonatologist is recognition of the need to develop a collaborative strategy with members of the pediatric nephrology, surgery, and urology teams for management of congenital structural abnormalities of the urinary tract that are present in the patient with severe CKD/ESRD, the majority of whom will ultimately require dialysis and transplantation (Sarwal, Salvatierra, 2004).

Outcomes

Hospitalization

There is emerging evidence to support the clinical expectation that a majority of neonates and infants with CKD or ESRD require frequent hospitalization throughout childhood. In one study of 18 children requiring chronic HD by 2 years of age, the median number of hospital admissions while receiving dialysis was 6 (range 3 to 16). Of those hospitalized, the median hospitalization rate per patient was 8.2 admissions per year with the duration of hospitalization ranging from 63 to 399 days (Shroff et al., 2003).

TABLE 90.13**Outcomes of Large Studies of Neonates and Infants With Severe Chronic Kidney Disease**

| Study Age Range | Carey et al. 2007 <2 years | Hijazi et al. 2009 <1 year | Mekahli et al. 2010 ≤2 years | Van Stralen et al. 2014 ≤1 month | Carey et al. 2015 <1 year |
|----------------------------|---|---|--|---|---|
| Population | ESRD <i>n</i> = 193 neonates <i>n</i> = 505 infants EC = 1992–1998 LC = 1999–2005 | ESRD <i>n</i> = 52 | GFR <20 mL/min per 1.73 m ² or requiring dialysis <i>n</i> = 101 | ESRD <i>n</i> = 264 | PD <i>n</i> = 241 (neonates) <i>n</i> = 387 (infants) EC = 1992–1999 LC = 2000–2012 |
| Database(s) | NAPRTCS | Single center | Single center | ESPN/ERA-EDTA, IPPN, Japanese, ANZDATA | NAPRTCS |
| Comorbidities | NR | 48.1% overall | 50.4% overall • 24% NDD • 4% pulmonary • 10% CV | 73% overall • 20% NDD • 12% pulmonary • 18% CV | NR |
| Growth | NR | Height SD: Z = −3.0 ± 1.5 (EC) Z = −1.4 ± 0.9 (LC) | <3rd% adult height M: 42% F: 63% | 63% GR | NR |
| Survival and hospital rate | Survival • 76% neonates • 80% infants Hospitalization • 80% neonates • 73% infants | Survival • 62% (1, 3 year) • 87% (5 year) If survived first year | w/out comorbidity 83.5% (2 year) 81.3% (5 year) w/comorbidity 78.4% (2 year) 72.3% (5 year) | Survival 81% (2 year) 76% (5 year) | 73% overall Neonates 70.0% (EC) 91% (LC) Infants 75.8% (EC) 84.6% (LC) |
| Transplant rates | Neonates 60% (EC) 80% (LC) | 75% | 70% | 21.9% (2 year) 54.9% (5 year) | Neonates 39% (EC) 68% (LC) Infants 53% (EC) 65% (LC) |
| Graft survival | NR | 79% (1 year) 68% (5 year) | 75% (10 year) 55% (15 year) | 84.2% (5 year) | 3-year survival Neonates 86.3% (EC) 84.2% (LC) Infants ~80% (EC) 92.1% (LC) |

ANZDATA, Australian and New Zealand Dialysis and Transplantation; Cr-EDTA GFR, chromium-51-EDTA glomerular filtration rate; CV, cardiovascular; EC, early cohort; ESRD, end-stage renal disease; ESPN/ERA-EDTA, European Society of Paediatric Nephrology/European Renal Association-European Dialysis and Transplant Association; F, female; GR, growth retardation; IPPN, International Pediatric Peritoneal Dialysis; LC, late cohort; M, male; NDD, neurodevelopmental delay; NR, not reported; PD, peritoneal dialysis; SD, standard deviation; Z, zone.

Another study divided 698 children requiring chronic dialysis within the first 2 years of life into those initiating dialysis by 1 month of age and those initiating dialysis between 1 month and 24 months of age. Approximately 80% of children in both groups required hospitalization at some point in the 13-year follow-up period. Among children ever hospitalized, those initiating dialysis as neonates were hospitalized more frequently than were children starting dialysis later (mean number of hospitalizations 54 vs 39; *P* < .001) and experienced longer hospital stays (Carey et al., 2007).

Survival

Long-term survival of neonates with ESRD appears to be approaching that of older infants and young children. In several recent studies examining medium-term survival of neonates, infants, or

young children who started dialysis, the overall survival rates ranged from 70%–87% (Table 90.13) (Carey et al., 2007; Hijazi et al., 2009; Mekahli et al., 2010; van Stralen et al., 2014; Carey et al., 2015). Neonates on dialysis had only slightly lower survival rates compared with older infants and children (Carey et al., 2007; Carey et al., 2015). The main reasons for death included infection and cardiovascular disease. Concomitant neurologic disease and other comorbidities were associated with increased mortality (Mekahli et al., 2010; van Stralen et al., 2014). Studies that examined multiple time periods showed improved survival for neonates and infants in more recent years (Carey et al., 2007; Carey et al., 2015). Although the recent large studies suggest a good medium-term survival and support the recommendation for RRT in these complex patients, more long-term data are clearly needed.

Ethics of Initiating or Withdrawing Renal Replacement Therapy

Decisions to withdraw or withhold treatment have to be made for many patients in neonatology units and for as many as 30%–58% of patients in pediatric intensive care units. The conceptual framework for medical decision making in seriously ill newborns attempts first to classify the anticipated therapy and outcomes as clearly beneficial, clearly futile, or of uncertain benefit. While families should be active participants in medical decision making, physicians may override a family's wishes in situations deemed clearly beneficial or clearly futile.

RRT for neonates and very young infants has long been considered of uncertain benefit due to unclear long-term outcomes. In turn, medical providers have often deferred to family members with regards to decisions about dialysis initiation and withdrawal in young patients. However, as dialysis has become more routinely offered to neonates and infants with ESRD and more published data on improved medium-term and long-term outcomes have become available, consideration has been given to classifying this therapy as clearly beneficial (Lantos and Warady, 2013). Nevertheless, few studies have directly examined the changing attitudes of medical providers on this issue. Geary and colleagues initially conducted an international survey on the attitudes of pediatric nephrologists regarding the management of ESRD during infancy nearly two decades ago (Geary, 1998). More than 200 physicians from eight countries replied to a series of questions pertaining to the provision of RRT to neonates younger than 1 month of age versus those 1 to 12 months old. At that time, 93% of respondents stated that they would offer dialysis to some patients less than 1 month of age, 41% would offer it to all patients less than one month of age, and 50% believed it was usually ethical for families to withhold RRT. In a follow-up study conducted by the same group using an almost identical survey 10 years later, 98% of pediatric nephrologists responded that they would offer dialysis to some patients less than 1 month of age, although only 30% of pediatric nephrologists would offer it to all patients less than one month of age. Additionally, there was a 25% increase compared with the earlier survey in those who thought it was the parents right to “usually” refuse RRT in neonates (Teh et al., 2011). In both studies, physicians responded that they more routinely provided dialysis to the older than 1-month to 12-month age group and thought it was less acceptable for families to refuse dialysis initiation for children of this age (Geary, 1998; Teh et al., 2011). The factors that most often influenced the decision to initiate or withhold RRT were the presence of coexistent serious medical disorders and the anticipation of significant morbidity for the child (Geary, 1998; Teh et al., 2011). Evidence for this age-related variation in

philosophy and practice has been seen in other surveys as well (Fauriel et al., 2004).

Most clinicians agree that there is more to their skill than the indiscriminant application of technology. Factors to consider when making the decision regarding initiation or withdrawal of RRT during infancy include quality of life concerns, allocation of resources, legal issues, and, most importantly, the opinions of the hospital team and the parents. Future research into the ethical issues surrounding infant dialysis requires separation of isolated renal failure from renal failure in the setting of comorbid conditions, the cost financially, socially, and emotionally, and research into the informed consent around the initiation of chronic dialysis (Lantos and Warady, 2013). The role of hospital ethics committees in the process remains extremely variable. In the end, clinicians and parents often struggle bravely to reach a compassionate decision with as much agreement as possible. Principles of practice that may provide valuable assistance in this process have been published (Watson, 2004).

Suggested Readings

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Glomerulonephropathies and Disorders of Tubular Function

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KEY POINTS

- Nephrotic syndrome (NS) is comprised of persistent heavy proteinuria, hypoalbuminemia, edema, and hyperlipidemia.
- Genetic abnormalities of structural or regulatory proteins within the glomerular basement membrane and/or podocyte lead to primary congenital NS (CNS).
- Treatment of CNS does not involve immunosuppression and is aimed at minimizing symptoms and preventing serious complications.
- Infections causing secondary NS include human immunodeficiency virus, syphilis, toxoplasmosis, hepatitis B, malaria, rubella, and cytomegalovirus.
- Renal tubular acidosis (RTA) causes a nonanion gap metabolic acidosis. It is important to evaluate for other sources of bicarbonate (HCO_3^-) loss before initiating a work-up for RTA and to note that immature tubular function in premature infants may cause a self-limited moderate metabolic acidosis in the first 2 weeks of life.
- Inherited renal tubulopathies are rare and can be distinguished from each other in part by differences in serum potassium levels, presence of metabolic acidosis or alkalosis, presence of hypertension, and urine findings (Table 91.1).
- Fanconi syndrome is a condition of diffuse proximal tubule dysfunction resulting in polyuria and wasting of HCO_3^- , amino acids, uric acid, phosphate, glucose, and low-molecular-weight proteins. Fanconi syndrome may be due to an isolated defect or can be a part of a broader genetic syndrome.
- Primary nephrogenic diabetes insipidus may present during pregnancy as polyhydramnios. In the newborn period, treatment should focus on maintaining adequate fluid balance.

Glomerulonephropathies

Generally, glomerulonephropathies are considered when nephrotic syndrome (NS)—the constellation of persistent heavy proteinuria, hypoalbuminemia, edema, and hyperlipidemia—are present. More than 4 mg/kg per hour of protein excretion on a 24-hour urine collection is consistent with nephrotic range proteinuria. Alternately, an untimed “spot” urine protein-to-creatinine ratio can be used when a 24-hour urine collection is impractical. A ratio of 3 mg protein/mg creatinine is consistent with nephrotic range proteinuria.

Congenital Nephrotic Syndrome

Congenital nephrotic syndrome (CNS) is defined as NS onset within the first 3 months of life. Infantile NS refers to NS onset within the first year of life. Both congenital and infantile NS can be divided into primary and secondary causes, with primary being the most common. Genetic abnormalities of structural or regulatory proteins within the glomerular basement membrane and/or podocyte lead to primary CNS (Cil et al., 2015). The podocytes are cells with extensive foot processes that wrap around the capillaries of the glomerulus to prevent loss of proteins from the capillary into the urinary space. Podocytes are anchored in place by many proteins that make up the slit diaphragm, located between podocytes (Fig. 91.1). When there are abnormalities of the podocyte or slit diaphragm proteins, large molecules, such as proteins, may leak into the urinary space leading to proteinuria. Secondary CNS can be a presentation of congenital or perinatal infections or related to underlying metabolic disease (Goldenberg et al., 2005). Diagnosis may be made prenatally when a positive family history is present or because of an elevated amniotic fluid alpha fetoprotein indicating fetal proteinuria. In infants, clinical concerns (e.g., edema, failure to thrive, developmental delay) or NS complications, such as thromboembolism or infection, may lead to work-up and diagnosis (Rheault, 2014). Although most children with CNS require renal transplantation at a young age, there are case reports of CNS with spontaneous resolution or delayed progression, likely related to mild genetic mutations (Wong et al., 2013).

Primary Congenital Nephrotic Syndromes

Finnish-Type Congenital Nephrotic Syndrome (MIM # 256300)

Mutations in the nephrin protein encoding gene *NPHS1* are the most common cause of autosomal recessive (AR) CNS. It is referred to as CNS of the Finnish type (CNF) owing to an incidence of approximately 1 in 8000 live births in the Finnish population (Jalanko, 2009). Nephrin is an essential component of the podocyte slit diaphragm (Hildebrandt, 2010). The classic presentation includes placental enlargement, premature delivery, and proteinuria at birth (Jalanko, 2009). Patients may have microscopic hematuria in addition to massive proteinuria (Fogo et al., 2015). Cardiac abnormalities, such as pulmonary stenosis or patent ductus arteriosus, have been reported in patients with *NPHS1* mutations, although severity and frequency are not well defined (Kari et al., 2014). Renal

TABLE 91.1 Characteristics of Renal Tubular Acidosis Types

| | Inheritance | Acid/Base | Potassium | Other Findings |
|--|---|---------------------|---------------------|--|
| Proximal Tubule | | | | |
| Proximal RTA (type 2) | AD or AR or acquired | Metabolic acidosis | Normal or decreased | |
| Fanconi syndrome (Diffuse proximal RTA) | Inheritance depends on underlying disease or acquired | Metabolic acidosis | Normal or decreased | Aminoaciduria Phosphaturia Glycosuria Uric aciduria Low-molecular-weight-proteinuria |
| Type 4 RTA | Inherited (variable) or acquired | Metabolic acidosis | Increased | |
| Loop of Henle | | | | |
| Barter syndrome | AR | Metabolic alkalosis | Decreased | Hypercalciuria |
| Distal Tubule | | | | |
| Distal RTA (type 1) | AD or AR or acquired | Metabolic acidosis | Normal or decreased | Hypercalciuria |
| Gitelman syndrome | AR | Metabolic alkalosis | Decreased | Hypomagnesemia |
| Gordon syndrome | AD | Metabolic acidosis | Increased | Hypertension Elevated serum aldosterone-to-renin ratio |
| Liddle syndrome | AD | Metabolic alkalosis | Decreased | Hypertension Low serum renin and aldosterone |
| Pseudohypoaldosteronism type 1 | AD or AR | Metabolic acidosis | Increased | Hyponatremia Elevated serum renin and aldosterone |

AD, Autosomal dominant; AR, autosomal recessive; RTA, renal tubular acidosis.

ultrasound images demonstrate large echogenic kidneys with poor corticomedullary differentiation. These imaging findings are similar among all forms of congenital and infantile NS (Jalanko, 2009). Renal biopsy is not always performed, and findings are nonspecific. Electron microscopy demonstrates diffuse foot process effacement (Fogo et al., 2015). The most common light microscopy findings are dilated proximal tubules and microcystic changes, but these are not diagnostic of a *NPHS1* mutation (Machuca et al., 2010).

Congenital Nephrotic Syndrome Type 2 (MIM #600995)

Podocin protein mutations in the *NPHS2* gene lead to the second most common form of AR CNS (Hildebrandt, 2010; Cil et al., 2015). This is the most common gene implicated in CNS in central European populations (Cil et al., 2015). Podocin is essential for targeting nephrin to the slit diaphragm of the podocyte, and protein absence leads to early onset, severe NS (Jalanko, 2009). Clinical presentation is often slightly later than for *NPHS1* mutations, with NS commonly presenting between 4 months and 1 year of age (Boute et al., 2000). Additionally, progression to end-stage renal disease (ESRD) is thought to occur more gradually, with a mean time of 6.6 years from diagnosis to development of ESRD (Hinkes et al., 2007). Ultrasound findings are similar to other forms of congenital and infantile NS. Renal biopsy often shows a pattern consistent with focal segmental glomerular sclerosis (Fogo et al., 2015).

Wilms Tumor Suppressor Gene Mutation Syndromes (MIM #194072, 136680, 194080)

Mutations of the Wilms tumor suppressor gene (*WT1*) may result in isolated diffuse mesangial sclerosis or be part of a syndrome associated with CNS such as Wilms tumor, aniridia, genitourinary abnormalities, and intellectual disabilities (WAGR), Frasier, or

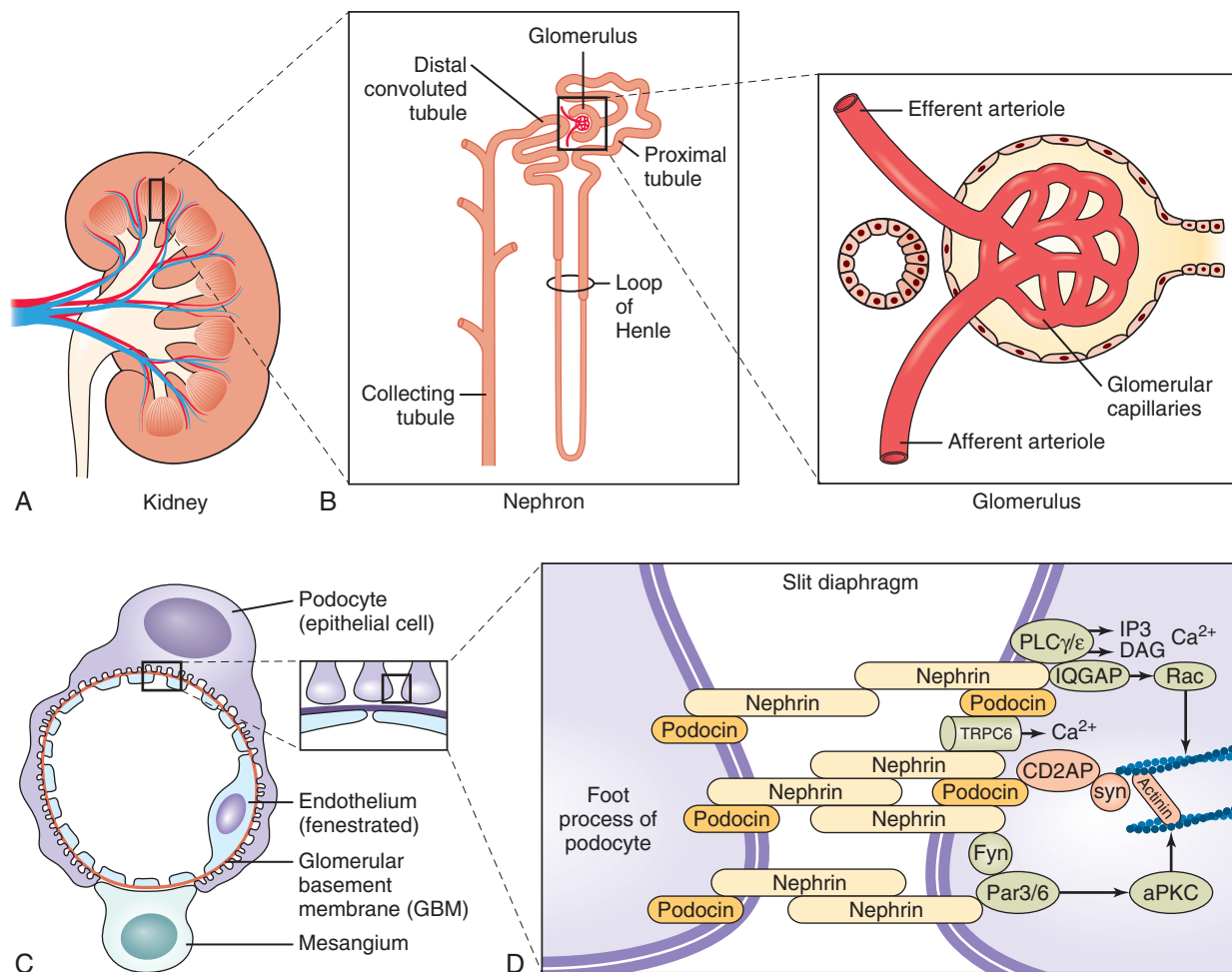
Denys–Drash syndrome. On average, ESRD occurs at 3 years of age but can occur in infancy, and disease severity is partially dependent on mutation type. Patients with *WT1* mutations are at risk for Wilms tumors and gonadoblastomas and should be screened for these malignancies. Patients should also be screened with karyotype testing and, possibly, further genitourinary imaging, as they may have ambiguous genitalia or other genitourinary tract anomalies such as cryptorchidism in males or uterine abnormalities in females (Lipska et al., 2014). The histologic findings on renal biopsy demonstrate either diffuse mesangial sclerosis, identified by mesangial collagen increase on light microscopy, or focal segmental glomerular sclerosis (FSGS) (Lipska et al., 2014; Fogo et al., 2015).

Pierson Syndrome (MIM #609049)

Pierson syndrome is an AR disorder caused by mutations in *LAMB2*, which codes for the beta 2 chain of laminin, a basement membrane protein. Children classically present with NS and microcoria (small pupils with inability to dilate). As laminin is present in multiple basement membrane structures throughout the body, patients may also have hypotonia, developmental delay, or other vision deficits (Wuhl et al., 2007). Age of presentation varies depending on the mutation type, but many present in the first year of life. Renal biopsies in these children most commonly show features of diffuse mesangial sclerosis with irregular lamellation seen on electron microscopy (Choi et al., 2008; Machuca et al., 2010).

Other Primary Causes of Congenital Nephrotic Syndrome (MIM #251300, 161200)

Galloway–Mowat syndrome is a rare AR condition in patients with NS and central nervous system anomalies, primarily microcephaly, with a genetic defect in the *WDR73* gene (Dietrich et al.,



• **Fig. 91.1** (A) The kidney contains approximately one million nephrons. (B) The nephron is composed of a glomerulus, proximal tubule, loop of Henle, distal tubule, and collecting duct. (C) The glomerular filtration barrier is composed of fenestrated endothelial cells, a basement membrane, and epithelial cells (podocytes). (D) Podocytes have structures called foot processes. The area between the foot processes is called the slit diaphragm. Proteins such as nephrin and podocin are essential for proper functioning of the slit diaphragm and filtration barrier. *actinin*, α -actinin-4; *aPKC*, atypical protein kinase C; *CD2AP*, CD2 adaptor protein; *DAG*, diacylglycerol; *IP3*, inositol 1,4,5 triphosphate; *IQGAP*, IQ motif containing GTPase activating protein 1; *PLC*, phospholipase C; *syn*, synaptopodin; *TRPC6*, transient receptor potential-like channel 6. (Adapted from Chiang CK, Inagi R. Glomerular diseases: genetic causes and future therapeutics. *Nat Rev Nephrol.* 2010;6:539–554.)

2008). Nail–patella syndrome is an autosomal dominant (AD) condition associated with *LMX1B* mutations leading to proteinuria, nail dysplasia, glaucoma, and bony anomalies classically identified by the presence of iliac horns (Bongers et al., 2002). This syndrome usually does not result in NS in infancy but may present early in life with proteinuria or bony abnormalities. *Lamb3* mutations have been found in individuals with NS and epidermolysis bullosa (Dietrich et al., 2008).

Management of Primary Congenital Nephrotic Syndromes

Primary CNS does not respond to the immunosuppressive treatments used for idiopathic NS. Given the abnormal or absent functional proteins in the basement membrane or slit diaphragm, this group of diseases is generally treated with symptomatic management targeted at minimizing protein loss. Definitive treatment is renal transplantation, but supportive therapies are used until the

child is of appropriate age and size for renal transplantation. The basic principles of management include maintenance of intravascular volume, minimization of proteinuria, adequate nutritional support, and maintaining electrolyte balance.

Albumin infusions are given to replace protein loss and restore intravascular volume. Diuretics are used in combination with intravenous albumin infusions to avoid severe edema. Treatment frequency varies based on patient characteristics. Medications to decrease the glomerular filtration rate by modulating renal blood flow can be used in an attempt to limit protein loss. Angiotensin-converting enzyme inhibitors and nonsteroidal antiinflammatory drugs (NSAIDs) can be used for this purpose either alone or in combination (Licht et al., 2000; Kovacevic et al., 2003). If medication management is insufficient to maintain stable serum albumin concentrations and prevent severe edema, then nephrectomy, unilateral or bilateral, would be indicated.

Actively nephrotic children are at risk for many serious complications and should be monitored vigilantly. Protein loss is not specific as the podocyte lesion allows varied protein sizes into the urine. Immunoglobulins, coagulation proteins, thyroid binding proteins, and other proteins are lost in the urine. Approximately 10% of children will experience thrombotic complications, and the majority will experience serious bacterial infections (Kim et al., 1998). All children will experience failure to thrive without nutritional supplementation, which often requires tube feedings. Management with intravenous immunoglobulins (IVIGs) is not routinely recommended because of the rapid loss of the IVIG proteins into the urine (Payne et al., 2013). No standard guidelines exist regarding when nephrotic children should receive anticoagulation. However, hypercoagulability is of particular concern in children requiring central venous access, and this subgroup should be placed on antithrombotic therapy. Hypothyroidism is common due to thyroid binding protein loss in the urine. Smaller studies report refractory anemia related to transferrin and transcobalamin loss (Toubiana et al., 2009). Ceruloplasmin loss in the urine leading to copper deficiency has been reported, causing neutropenia and refractory anemia (Ulinski et al., 2009).

Most children progress to renal transplantation. At present, patients with CNS are expected to do as well as children with ERSD due to other etiologies (Jalanko, 2009). A small percentage of patients, particularly with CNF, do have a return of proteinuria after transplantation because of antibody formation against the previously absent nephrin protein (Mejía et al., 2015).

Secondary Causes of Congenital Nephrotic Syndrome

Although primary forms of CNS are most common, clinicians should consider secondary causes in children with negative genetic testing or clinical findings that point to other etiologies. For example, children with signs of metabolic disease in addition to their proteinuria should be evaluated for mitochondrial cytopathies. Those with nipple inversion and other dysmorphisms should be evaluated for congenital disorders of glycosylation (Park et al., 2015). Congenital and perinatal infections may cause CNS, a finding more common in developing countries. Implicated infections include human immunodeficiency virus, syphilis, toxoplasmosis, hepatitis B, malaria, rubella, and cytomegalovirus. Infection-related NS may respond with appropriate antimicrobial treatment, depending on the degree of glomerular damage.

Other Glomerular Diseases

Neonatal glomerulonephritis (GN) is uncommon and primarily described in case reports. GN is defined by proteinuria and hematuria accompanied by varying degrees of acute kidney injury, oliguria, and hypertension. Rarely, maternal antibody transfer can lead to membranous nephropathy in a neonate because of absence of maternal neutral endopeptidase and subsequent antibody production following exposure to fetal neutral endopeptidase. The infant may experience transient glomerulopathy or may develop chronic kidney disease (Debiec et al., 2004). Additionally, nephritis may be the result of transfer of maternal antibodies related to systemic lupus erythematosus to the infant (Rheault, 2014).

Renal Tubular Disorders

The renal tubule regulates and modifies the glomerular filtrate to avoid excess salt and water loss in the urine. Defects along the tubule lead to varying pathology. The proximal tubule is in charge

of bulk water and electrolyte reabsorption. The distal nephron delicately regulates the final urinary product with close regulation of acid–base balance and water reabsorption. In neonates, genetic causes of tubular dysfunction predominate; however, medications and other secondary causes of tubular dysfunction can occur. Adequate evaluation of renal tubular disorders in neonates can be complicated by the use of intravenous fluids, parenteral nutrition, or electrolyte supplementation, as these modify the serum and urine studies. In addition, the renal tubules of premature infants continue to mature after birth, particularly with improved sodium reabsorption over the first month of life (Gubhaju et al., 2014). This immature tubular function may cause a self-limited moderate metabolic acidosis in the first 2 weeks of life (Bourchier and Weston, 2015).

Renal Tubular Acidosis

Renal tubular acidosis (RTA) is suspected in children with a nonanion gap hyperchloremic metabolic acidosis. Type 1 RTA is due to a distal nephron defect, type 2 is due to a proximal tubule defect, type 3 is a mix of type 1 and type 2 defects, and type 4 is due to hypoaldosteronism or aldosterone resistance.

Children with RTA present with failure to thrive, emesis, and polyuria. It is important to evaluate for other sources of bicarbonate (HCO_3^-) loss, such as diarrhea, as this should be addressed before pursuing a work-up for RTA. Baseline testing of serum electrolytes, blood gases, and a urine sample should be obtained while the child is acidotic. Calculation of a serum anion gap is helpful to determine if a high anion gap acidosis or a normal anion gap acidosis is present, as RTA is associated with a normal anion gap acidosis. The anion gap is a calculation of serum cations minus serum anions. A high anion gap usually indicates increased unmeasured anions, whereas a normal anion gap acidosis is usually due to increased serum chloride. The anion gap is calculated from serum chemistries ($[\text{sodium } \{\text{Na}^+\}] - ([\text{chloride } \{\text{Cl}^-\}] + [\text{HCO}_3^-])$), and normal values are between 7 and 13 milliequivalent (mEq)/L. Of note, sometimes potassium (K^+) is used in the equation, which increases the normal range to 11–14 mEq/L. The presence of an anion gap acidosis should lead clinicians away from the diagnosis of RTA. Urinary studies will help differentiate between the types of RTA. The urinary anion gap is used to approximate urinary ammonium (NH_4^+) concentration, and it is calculated by $(\text{U}_{\text{Na}^+} + \text{U}_{\text{K}^+} - \text{U}_{\text{Cl}^-})$. Urine anion gap is negative in the setting of high urinary NH_4^+ concentration, as NH_4^+ excretion leads to increased chloride anion excretion, and the excess chloride is measured in the urinary anion gap. The urinary anion gap is positive with inadequate NH_4^+ concentration in the urine; however, in the setting of volume depletion and low urine sodium (≤ 25 mmol/L) the urine anion gap is also positive as the kidney reabsorbs chloride and has limited ammonia excretion (Sulyok and Guignard, 1990; Kim, 2011). This volume depletion setting may mimic the serum and urine anion gap findings of RTA. Urinary osmolar gap $(\text{U}_{\text{Osm}} - 2\text{U}_{\text{Na}^+} + 2\text{U}_{\text{K}^+} + (\text{U}_{\text{urea}} (\text{mg/dL})/2.8) + \text{U}_{\text{glucose}} (\text{mg/dL})/18)$ also helps determine if adequate NH_4^+ excretion is present. An elevated urinary osmolar gap (>1000 mOsm/kg) suggests high NH_4^+ excretion (Kraut and Madias, 2012).

Fanconi Syndrome

Fanconi syndrome is a condition of diffuse proximal tubule dysfunction. Children with Fanconi syndrome have polyuria and wasting of HCO_3^- , amino acids, uric acid, phosphate, glucose, and low-molecular-weight proteins. Fanconi syndrome may be an isolated defect or part of a broader genetic syndrome.

Nephropathic Cystinosis (MIM #219800)

Cystinosis is the most common cause of primary proximal tubular dysfunction in children. It is an AR lysosomal storage disease caused by a defect in the transporter cystinosin. This protein is responsible for cystine transport through the lysosome, and mutations cause cystine accumulation within the proximal tubular cells and throughout the body. The renal manifestations of cystinosis present early in life and often lead to diagnosis of the disease (Emma et al., 2014). Additional clinical features include corneal deposits, hepatomegaly, insulin dependent diabetes mellitus, weakness, infertility, and hypogonadism. Corneal deposits initially cause light sensitivity and later lead to significant visual impairment.

Cystinosis diagnosis is made by measuring leukocyte cysteine levels, detecting corneal deposits, or genetic testing. Genetic testing can also be done prenatally by chorionic villi sampling (Jackson and Young, 2005). Discovery of cysteamine in 1976, now the mainstay of treatment, was a breakthrough in the treatment of patients with cystinosis (Goodyer, 2011). Cysteamine decreases cystine concentration within cells, and although cysteamine therapy cannot reverse renal tubular dysfunction, it can help preserve glomerular filtration rate, delaying the onset of ESRD (Nesterova et al., 2015). Measurement of leukocyte cystine depletion is used to monitor efficacy of therapy. Compliance with treatment is difficult as the medication has many side effects such as gastrointestinal upset, bad breath, body odor, and skin lesions at high doses. Additionally, it requires dosing multiple times per day, depending on the formulation. Cysteamine eye drops are used to limit corneal deposits, and these require hourly dosing.

Other Causes of Fanconi Syndrome

Tyrosinemia (MIM #276700) and classic galactosemia (MIM #230400) are both part of the uniform newborn screening panel performed in the United States. Therefore they are often diagnosed early in life. Tyrosinemia is an AR disorder where toxic metabolites accumulate in the liver and kidney. Early therapy with nitisinone to reduce the production of toxic metabolites can improve renal proximal tubular dysfunction (Maiorana et al., 2014). Classic galactosemia is also AR and presents early in life with emesis, lethargy, hepatomegaly, sepsis, and failure to thrive. Cataracts may also be present in infancy. Proximal tubular dysfunction can accompany galactosemia and exacerbate the clinical picture, although dietary modification can prevent worsening of tubular dysfunction (Bosch, 2006).

Hereditary fructose intolerance (MIM #229600) does not usually present until fructose or sucrose is added to an infant's diet. Infants present with emesis and hypoglycemia and on subsequent evaluation may be found to have proximal tubular dysfunction due to deficient aldolase B within the kidney. Early dietary modification can prevent renal complications (Ali et al., 1998).

Lowe syndrome (MIM #309000), also known as the oculocerebrorenal syndrome, is a rare X-linked syndrome. Congenital cataracts and hypotonia are often detected at birth, with proximal tubulopathy developing over subsequent weeks to months (Loi, 2006).

Dent disease (MIM #300009) is another rare X-linked recessive proximal tubulopathy. It is characterized by hypercalciuria and recurrent nephrolithiasis. Urinary phosphate losses can lead to rickets and significant bone abnormalities in infancy (Devuyst and Thakker, 2010).

Fanconi syndrome is also a defining feature of Fanconi-Bickel syndrome (MIM #227810), which is characterized by hepatic and

renal dysfunction caused by *GLUT2* mutations (Bockenhauer and Bichet, 2015). This generally presents after a few months of life but can be seen in early infancy.

In addition to the variety of genetic syndromes described above, many medications can lead to a proximal tubular dysfunction. Common offending medications include tenofovir, ifosfamide, carbonic anhydrase inhibitors, and aminoglycosides.

Isolated Proximal Renal Tubular Acidosis (Type 2 RTA)

Proximal RTA is not often seen in isolation and usually accompanies the more diffuse proximal tubular dysfunction, Fanconi syndrome, described earlier. The proximal tubule generally reabsorbs 80% of filtered HCO_3^- ; therefore defects lead to massive bicarbonaturia and subsequent acidosis. Serum potassium may be normal or decreased, due to impaired proximal potassium reabsorption in the setting of metabolic acidosis.

Inherited isolated proximal RTA is rare. It can be associated with ocular abnormalities and mental retardation (Santos et al., 2015). Age of presentation is variable. Proximal RTA can be distinguished from distal RTA by the absence of hypercalciuria and absence of nephrocalcinosis. In proximal RTA, the urinary pH is often inappropriately high (>5.5) due to bicarbonaturia; however, once the serum HCO_3^- drops, less HCO_3^- will be filtered at the glomerulus, and urinary pH will also drop (<5.5). Treatment is with alkali replacement to correct the metabolic acidosis; however, large doses of HCO_3^- supplementation are often needed to maintain balance. As this therapy can often worsen hypokalemia, potassium supplementation is also needed.

Distal Renal Tubular Acidosis (Type 1 RTA)

Distal RTA is caused by an inability of the collecting duct to excrete adequate hydrogen (H^+) ions because of poor NH_4^+ excretion. Inherited distal RTA typically presents early in life with hyperchloremic metabolic acidosis, failure to thrive, hypercalciuria, and nephrocalcinosis that is usually seen on ultrasound within the first month of life (Santos et al., 2015). Patients may be hypokalemic due to decreased distal hydrogen ion secretion and subsequent increased distal potassium excretion to maintain electroneutrality within the urinary filtrate. Specific AR mutations in the H^+ adenosine triphosphatase have been associated with sensorineural hearing loss as well as distal RTA (Vargas-Poussou et al., 2006). Individuals with distal RTA typically have an inappropriately high urinary pH, generally greater than 5.5. The urine anion gap is usually positive, and the urine osmolar gap is low, representing inadequate NH_4^+ excretion. Treatment is with alkali supplementation.

Hyperkalemic Tubulopathies (Type 4 RTA)

Hypoaldosteronism

Clinical hypoaldosteronism may be due to either a deficiency in aldosterone production or an inability of the renal tubules to respond to aldosterone (pseudohypoaldosteronism). Aldosterone deficiency can be primary in cases of congenital adrenal insufficiency or secondary due to drugs or infection and is outside the scope of this chapter.

Pseudohypoaldosteronism Type 1

Pseudohypoaldosteronism type 1 can be AD or AR and involves a loss of function mutation of the ENaC channel. This can present in infancy with volume depletion, hyponatremia, hyperkalemia, and acidosis. Because of mineralocorticoid resistance, patients have symptoms and laboratory results consistent with hypoaldosteronism

but with elevated renin and aldosterone levels. Management requires treatment with Na^+ supplementation and K^+ restriction.

Gordon Syndrome (Pseudohypoaldosteronism Type 2)

Gordon syndrome is an AD hypertensive condition that traditionally presents at an older age with hyperkalemia and metabolic acidosis. Hypertension is due to volume overload through mutations in the distal convoluted tubule that increase Na^+ and Cl^- reabsorption. Although renin levels are depressed, aldosterone is not suppressed and therefore the aldosterone-to-renin ratio is elevated, which can help in the diagnosis. Low-dose diuretics are the mainstay of therapy (Simonetti et al., 2012).

Hypokalemic Tubulopathies

Bartter Syndrome (MIM #601678, 241200, 607364, 613090, 602522, 601198)

Bartter syndrome is caused by AR mutations in the thick ascending limb (TAL) of the loop of Henle. There are many known types of Bartter syndrome. To date, mutations in the genes *SLC12A1*, *KCNJ1*, *CLCNKB*, *CLCNKA*, *BSND*, and *CASR*, have been found. Bartter syndrome is extremely rare, with a prevalence of 1 in 1,000,000 based on adult studies (Ji et al., 2008). Bartter syndrome often comes to clinical attention based on poor growth and development, typically at a young age. Impairment of sodium chloride reabsorption in the TAL leads to decreased urinary concentrating ability, polyuria, volume depletion, and renin and aldosterone system activation. The secondary aldosterone activation increases Na^+ reabsorption and K^+ and H^+ ion excretion. This physiology can be thought of as analogous to the effect seen with loop diuretics.

Laboratory evaluation will demonstrate hypokalemia, metabolic alkalosis, hypochloremia, and hypercalciuria. Serum magnesium is often normal. Nephrocalcinosis may develop in infancy because of high urinary calcium. Sometimes, differentiation of Bartter syndrome from electrolyte abnormalities related to volume depletion can be difficult. Urinary Cl^- measurement can be very useful, as this should be low (<35 mEq/L) in patients with volume depletion (Veldhuis et al., 1979). Bartter syndrome patients uniquely have elevated urinary Cl^- in the presence of hypochloremia.

Bartter syndrome type IV is uniquely associated with sensorineural hearing loss, as the barttin protein is present in the kidney and the inner ear (Landau et al., 1995). Genetic testing can confirm the diagnosis and specific mutation type; however, the diagnosis is primarily made clinically. Types I, II, and IV may be detected prenatally as part of the work-up for polyhydramnios. Amniotic fluid alpha fetoprotein should be low in cases of suspected Bartter syndrome (Allaf et al., 2015).

Early fluid management is with adequate volume and electrolyte repletion, necessary to allow appropriate growth (Azzi et al., 2015). The TAL defect leads to an upregulation of prostaglandin- E_2 , therefore NSAIDs can help counteract this problem and limit fluid and electrolyte losses. Spironolactone and amiloride may also be used to limit distal potassium loss and minimize the need for large-dose potassium supplements.

There has recently been a new mutation described in melanoma-associated antigen D2 (*MAGE-D2*) on the X chromosome, which leads to a severe but transient form of neonatal Bartter syndrome. This mutation has been associated with perinatal death (Laghmani et al., 2016). These patients have an earlier, more severe presentation with polyhydramnios around the 20th week of gestation. Because of massive polyhydramnios, infants are often born premature. In

the small group of infants studied, polyuria resolved at a median of 4.5 weeks after birth (Laghmani et al., 2016).

Gitelman Syndrome (MIM #263800)

Gitelman syndrome is AR with defects found in the *SLC12A3* gene, which codes for the sodium chloride cotransporter in the distal convoluted tubule. Gitelman syndrome presents with hypokalemia and metabolic alkalosis similar to Bartter syndrome. However, in contrast to Bartter syndrome, it typically presents later in life and with hypomagnesemia and hypocalciuria. This condition can be thought of as analogous to the laboratory findings seen in patients being treated with a thiazide diuretic. Patients commonly present with muscle weakness, paresthesia, paralysis, or tetany. Diagnosis is primarily made based on serum and urine electrolyte measurements and can be confirmed with genetic testing. Treatment involves NSAIDs and drugs that block distal potassium-sodium exchange, combined with potassium and magnesium supplementation.

Liddle Syndrome (MIM #177200)

Liddle syndrome is an AD condition where children are hypertensive because of a distal tubule gain of function mutation in epithelial sodium channel (ENaC). A mutation in this channel, which is responsible for distal sodium reabsorption, leads to excess sodium and subsequent water reabsorption. Consequently, Liddle syndrome is associated with low renin and aldosterone levels, and, classically, hypokalemia and metabolic alkalosis accompany this disease. Triamterene is the therapy of choice as it directly inhibits ENaC channels (Simonetti et al., 2012). Low sodium diet is also beneficial in minimizing the effects of ENaC activation.

Other Tubulopathies

Nephrogenic Diabetes Insipidus (MIM # 304800, 125800)

In children, nephrogenic diabetes insipidus (NDI) is more often primary than secondary. Arginine vasopressin receptor type 2 (*AVPR2*) mutations are inherited in an X-linked recessive pattern and are the most common genetic defect, accounting for 90% of primary NDI (Wesche et al., 2012). Typically, arginine vasopressin is released from the pituitary in response to volume depletion and signals increased water reabsorption. *AVPR2* encodes for arginine vasopressin receptor-2, the receptor for arginine vasopressin on the basolateral surface of collecting duct cells. Receptor activation leads to translocation of aquaporin channels to the apical surface of the nephron for water reabsorption. Mutations in *AVPR2* result in arginine vasopressin resistance because of an inability to appropriately interact with circulating vasopressin (Bockenhauer and Bichet, 2015). Defects in the aquaporin channel are inherited in an AR pattern and are much less common (Wesche et al., 2012).

Children with genetic forms of NDI present at a young age with polyuria, failure to thrive, and hypernatremic volume depletion. Infants and small children that do not have the ability to increase their own water intake based on thirst responses are at greatest risk of complications, particularly at times of illness. Long-term complications include developmental delay, growth failure, and chronic kidney disease, all thought to be secondary to repeated volume and Na^+ depletion and rapid fluid shifts. Prenatal polyhydramnios may be appreciated during pregnancy.

Imaging often demonstrates a dilated urinary system because of the high volumes of urine. Elevated arginine vasopressin hormone levels in the setting of hypernatremic volume depletion and polyuria can help differentiate central diabetes insipidus from NDI, as can a

desmopressin challenge. Definitive diagnosis is made through genetic testing.

Treatment focuses on providing adequate fluids in the newborn period. Decreasing the osmolality of the infant's formula helps maintain balance as these children have a limited ability to excrete osmotic loads. Higher osmolality necessitates increased urine volume. Since the osmotic and salt load of breast milk are much lower than cow milk-based formula, breastfed infants often present later than formula-fed infants (Wesche et al., 2012). Thiazide diuretics and nonsteroidal prostaglandin-synthetase inhibitors aid in management. Thiazide diuretics block sodium chloride reabsorption in the distal tubule leading to mild volume depletion, stimulating upregulation of proximal tubular sodium and water reabsorption to overcome some of the massive water loss in the distal nephron. The mechanism of how nonsteroidal prostaglandin-synthetase inhibitors decrease polyuria is not well understood, but they are particularly effective when combined with a thiazide diuretic. Gastrointestinal upset may limit usage however (Pattaragarn and Alon, 2003). Recently, sildenafil has been presented as a possible treatment to reduce proteinuria in therapy-resistant patients (Assadi and Sharbaf, 2015). These current therapies are primarily aimed at symptomatic relief, but new research is investigating protein folding and increased protein expression, to better target the cause of disease (Wesche et al., 2012).

Suggested Readings

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Complete references used in this text can be found online www.expertconsult.com

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Urinary Tract Infections and Vesicoureteral Reflux

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KEY POINTS

- The presentation of urinary tract infections (UTIs) in neonates differs from that seen in older children.
- The type and route of infection also differ in neonates, when compared with older children.
- An appropriate urine sample for diagnosis is needed, but treatment should not be delayed.
- A neonate in whom a UTI is suspected should be evaluated for sepsis, including invasive cultures.
- After a documented neonatal UTI, a radiologic work-up is warranted to detect anatomic anomalies.
- Vesicoureteral reflux (VUR) is diagnosed in 30%–50% of neonates with proven UTI.
- Voiding cystourethrogram is the gold standard for the diagnosis of VUR and should be performed on high-risk neonates.
- Treatment of VUR should be tailored to each patient's individual risk of UTI recurrence, with the goals of preventing future UTIs and renal scar formation.

Urinary Tract Infection

Urinary tract infections (UTIs) in the newborn period are common and differ in their epidemiology, risk factors, presentation, treatment, and prognosis compared with those of older infants and children. This chapter will discuss UTIs in neonates, with an emphasis on vesicoureteral reflux (VUR).

Epidemiology

Neonates, by virtue of their immature immune system, are at a higher risk for UTIs (Hanson, 1976). In the United States the incidence of UTI within the first 3 days of life is estimated at 0%–1% (Morley et al., 2012; Bonadio and Maida, 2014), while in developing countries it is as high as 1.8% (Samayam and Ravi Chander, 2012; Riskin et al., 2013). UTI is the second most common bacterial infection in children after otitis media (Bachur and Harper, 2001).

In febrile children less than 2 months of age without an obvious source of infection, the prevalence of UTI is 5%–20% (Hoberman et al., 1993; Giorgi et al., 2005; Shaikh et al., 2008). There is a

male predominance in the neonatal period, with males comprising 70%–90% of all cases of neonatal UTI before the age of 6 months (El-Dahr and Lewy, 1992; Wang et al., 1994; Kanellopoulos et al., 2006) irrespective of their degree of prematurity (Eliakim et al., 1997). Hispanics and whites are more likely to be diagnosed with a UTI than children of African descent (Airede, 1992; Chen and Baker, 2006).

Pathophysiology

The natural pathophysiology of UTI is dependent on the migration of uropathogenic bacteria through the fecal–perineal–urethral route (Gruneberg, 1969; Yamamoto et al., 1997) with subsequent entry into the bladder. Iatrogenic instrumentation and hematogenous seeding are alternative modes of entry into the urinary system. Once in the bladder, these uropathogens may ascend into the ureter and kidney in a retrograde manner. The effectiveness of this process is determined by both pathogen and host factors.

Host factors in the neonate often relate to anatomy and involve urogenital malformations such as posterior urethral valves, VUR, phimosis, ureteropelvic junction obstruction, and neurogenic bladder dysfunction secondary to spinal dysraphism. These conditions ultimately disturb the washout effect of antegrade urinary flow that would normally clear the uropathogens (Cox and Hinman, 1961). Additional host factors include neonatal immune function and the antimicrobial properties of urine such as low pH (Sobel, 1997).

Uropathogens have a number of virulence factors that are used to overcome the defense of the host. *Escherichia coli* serotypes with P-fimbriae are commonly isolated in infected urine (Johnson, 2003). These are adhesion proteins at the tip of the bacterium's attachment structures that enhance binding to receptors on the host urothelium (Sussman and Gally, 1999; Wullt et al., 2000; Bower et al., 2005). These increase the bacteria's ability to ascend the urinary tract, even in the absence of VUR (Pohl, 2007).

Epidemiologic studies have shown that 76%–94% of pyelonephritic strains of *E. coli* are P-fimbriated, compared with 19%–23% of strains causing cystitis and 14%–18% of strains isolated from patients with asymptomatic bacteriuria (Vaisanen-Rhen et al., 1984; Tomisawa et al., 1989). However, in the presence of VUR, P-fimbriated *E. coli* is not necessary for infection of the upper tracts. In one study, P-fimbriated *E. coli* was isolated in only 36% of girls with VUR and recurrent pyelonephritis, as compared with

71% of girls with pyelonephritis without VUR (Lomberg et al., 1989).

Other virulence factors from uropathogenic *E. coli* include the release of alpha hemolysin and cytotoxic necrotizing factor-1 (Uhlen et al., 2000; Guyer et al., 2002; Toth et al., 2003), as well as the ability to form a protective glycosylated polysaccharide capsule (Russo et al., 1996).

Presentation

The signs and symptoms of UTI in newborns differ from those of their older counterparts. The familiar symptoms of dysuria, frequency, urgency, malodorous urine, incontinence, suprapubic pain, and hematuria are often absent or not recognized. The clinician must retain a high index of suspicion for the diagnosis of a UTI in a neonate. In full-term infants the most common presentation is a fever of greater than 38.5°C (Littlewood, 1972; Kanellopoulos et al., 2006) followed by poor feeding, tachypnea, and lethargy. In contrast, premature infants commonly present with apnea, hypoxia or tachypnea, and fevers with a temperature greater than 39°C (Milas et al., 2013). Other nonspecific signs and symptoms, such as abdominal distention, diarrhea, vomiting, and failure to thrive, have also been described in infants with a UTI (Arshad and Seed, 2015).

Neonatal jaundice, especially with an onset after 8 days of life, has been associated with neonatal UTIs (Garcia and Nager, 2002; Milas et al., 2013). Mutlu et al. (2014) found that the incidence of UTI in children with hyperbilirubinemia was 18%. This is an important association, as Xinias et al. (2009) showed that approximately 50% of neonates with UTI-induced jaundice had renal cortical changes on dimercaptosuccinic (DMSA) scan.

Bacteriology

Neonatal UTIs are commonly caused by gram-negative rods, with *E. coli* (40%–72%) and *Klebsiella* species (7%–40%) responsible for over 80% of cases (Chen et al., 2011; Shahian et al., 2012). Enterococcus, a gram-positive coccus, is the third most common organism, with an incidence of 10%–16% (Bonadio and Maida, 2014; Arshad and Seed, 2015).

In neonates with UTIs, group B streptococci have been found to be more common than in older children (Wu et al., 2004). Additionally, other gram-positive bacteria, such as coagulase-negative staphylococci, have been associated with UTIs in premature infants, although this remains controversial (Jean-Baptiste et al., 2011). Candida, as a causative agent for UTIs, has a reported incidence of 25%–42% in premature children admitted to the neonatal intensive care unit (Phillips and Karlowicz, 1997; Benjamin et al., 2010).

Risk Factors

The risk factors for UTIs in neonates are circumcision status in males, a maternal history of UTI, and anatomic anomalies.

The decreased risk for UTI in circumcised males is supported by two metaanalyses, conducted by Singh-Grewal et al. (2005) and Shaikh et al. (2008). In febrile neonates the incidence of UTI in uncircumcised males is 20%–21% compared with 2% in circumcised males and 5%–8% for females (Zorc et al., 2005; Shaikh et al., 2008). This phenomenon is supported by studies showing a higher concentration of uropathogenic bacteria in the periurethral region of uncircumcised males (Wiswell et al., 1988).

Laway et al. (2012) prospectively studied the effects of circumcision on the colonization of the periurethral area of 124 children. The authors found that the periurethral region was devoid of *E. coli*, klebsiella, proteus, pseudomonas, and enterococci after the children underwent circumcision, whereas these uropathogenic bacteria were present in 68% preoperatively. The American Academy of Pediatrics (AAP) currently does not support the recommendation of routine neonatal circumcisions, following its review of the current scientific literature (Sorokan et al., 2015). Despite this, the authors believe that circumcision or treatment of phimosis in high-risk children, such as those with high-grade VUR or those with posterior urethral valves, may be of benefit.

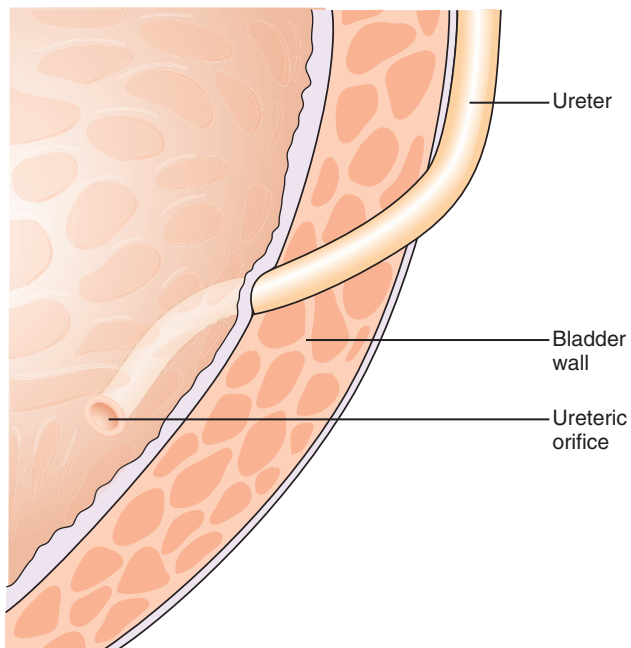
A maternal history of UTI is a risk factor for neonatal UTI. Milas et al. (2013) studied 1200 newborns for a 6-month period and showed that neonates with UTIs were more likely to have mothers that were diagnosed with a UTI during the pregnancy (22.2% vs 5.2%, $P < .001$). This was further supported by a cross-sectional study by Khalesi et al. (2014) who demonstrated a 5.9-fold increased risk for neonatal UTI in newborns where there was a history of maternal UTIs.

The overall reported incidence of genitourinary abnormalities in neonates is 5% (Bonadio and Maida, 2014). VUR is a common renal abnormality predisposing neonates to UTIs and pyelonephritis. Cleper et al. (2004) retrospectively studied 64 neonates with UTIs and found the incidence of VUR to be 20.3%, of which 31% represented high-grade VUR. However, other studies have reported rates between 20%–50% (White, 1989; Gerard et al., 1998). In male neonates with UTI, the most common anomaly was VUR (Goldman et al., 2000). In neonates with UTI the incidence of VUR increases to 30%–50% (Ginsburg and McCracken, 1982; Bonadio and Maida, 2014). Although other anatomic abnormalities, such as posterior urethral valves, congenital urethral strictures, ureteroceles, ureteropelvic junction obstruction, and neurologic bladder dysfunction, increase the risk of UTIs, these are less common than VUR. An obstructive pathology represents less than 1%–2% of the abnormalities found in neonates with UTIs (Hoberman and Wald, 1997). As such, the discussion of this chapter will focus on VUR.

VUR is defined as the retrograde flow of urine from the bladder to the upper urinary tract. Primary VUR is thought to result from the abnormal structure and function of the ureterovesical junction, whereas secondary VUR is acquired as a result of increased intravesical pressure. Normally the ureters enter the bladder obliquely, where a certain length of the ureter courses intramurally and submucosally. This creates a flap-valve mechanism where the distal ureter is compressed as the bladder fills with urine, thus normally preventing urine backflow. Fig. 92.1 demonstrates this relationship.

The lateral displacement of the ureteric orifice in the bladder in patients with VUR is thought to occur as a result of an abnormal ureteric bud origin along the mesonephric duct, where the extent of lateral displacement correlates with the degree of associated renal dysplasia (Mackie and Stephens, 1975). The International Reflux Study grading system is the most commonly accepted (Committee, 1981). Fig. 92.2 describes the appearance of each grade along with an example of each on voiding cystourethrogram (VCUG).

The bacterial etiology in neonates with VUR is distinct, with the presence of species such as *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Morganella morganii* (Zorc et al., 2005; Kanellopoulos et al., 2006). In addition, *Klebsiella pneumoniae* has a higher incidence in neonates with VUR presenting



• **Fig. 92.1** Normal Course of the Ureter in the Bladder. Notice how the ureter courses through the bladder wall (intramural portion) and under the mucosa (submucosal portion) to expel urine into the bladder through the ureteric orifice.

with a UTI compared with those without VUR (Cleper et al., 2004).

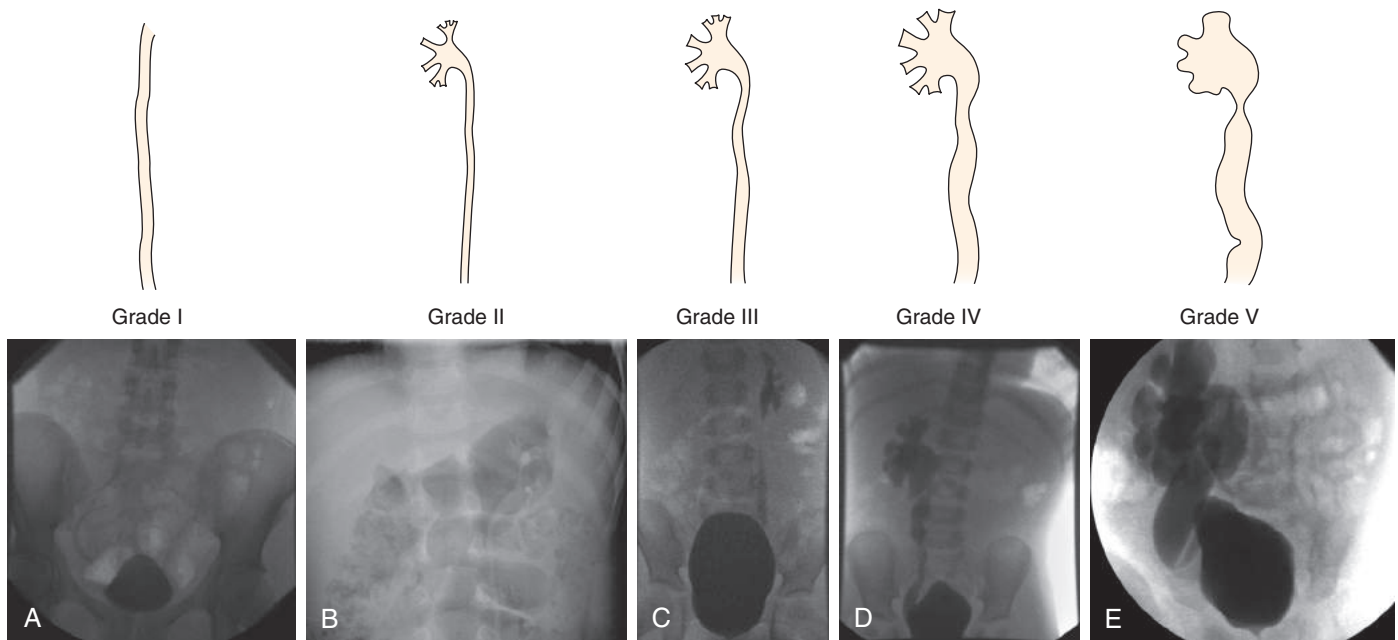
Diagnosis

The diagnosis of UTI in neonates differs from older children. An initial assessment should involve a detailed history including birth and family history and findings on prenatal imaging, if performed. The focused physical examination should include vital signs, general appearance, and abdominal and genital examination. In girls, special attention should be paid to the presence of labial adhesions or intravaginal foreign bodies (Piippo et al., 2000). In boys, special attention should be paid to the presence of phimosis or meatal stenosis, and testicular examination for signs of epididymo-orchitis should be conducted (Merlini et al., 1998).

In febrile newborns with a UTI, 20%–30% have associated bacteremia (Littlewood, 1972; Bachur and Caputo, 1995; Ismaili et al., 2011). As such, a comprehensive work-up for sepsis should be performed in a febrile neonate if there is suspicion of a UTI.

Urine Sample

The easiest method of obtaining a specimen from neonates is with a bag applied to the perineum. However, this carries a high contamination rate with a false-positive urine culture rate of 75%, and its only validity is to rule out a UTI in the event of a negative culture (Leong and Tan, 1976). As always, the culture result must be correlated to a urinalysis, looking for signs of inflammation.



• **Fig. 92.2** International Reflux Study Grading With Voiding Cystourethrogram Examples. (A) In grade I, urine refluxes only into the ureter without reaching the renal pelvis or calyces (notice that it only appears upon voiding in this voiding cystourethrogram example); (B) In grade II, urine refluxes into the ureter, renal pelvis, and calyces, without any dilatation of the collecting system (notice the sharp contours of the fornices); (C) In grade III, urine refluxes into a mildly dilated and tortuous ureter, the renal pelvis, and calyces, with mild blunting of the fornices (notice how the forniceal contours are less sharp); (D) In grade IV, urine refluxes into a moderately dilated and tortuous ureter, the renal pelvis, and calyces, with complete obliteration of forniceal angles but maintenance of papillary impressions; (E) In grade V, urine refluxes into a grossly dilated and tortuous ureter, the renal pelvis, and calyces, with blown fornices where papillary impressions are no longer visible.

A superior method in neonates is urethral catheterization. This yields a sensitivity of 95% and a specificity of 99% for urine culture (Pryles et al., 1959; Pollack et al., 1994). An even more accurate, although more invasive, method is suprapubic aspiration, with a contamination rate of 1% (Tosif et al., 2012). This may be a more appropriate option for boys with tight phimosis and girls with labial adhesions (Tobiansky and Evans, 1998). Topical anesthetics such as Eutectic Mixture of Local Anesthetic may be used to reduce pain associated with suprapubic aspiration in children (Dutta, 1999).

Dipstick urinalysis is an inexpensive and rapid screening tool and tests for urine nitrites and leukocyte esterase. Nitrites in the urine imply the presence of nitrate reductase, which is common among gram-negative uropathogens. Positive detection of leukocyte esterase, which is released by white blood cells (WBCs), is a surrogate for pyuria. In a retrospective study of 13,030 febrile newborns, the sensitivity and specificity of a dipstick urinalysis were 91.7% and 90.4%, respectively. This was comparable with microscopic urinalyses obtained from the same sample population (Glissmeyer et al., 2014).

Microscopic analysis completes the simple dipstick analysis by quantifying the WBCs, red blood cells, and presence of bacteria. Pyuria is the presence of 5 or more WBCs per high power field (HPF) in a centrifuged sample or greater than 10 WBC/HPF in a noncentrifuged sample if a counting chamber is used (Finnell et al., 2011), although these definitions may be less reliable in neonates (Crain and Gershel, 1990).

A urine culture is considered positive when there are 50,000 or more colony-forming units (cfu)/mL of a single bacterial strain on a catheterized or suprapubic specimen or 100,000 or more cfu/mL when a voided sample is cultured (Mori et al., 2010). In combination with a positive dipstick or microscopic urinalysis, a definition of 10,000 or more cfu/mL may be used (Hoberman and Wald, 1997).

Imaging

The optimal approach for imaging in a febrile neonate with a UTI is controversial. Although clear guidelines are set by the AAP for children aged 2 to 24 months, there are no recommendations for neonates (Roberts, 2012). Traditionally, routine imaging with ultrasonography, VCUGs, and renal scans (DMSA) were all recommended for neonates with a febrile UTI (Drew and Acton, 1976). However, recently there has been a shift toward more selective use for DMSA scans and VCUGs because of their ionizing radiation, invasiveness, and cost.

A renal bladder ultrasound in the acute phase is the first-line imaging tool for the investigation of a neonate with a UTI. It is safe, noninvasive, inexpensive, and readily available and should be routinely performed to rule out obstruction, perinephric abscess, and other congenital abnormalities. However, in male neonates with febrile UTIs, Goldman et al. (2000) found VUR in 30% of those with normal ultrasounds. The sensitivity and specificity of predicting all VUR based on an abnormal renal bladder ultrasound are 67% and 77%, respectively (Ismaili et al., 2011). In the same study the sensitivity and specificity increased to 100% and 78%, respectively, when predicting for dilating VUR.

The arguments for routine VCUG in neonates with a febrile UTI revolve around the higher incidence of VUR in this population, which requires aggressive and timely management (Goldman et al., 2000; Cleper et al., 2004). The discussion against the routine use of VCUG is based on evidence that renal scarring is more common with grades IV and V VUR (Ismaili et al., 2011), while the majority

of VUR detected on routine VCUG is grade I–III (Siomou et al., 2009).

It is the author's opinion that the decision to perform a VCUG should be selective, with a thorough discussion with the parents regarding the risks, benefits, and alternatives to the imaging modality. A VCUG should be performed in high-risk neonates such as those with UTIs caused by bacteria other than *E. coli*, those with renal bladder ultrasound abnormalities, and uncircumcised males. If a decision is made to perform a VCUG, it should be performed 2 to 4 weeks after the infection to ensure that the infection is appropriately treated before instrumentation (Cleper et al., 2004).

The debate surrounding the routine use of a DMSA scan is two-fold—first, whether it predicts VUR and second, if it should be performed at all in neonates. Siomou et al. (2009) prospectively followed 72 neonates after their first febrile UTI and found that an early DMSA scan (within the first 72 hours) had poor sensitivity and specificity of 29% and 82%, respectively, for predicting VUR grades III and higher (Siomou et al., 2009). However, the same study showed that an early DMSA scan had a sensitivity and specificity of 100% and 87%, respectively, for predicting permanent renal scarring.

The decision for a DMSA scan should be shared with the parents, taking into account the risk for renal scarring based on the ultrasound findings and grade of VUR (i.e., abnormal renal parenchyma or VUR grade \geq III). The findings of a DMSA scan may contribute to the management plan, providing evidence for or against more aggressive treatment.

Treatment of Urinary Tract Infection

For a febrile neonate a septic work-up should be performed, including a lumbar puncture, complete blood count, urinalysis, urine culture, and blood culture. UTIs in the newborn are considered complicated and are associated with higher incidences of bacteremia (16%–31%) (Ginsburg and McCracken, 1982; Lin et al., 2000). As such, empiric therapy with broad-spectrum antibiotics should be initiated without delay, after blood, urine, and cerebrospinal fluid (CSF) cultures are obtained, to prevent sequelae such as urosepsis and renal scarring.

Candiduria in the neonate indicates systemic disease, and its presence will require consultation with a pediatric infectious disease specialist for further work-up and management.

Local antibiotic resistance patterns and maternal antibiotics history should be used to determine choice of antimicrobial medication for empiric therapy (Allen et al., 1999; Glasgow et al., 2005). The antibiotic should then be changed to one with less broad activity, according to the bacterium's antibiotic sensitivities (Shaikh et al., 2007).

Parenteral ampicillin (50 mg/kg every 8 hours) and gentamicin (2 mg/kg every 12 hours) form the recommended empiric regime for neonates weighing 2 kg or more. In preterm neonates the dose of ampicillin is 150 mg/kg every 12 hours (Pickering et al., 2012). An alternative regime described by the European Association of Urology includes ampicillin with ceftazidime (30–50 mg/kg every 8–12 hours for neonates \geq 2 kg) (Stein et al., 2015).

In young neonates, parenteral therapy should be continued for 7–14 days followed by oral therapy for a total of 14–21 days (Stein et al., 2015). For older and more mature neonates with negative blood and CSF cultures, parenteral antibiotics should be continued for 3–4 days followed by oral therapy for a total of 7–14 days (Pickering et al., 2012).

TABLE 92.1 Randomized Controlled Trials Evaluating the Effect of Continuous Antibiotic Prophylaxis on Urinary Tract Infection Recurrence Rates and Renal Scar Formation

| Authors | Publication Date | PATIENTS INCLUDED | | Randomization | Rate of UTI Recurrence | Renal Scar Formation |
|---------------------------|------------------|-------------------|--------------------|----------------------------------|--|--------------------------|
| | | Age | VUR Status (Grade) | | | |
| Jodal et al. | 2006 | <11 years | III–IV | CAP vs ureteral reimplantation | CAP = reimplantation Febrile CAP > girls > boys | CAP = reimplantation |
| Garin et al. | 2006 | 3 months–18 years | 0–III | CAP vs surveillance | CAP = surveillance | CAP = surveillance |
| Roussey-Kesler et al. | 2007 | 1 month–3 years | I–III | CAP vs surveillance | CAP = surveillance (except boys with grade III reflux: CAP < surveillance) | |
| Montini et al. | 2008 | 2 months–7 years | 0–3 | CAP vs surveillance | CAP = surveillance Grade III = independent predictor febrile recurrence | CAP = surveillance |
| Pennesi et al. | 2009 | <30 months | II–IV | CAP vs surveillance | CAP = surveillance | CAP = surveillance |
| Craig et al. | 2009 | <18 years | 0–IV (60% 0–II) | CAP vs placebo | CAP < placebo ARR 6% NNT 14 | CAP = placebo |
| Brandstrom et al. | 2011 | 1–2 years | III–IV | CAP vs endoscopy vs surveillance | CAP < surveillance girls Endoscopic < surveillance girls Not boys | CAP < surveillance girls |
| RIVUR Trial Investigators | 2014 | <6 years, 8% boys | I–IV (92% I–III) | CAP vs placebo | CAP < placebo ARR 12% NNT 8 CAP > placebo resistance | CAP = placebo |

= signifies that these treatments were shown to be equivalent; > signifies that the first listed treatment yields more of the outcome than the second one; < signifies that the first listed treatment yields less of the outcome than the second one.

ARR, Absolute risk reduction; CAP, continuous antibiotic prophylaxis; NNT, number needed to treat; RIVUR, randomized intervention for children with vesicoureteral reflux; UTI, urinary tract infection; VUR, vesicoureteral reflux.

Antibiotic Resistance

In the United States the incidence of ampicillin- and gentamicin-resistant *E. coli* in the neonatal population reaches upwards of 75% and 17%, respectively (Hasvold et al., 2013; Shakir et al., 2014).

Treatment of Vesicoureteral Reflux

Surgical intervention is rarely required in the neonatal period, excluding obstructive abnormalities. If surgery is required, it is reserved for infants with high-grade VUR associated with recurrent breakthrough UTIs and renal scarring. A comprehensive discussion regarding the surgical management of VUR after the neonatal period is beyond the scope of this chapter.

Overall, the management of VUR diagnosed in the neonate after a UTI after the initial evaluation includes close follow-up with renal bladder ultrasounds and selective use of continuous antibiotic prophylaxis (CAP).

Continuous Antibiotic Prophylaxis

The basis for CAP stems from studies in the 1970s showing that the use of long-term antibiotics reduces the risk of repeat positive urine cultures, in comparison with no prophylaxis (Savage et al., 1975; Smellie et al., 1978). In the last 10 years a number of randomized controlled trials (RCTs) have been conducted to elucidate the

role of CAP in preventing UTI recurrences and renal scars. However, evidence for the efficacy of CAP in the neonatal population is lacking. The following discussion is based on study populations older than 1 month of age.

Table 92.1 summarizes these RCTs and their findings. Most fail to show a significant difference in UTI recurrence rate and renal scar formation between those treated with CAP, surveillance, or ureteral reimplantation (Garin et al., 2006; Jodal et al., 2006; Montini et al., 2008; Pennesi et al., 2008; Roussey-Kesler et al., 2008). A Cochrane metaanalysis in 2011 also showed no significant difference in the rate of UTI recurrence between those receiving or not receiving CAP (Nagler et al., 2011). This was further supported by a metaanalysis performed by the AAP using data from six randomized RCTs conducted between 2006 and 2010 encompassing 1091 children with VUR. A two-tailed χ^2 test comparing CAP versus no prophylaxis (surveillance or no treatment) for VUR grades I, II, III, and IV showed no statistically significant benefit from CAP ($P = 1.00, .95, .29$, and $.14$, respectively) (Roberts, 2012).

In 2014 the findings of the Randomized Intervention for Children with Vesicoureteral Reflux (RIVUR) trial were published. RIVUR is the largest randomized, placebo-controlled, double-blind, multicenter study to date investigating the efficacy of CAP compared with placebo in young children with VUR (Carpenter et al., 2013; RIVUR Trial Investigators et al., 2014). This trial, sponsored by

TABLE 92.2 Risk of Breakthrough Urinary Tract Infection Stratification Groups

| Risk Category | Defining Risk Factors | % Found in Population | % Two-Year Risk of Breakthrough UTI | Treatment Suggested |
|-------------------|---|-----------------------|-------------------------------------|-----------------------------------|
| Low risk | <ul style="list-style-type: none"> VUR grade I–III in female without BBD Circumcised male | 67.0 | 8.6 | Surveillance |
| Intermediate risk | <ul style="list-style-type: none"> VUR grade I–III female with BBD Uncircumcised male VUR grade IV–V female presented as prenatal hydronephrosis | 27.0 | 27.0 | CAP |
| High risk | <ul style="list-style-type: none"> VUR grade IV–V female presented as UTI | 6.0 | 62.0 | Endoscopic or surgical correction |

BBD, Bladder bowel dysfunction; CAP, continuous antibiotic prophylaxis; UTI, urinary tract infection; VUR, vesicoureteral reflux.

Data from Hidas G, Billimek J, Nam A, et al. Predicting the risk of breakthrough urinary tract infections: primary vesicoureteral reflux. *J Urol*. 2015;194:1396–1401.

the National Institute of Diabetes and Digestive and Kidney Diseases, showed a significantly lower rate of UTI recurrence in children receiving CAP compared with placebo (12.8% vs 25.4%, $P < 0.001$). Furthermore, CAP reduced the risk of UTI recurrence by 50% (hazard ratio 0.50, 95% confidence interval 0.34–0.74) (RIVUR Trial Investigators et al., 2014).

Nevertheless, the results and implications of the RIVUR trial have been widely debated. Critique of the trial includes the homogeneity of the study population. Females and those with VUR grades I–III comprised 91.9% and 91.7% of the study population, respectively (Mattoo et al., 2015). The AAP practice guidelines were recently reaffirmed by the AAP, which suggests that the results of the RIVUR trial may not be clinically significant (Subcommittee on Urinary Tract Infection et al., 2011). The authors acknowledged that a benefit from CAP was demonstrated; however, the number needed to treat (NNT) of 8 translates to a total of 5840 doses of antibiotic to prevent one UTI recurrence.

A lack of evidence for a role for CAP in the neonatal population, coupled with the contentious nature of the current debate over the efficacy of CAP in older children, means that the authors do not have specific recommendations regarding the initiation of CAP in neonates with low-grade VUR.

The decision to start CAP should be made on an individual and selective basis, based on risk for UTI recurrence, in consultation with parents. The use of a clinical calculator (iReflux) developed by Hidas et al. (2015) for determining a child's individualized risk for UTI recurrence may help in this decision. However, it is the author's opinion that in children with high-grade VUR (grades III–V), especially in uncircumcised males, CAP should be initiated (amoxicillin or cephalexin 25 mg/kg daily) (Table 92.2).

Conclusion

In summary, UTIs in neonates can be a serious cause of morbidity, and prompt diagnosis and treatment should be initiated. The presentation, route of infection, work-up, treatment, and spectrum of infecting bacteria are different in neonates compared with older infants and children. The days of a singular approach to treating neonates and older children with UTI and VUR have long disappeared. A new, tailored approach that closely evaluates the known and multiple risk factors that increase a child's risk of recurrence and weighs the effects of concomitant anatomic variations, is necessary to treat each child uniquely and optimally.

Suggested Readings

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Neonatal Hypertension

JOSEPH T. FLYNN

KEY POINTS

- There are numerous influences on normal blood pressure in neonates, including gestational age, birthweight, and maternal factors such as preeclampsia. This makes it difficult to define normal and abnormal blood pressure (BP).
- As in older children, identification of hypertension (HTN) in the neonate is dependent on correct BP measurement technique.
- While the differential diagnosis of systemic HTN in the neonate is broad, common causes include catheter-related thromboembolic phenomena, chronic lung disease, kidney disease, and iatrogenic causes. A focused diagnostic evaluation should lead to correct identification of the underlying cause in most neonates.
- Therapy for HTN in the neonate is largely empiric because of a lack of data on outcomes of elevated BP in neonates and exclusion of neonates from antihypertensive drug trials.

As in other areas of neonatology, advances in the ability to identify, evaluate, and care for premature infants have led to an increased awareness of systemic hypertension in neonates, especially in the neonatal intensive care unit (NICU). However, there is also uncertainty regarding normative blood pressure (BP) in neonates and the best therapeutic approach, mostly because of a lack of rigorous evidence. This chapter will review the many factors that influence BP in the neonate, present what we know about normal neonatal BP, and then move on to a discussion of differential diagnosis of hypertension (HTN), the optimal diagnostic evaluation, and antihypertensive therapy.

Factors That Influence Neonatal Blood Pressure

Many studies have examined the factors that influence BP patterns in normal and premature infants (Versmold et al., 1981; Tan, 1988; Hegyi et al., 1994). Zubrow et al. (1995) examined BPs obtained in more than 600 infants of various birthweights (BW) and gestational ages (GA)s admitted to 14 Philadelphia-area NICUs. They found that BP at birth is closely correlated with GA (Fig. 93.1) and BW (Fig. 93.2). After delivery there is a predictable increase in BP in the first 5 days of life that is independent of these factors. Thereafter, BP continues to rise gradually, with the most important determining factor in the study of Zubrow et al. being postmenstrual (postconceptual) age (Fig. 93.3). A more recent

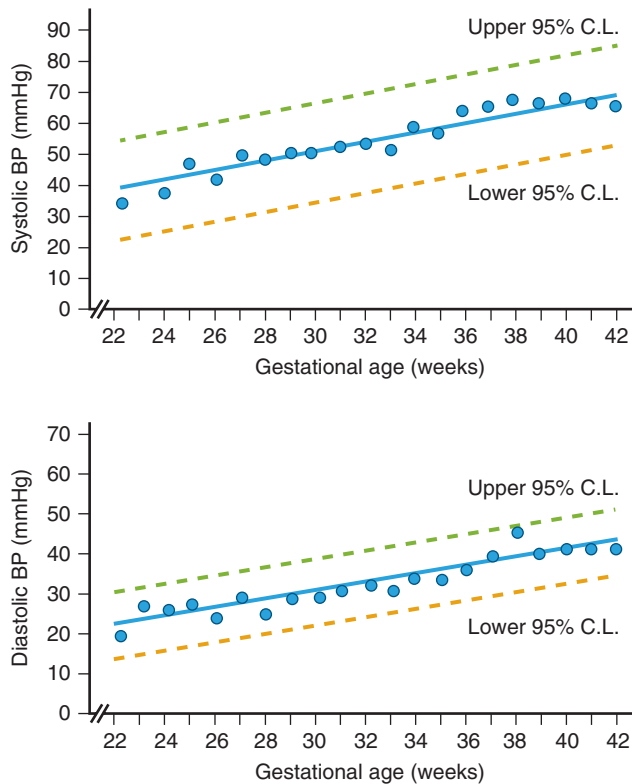
study of stable NICU infants showed a similar pattern, with BPs in each GA category of premature infants increasing at a faster rate during the first week of life, with subsequent slowing (Pejovic et al., 2007); the rate of rise was more rapid in preterm infants than in term infants. Dionne et al. (2012) have summarized available BP data on preterm neonates and have published a table of BPs that is helpful in categorizing an infant's BP as normal or elevated (Table 93.1).

Also important are maternal factors that may influence a neonate's BP. There has been much controversy in the literature regarding the effect of prenatal steroid therapy on infant BP. A randomized controlled trial of corticosteroids versus placebo (Mildenhall et al., 2009) showed no difference in infant BPs between groups. There is some suggestion in the literature that chorioamnionitis and HELLP (hemolysis, elevated liver enzyme levels, low platelet count) syndrome may be associated with lower infant BPs. Higher BPs have been correlated with maternal body mass index greater than 30 kg/m² and low socioeconomic status in a study of Nigerian infants (Sadoh et al., 2010) and in an Australian study of premature infants born to mothers with diabetes or neonates with abnormal uteroplacental perfusion as evidenced by placental pathology (Kent et al., 2009). While these factors may not have caused hypotension or HTN per se, it is clear that many prenatal and postnatal processes combine to influence BP in the newborn period.

Definition of Hypertension

While the definition of HTN in an older infant or child is clear (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004), it is more difficult to define HTN in newborns and preterm infants given the changes in BP that normally occur in the first few weeks of life as discussed earlier. After 1 month of age, HTN is defined as systolic and/or diastolic BP equal to or greater than the 95th percentile for that infant's age and sex. Normative values for infants aged 12 months or younger can be found in the curves published in the 1987 Second Task Force report (National Heart Lung and Blood Institute Task Force on Blood Pressure Control in Children, 1987) (Fig. 93.4).

Whether a similar definition can be used for premature neonates is less clear. Dionne et al. (2012) have compiled available data on neonatal BP, and their summary table of BPs (Table 93.1) includes values for the 95th and 99th percentiles for infants of up to 44 weeks' postmenstrual age. They propose that similar criteria for defining HTN in premature neonates as those used in older children can be followed, using the values in Table 93.1 as a guide. It may



• **Fig. 93.1** Correlation between gestational age and neonatal blood pressure (BP). C.L., Confidence limit. (From Zubrow AB, Hulman S, Kushner H, Falkner B. Determinants of blood pressure in infants admitted to neonatal intensive care units: a prospective multicenter study. *J Perinatol.* 1995;15:470–479.)

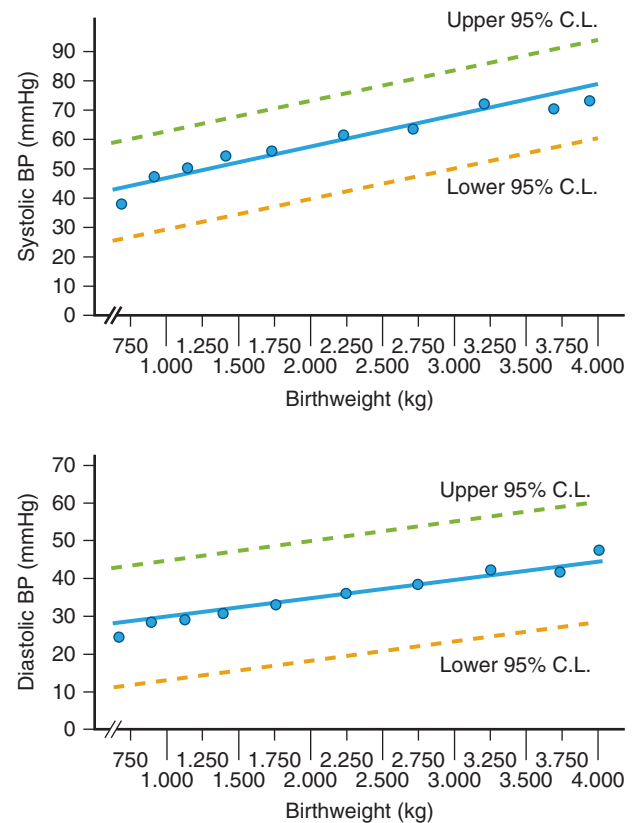
not be possible to be certain whether this approach is a valid one given the complete lack of any outcome data for hypertensive neonates.

As with older children, the diagnosis of HTN should not be made on the basis of a single reading. If the infant is critically ill and continuous BP monitoring reveals sustained BP elevation over several hours, then HTN should be diagnosed, and appropriate investigation and intervention should be initiated. For less critically ill infants still in the NICU, a pattern of elevated readings over 1 to 2 days should be sufficient to make the diagnosis of HTN. For older infants/NICU graduates who are being followed up as outpatients, at least three elevated readings should be documented over 1 to 2 weeks before a diagnosis of HTN is made (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004).

Incidence of Hypertension

Although one recent series found that 28% of very low birth weight (VLBW) infants had at least one elevated BP reading documented during their NICU stay, the actual incidence of HTN in neonates is very low, ranging from 0.2% in healthy newborns to between 0.7% and 2.5% in high-risk newborns (Dionne et al., 2012). The most recently published study demonstrated a 1.3% prevalence of HTN requiring treatment among infants admitted to a teaching hospital NICU (Sahu et al., 2013).

Certain categories of infants are at significantly higher risk, however. For example, HTN is relatively common in patients with a history of umbilical artery (UA) catheterization (3%) and those



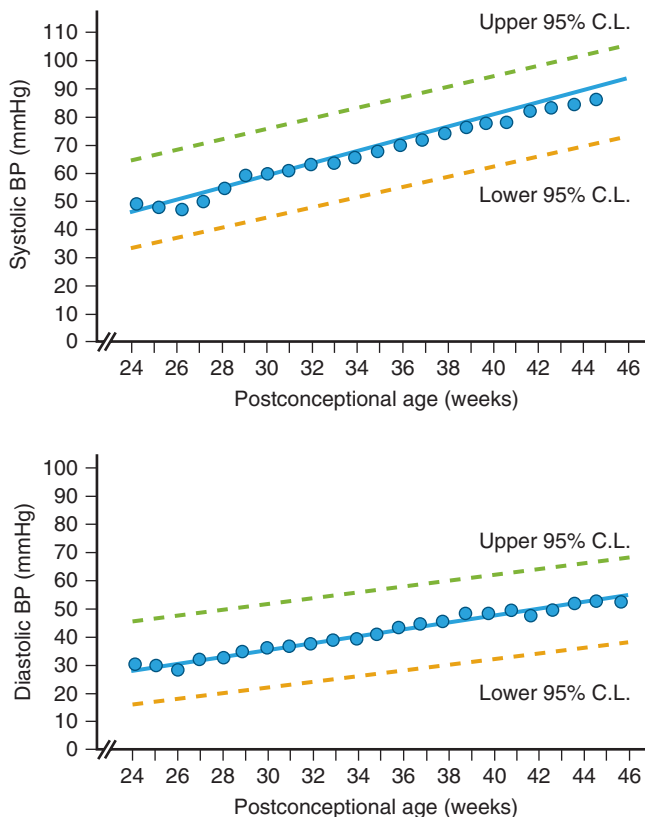
• **Fig. 93.2** Correlation between birthweight and neonatal blood pressure (BP). C.L., Confidence limit. (From Zubrow AB, Hulman S, Kushner H, Falkner B. Determinants of blood pressure in infants admitted to neonatal intensive care units: a prospective multicenter study. *J Perinatol.* 1995; 15:470–479.)

with bronchopulmonary dysplasia (BPD) (as high as 43%). In one series it was also associated with patent ductus arteriosus and intraventricular hemorrhage. On the other hand, HTN is so uncommon in otherwise healthy term infants that routine BP determination is not even recommended (American Academy of Pediatrics Committee on Fetus and Newborn, 1993).

Fewer data are available on the incidence of sustained HTN in NICU graduates. In their classic study, Sheftel et al. (1983) performed BP measurement in infants followed up in a neonatal follow-up clinic and found that 8.9% were hypertensive according to criteria used at that time. A later report by some of the same authors demonstrated an incidence of 2.6% (Friedman and Hustead, 1987). Secondary causes such as those discussed later were found in most cases. Recently, an incidence of elevated BP of 2.9% was seen in a cohort of infants from the neonatology follow-up clinic at the University of Iowa Children's Hospital (Dagle et al., 2011); elevated BP was associated with the cytochrome P450 genotype (*CYP2D6*) in that cohort. Given these data, it is recommended that BP screening be incorporated into the long-term follow-up of NICU graduates.

Etiology and Pathophysiology

While the differential diagnosis of HTN in the neonate or older infant is extensive (Box 93.1), the most important categories of causes of neonatal HTN include renovascular HTN, congenital and acquired renal disease, and BPD.



• **Fig. 93.3** Correlation between postmenstrual (postconceptual) age and neonatal blood pressure (BP). C.L., Confidence limit. (From Zubrow AB, Hulman S, Kushner H, Falkner B. Determinants of blood pressure in infants admitted to neonatal intensive care units: a prospective multicenter study. *J Perinatol.* 1995;15:470–479.)

Renovascular Causes

The most common cause of neonatal renovascular HTN is aortic or renal thromboembolism related to UA catheterization (Bauer et al., 1975). HTN may develop either while the catheter is in place or long after its removal and may be associated with a history of renal insufficiency or gross hematuria. Associated signs may include acute kidney injury (AKI) in patients with bilateral involvement, hematuria, and loss of femoral pulses and blood flow to the lower extremities in patients with extensive aortic thrombosis.

Studies using ultrasound report an incidence of UA-related thromboembolism ranging from 14% to 35%, whereas studies using angiography document incidences up to 64%. Autopsy studies have shown an incidence of UA-related thromboembolism between 9% and 28%, although major clinical symptoms of UA-related thromboembolism occur in 1%–3% of infants (Andrew et al., 2001). Trauma at the time of insertion of an UA catheter caused by endothelial injury is postulated to be the cause of aortic thrombus formation, with clot embolization to the kidneys, causing localized areas of infarction and increased renin release.

Although several studies that have examined the duration of line placement and line position (“low” vs “high”) as factors involved in thrombus formation, the data have not been conclusive. The Cochrane Neonatal Group has attempted to resolve this controversy (Barrington, 2000). It analyzed 11 randomized clinical trials and one study using alternate assignments to compare the incidence of morbidity and mortality for high versus low catheter tip placement. The placement of a catheter tip was defined as high when

• BOX 93.1 Differential Diagnosis of Hypertension in the Neonate

Renovascular

Thromboembolism
Renal artery stenosis
Midaortic coarctation
Renal venous thrombosis
Compression of renal artery
Idiopathic arterial calcification
Congenital rubella syndrome

Renal Parenchymal Disease

Congenital/chronic
Polycystic kidney disease
Multicystic dysplastic kidney
Tuberous sclerosis
Ureteropelvic junction obstruction
Renal hypodysplasia
Congenital nephrotic syndrome
Renal tubular dysgenesis
Acquired/acute
Acute tubular necrosis
Cortical necrosis
Interstitial nephritis
Hemolytic–uremic syndrome
Obstruction (stones, tumors)

Pulmonary

Bronchopulmonary dysplasia
Pneumothorax

Cardiac

Thoracic aortic coarctation

Endocrine

Congenital adrenal hyperplasia
Hyperaldosteronism
Hyperthyroidism
Pseudohypoaldosteronism type II

Medication Related

Infant
Dexamethasone
Adrenergic agents
Vitamin D intoxication
Theophylline
Caffeine
Pancuronium
Phenylephrine
Maternal
Cocaine
Heroin

Neoplasia

Wilms tumor
Mesoblastic nephroma
Neuroblastoma
Pheochromocytoma

Neurologic

Pain
Intracranial hypertension
Seizures
Familial dysautonomia
Subdural hematoma

Miscellaneous

Total parenteral nutrition
Closure of abdominal wall defect
Adrenal hemorrhage
Hypercalcemia
Traction
Extracorporeal membrane oxygenation
Birth asphyxia
Fluid overload

it was located in the descending aorta above the diaphragm and low when it was placed in the descending aorta above the bifurcation but below the renal arteries. The reviewers concluded that high catheter position causes fewer clinically obvious ischemic complications. With regard to HTN, however, it was concluded that it seems to appear with equal frequency among infants with high or low catheter placement (Barrington, 2000).

Congenital vascular anomalies responsible for neonatal renovascular HTN include stenosis or hypoplasia of the renal artery and segmental intimal hyperplasia. These conditions may involve the aorta and the renal arteries. Unilateral renal artery stenosis may cause a reversible syndrome characterized by hypokalemic alkalosis, hyponatremia, and increased echogenicity of the contralateral kidney (Castello Girona et al., 1996).

HTN may rarely result from two types of infiltration of the arterial wall. Idiopathic arterial calcification of infancy is characterized by calcium deposits in all layers of the arteries, including the aorta and the coronary arteries, and in the heart valves (Chong and Hutchins, 2008). Some of these deposits may be visible on a plain radiograph. Most cases have been diagnosed at autopsy. HTN typically fails to respond to standard antihypertensive medication

TABLE 93.1**Blood Pressure (mmHg) Percentiles for Neonates up to 44 Weeks' Postconceptual Age**

| Postconceptual Age | 50th Percentile | 95th Percentile | 99th Percentile | Postconceptual Age | 50th Percentile | 95th Percentile | 99th Percentile |
|--------------------|-----------------|-----------------|-----------------|--------------------|-----------------|-----------------|-----------------|
| 44 Weeks | | | | 34 Weeks | | | |
| SBP | 88 | 105 | 110 | SBP | 70 | 85 | 90 |
| DBP | 50 | 68 | 73 | DBP | 40 | 55 | 60 |
| MAP | 63 | 80 | 85 | MAP | 50 | 65 | 70 |
| 42 Weeks | | | | 32 Weeks | | | |
| SBP | 85 | 98 | 102 | SBP | 68 | 83 | 88 |
| DBP | 50 | 65 | 70 | DBP | 40 | 55 | 60 |
| MAP | 62 | 76 | 81 | MAP | 49 | 64 | 69 |
| 40 Weeks | | | | 30 Weeks | | | |
| SBP | 80 | 95 | 100 | SBP | 65 | 80 | 85 |
| DBP | 50 | 65 | 70 | DBP | 40 | 55 | 60 |
| MAP | 60 | 75 | 80 | MAP | 48 | 63 | 68 |
| 38 Weeks | | | | 28 Weeks | | | |
| SBP | 77 | 92 | 97 | SBP | 60 | 75 | 80 |
| DBP | 50 | 65 | 70 | DBP | 38 | 50 | 54 |
| MAP | 59 | 74 | 79 | MAP | 45 | 58 | 63 |
| 36 Weeks | | | | 26 Weeks | | | |
| SBP | 72 | 87 | 92 | SBP | 55 | 72 | 77 |
| DBP | 50 | 65 | 70 | DBP | 30 | 50 | 56 |
| MAP | 57 | 72 | 77 | MAP | 38 | 57 | 63 |

This table provides estimated values for blood pressure percentiles after 2 weeks of age in infants from 26 to 44 weeks' postconceptual age. The 95th and 99th percentile values are intended to serve as a reference to identify infants with persistently elevated blood pressure.

DBP, Diastolic blood pressure; MAP, mean arterial pressure; SBP, systolic blood pressure.

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or even to nephrectomy; biphosphonate, calcium antagonists, or prostaglandin infusion may be successful (Ciana et al., 1997), although reported mortality rates are high. Peritoneal dialysis has also been used for management of HTN in at least one recent report (Stojanovic et al., 2013). Galactosialidosis may result in intimal infiltration by sialyloligosaccharides and lead to hyperreninemic HTN (Nordborg et al., 1997).

Other causes of renovascular HTN include neonatal renal arterial embolism in the absence of UA catheterization, intramural hematoma of the renal artery, renal venous thrombosis, and external compression of the renal artery by a hydronephrotic kidney, adrenal hemorrhage, and urinoma. Finally, a neonate with HTN as a result of an aneurysm of the abdominal aorta has been reported (Kim et al., 2001); this fortunately rare condition may present with intractable congestive heart failure.

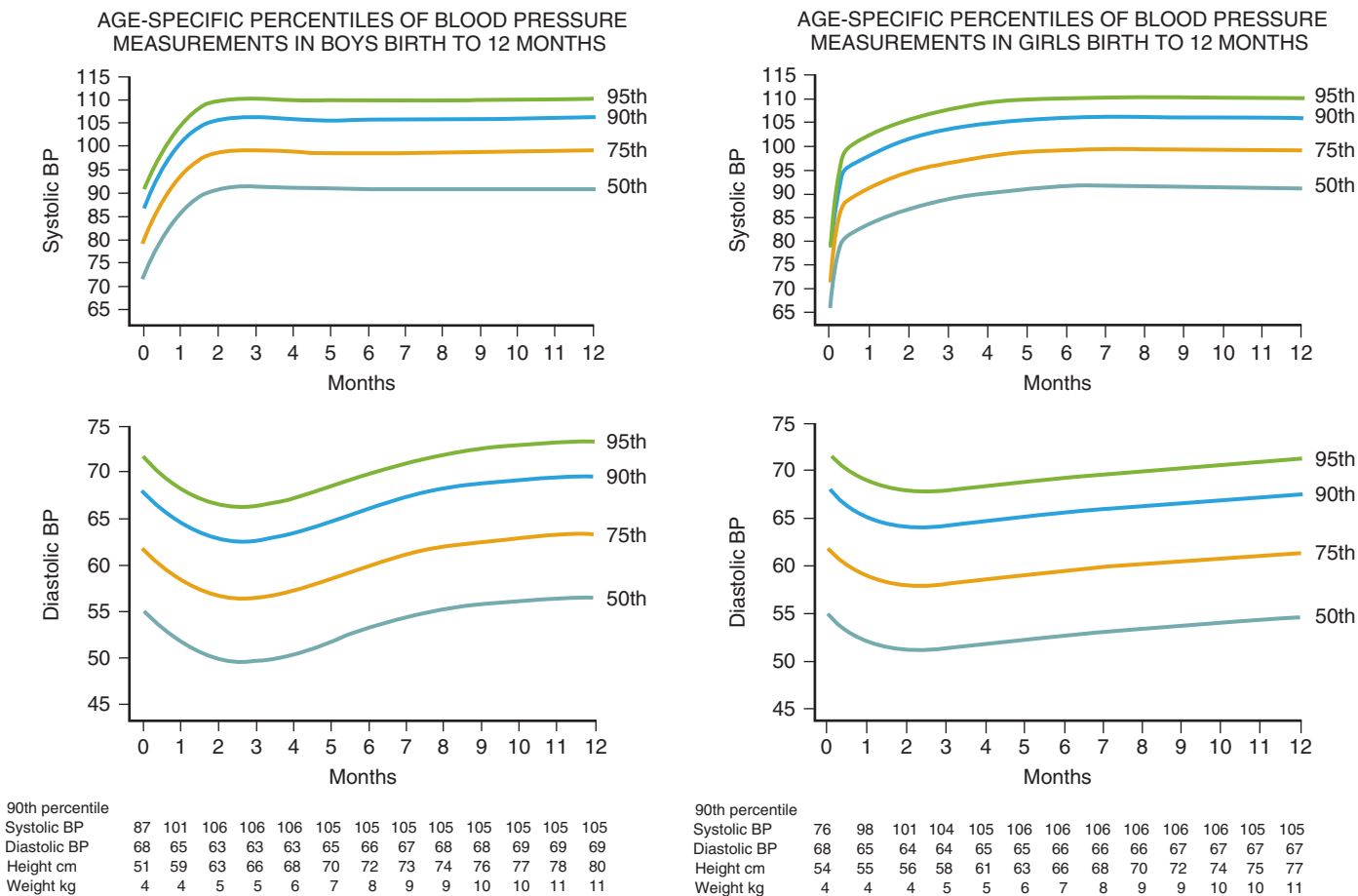
Bronchopulmonary Dysplasia

Many infants with BPD develop HTN. This phenomenon was first described in the mid-1980s by Abman et al. (1984). In a study of 65 infants discharged from a NICU, the instance of HTN in infants with BPD was 43%, versus 4.5% in infants without

BPD. The investigators were unable to identify a clear cause of HTN but postulated that hypoxemia might be involved. More than half of the infants with BPD who developed HTN did not display it until after discharge from the NICU, further highlighting the need for measurement of BP in NICU "graduates," whether or not they have lung disease.

The findings of Abman et al. have been reproduced by other investigators, including Alagappan and Malloy (1998), who found that HTN was twice as common in VLBW infants with BPD compared with all VLBW infants. Because all of the hypertensive infants required supplemental oxygen and aminophylline, development of HTN appeared to be correlated with the severity of pulmonary disease. Anderson et al. (1993) have demonstrated that the more severe the BPD (defined as a greater need for diuretics and bronchodilators), the higher the likelihood of the development of increased BP. BPD was also noted as a common diagnosis in a recently published case series (Sahu et al., 2013), and infants from the Iowa cohort mentioned previously (Dagle et al., 2011) were more likely to have elevated BP if they required ongoing oxygen therapy after NICU discharge.

These observations reinforce the impression that infants with severe BPD are clearly at increased risk and need close monitoring



• **Fig. 93.4** Normative curves for blood pressure (BP) in infants aged 1 to 2 months. (Data from National Heart, Lung, and Blood Institute Task Force on Blood Pressure Control in Children. Report of the Second Task Force on blood pressure control in children—1987. *Pediatrics*. 1987;79:1–25.)

for the development of HTN. This is especially true in infants who require ongoing treatment with theophylline preparations and/or corticosteroids; as many as 30% of infants receiving dexamethasone for BPD manifest HTN (Ferrara et al., 1990).

Congenital and Acquired Renal Disease

HTN is a common complication of congenital renal anomalies and diseases such as Ask-Upmark kidney, renal hypodysplasia, and obstructive lesions causing hydronephrosis. It is well known that both autosomal dominant polycystic kidney disease and autosomal recessive polycystic kidney disease (ARPKD) may present in the newborn period with severe nephromegaly and HTN (Zerres et al., 1996; Guay-Woodford et al., 2003; Verghese and Miyashita, 2014). With ARPKD the median age of onset of HTN has been reported to be 16 days; most affected infants will be discovered to be hypertensive during the first year of life (Zerres et al., 1996). The most severely affected infants with ARPKD are at risk of development of congestive heart failure as a result of severe, malignant HTN. Bilateral nephrectomy may be lifesaving in such infants.

Although much less common than in polycystic kidney disease, HTN has also been reported in infants with multicystic dysplastic kidneys (Susskind et al., 1989). This is somewhat paradoxical,

as such kidneys are usually thought to be nonfunctioning. The case has been made that HTN in such patients is the result of another coexisting abnormality such as parenchymal scarring (Husmann, 1998). Another possible explanation is increased renin production by macrophages within the dysplastic kidney (Liapis et al., 2002).

Renal obstruction may be accompanied by HTN, even when there is no compression of the renal artery. This has been seen, for example, in infants with congenital ureteropelvic junction obstruction and sometimes may persist following surgical correction of the obstruction. HTN has also been described in infants with congenital primary megaureter. Ureteral obstruction by other intra-abdominal masses may also be accompanied by HTN. The mechanism of HTN in such instances is unclear, although activation of the renin–angiotensin–aldosterone system (RAAS) may be involved (Cadnapaphornchai et al., 1978).

HTN as a result of acquired renal disease occurs less commonly in the NICU than that as a result of congenital renal abnormalities. However, AKI from nephrotoxic or hypoxic acute tubular necrosis, interstitial nephritis, or cortical necrosis may be accompanied by significant HTN, usually as a result of fluid and sodium overload or activation of RAAS. Atypical hemolytic–uremic syndrome, which has been described in both term and preterm infants (Wilson and

TABLE 93.2 Major Features of Monogenic Forms of Hypertension

| | Inheritance Pattern | Age | Potassium Level | Renin Level | Aldosterone Level | Genetic Defect | Therapy |
|---|---------------------|---------|---------------------|-------------|---------------------|--|---|
| Apparent mineralocorticoid excess | AR | I, C, A | Decreased or normal | Decreased | Decreased | Loss of function in 11- β -HSD2 gene | Spironolactone, eplerenone |
| Glucocorticoid remediable aldosteronism | AD | I, C | Decreased or normal | Decreased | Decreased or normal | Chimeric gene; fusion of aldosterone synthase and 11- β -hydroxylase genes | Amiloride, triamterene, glucocorticoids |
| Congenital adrenal hyperplasia | AR | I | Decreased or normal | Decreased | Decreased | Loss of function in 11- β -hydroxylase or 17 α -hydroxylase gene | Spironolactone, eplerenone |
| Liddle syndrome | AD | C, A | Decreased or normal | Decreased | Decreased | Gain of function in ENaC gene | Amiloride, triamterene |
| Gordon syndrome | AD | A, C | Increased or normal | Decreased | Increased or normal | Gain of function in WNK1 gene; loss of function in WNK4 gene | Thiazide |

A, Adulthood; AD, autosomal dominant; AR, autosomal recessive; C, childhood; ENaC, epithelial sodium channel; 11- β -HSD2, 11- β -hydroxysteroid dehydrogenase2; I, infancy; WNK, WNK lysine-deficient protein kinase.

Flynn, 1998), is usually also accompanied by HTN. Such HTN may be extremely difficult to control, requiring treatment with multiple agents.

Genetic Causes

Genetic forms of HTN that may present in the neonatal period fall into two broad categories: namely, HTN resulting from a single-gene disorder and HTN occurring as one feature of a malformation syndrome. Single-gene disorders causing HTN with reported cases in infancy include Liddle syndrome, glucocorticoid-remediable aldosteronism, and Gordon syndrome (pseudohypoaldosteronism type II). A summary of the major features of these disorders is presented in Table 93.2; for a detailed discussion of monogenic HTN, the reader should consult other references (Simonetti et al., 2012; Toka et al., 2013).

Malformation syndromes that may cause HTN include Williams syndrome (renal artery stenosis), Turner syndrome (aortic coarctation), neurofibromatosis (renal artery stenosis, coarctation), and Cockayne syndrome. Usually the HTN in these syndromes presents beyond the neonatal period, but infantile presentations with HTN have been described.

Miscellaneous Causes

Coarctation of the thoracic aorta (see Chapter 55) has been reported in numerous case series of neonatal HTN. Although usually detected in the newborn period on the basis of decreased pulses and lower BPs in the lower extremities compared with the upper extremities, the similarity of upper and lower extremity BP readings in early infancy means that echocardiography is needed for definitive diagnosis (Crossland et al., 2004). HTN may persist or recur in these patients even after surgical repair of the coarctation (O'Sullivan et al., 2002). Repair early in infancy seems to lead to an improved long-term outcome compared with delayed repair.

Endocrine disorders, particularly congenital adrenal hyperplasia, hyperaldosteronism, and hyperthyroidism, constitute easily recognizable clinical entities that have been reported to cause HTN in

neonates (White, 1996). Several adrenal disturbances can induce HTN directly; they should be differentiated from Liddle syndrome. Hyperthyroidism is associated with systolic HTN and sustained tachycardia and, sometimes, with episodes of supraventricular tachycardia (Schonwetter et al., 1983).

Tumors, including neuroblastoma, Wilms tumor, and mesoblastic nephroma, may present in the neonatal period and may produce HTN, either because of compression of the renal vessels or ureters or because of production of vasoactive substances such as renin or catecholamines.

Neurologic causes of HTN include intracranial HTN, drug withdrawal, seizures, pain, and familial dysautonomia. Seizures are common complications of severe HTN; in turn, BP may increase transiently during seizure episodes (Perlman and Volpe, 1983). Appropriate pain relief should be given before and after surgical procedures.

Iatrogenic causes of neonatal HTN are common and usually obvious but are important to consider. If the infant is hypervolemic secondary to excessive administration of sodium or fluids, intake should be restricted, and a diuretic should be administered. It is imperative to eliminate hidden sources of sodium, such as isotonic saline used to flush an arterial line and sodium-containing medications (e.g., antibiotics). If HTN is induced by a medication, one may consider withholding it, decreasing the dose, or using an infusion instead of repeated injections. As noted earlier, dexamethasone may cause BP elevation relatively commonly (Ferrara et al., 1990; Smets and Vanhaesebrouck, 1996); if this occurs, a decision must be made regarding the possible benefits of continued steroid treatment versus the risks of HTN. HTN induced by pancuronium is probably related to catecholamine release; BP may normalize after substitution of vecuronium for pancuronium.

HTN develops in 11%–92% of neonates receiving extracorporeal membrane oxygenation (Boedy et al., 1990; Blowey et al., 2011) and may result in serious complications, including intracranial hemorrhage and increased mortality. Despite extensive investigation, the exact pathogenesis of this form of HTN remains poorly understood. Fluid overload, altered renal sodium and water handling,

and altered baroreceptor function have all been proposed as causative factors. Nicardipine infusions are commonly used to treat this form of HTN.

HTN may develop after surgery. Of four patients who developed HTN after surgical repair of an abdominal wall defect, three had edema of the lower extremities and normal plasma renin activity (PRA), and one had evidence of ureteropelvic junction obstruction and high PRA (Adelman and Sherman, 1980). The duration of HTN in these patients ranged from 12 days to 6 months. A more recent case series demonstrated that HTN is more frequent and more persistent in patients with omphalocele than in patients with gastroschisis (Cachat et al., 2006). HTN appearing after primary closure for bladder exstrophy may be related to traction for skeletal immobilization (Husmann et al., 1993).

Diagnostic Evaluation

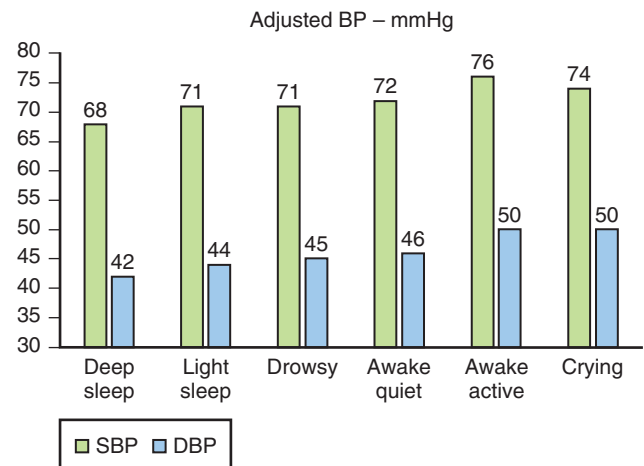
The first step in the evaluation is to make sure the BP is being measured correctly; this is discussed in detail in the next section. After that it is important to determine whether the infant is persistently hypertensive or if the BP rises only during periods of agitation, pain, crying, feeding, or performance of procedures. Only in infants with correctly measured BP and persistent BP elevation should the “diagnosis” of HTN be made and diagnostic work-up be initiated.

Blood Pressure Measurement

Some neonates have their BP measured directly through an indwelling UA catheter; this technique provides the most accurate method of measuring BP, except for minor artifacts from air bubbles or blood clots in the tubing (Rothe and Kim, 1980). The most commonly used indirect method of BP measurement is the oscillometric technique, which directly measures mean arterial pressure (MAP) on the basis of the oscillations of the arterial wall as the cuff is deflated; systolic and diastolic BP are then back-calculated from the MAP with use of manufacturer-specific algorithms. Although the readings obtained by oscillometric devices may differ between 1 and 5 mmHg compared with directly measured BP, these devices are usually sufficiently accurate for routine clinical use (Low et al., 1995). Of note, shock may lead to inaccurate oscillometric measurements (Kimble et al., 1981). Despite these issues, oscillometric devices are clearly useful for measuring BP in infants without indwelling arterial catheters (especially those who require frequently repeated BP measurement) and in infants who have been discharged from the nursery.

Selection of a proper sized cuff is crucial for correct indirect BP measurement. The cuff bladder length should encircle 80%–100% of the arm circumference; a cuff bladder with a width-to-length ratio of 0.45–0.55 is recommended (Kimble et al., 1981; Sonesson and Broberger, 1987). A full range of “neonatal” cuff sizes will need to be available for outpatient follow-up of NICU graduates. If BP is measured in the calf, it is important to use a wide enough cuff; cuffs designed for use in the upper arm may be too narrow, resulting in a falsely high BP reading. Calf BPs are generally the same as upper arm BPs in neonates, and this similarity has been shown to persist for as long as the first 6 months of life. This has implications for the diagnosis of coarctation of the aorta in neonates (Park and Lee, 1989; Crapanzano et al., 1996; Crossland et al., 2004).

BP increases when an infant is awake, in the knee–chest position, with crying, pain, and during physical examination and procedures,



• **Fig. 93.5** Effect of infant state on blood pressure (BP). DBP, Diastolic blood pressure; SBP, systolic blood pressure. (From Satoh M, Inoue R, Tada H, et al. Reference values and associated factors for Japanese newborns' blood pressure and pulse rate: the babies' and their parents' longitudinal observation in Suzuki Memorial Hospital on intrauterine period (BOSHI) study. *J Hypertens*. 2016;34:1578–1585.)

or during feeding (Spahr et al., 1981; Sinkin et al., 1985; Park and Lee, 1989). A recent study from Japan of BP in 3148 newborns demonstrated the effects on BP of various states of activity (Fig. 93.5; Satoh et al., 2016). Even pacifier use during sleep has been shown to increase BP (Yiallourou et al., 2014). Because of these factors, it is important to follow a standard approach for BP measurement in neonates; the protocol described by Nwankwo et al. (1997) is appropriate. Measurements in NICU graduates should be obtained only when the infant is asleep or calm (Duncan et al., 2008).

History and Physical Examination

In most neonates suspected of having HTN, a relatively focused history can be obtained, paying attention to determining whether there were any relevant prenatal exposures and to the particulars of the infant's NICU/nursery course and any concurrent medical conditions/complications of prematurity. The procedures that the infant has undergone (e.g., umbilical catheter placement) should be reviewed, and the current medication list should be scrutinized. Easily identifiable causes of HTN such as fluid overload or medication-induced HTN should be able to be identified at this stage, and appropriate countermeasures should be taken to correct the problem.

The physical examination should similarly focus on identifying obvious problems that may be causing the BP elevation. BP readings should be obtained in all four extremities to rule out coarctation of the thoracic aorta. The general appearance of the infant should be assessed, with particular attention paid to the presence of dysmorphic features. Careful cardiac and abdominal examinations should be performed, as specific findings (heart murmur, unilateral flank mass, ambiguous genitalia, etc.) can help direct subsequent diagnostic testing.

Diagnostic Testing

If the infant's HTN appears to be iatrogenic or secondary to drug withdrawal, specific therapy can be instituted without performance

TABLE 93.3 Diagnostic Studies

| Generally Useful | Useful in Selected Infants |
|--|---|
| Urinalysis (with or without culture) | Thyroid studies |
| Complete blood count and platelet count | Aldosterone |
| Electrolytes | Cortisol |
| Blood urea nitrogen, creatinine | Urine vanillylmandelic acid/homovanillic acid |
| Calcium | Plasma catecholamines/metanephrines |
| Plasma renin | Echocardiogram |
| Chest X-ray | Abdominal/pelvic ultrasonography |
| Renal ultrasonography with Doppler measurement | Voiding cystourethrogram |
| | Renal angiography |
| | Nuclear scan (DTPA/MAG-3) |

DTPA, Diethylenetriaminepentaacetic acid; MAG-3, mercaptoacetyltriglycine.

of additional investigation. If no cause is evident, then urinalysis and screening chemistry tests should be performed (Table 93.3). If a renal or renovascular cause is suspected, the work-up will usually include ultrasonography of the kidneys, adrenal glands, aorta, and bladder, with a flow study (i.e., Doppler ultrasonography) of the aorta and the renal arteries and renal veins. Usually a chest X-ray is useful if one has not been obtained recently. An echocardiogram will be needed to confirm the diagnosis of aortic coarctation. Nuclear renal scans, angiography, magnetic resonance imaging, or computed tomography may be indicated in specific patients. If there is any suspicion of hydronephrosis or vesicoureteral reflux, urine obtained by suprapubic aspiration or bladder catheterization should be sent for bacterial and fungal culture.

PRA should be measured as part of the work-up in most hypertensive infants. The PRA is most helpful if it is extremely low—in such cases, a single-gene disorder affecting renal sodium transport should be suspected (see the preceding discussion on genetic causes). Elevated PRA is less helpful as it may be secondary to the administration of diuretics or adrenergic medications or may be secondary to severe respiratory disease; mild elevations of PRA may be seen in normal infants.

Other blood studies listed in Table 93.3 should be reserved for selected infants when there is a suggestion from the history, physical examination, and/or screening studies that a specific secondary cause of HTN may be present. Usually it is best to obtain the assistance of the appropriate subspecialist (nephrologist, endocrinologist, oncologist, etc.) before such studies are ordered.

Treatment

Treatment of neonatal HTN should begin with correction of any iatrogenic causes such as fluid overload or medications that increase BP. After that, a decision needs to be made as to whether antihypertensive medications are indicated. Clearly infants with severe, symptomatic HTN should receive immediate treatment. However, in those with less severe HTN, particularly those in whom the high BP does not appear to be causing any immediate problem, the necessity of initiating treatment with antihypertensive medications is often unclear. We do not know whether HTN in such infants affects long-term outcomes, and we certainly do not know at what BP target-organ effects begin to appear. Furthermore, no clinical trials of antihypertensive medications have ever been

conducted in neonates, so there are no data on efficacy or safety in this population. Thus a great deal of individual judgment must be brought to bear in deciding to treat HTN in the NICU.

That said, we would certainly recommend that critically ill infants with acute onset of severe HTN should be treated with an intravenous agent administered by continuous infusion, as this will allow the greatest control of the rate and magnitude of the BP reduction. These infants should have their BP lowered by no more than 25% in the first 8 hours to prevent cerebral ischemia (Flynn and Tullus, 2009). On the other hand, relatively well infants with mild HTN may be treated with oral antihypertensive agents. Recommended doses for intravenous and oral antihypertensive drugs in neonates can be found in Table 93.4.

The choice of oral antihypertensive medication to use in hypertensive neonates is somewhat controversial. Available data indicate that hypertensive infants are treated with a wide variety of classes of antihypertensive agents (Blowey et al., 2011). Whereas angiotensin-converting enzyme (ACE) inhibitors are considered the drugs of choice for adults and children with renal forms of HTN and although there is a long history of their use in neonatal HTN, many neonatologists and pediatric nephrologists have serious concerns about the potential major side effects, such as excessive hypotension, AKI (Gantenbein et al., 2008), and neurologic abnormalities. There may also be adverse effects on the completion of renal development in premature neonates. Other medications, such as a β -blocker or, in the case of a hypertensive crisis, a potent vasodilator, should be tried first. The advantage of a β -blocker such as propranolol is that it reduces the secretion of renin and the release of norepinephrine; however, it may also cause bronchoconstriction or hypoglycemia, making its use problematic in some infants.

Of the available vasodilators, the calcium channel blockers isradipine and amlodipine have found widespread use in neonates (Dionne et al., 2012). Older vasodilating agents such as hydralazine and minoxidil may also be useful in selected infants or when the newer agents are not available. Clonidine is yet another potential choice. All oral antihypertensive medications excepting propranolol need to be compounded into suspensions by the hospital pharmacy.

Of the many intravenous antihypertensive agents available, nicardipine has emerged as the most useful for management of severe neonatal HTN (Gouyon et al., 1997; Milou et al., 2000). It can be precisely titrated to the desired antihypertensive effect, and its use may be continued for prolonged periods without loss of antihypertensive efficacy. A possible issue is the need to infuse nicardipine through a central line. Alternative agents that may be given by continuous infusion include esmolol, hydralazine, labetalol, and sodium nitroprusside. Esmolol and labetalol may be contraindicated in infants with lung disease, and nitroprusside can be used only for limited periods (usually <72 hours) because of the accumulation of thiocyanate.

Intravenous antihypertensives that can be administered by intermittent bolus injection include hydralazine and labetalol. The intravenous ACE inhibitor enalaprilat has been reported to be effective in cases of severe neonatal HTN in one small case series (Wells et al., 1990); however, our anecdotal experience suggests that this agent may cause sudden, oliguric acute renal failure similar to that reported for orally administered enalapril (Dutta and Narang, 2003). Given this, we do not recommend use of enalaprilat in neonates.

Surgery is indicated for treatment of neonatal HTN in a limited set of circumstances (Rajpoot et al., 1999). In particular, HTN

TABLE 93.4 Recommended Dosing of Selected Antihypertensive Agents in Neonates

| Class | Drug | Route | Dose | Interval | Comments |
|--------------------------|----------------------|-------|--|----------|--|
| ACE inhibitors | Captopril | Oral | <3 months: 0.01–0.5 mg/kg/dose Maximum 2 mg/kg per day >3 months: 0.15–0.3 mg/kg per dose Maximum 6 mg/kg per day | TID | 1. First dose may cause rapid drop in BP, especially if receiving diuretics. 2. Monitor serum creatinine and K ⁺ levels. |
| | Enalapril | Oral | 0.08–0.6 mg/kg per day | BID | |
| | Lisinopril | Oral | 0.07–0.6 mg/kg per day | QD | |
| α and β antagonists | Labetalol | Oral | 0.5–1.0 mg/kg/dose up to 10 mg/kg per day | BID–TID | Heart failure, BPD relative contraindications |
| | | IV | 0.20–1.0 mg/kg per dose (bolus) | Q4h–Q6h | |
| | Carvedilol | IV | 0.25–3.0 mg/kg per hour | Infusion | |
| β antagonists | Esmolol | Oral | 0.1 mg/kg/dose up to 0.5 mg/kg per dose | BID | May be useful in heart failure |
| | | IV | 100–500 µg/kg per min | Infusion | |
| | Propranolol | Oral | 0.5–1.0 mg/kg per dose Maximum 8–10 mg/kg per day | TID | Very short acting—constant infusion necessary Monitor heart rate; avoid in BPD |
| Calcium channel blockers | Amlodipine | Oral | 0.05–0.3 mg/kg per dose up to 0.6 mg/kg per day | QD | All may cause mild reflex tachycardia. |
| | Isradipine | Oral | 0.05–0.15 mg/kg per dose up to 0.8 mg/kg per day | QID | |
| | Nicardipine | IV | 0.5–4 µg/kg per min | Infusion | |
| Central α agonist | Clonidine | Oral | 5–10 µg/kg per day up to 25 µg/kg per day | TID | May cause mild sedation |
| Diuretics | Chlorothiazide | Oral | 5–15 mg/kg per dose | BID | Monitor electrolyte levels. |
| | Hydrochlorothiazide | Oral | 1–3 mg/kg per dose | QD–BID | |
| | Spironolactone | Oral | 0.5–1.5 mg/kg per dose | BID | |
| Vasodilators | Hydralazine | Oral | 0.25–1.0 mg/kg per dose up to 7.5 mg/kg per day | TID–QID | Tachycardia and fluid retention are common side effects. |
| | | IV | 0.15–0.6 mg/kg per dose | Q4h | |
| | Minoxidil | Oral | 0.1–0.2 mg/kg per dose | BID–TID | Tachycardia and fluid retention are common side effects; prolonged use causes hypertrichosis. |
| | Sodium nitroprusside | IV | 0.5–10 µg/kg per min | Infusion | Thiocyanate toxicity can occur with prolonged (>72 h) use or in renal failure. |

Use of any of these medications in neonates should be considered off-label.

ACE, Angiotensin-converting enzyme; BID, twice daily; BP, blood pressure; BPD, bronchopulmonary dysplasia; IV, intravenous; K⁺, potassium; QD, once daily; Q4h, every 4 hours; Q6h every 6 hours; QID, four times daily; TID, three times daily.

caused by urologic causes such as ureteropelvic junction obstruction or aortic coarctation is best approached surgically. On the other hand, most infants with renal artery stenosis will need to be treated medically until they have grown sufficiently to undergo definitive repair of the vascular abnormalities, although case reports of successful intervention in infancy have been published (Peco-Antic et al., 2006). Infants with HTN secondary to Wilms tumor or neuroblastoma will require surgical tumor removal, possibly following chemotherapy. A case has also been made by some authors for prophylactic removal of multicystic dysplastic kidneys because of the risk of development of HTN (Webb et al., 1997), although this is controversial.

Prognosis

The prognosis of neonatal HTN depends on the cause, timing of the diagnosis, presence of complications, and response to therapy. Patients with severe HTN and neurologic, cardiovascular, or renal

decompensation have a high mortality rate. As previously mentioned, the mortality rate of patients with idiopathic calcification of the arteries or with massive aortic thrombosis remains high despite aggressive therapy. Although data are limited, the long-term prognosis for newborns with HTN related to thromboembolism or BPD appears to be good, often with progressive resolution of the HTN within 6 to 12 months of age (Seliem et al., 2007). On the other hand, patients with parenchymal renal disease, especially those with persistent renal dysfunction following NICU discharge, are likely to have persistent HTN throughout childhood. Infants who undergo repair of aortic coarctation are at long-term risk of persistent or recurrent HTN later in childhood and require continued follow-up (O'Sullivan et al., 2002).

Suggested Readings

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- Complete references used in this text can be found online at www.expertconsult.com

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Developmental Endocrinology

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KEY POINTS

- Endocrine organ function in neonates is dependent on organ development as well as the maternal, placental, and fetal hormone milieu.
- Fetal adaptation or maladaptation to the hormonal milieu may contribute to neonatal disease.
- Developmental disorders of endocrine organs often manifest themselves in the neonatal period.
- The fetal adrenal gland produces large amounts of androgens to be used by the placenta for estrogen biosynthesis.
- Maternal cortisol is inactivated by placental enzymes that convert cortisol to inactive cortisone.
- Neonatal hypopituitarism may be due to a number of specific gene mutations with or without defects in other cranial structures.
- The fetal adrenal gland increases in size during gestation to 10–20 times the size of adult adrenal glands. Rapid involution and decrease in size occur after birth.
- The thyroid emerges from the pharyngeal floor and then migrates caudally to its final destination in the anterior neck. Abnormalities in thyroid descent result in ectopic thyroid, thyroglossal duct, or cysts.
- Pancreatic β -cell differentiation and proliferation are dependent on insulin-like growth factor 2.
- The placenta actively transports maternal calcium, phosphorus, and magnesium to maintain high fetal serum levels necessary for the developing skeleton.

Like development of other fetal organ systems, development of the endocrine system involves integration of complex genetic, cellular, and hormonal cues as well as the coordinated action of transcription factors, signaling molecules, and epigenetic regulation. Additionally, endocrine communication is necessary for the vital maternal–placental–fetal interaction because endocrine signals are the tools that cells and organs use to communicate with each other. In this chapter, we will first discuss basic concepts of hormonal systems and then discuss the unique hormonal milieu of the placenta that allows maternal–fetal communication and fetal growth. The development of each of the classic endocrine organs will then be discussed. Finally, the developmental origins of the adult disease hypothesis will be discussed.

Endocrine Systems

A few key concepts of cell communication will be presented here to assist in the building of a foundation of knowledge to understand hormonal abnormalities in neonates.

In the process of communication, cells emit signals that act locally or distally. If a cell emits signals that are received by the cell itself, this is termed *autocrine regulation*. If a cell emits signals that are received by neighboring cells, this is termed *paracrine regulation*. Finally, if the cell signal travels through the bloodstream to distant cells and organs, this is termed *endocrine regulation*.

A feature of endocrine systems is the fact that the hormonal signals are present in the bloodstream at very low concentrations, and even small changes in hormone concentration can elicit a robust response from receiving cells. The released endocrine hormone can be either water-soluble peptide hormones that must bind to receptors on the extracellular surface of the cell or fat-soluble steroid-like molecules that can pass through the cell membrane directly into the nucleus of the cell to bind its receptor, to regulate gene transcription. On binding to their specific cell membrane receptor, peptide hormones activate downstream signaling transduction pathways in the cell to induce biological responses. These responses can be immediate; for instance, thyroid-stimulating hormone (TSH), when bound to the TSH receptor on thyroid follicular cells, cause iodine transport into the cell to increase. It can also induce cell responses that are relatively delayed, such as an increase in gene transcription of enzymes involved in thyroglobulin production. In contrast, steroid-like molecules, such as cortisol, when bound to their respective receptors induce a biological response by causing an increase in transcription of various genes. These induce longer term, broader changes in the target cell function by modulating which genes the cells express and thus the proteins they produce.

Many hormonal systems exhibit a hierarchy of hormone signals that allow strict regulation of system function. The hypothalamic–pituitary–end gland system is an example of this multitier hormonal system. In this system, hormone released by the upstream hypothalamus travels through the portal circulation of the pituitary and binds to the corresponding receptor on the dedicated pituitary cell. Once bound, this receptor induces the cell to synthesize and release the corresponding pituitary hormone. This hormone in turn then travels through the systemic circulation to the endocrine gland to bind to the hormone’s dedicated receptor on the target

gland. Binding of the hormone to its receptor induces the target organ to then release its dedicated end hormone. This hormone then travels through the bloodstream to multiple organs to affect cell function. End hormones use negative feedback to regulate their own production. That is, the hypothalamus and pituitary sense the end hormone concentration, adjusting release of the upstream, regulatory hormone cascade to fine-tune end hormone production. Negative feedback is a unique and consistent property of hormonal systems and is useful in interpreting adequate response of a hormonal cascade to perturbation in the system.

Endocrine Organ Development and Perinatal Transition

Early in fetal development, fetal hormonal gland development is driven by fetal genotype. However, later in fetal development, the hormonal responsiveness of target tissues is dependent not only on fetal genotype but also on the complex fetal hormonal milieu, which comprises fetal, maternal, and placental hormones. Placental hormones may be influenced by maternal and fetal genotype, as well as maternal prepregnancy and pregnancy health and nutrition. Thus the fetal hormonal environment is dependent on a complex interplay between fetal and maternal factors.

In evaluating hormonal disorders in newborns, one needs to consider both maternal and fetal factors and keep the following principles in mind. First, human fetal endocrine organ development begins predominantly independently of maternal hormones. This is possible because the placenta is a barrier to many (but not all) maternal hormones, including steroids, peptides, and glycoproteins. Second, although endocrine organ development may be normal, perturbations in the maternal hormonal milieu may still affect fetal development for the rare hormone that crosses the placenta. This finding is found in disorders of thyroid hormone production. Maternal–fetal transfer of thyroxine (T_4) may result in 25%–50% of neonatal plasma levels of T_4 (Vulsma et al., 1989). This transfer allows children with athyrosis to have good neurodevelopmental outcomes if treatment is initiated within 2 weeks of birth, as the maternal thyroid hormone crossed the placenta and allowed normal fetal neurodevelopment and growth. In contrast, children born to mothers with untreated or undertreated hypothyroidism during pregnancy have poor neurodevelopmental outcomes because of the hypothyroxinemia early in gestation before T_4 production by the fetal thyroid (Haddow et al., 1999; Yasuda et al., 1999). Third, alterations in transplacental substrate transfer can modify late development of the fetal and thus neonatal hormonal pathway and feedback mechanisms. This can be seen in neonates born from pregnancies with uncontrolled diabetes, in which the transplacental passage of glucose induces robust insulin release and subsequent β -cell hypertrophy in the still developing pancreas. This leads to transient neonatal hyperinsulinemic hypoglycemia as a consequence of the abrupt fall in glucose supply at birth. This is also seen in the placental transfer of hormonal agents or maternal antibodies that affect neonatal endocrine gland function. Examples include the transplacental crossing of maternal TSH antibodies causing neonatal hyperthyroidism. Fourth, knowledge of endocrine developmental biology has been obtained from large animal fetal physiology models and gene manipulation studies in mice. When comparing ontogenic studies performed in different species, one must consider similarities and differences from human fetuses and newborns. The maturational state at birth differs widely among species. For many species, including rodents

and some large animals, the maturational state of the newborn is relatively immature (altricial). Organ systems in humans, in contrast, are relatively more developed at birth (precocial). This is especially true in neuroendocrine and most endocrine systems. Thus study of hormonal physiology in newborn mice may yield insights, but they may not be immediately applicable to human newborns.

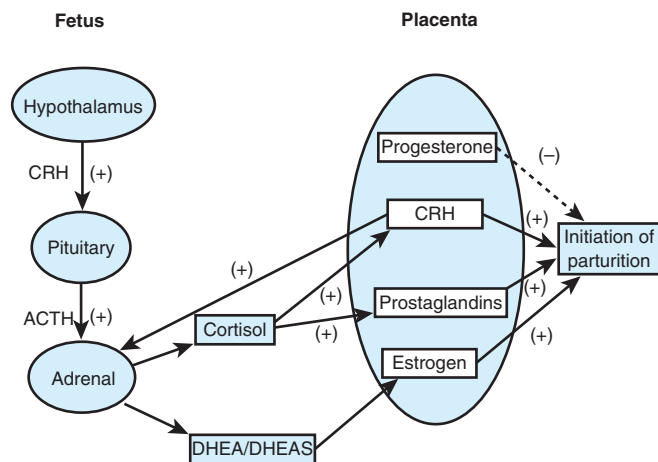
The Maternal–Placental–Fetal Unit

During pregnancy the mother, fetus, and placenta function in concert as a steroidogenic unit for estrogen and progesterone production. Most steroidogenic activity is exerted in the fetal zone (FZ) of the human fetal adrenal (HFA) gland, where large amounts of adrenal androgens are produced to be used by the placenta for estrogen biosynthesis. Estrogen promotes placental trophoblast differentiation into syncytiotrophoblast and upregulates key enzymes in progesterone biosynthesis (Mesiano and Jaffe, 1997).

Cortisol may act as a “two-edged sword” for the fetus; it promotes maturation of fetal organs necessary for extrauterine life, but it can also adversely influence fetal growth and postnatal development. Therefore cortisol production in the fetoplacental unit is strictly regulated to protect the fetus from hypercortisolism effects. Fetal access to maternal glucocorticoid is restricted, and a maternal–fetal gradient is maintained by the enzyme 11β -hydroxysteroid dehydrogenase type 2, which converts cortisol to inactive cortisone. Maternal cortisol levels are usually 5 to 10 times higher than fetal cortisol levels. Fetal protection is also achieved through other mechanisms in the HFA by regulation of 3β -hydroxysteroid dehydrogenase/ Δ^{4-5} isomerase type 2, the key steroidogenic enzyme for cortisol biosynthesis, and in fetal membranes by control of 11β -hydroxysteroid dehydrogenase type 1 activity, which converts inactive cortisone to cortisol (Ishimoto and Jaffe, 2011). A single course of glucocorticoids is universally accepted as a standard therapy for pregnant women at risk of preterm delivery to accelerate fetal lung maturation and reduce morbidity and mortality in preterm infants (Roberts and Dalziel, 2006). However, multiple courses of glucocorticoids prenatally may be associated with decreased weight, length, and head circumference at birth (Murphy et al., 2008).

Placental corticotropin-releasing hormone (CRH) is one of the key determinants of the timing of parturition. Near term, its level increases and directly stimulates the HFA by increasing its responsiveness to adrenocorticotrophic hormone (ACTH) and secretion of cortisol and dehydroepiandrosterone (DHEA) and its sulfonated form, precursors of placental estrogen. Cortisol, in turn, stimulates placental CRH production, forming a positive feedback loop and generating more cortisol and estrogen near term. Estrogen upregulates contraction-associated proteins and transforms the myometrium into a contractile state, preparing for successful uterine contractions and parturition. Cortisol also promotes maturation of fetal organs (e.g., the lung) and stimulates production of prostaglandins necessary for parturition. Increased levels of estrogen, cortisol, and CRH, together with functional progesterone withdrawal, are thought to contribute to the initiation of parturition (Sirianni et al., 2005; Mendelson, 2009; Fig. 94.1).

From this perspective, one would expect that the lack of the increased cortisol production observed near term in preterm infants may have adverse consequences. This is not confirmed; some studies showed no adverse effects related to low cortisol levels in preterm infants (Aucott et al., 2008), while others correlated low cortisol levels to increased severity of illness, hypotension, mortality, and



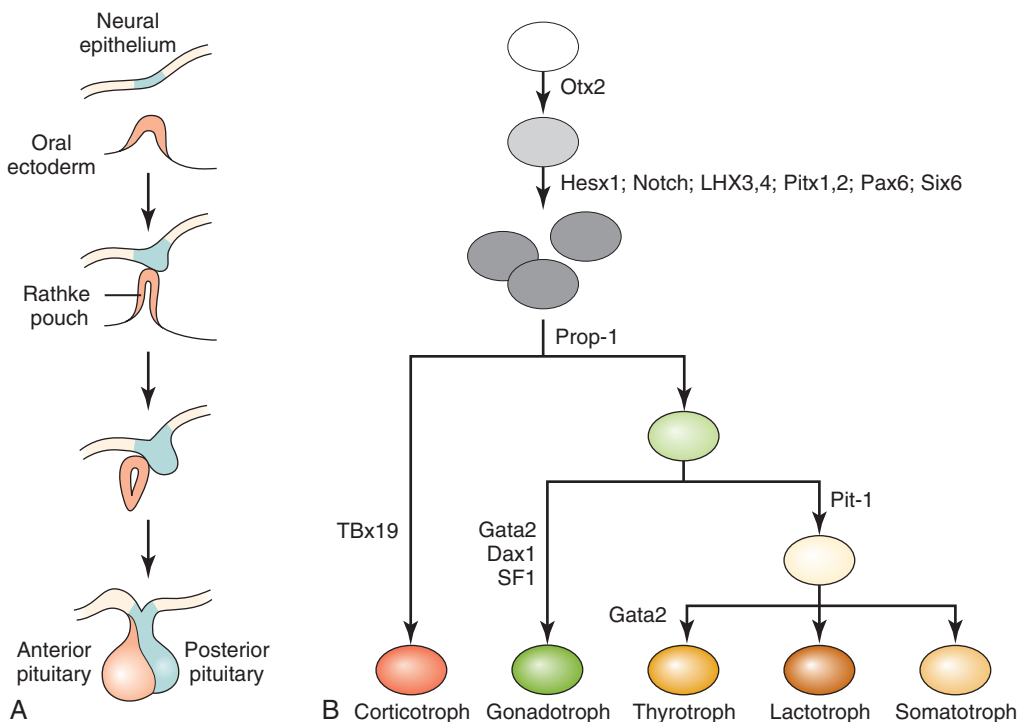
• **Fig. 94.1** Endocrine Cascades in the Fetoplacental Unit That Lead to Initiation of Parturition. Near term, production of placental corticotropin-releasing hormone (CRH) increases and stimulates fetal adrenal glands to produce dehydroepiandrosterone (DHEA)/dehydroepiandrosterone sulfate (DHEAS) and cortisol. Increased cortisol production stimulates production of placental CRH through a positive feedback loop. DHEA/DHEAS is converted by the placenta to estrogen, promoting initiation of parturition through upregulation of contraction-associated proteins. Cortisol and CRH also stimulate production of prostaglandins necessary for contractions. When “functional withdrawal” of progesterone occurs, coupled with these changes, parturition is initiated. ACTH, Adrenocorticotropic hormone.

development of bronchopulmonary dysplasia (Watterberg et al., 2007). Selective hydrocortisone supplementation for at-risk infants may be used for care of these infants.

Umbilical plasma estradiol and progesterone levels are quite high and fall by approximately 100-fold during the first day after birth. The consequences of estradiol and progesterone withdrawal earlier in premature infants remain largely unknown. Pilot studies of estradiol and progesterone supplementation in extremely low-birthweight infants have shown trends toward increased bone mineralization and a decrease in the incidence of chronic lung disease (Trotter et al., 2001, 2007). More powered clinical trials are needed to determine the benefits and risks of hormonal replacement in preterm infants.

Hypothalamic and Pituitary Development

The anterior and posterior pituitary glands have different embryonic origins. The anterior lobe arises from oral ectoderm, and the posterior pituitary arises from the infundibulum of the developing central nervous system. A section of the oral ectoderm thickens very early in development, forming an invagination termed a *Rathke pouch*. As the Rathke pouch invaginates further, the adjacent neural ectoderm evaginates to form the infundibulum (Fig. 94.2A). With further evagination, the Rathke pouch and the infundibulum directly contact each other. This close contact is important for subsequent anterior and posterior pituitary development. The Rathke pouch later “pinches off” from the remaining oral ectoderm



• **Fig. 94.2** Pituitary Gland Development. (A) Simplified schematic of anterior pituitary development from oral ectoderm via evagination into the Rathke pouch, with subsequent pinching off of tissue to separate from oral ectoderm and eventually form the anterior pituitary. (B) The transcription factor cascade implicated in pituitary development and cell differentiation. Dax1, Dosage-sensitive sex reversal, adrenal hypoplasia congenital, X-chromosome factor; Gata2, GATA binding protein; Hesx1, homeobox1; LHX3,4, LIM homeobox3,4; Pax6, paired box 6; Pitx1,2, paired-like homeodomain 1, 2; Prop-1, prophet of Pit-1; Otx2, orthodenticle homeobox 2; SF1, steroidogenic factor 1; Six6, sine oculis-related homeobox 6; TBx19, T-box 19.

TABLE 94.1**Genes Important in Pituitary Development and Implicated in Human Disease**

| Gene | Involved Pituitary Cell Types | Extrapituitary Phenotype | Radiology Findings | Mode of Inheritance |
|----------------------|---|---|---|---------------------|
| <i>HESX1</i> | Somatotrophs, thyrotrophs, gonadotrophs, posterior pituitary cells | Septo-optic dysplasia/optic nerve hypoplasia Midline forebrain abnormalities | Pituitary hypoplasia EPP | AD or AR |
| <i>LHX3</i> | Somatotrophs, lactotrophs, thyrotrophs, gonadotrophs, corticotrophs (variable) | Rigid cervical spine with limited neck rotation | Pituitary hypoplasia | AR |
| <i>LHX4</i> | Somatotrophs, lactotrophs, thyrotrophs, gonadotrophs, corticotrophs | Abnormality of skull base Cerebellar abnormalities | Pituitary hypoplasia EPP | AD |
| <i>SIX6</i> | Somatotrophs, gonadotrophs | Bilateral anophthalmia Associated with deletion of chromosome band 14q22-23 | Pituitary hypoplasia | Haploinsufficiency |
| <i>PITX2</i> | Somatotrophs, lactotrophs, thyrotrophs, gonadotrophs | Reiger syndrome Dental hypoplasia Craniofacial dysmorphism | Pituitary hypoplasia | AD |
| <i>PROP1</i> | Somatotrophs, lactotrophs, thyrotrophs, gonadotrophs, corticotrophs (later in life) | May have normal puberty (variable gonadotroph function) | Pituitary hypoplasia | AR |
| <i>POU1F1 (PIT1)</i> | Somatotrophs, lactotrophs, thyrotrophs, | | Pituitary hypoplasia | AD/AR |
| <i>OTX2</i> | Somatotrophs, thyrotrophs, gonadotrophs | Microphthalmia or anophthalmia | Pituitary hypoplasia EPP | Unknown |
| <i>SOX2</i> | Somatotrophs, gonadotrophs | Microphthalmia or anophthalmia Sensorineural defects Esophageal atresia | Pituitary hypoplasia | De novo |
| <i>SOX3</i> | Somatotrophs, lactotrophs, thyrotrophs, gonadotrophs, corticotrophs | Duplication of Xq26-27 in affected males | Pituitary hypoplasia EPP Abnormal corpus callosum | X-linked recessive |

AD, Autosomal dominant; *AR*, autosomal recessive; *EPP*, ectopic posterior pituitary.

to form the anterior pituitary gland. In humans this occurs by 5 to 6 weeks' gestation.

The hypothalamus comprises a diverse collection of neurons that regulate pituitary hormone release, thirst, body temperature, blood pressure, and serum osmolality. Although the location of the diverse neurons within the hypothalamus is well delineated, the development of these diverse neuron populations within the hypothalamus has yet to be elucidated. The complexity of the anatomy and neuronal cell types makes it difficult to elucidate the developmental cascade of events. Nevertheless, it is known that the hypothalamic nuclei, which contain the individual neuron types, are fully developed with projections to the median eminence (with subsequent release of hormone into the pituitary portal circulation) by 15 to 18 weeks' gestation (Kelberman et al., 2009).

In contrast, knowledge regarding differentiation of the anterior pituitary is well delineated. Differentiation of Rathke pouch cell progenitors into the pituitary cell types (corticotrophs, gonadotrophs, lactotrophs, thyrotrophs, and somatotrophs) is tightly regulated by a cascading series of transcription factors (see Fig. 94.2B). Knowledge of the series of events is from knockout studies in mice as well as genetic studies in humans with congenital hypopituitarism. The pituitary cell types arise in a temporally and spatially specific pattern as directed by the transcription factors, occurring between week 7 and week 16 of human gestation. If one transcription factor of the developmental series malfunctions or is expressed out of series, as is seen in clinical syndromes of

hypopituitarism (Table 94.1), then a very typical pattern of pituitary hormone deficiency is manifest (Kelberman et al., 2009; Romero et al., 2009).

Each mature pituitary cell type synthesizes and secretes a corresponding hormone that is regulated by a hypothalamic peptide: corticotrophs secrete ACTH in response to CRH; gonadotrophs secrete luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in response to gonadotropin-releasing hormone (GnRH); lactotrophs secrete prolactin in response to dopamine; thyrotrophs secrete thyrotropin (TSH) in response to thyrotropin releasing hormone (TRH); and somatotrophs secrete growth hormone in response to growth hormone-releasing hormone and somatostatin.

The mature pituitary cell types contain secretory granules by 10 to 12 weeks' gestation, and the hormones can be measured by 12 to 17 weeks' gestation (Kelberman et al., 2009). The pituitary portal circulation is mature by 12 to 17 weeks, and thus regulation of pituitary hormone release by the corresponding hypothalamic peptide may be operational by that time. However, the negative feedback mechanisms that finely tune hypothalamic-pituitary-end hormone release may not be fully mature until later in gestation. At birth the negative feedback mechanisms are fully mature, which is crucial in the diagnostic evaluation of infants with suspected pituitary hormone disorders.

The neurohypophysis (posterior pituitary), formed by the evagination of the neuroectoderm, is fully developed by 10 to 12 weeks' gestation and contains granules of arginine vasopressin (AVP)

and oxytocin (Leake and Fisher, 1985). The neurohypophysis comprises the axonal terminals of hypothalamic neurons whose cell bodies are in the mature paraventricular nuclei and supraoptic nuclei. These neurons generate AVP or oxytocin separately and thus are regulated by separate factors. AVP-releasing neurons integrate signals from plasma osmolality sensors and baroreceptors in the carotid sinus and aortic arch to release AVP and thus regulate serum osmolality and systemic blood pressure. AVP acts on the renal collecting duct via V_2 receptors to induce water reabsorption. AVP also acts on V_{1A} receptors on endothelial cells to increase arterial and venous constriction and thus increase blood pressure. Serum osmolality is tightly controlled between 280 and 295 milliosmoles (mOsm)/kg water in adults (Robertson et al., 1976). With even a slight increase in serum osmolality, AVP release increases exponentially to induce renal water reabsorption to a maximum urine osmolality of 800–1200 mOsm/kg water (maximum urine osmolality depends on the osmolality of the upstream kidney medulla). In neonates, however, the urine-concentrating ability of the kidney is impaired to a maximum of 300 mOsm/kg water because of the relative immaturity of the renal tubules rather than lack of AVP release; AVP is present in the fetus and neonate, and V_2 receptors are functional (Baum et al., 1998).

Diseases of Hypothalamic or Pituitary Maldevelopment

Given the overlapping developmental window of important cranial midline structures, a diagnosis of congenital hypopituitarism in a neonate should be considered if there are intracranial or extracranial midline defects. For instance, hypopituitarism has been reported in children with cleft lip or palate or central incisors. Hypopituitarism and diabetes insipidus can be encountered in neonates with holoprosencephaly. Children who have intracranial abnormalities along the spectrum of optic nerve hypoplasia, with or without septo-optic dysplasia, commonly have congenital hypopituitarism.

Neonates with congenital hypopituitarism because of a mutation in a gene important in pituitary differentiation may or may not have obvious defects in midline structures (Table 94.1). In the absence of these clinical signs, hypopituitarism may be suspected in neonates with micropenis and normal testicular descent (caused by growth hormone with or without gonadotropin deficiency), prolonged hypoglycemia (caused by combined growth hormone and cortisol deficiency), or rarely, cholestatic giant cell hepatitis (caused by combined growth hormone, thyroid hormone, and cortisol deficiency). As fetal growth is independent of growth hormone, children with congenital hypopituitarism are of normal size and weight at birth. If a newborn screen uses T_4 levels as the screen for congenital hypothyroidism, then low T_4 levels may be a diagnostic clue; if a newborn screen measures thyrotropin (also known as thyroid stimulating hormone, TSH) to screen a newborn for congenital hypothyroidism, the central hypothyroidism of hypopituitarism will not be detected. After 2 months of life, a clue to optic nerve hypoplasia may be a disconjugate gaze. If diabetes insipidus is diagnosed, then full evaluation of the remaining pituitary hormones is mandatory.

Adrenal Gland Development

Cells of the adrenal cortex arise from the intermediate mesoderm and can be recognized at the upper pole of the mesonephros by 3 to 4 weeks' gestation. Cells of the adrenal medulla are derived

from the ectoderm, and they infiltrate the adrenal cortex cells at 7 to 8 weeks' gestation. Encapsulation of the adrenal gland occurs at 9 weeks' gestation, resulting in the formation of a distinct organ above the developing kidneys. The cortical and medullary cells proliferate rapidly and sort into a central medulla and surrounding cortex (Ishimoto and Jaffe, 2011; Dattani and Gevers, 2016).

The HFA cortex acquires two distinct zones: the inner FZ and the outer definitive zone (DZ). DZ cells are in an undifferentiated proliferative state, whereas FZ cells are differentiated, steroidogenic cells. Current data suggest that the HFA cortex is a dynamic organ in which cells proliferate in the periphery (i.e., the DZ) and migrate centripetally, differentiating to form the inner FZ that is unique to primate gestation (Spencer et al., 1999). There is a third zone between the DZ and FZ, named the *transitional zone* (TZ). Cells in this zone show intermediate characteristics and have the capacity to produce cortisol, being analogous to the zona fasciculata cells of the adult adrenal cortex. By the 30th week of gestation, the HFA cortex manifests a rudimentary form of the adult adrenal cortex; the DZ and TZ begin to resemble the zona glomerulosa and the zona fasciculata, respectively. The third definitive cortical layer, the zona reticularis, is absent at birth and starts developing at 3 years of age to form an extragonadal source of sex steroids. The cells of the inner FZ are the primary site for steroidogenesis during gestation, producing an abundant amount of adrenal androgens, which are the substrates for placental estrogen production.

The weight of the adrenal gland increases 10-fold from 8 to 10 weeks' gestation, and the gland continues to grow rapidly thereafter until term. By 30 weeks, it achieves a relative size 10 to 20 times that of the adult adrenal gland. A further doubling in weight occurs thereafter. Soon after birth, the HFA undergoes rapid involution with rapid disappearance of the FZ and a decrease in androgen secretion. As a consequence, the total weight of the glands decreases by approximately 50%. Remodeling of the postnatal adrenal gland involves a combination of FZ regression and development of the zona glomerulosa and zona fasciculata. During the first year, the fetal cortex regresses, and adrenal mass diminishes to 2–3 g.

The early stages of adrenal development are regulated by a number of transcription factors, mainly steroidogenic factor 1 (SF1) and DAX1 (dosage-sensitive sex reversal, adrenal hypoplasia congenital, X-chromosome factor). SF1-knockout mice manifest adrenal and gonadal agenesis and gonadotropin deficiency (Parker et al., 2002). Inactivating DAX1 mutations are associated with adrenal hypoplasia and gonadotropin deficiency in mice and humans (McCabe, 2007).

ACTH secreted by the fetal pituitary is the primary regulator of the development and function of the HFA. There are other ACTH-independent regulators; these include local peptide growth factors and placenta-derived factors, such as CRH and estrogens. The steroidogenic activity in the HFA is characterized by early transient cortisol biosynthesis (Goto et al., 2006). The hypothalamic–pituitary–adrenal axis is sensitive to glucocorticoid-mediated feedback at this time, and therefore 46,XX females with steroidogenic defects (such as congenital adrenal hyperplasia) who lack cortisol will have an elevated ACTH level that stimulates overproduction of androgens, resulting in virilization of the female genitalia. The cortisol production is suppressed thereafter until late gestation (because of the lack of 3 β -hydroxysteroid dehydrogenase type 2 expression). Near term, the fetal cortisol production rate increases and is similar to that in adults, per unit body weight. There is extensive production of DHEA and its sulfate, precursors of placental estrogen, during most of gestation. This begins at around 8 to 10

weeks' gestation and increases significantly during the second and third trimesters. Aldosterone synthesis in the HFA may be suppressed during midgestation because of the probable lack of *CYP11B2* expression but likely becomes active by term because 80% of aldosterone in human fetal blood at term appears to originate from the HFA.

The adrenal medulla functions as a classic endocrine (ductless) gland that secretes hormones directly into the bloodstream. It also participates in sympathetic control via preganglionic sympathetic nerve fibers. Pheochromocytoblasts give rise to the medullary pheochromocytes, which are epinephrine- and norepinephrine-secreting homologues of sympathetic postganglionic cells. By 3 months' gestation, adrenal pheochromocytes secrete epinephrine and norepinephrine into the medullary sinusoids and then into the systemic circulation (Unsicker et al., 2005). The hypothalamic–pituitary–medullary adrenal axis becomes sufficiently functional by midgestation so that fetal stress responses can be independent of those of the mother (Gitau et al., 2001). This fetal catecholamine stress response contrasts with the fetal cortisol output capacity, which is minimally present before midgestation.

Thyroid Gland Development

Thyroid hormones T_4 and triiodothyronine are essential for the development and maintenance of normal fetal physiologic processes, especially those of the central nervous system, where thyroid hormones assist in brain maturation throughout gestation (Neale et al., 2007). Thyroid hormones regulate genes involved in myelination and neuronal/glial cell differentiation (Bernal, 2005). Delivery of thyroid hormones to the fetal brain is a complex process requiring, at different times, expression of brain thyroid hormone receptors, placental thyroid hormone and iodide transport, a feedback system that involves the hypothalamic–pituitary–thyroid (HPT) axis, and thyroid hormone metabolism by liver and brain deiodinase enzymes (deiodinase type 2 and deiodinase type 3), which ensure basal levels are sustained (Zoeller et al., 2007).

The thyroid is the first endocrine gland to develop in the embryo. It is derived from contributions of two anlagen: a midline thickening of the pharyngeal floor (median anlage) that gives rise to the T_4 -producing follicular cells and paired caudal extensions of the fourth pharyngobranchial pouches (lateral anlagen) that act as the precursor to the parafollicular calcitonin-secreting cells (C cells). The thyroid begins its development by 24 days' gestation when the median anlage forms the thyroid diverticulum that extends from the floor of the buccal cavity to the fourth branchial arch. At 24 to 32 days the median anlage becomes a bilobed structure. By 50 days the median and lateral anlagen have fused and the buccal stalk has ruptured. During this period the thyroid gland migrates caudally from the pharyngeal floor to the developing hyoid bone and laryngeal cartilages, where it reaches its final location in the anterior neck below the thyroid cartilage. During its descent the thyroid gland retains an attachment to the pharynx by a narrow epithelial stalk known as the *thyroglossal duct* that disappears by 7 weeks' gestation. Abnormalities in thyroid descent result in ectopic thyroid, persistent thyroglossal duct, or cyst. The genes involved in thyroid gland development include *HEX*, *TTF1*, *FOXE1*, *NKX2-5*, and *PAX8*. Defects in these genes can result in varied abnormalities in thyroid gland development and congenital hypothyroidism (Kratzsch and Pulzer, 2008).

By 10 to 11 weeks, clusters of endodermal epithelial cells form single layers around lumens, the thyroid follicles, in which colloid begins to appear. At 4 weeks the fetal gland is able to synthesize

thyroglobulin; 6 to 8 weeks later the thyroid synthesizes thyroid hormones mediated by the incorporation of iodine. This early growth and development appears to be independent of TSH, as secretion of TSH cannot be shown before 10 to 12 weeks. As a consequence of maturation of the hypothalamic–pituitary–thyroid axis, TSH levels increase rapidly thereafter. Similarly, total T_4 and free T_4 levels increase significantly in the second and third trimesters. The fetal pituitary–thyroid feedback mechanism appears to be fully responsive by 18–20 weeks. Hypothyroidism will result in elevated fetal TSH production, whereas hyperthyroidism caused by maternal Graves disease (due to transplacental passage of maternal thyroid-stimulating autoantibodies) will cause suppressed TSH production.

Prematurity can result in low thyroid hormone levels, and the severity correlates inversely with the gestational age because of the immaturity of the hypothalamic–pituitary–thyroid system. Fifty percent of preterm infants born before 28 weeks' gestation will have transient hypothyroxinemia of prematurity identified by low T_4 and normal TSH levels that do not require treatment. Very low-birthweight infants are at higher risk of developing primary hypothyroidism and can have a delayed TSH level rise. Thyroid hormone treatment is indicated in these infants, although it can be a transient problem (Dattani and Gevers, 2016).

Small amounts of maternal thyroid hormones cross the placenta, and adequate functioning of both maternal and fetal thyroid glands is important for normal fetal development. In congenital hypothyroidism because of thyroid agenesis (athyreosis), maternal thyroid hormones lessen the impact on fetal neurologic development. Early neonatal diagnosis and T_4 treatment permit normalized growth and development. In contrast, when the mother is hypothyroid throughout gestation, the developmental consequences are more severe (Glinioer and Delange, 2000).

Reproductive Axis Development

Similar to other hypothalamic–pituitary–end gland systems, early gonadal development is completely separate from neuroendocrine development and thus will be discussed separately. GnRH neurons, the hypothalamic neurons that induce gonadotrophs to synthesize and secrete LH and FSH, are unique among hypothalamic neurons in that they arise outside the neuroectoderm in the olfactory pit. This occurs at week 6 after conception. The GnRH neuron begins to synthesize and secrete GnRH shortly thereafter. The neurons then migrate via the forebrain, arriving at the hypothalamus by week 9 (Schwanzel-Fukuda et al., 1996). The location, number of GnRH neurons, and projection to the median eminence are similar to those in adult animals. Migration is supported by the signaling protein products of the genes *ANOS1* (anosmin 1), *PROK1* (prokineticin 1), and *FGF8* (fibroblast growth factor 8), as well as receptor encoding genes such as *FGFR1*, *PROKR2*, and *CHD7* (Bhagavath and Layman, 2007).

Before gestational week 6 the male and female genital duct systems coexist with an indifferent (bipotential) gonad. The specifics of sex-specific gonadal differentiation and associated disorders are discussed in Chapter 97. In brief, the presence or absence of the Y chromosome, and thus the gene *SRY*, is the determining factor directing development of the bipotential gonad into either testes or ovaries. *SRY* is expressed by week 6 of gestation. *SRY* directs the expression of many genes that direct the differentiation of the bipotential gonad into the Leydig (testosterone-producing) cells of the testes as well as the Sertoli cells (Achermann and Hughes, 2016). *SRY* also induces expression of the gene *SF1*. *SF1* in turn

acts to induce expression of anti-müllerian hormone, which induces regression of the female (müllerian) duct structures. Müllerian structures include the fallopian tubes, uterus, and upper third of the vagina. In the presence of two normal X chromosomes, ovarian differentiation commences, although less is known about ovarian development. Formation of the bipotential gonad into testes or ovaries is complete by week 8 of gestation, while differentiation of the genital duct systems into the male or female internal reproductive organs is complete by week 9 of gestation (Achermann and Hughes, 2016).

External development of male or female structures is determined by the presence or absence of testosterone (and thus the *SRY* gene). Further conversion of testosterone to dihydrotestosterone (DHT) is necessary for normal development of the penis and prostate gland. In the absence of DHT, the lower two-thirds of the vagina and the labia minora and majora develop. Placental human chorionic gonadotropin directs testosterone secretion by the ever-expanding Leydig cell population after week 14 of gestation. The testosterone formed in effect directs formation of the external genitalia. LH and FSH reach peak levels at midgestation, about 20 to 24 weeks. The testes and ovaries are responsive to LH and FSH by week 20, and some primary follicles are seen in the ovaries. However, placental estrogen predominates, so estrogen secretion by the fetal ovary is thought to be minimal. LH-directed testosterone synthesis is required for normal testicular descent from the abdomen into the scrotal sac in late gestation as well as for further lengthening the penis in late gestation. By the last half of the third trimester, placental estrogen production provides negative feedback to the axis to cause LH and FSH levels to decrease (Kaplan et al., 1976).

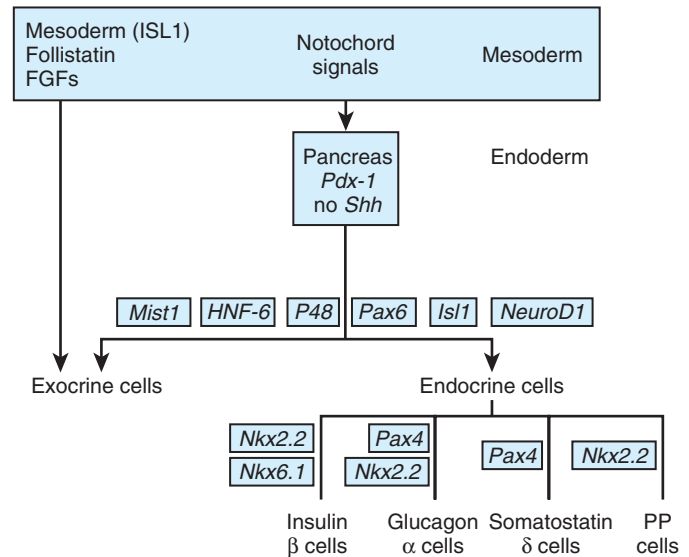
Development of the Endocrine Pancreas

The pancreas is derived from two buds, dorsal and ventral, that arise from the distal foregut endoderm. It contains a distinctive combination of cell lineages. The exocrine tissue comprises acinar cells that secrete digestive fluid and a duct system by which the fluid drains into the intestine. The endocrine part is arranged as discrete islets of Langerhans, which contain distinct cell types secreting different hormones into the circulation (α cells, glucagon; β cells, insulin; δ cells, somatostatin; ϵ cells, ghrelin; and γ cells, pancreatic polypeptide) (Jennings et al., 2015).

Human pancreas formation is first evident at 26 days' gestation, and it begins with the dorsal bud formation, which is followed by the appearance of two ventral buds at 30 days. The left ventral bud gradually regresses, whereas the right ventral bud migrates posteriorly and fuses with the dorsal bud on gut rotation at 6 to 7 weeks' gestation. Failure of the left ventral bud to regress could lead to an annular pancreas. The dorsal pancreas gives rise to most of the pancreas, including the upper part of the head, the isthmus, the body, and the tail of the pancreas. The right ventral bud gives rise to the inferior part of the head (Pan and Brissova, 2014).

Starting from day 45 to day 47 of gestation, the pancreatic epithelium undergoes active growth and branching morphogenesis to give rise to endocrine and acinar cells under the influence of locally acting signals and activation of lineage-specific transcription factors (Peters et al., 2000). During this process, pancreatic epithelium is embedded in the loose mesenchyme and is surrounded by a dense peripancreatic mesenchyme. At approximately 7 to 8 weeks the epithelium begins to ramify and starts to form a lobular pattern (Pan and Brissova, 2014).

The first endocrine cells that appear are insulin-expressing cells at 7.5 weeks, and they remain the most prevalent endocrine cell



• **Fig. 94.3** Molecular Control of Cell Fate Choices in the Pancreas. *Pax6* and *Isl1* are expressed early, and their disruption leads to the reduction or absence of endocrine differentiation, which suggests that they could be expressed in endocrine progenitors able to give rise to all cell types. In *Isl1*-mutant mice, *Pax6* is not expressed, which suggests *Pax6* is downstream of *Isl1*. Although *Pax4*, *NeuroD1*, *Nkx2.2*, and *Nkx6.1* are expressed as early as *Pax6* and *Isl1*, they affect the differentiation of only a subset of lineages. *Pax4* is required for glucagon-producing and somatostatin-producing cell differentiation. *Nkx2.2* is expressed in all islet cells, except somatostatin-producing cells, and its inactivation leads to the absence of the cell types where it is expressed. *Nkx6.1* is itself required for insulin cell differentiation. Although *Mist1* and *Onecut1* (also known as *Hnf6*) are expressed in endocrine cell lineages, their function has not yet been assessed by inactivation experiments. *FGFs*, Fibroblast growth factors; *PP*, pancreatic polypeptide. (From Grapin-Botton A, Melton DA. Endoderm development: from patterning to organogenesis, *Trends Genet.* 2000;16:124–130.)

type during the first trimester (Piper et al., 2004). This is followed by the appearance of glucagon- and somatostatin-expressing cells at week 8 and pancreatic polypeptide-expressing and ghrelin-expressing cells at week 9. Ongoing β -cell proliferation and differentiation are dependent on insulin-like growth factor type 2 (*IGF2*) expression. Islet formation begins by week 12. Islet volume increases from about 4% to 13% of total pancreatic tissue by term (Peters et al., 2000). Targeted gene deletion studies in mice have demonstrated critical roles for several transcription factors in pancreatic endocrine development (Fig. 94.3).

Neonatal insulin kinetics and end-organ sensitivity to insulin appear to be established during the third trimester in preparation for extrauterine fuel metabolism. The maternal environment and fetal genome appear to influence the number and/or function of pancreatic β cells in early life, with lifelong implications for postnatal diabetes. In contrast, insulin gene-knockout mice and human newborns with pancreatic agenesis experience severe intrauterine growth restriction (IUGR), consistent with insulin's role in mitogenesis and growth.

Development of the Parathyroid Glands and Fetal Mineral Homeostasis

The parathyroid glands arise from the third and fourth pharyngeal pouches. The third pouches develop into the inferior parathyroid

glands, and the fourth pouches develop into the superior glands. Fetal parathyroid glands produce low amounts of parathyroid hormone (PTH) throughout gestation that can be detected as of 10 weeks. It is unclear if they also make PTH-related protein (PTHrP), which is mainly produced in the placenta. Other hormones involved in mineral homeostasis include calcitonin, which is detectable in fetal thyroidal C cells as early as 14 weeks. The fetal renal tubules can synthesize the active form of vitamin D, 1,25-dihydroxyvitamin D (calcitriol), as early as the second half of gestation, but the fetal levels remain low.

Mineral and bone metabolism is regulated differently in utero compared with the adult. The placenta meets the fetal mineral need by actively transporting calcium, phosphorus, and magnesium from the maternal circulation to maintain higher fetal concentrations compared with maternal levels. These high levels are necessary for the developing skeleton to accrete a normal amount of mineral by term. A human fetus typically accumulates approximately 30 g of calcium by term, with 80% of that mineral content obtained in the third trimester.

PTH and PTHrP are critical for fetal bone development, regulation of serum minerals, and placental mineral transfer, while calcitriol and calcitonin are not required. PTH and calcitriol circulate at low concentrations in the fetal circulation. PTH is suppressed by the high fetal serum calcium level. Low calcitriol concentration is caused by low PTH concentration, high calcium concentration, and rapid clearance (Kovacs, 2014).

Hormonal Regulation of Fetal Growth

Hormones that play an important role in postnatal growth, such as thyroid hormone, growth hormone, and gonadal steroids, play a very limited role in fetal growth. As such, neonates with these hormonal disorders are generally born with normal weight and size. Fetal growth is driven by nutritionally dependent growth factors such as insulin, or other growth factor systems such as IGF1, IGF2, and the epidermal growth factor (EGF)–transforming growth factor (TGF) system.

Insulin

Insulin is an important fetal growth factor, as evident by the hyperinsulinemia and macrosomia experienced by infants born to women with uncontrolled diabetes mellitus. In contrast, infants with pituitary agenesis and corresponding lack of insulin are small at birth, with minimal muscle bulk and adipose tissue (Schwitzgebel et al., 2003).

Insulin-Like Growth Factor 1

The importance of IGF-1 in fetal growth is derived from studies of humans and mice with *IGF1* or IGF-1 receptor (*IGF1R*) mutations. Humans with mutations in these genes have IUGR, microcephaly, hypoglycemia, and severe developmental delay (Cooke et al., 2016). Mice null for the *IGF1* gene or the *IGF1R* gene are 60% of normal weight and die shortly after birth because of impaired lung maturation.

Insulin-Like Growth Factor 2

The importance of IGF-2 to fetal somatic and organ growth is manifest by the phenotypes of Beckwith–Wiedemann syndrome (BWS) and Russell–Silver syndrome (RSS). The *IGF2* gene is

normally imprinted, such that only the paternal allele is active. In BWS, there is either loss of imprinting, such that the maternal allele is also active, or there are two copies of the active, paternal allele (uniparental disomy). Infants with BWS have *IGF2* overexpression and have resultant fetal and postnatal overgrowth. This overgrowth is manifest as macrosomia, enlarged tongue, β -cell hypertrophy with hyperinsulinemic hypoglycemia, and an increased risk of certain childhood cancers. In contrast, RSS may be due to duplication of the maternal allele or silencing of the paternal allele via imprinting. These infants have IUGR with postnatal growth retardation, with adult height -4 standard deviations from the mean (Cooke et al., 2016). Other clues to the diagnosis of RSS include hemihypertrophy, clinodactyly, triangular facies, and normal head circumference.

Epidermal Growth Factor–Transforming Growth Factor System

The role for the EGF–TGF system in fetal and somatic growth is derived from rodent studies and in vitro studies. EGF and TGF have 40% homology in amino acid structure to each other and can bind each other's receptor. EGF, TGF, and their receptors are expressed in all mammalian tissues studied, including placenta, and the expression increases with gestation in fetal rodents and humans. In vitro, treatment with EGF or TGF induces neuronal cell proliferation as well as placental cell proliferation. Embryos of mice null for EGF/TGF or the receptors are smaller than wild-type embryos (Dattani and Gevers, 2016). In late gestation, they have maldeveloped midbrain, hindbrain, and ventral forebrain.

Placental Factors

Human placental lactogen is a major regulator of maternal glucose, amino acid, and lipid metabolism during pregnancy, allowing mobilization of these fuel sources for use by the fetus (Newbern and Freemark, 2011). Alterations in maternal nutrition induced by starvation or disease also affect fetal growth. Uterine vasoconstriction or vascular insufficiency of the placenta is also associated with poor fetal growth and neonatal IUGR. Vascular compromise of the placenta may be due to maternal hypertension, drug exposure, infection, or placental abruption.

Developmental Origin of Health and Disease

The developmental origin of health and disease hypothesis is the theory that the perinatal environment (conception to infancy into toddlerhood) directly influences the development of metabolic and cardiovascular disease during adulthood. This hypothesis, also termed the *Barker hypothesis*, was first proposed in the 1990s by David Barker as he attempted to account for the higher rates of heart disease in poorer areas of Britain (Barker, 2007). He noted that the geographic areas with higher heart disease rates had higher rates of neonatal deaths in previous decades even accounting for differences in smoking rates. Using longitudinal data from the 15 boroughs of London, he found that the boroughs with the highest neonatal mortality in 1910s to 1925 also had the highest rates of cardiovascular disease. Delving into birthweight data, Barker found low birthweight was associated with an increased risk of death from coronary heart disease. Many studies since Barker's original studies have replicated his results in men and women in Europe, North America, South America, and India. In addition to heart disease, low birthweight has been associated with increased risk

of type 2 diabetes, cerebrovascular disease, hypertension, dyslipidemia, altered puberty, and obesity in adulthood.

The association of low birthweight with metabolic disease led to the proposal that a nutrient supply perceived as limited by the fetus yields a fetal/neonatal “thrifty phenotype” that would allow survival in a nutrient-limited extrauterine environment (Hales and Barker, 2001). The “programming” for an extrauterine life of limited nutrients proves maladaptive in the abundant postnatal nutrition environment. As a corollary to low birthweight, epidemiologic studies have now found that excess maternal weight during pregnancy or excessive weight gain in early infancy (whether experienced by low-birthweight or normal-weight neonates) is also associated with increased risk of metabolic diseases later in life. At highest risk of metabolic disease as adults are individuals with low birthweight and excessive weight gain in infancy and childhood.

Other in utero exposures that have been associated with development of adult disease include increased risk of obesity and mental disorders in offspring of pregnancies complicated by maternal depression, increased risk of metabolic disorders in offspring of mothers with hyperandrogenemia, and increased risk of hypertension in offspring of pregnancies complicated by preeclampsia (Padmanabhan et al., 2016). Emerging epidemiologic data suggest that perinatal exposure to certain endocrine disrupting chemicals (EDCs) is associated with obesity, metabolic disease, cancer, and reproductive defects in offspring. EDCs are chemicals that mimic hormone actions and thus could change maternal, placental, and fetal hormonal milieus. EDCs are categorized into persistent organic pollutants (POPs), which linger in the environment for decades, or short-lived pollutants used in manufacturing. Examples of POPs include organochlorine pesticides such as dichlorodiphenyltrichloroethane, industrial by-products such as polychlorinated biphenyls, and flame retardants such as polybrominated diphenyl ethers. Examples of short-lived pollutants include phthalates and bisphenols, used in plastics and adhesives. EDCs are ubiquitous in the environment, accounting for daily exposure to minuscule amounts. EDC doses and developmental exposure windows that are disease causing are under dispute for many of the EDCs. Controlled exposure studies that would provide a causal relationship between EDC

exposure and human disease are not ethically feasible, and thus disease risk must be surmised from the body of evidence provided by epidemiologic and animal exposure studies.

A number of mechanisms have been proposed to program later disease by environmental influences. These include epigenetic regulation of gene expression; oxidative stress of tissues resulting in damage to DNA, lipids, and proteins; and direct hormonal effects on development. Epigenetic changes that affect gene expression include DNA methylation, histone modification, chromatin packing, and micro-ribonucleic acid expression. These changes are malleable or permanent and can accumulate over time to influence gene expression in the short term or long term. Oxidative stress may lead to inflammation of tissues and chronic maladaptation (Padmanabhan et al., 2016). Finally, an example of direct hormonal effects on development is the direct effect of intrauterine testosterone on gonadal development and, in females, later development of obesity.

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Disorders of Calcium and Phosphorus Metabolism

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KEY POINTS

- Neonatal hypercalcemia may be asymptomatic if there are only mild elevations in calcium level or may lead to severe symptoms such as failure to thrive, polyuria, lethargy, and seizures.
- The causes of hypercalcemia can differ but are due to dysregulation of calcium regulatory systems.
- Treatment of hypercalcemia includes hydration and diuretics, with the use of other agents such as glucocorticoids, calcitonin, calcimimetics, and bisphosphonates in severe cases.
- Metabolic bone disease of prematurity is caused by deficiencies in dietary phosphate and calcium, early withdrawal of placental estradiol and progesterone, lack of mobility, and therapy with medications that can increase urinary calcium excretion and contribute to serum mineral imbalance and osteopenia.
- Osteopenia in preterm infants usually appears between 6 and 12 weeks of age, and fractures may be seen. The incidence and severity increase with decreasing gestational age and birthweight and are more common in preterm infants having a complicated medical course and delayed nutrition.

Homeostatic Control of Calcium and Magnesium

Calcium plays two important physiologic roles. Calcium salts in bone provide structural integrity. Calcium ions present in the cytosol and extracellular fluid (ECF) are essential for maintenance and control of many biological processes, including cell–cell communication, cell aggregation and division, coagulation, neuromuscular excitability, membrane integrity and permeability, enzyme activity, and secretion. This functional diversity is made possible by the maintenance of a large electrochemical gradient between the ECF ionized calcium (Ca^{2+}) concentration, which is in the 1-mmol/L range, and the resting intracellular (cytosolic) Ca^{2+} concentration, which is about 0.1 $\mu\text{mol/L}$.

Significant alterations in serum calcium concentration occur frequently in the neonatal period. It is important to evaluate these potential derangements in light of normal dynamic changes that

occur during the perinatal transition. After the first 2 to 3 days, normal total serum calcium concentrations vary only slightly with age and a range between 8.8 and 10.6 mg/dL (2.2–2.6 mmol/L), with an ionized serum calcium concentration of 4 to 5.6 mg/dL (1–1.4 mmol/L). The metric measure unit conversion factors are 0.2595 and 0.2495, respectively, as used everywhere other than the United States.

Approximately 55%–60% of the total plasma calcium is diffusible (or ultrafilterable), the remainder being protein bound. Most diffusible calcium is ionized, but about 5% of total circulating calcium is complexed to plasma anions, such as phosphates, citrate, and bicarbonate. Ca^{2+} is the only biologically available fraction of ECF calcium. It is subject to precise metabolic control based on the integrated regulation of calcium fluxes with respect to the intestine, kidneys, and bone. The precise regulation of circulating Ca^{2+} is controlled by calcium itself, through a calcium receptor and several hormones, the most important of which are parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D ($1,25[\text{OH}]_2\text{D}$) (Carmeliet et al., 2003; Ramasamy, 2008; Allgrove and Shaw, 2009).

Hypoalbuminemia leads to a decline in total serum calcium, but proportionate increases in the ionized fraction usually maintain serum Ca^{2+} concentration within the normal range. Acute alkalosis (e.g., hyperventilation or bicarbonate infusion) or rapid administration of citrate-buffered blood (e.g., during exchange transfusion, initiation of extracorporeal membrane oxygenation [ECMO], cardioplegia, or organ transplant) may acutely lower serum Ca^{2+} concentration by increasing albumin binding or citrate chelation. These conditions can produce transient clinical manifestations of hypocalcemia but do not lower the total serum calcium concentration (Clase et al., 2000). Generally, for routine clinical purposes, measurement of total serum calcium concentration often suffices, and the correction formula in the setting of hypoalbuminemia for total serum calcium concentration measured as mg/dL is:

$$\begin{aligned} \text{Corrected Ca Level} \\ = [0.8 \times (\text{Normal Albumin Level} - \text{Patient's Albumin Level})] \\ + \text{Serum Ca Level} \end{aligned}$$

Large amounts of calcium exchange occurs in kidney, bone, and intestine. Although the intestine has considerable calcium absorptive capacity, renal tubular calcium reabsorption usually exceeds intestinal

absorption by at least 40-fold. Most of the tubular Ca^{2+} load is reabsorbed in the proximal tubule and thick ascending limb of the loop of Henle via paracellular, passive flux (coupled with sodium reabsorption) driven by the existing electrochemical gradient. A transcellular pathway in the distal nephron tightly regulates the rest of urinary Ca^{2+} reabsorption. Calcitropic hormones regulate the distal Ca^{2+} -selective, Na^+ -independent channels. More than 98% of total body calcium is deposited in the skeleton as hydroxyapatite ($\text{Ca}_5[\text{OH}][\text{PO}_4]_3$); the ECF and soft tissues contain the remainder. A small fraction of skeletal calcium freely exchanges with the ECF and serves as an important buffer of circulating calcium. Consequently, decreased skeletal calcium is a hallmark of most metabolic bone diseases.

Magnesium homeostasis is largely mediated through the kidneys. Approximately 80% of total plasma magnesium is filtered through the glomerulus and is reabsorbed mainly in cortical segments of the thick ascending limb of the loop of Henle. Once the maximal tubular reabsorption is exceeded, filtered magnesium is excreted into the urine. Hormones regulate magnesium reabsorption by changing the transepithelial voltage and paracellular permeability of tubular cells. Magnesium is required to maintain normal PTH secretory responses.

Homeostatic Control of Phosphorus

Blood inorganic phosphate concentration varies with age. It is highest during infancy and gradually declines to adulthood. Approximately 10% of plasma inorganic phosphate is noncovalently bound to protein, whereas 90% circulates as ions or as complexes with sodium, calcium, or magnesium. About 80%–85% of total body phosphorus contributes to mechanical support as part of the hydroxyapatite lattice of bone. The remainder is distributed in the ECF, largely as inorganic ions or complexes, and in soft tissues as phosphate esters. Intracellular phosphate esters and phosphorylated intermediates regulate cell metabolism and gene expression (via phosphorylase, kinase, and phosphatase activities) and generate and transfer cellular energy (i.e., via adenosine triphosphate). Cytosolic and ECF phosphorus levels (approximately 0.1 and 0.2 mmol/L, equivalent to 0.31 and 0.62 mg/dL respectively) are less stringently regulated than are levels of Ca^{2+} and magnesium (Mg^{2+}).

Dietary phosphate is generally absorbed in proportion to its content in food. Although phosphorus and calcium can be absorbed along the entire length of the small intestine, most phosphate absorption occurs in the jejunum and ileum, whereas most calcium absorption occurs in the duodenum. The renal proximal tubule is the principal regulatory site for phosphorus homeostasis (Silver and Naveh-Many, 2009). Renal regulation is accomplished primarily by variation of the threshold for phosphate reabsorption (the tubular maximum for inorganic phosphate [TmP]/glomerular filtration rate [GFR]). Hormones (PTH, PTH-related protein [PTHrP], growth hormone) and dietary phosphate reset this theoretical threshold by regulating apical tubular Na^+ - TmP cotransporters (Murer and Biber, 1995). Essentially, TmP/GFR is the “setpoint” that defines the fasting serum phosphorus concentration. At lower serum phosphorus levels, most filtered phosphorus is reabsorbed; at higher levels, most filtered phosphorus is excreted. To assess TmP/GFR , a fasting urine specimen is obtained for measurement of phosphorus and creatinine along with simultaneous determination of serum phosphorus and creatinine. A nomogram has been constructed so that TmP/GFR can easily be derived from these values (Walton and Bijvoet, 1975).

The higher serum phosphate levels in infants (e.g., 4.5–9.3 mg/dL) compared with those in adults (3.0–4.5 mg/dL) reflect infants’ greater tubular phosphate resorption. This adaptation permits avid tubular phosphate conservation despite high ambient serum phosphate levels. For this reason, neonatal disorders of chronic hypophosphatemia and/or phosphorus depletion usually result from inadequate dietary supply (as in preterm infants) or intrinsic (e.g., familial hypophosphatemic rickets) or extrinsic (e.g., hyperparathyroidism) alterations in TmP/GFR . Similarly, chronic hyperphosphatemia usually implies either intrinsic (e.g., renal insufficiency) or extrinsic (e.g., hypoparathyroidism) abnormalities in TmP/GFR .

Parathyroid–Renal Hormonal Axis

In mammals, calcium and phosphate homeostasis is controlled by a parathyroid–renal hormonal axis involving PTH and $1,25(\text{OH})_2\text{D}$. The influence of these two hormones on bone deposition, mobilization of mineral, and regulation of intestinal and renal absorption is depicted in Fig. 95.1. Deficiency or excess of either hormone causes hypocalcemia or hypercalcemia, respectively.

Parathyroid Hormones

PTH mobilizes calcium and phosphorus from bone, stimulates calcium reabsorption in kidneys, inhibits phosphorus reabsorption by reducing TmP/GFR , and stimulates the renal synthesis of $1,25(\text{OH})_2\text{D}$, which participates with PTH in calcium reabsorption in kidneys, increases the efficiency of intestinal absorption of calcium and phosphorus, and mobilizes calcium from bones. Therefore PTH secretion causes the serum calcium concentration to rise and the serum phosphorus concentration to be maintained or decline.

PTH is a 9500-Da, single-chain polypeptide. It is synthesized by the four parathyroid glands embedded within the thyroid gland poles, which are derived from the embryonic third and fourth pharyngeal pouches. The messenger ribonucleic acid (mRNA) for PTH (preproPTH) encodes the 84 amino acids of the mature peptide, an amino-terminal (N-terminal) “pre” sequence of 25 amino acids, and a basic “pro” hexapeptide, which is clipped intracellularly. After secretion, the intact PTH molecule, PTH (1–84), is further metabolized and rapidly cleared from the circulation, with a half-life of less than 4 minutes. The N-terminal region of the PTH molecule, PTH(1–34), binds the PTH receptor and shows full biological activity, whereas the carboxyl terminal (C terminal) has specific, albeit poorly understood, activities in osteoclasts and osteoclastic precursors.

Secretion of PTH fragments by the parathyroid glands and prolonged clearance of the C-terminal PTH metabolites add considerable immunoheterogeneity to circulating PTH. The numerous inconsistencies found in reports on PTH pathophysiology until the late 1980s are due to use of earlier generation “C-terminal” and “midmolecule” PTH assays. In contrast, current two-site “intact PTH” assays are sufficiently sensitive and specific to detect physiologic levels of biologically active PTH(1–84) and to distinguish hypoparathyroid from euparathyroid states. The normal circulating levels of intact PTH range from approximately 10 to 60 picogram (pg)/mL; the maximally stimulated (hypocalcemic) and maximally suppressed (hypercalcemic) levels for normal parathyroid function are about 100 to 150 pg/mL and 2 to 5 pg/mL, respectively.

Parathyroid cells are exquisitely responsive to changes in ambient Ca^{2+} concentration. PTH secretion may be described as an inverse sigmoid hysteretic relationship between serum PTH and Ca^{2+} with

TABLE 95.1 Other Hormones

| | Serum Calcium Concentration | Intestinal Calcium Absorption | Renal Calcium Excretion | Bone Effects |
|------------------------------|-----------------------------|-------------------------------|-------------------------|---------------------------|
| Glucocorticoids | Increase | Decrease | Increase | |
| Growth hormone | | Increase | | |
| Insulin-like growth factor 1 | | | | Protein synthesis in bone |
| Insulin | | | | Bone loss |
| Thyroid hormone excess | Increase | | Increase | Osteoporosis |

or provitamin D, the immediate precursor of cholesterol, to form a sterol, previtamin D. Previtamin D in the skin undergoes isomerization to the biologically inert vitamin D. Vitamin D enters the circulation bound to vitamin D-binding protein and is transported to the liver, where a mitochondrial cytochrome P450 vitamin D 25-hydroxylase produces 25(OH)D. 25(OH)D (provitamin D) is the major circulating vitamin D metabolite. Because activity of hepatic 25-hydroxylase is not tightly regulated, measurement of serum 25(OH)D is a useful assessment of vitamin D stores. In renal proximal tubule cells, mitochondrial 25(OH)D 1 α -hydroxylase metabolizes 25(OH)D to the biologically active hormone, 1,25(OH)₂D. The normal circulating level of 25(OH)D is approximately 10 to 50 ng/mL. The normal circulating concentration of 1,25(OH)₂D ranges from 30 to 75 pg/mL, or about 1/1000 that of 25(OH)D.

Serum 25(OH)D levels are increased by sunlight exposure and by vitamin D ingestion and are decreased in vitamin D deficiency and in hepatobiliary disorders. Circulating 1,25(OH)₂D levels are increased by hyperparathyroidism and phosphate depletion and are reduced in hypoparathyroidism. 1,25(OH)₂D is biologically inactivated through a series of reactions beginning with 24-hydroxylation. 1,25(OH)₂D induces the 24-hydroxylase in vitamin D target cells. Hypocalcemia, by increasing PTH levels, suppresses this enzyme. 24-Hydroxylase metabolizes 25(OH)D as well as 1,25(OH)₂D. In vitamin D-sufficient states, the kidney preferentially 24-hydroxylates the prohormone, 25(OH)D, to 24,25-dihydroxyvitamin D (24,25[OH]₂D). In contrast, when vitamin D action is required, 25(OH)D 1 α -hydroxylase is preferentially activated for 1,25(OH)₂D synthesis.

The parathyroid-renal (PTH-1,25[OH]₂D) axis, reminiscent of the hypothalamic-pituitary-adrenal axis, is the principal means for systemic response to a sustained or major hypocalcemic challenge. In this long-loop feedback system, 1,25(OH)₂D-mediated calcium absorption provides the ultimate feedback on PTH secretion. PTH secreted in response to hypocalcemia is the principal regulator of renal production of 1,25(OH)₂D, which, in turn, feeds back to suppress PTH gene expression (see Fig. 95.1). Hypocalcemia directly stimulates PTH mRNA transcription. PTH regulates minute-to-minute perturbations of ECF Ca²⁺ concentration. Maximal adjustments of intestinal calcium absorption via the PTH-1,25(OH)₂D axis require 1 to 2 days to become fully operative, so 1,25(OH)₂D effects come into play only when a hypocalcemic stress persists.

Calcitonin

Calcitonin, a peptide hormone synthesized by thyroid parafollicular C cells (also known as *clear cells*) has an antihypercalcemic

effect—that is, opposite that of PTH. Human calcitonin is a 32-amino-acid chain with a 1,7-disulfide bridge and a C-terminal prolinamide. Alternative splicing of several transcripts from the calcitonin gene produces several polypeptide products, some of which have uncertain calcitropic importance. The primary stimulus for calcitonin secretion is a rise in circulating calcium concentration. Calcitonin lowers serum calcium and phosphorus concentrations mainly by inhibiting bone resorption and by increasing calcium excretion in the kidneys (Table 95.1).

Glucocorticoids lower serum calcium concentration by inhibiting osteoclast formation and activity, but use for a long time causes osteoporosis by decreasing bone formation and increasing bone resorption. They also decrease intestinal absorption and increase renal excretion of calcium and phosphorus. Because of these mechanisms glucocorticoids depress hypercalcemia in vitamin D intoxication and subcutaneous fat necrosis.

Growth hormone increases calcium excretion in the kidney and intestinal absorption. Insulin-like growth factor 1, generated by growth hormone action, stimulates protein synthesis in bone. Insulin increases bone formation, with observed bone loss in patients with uncontrolled diabetes mellitus.

Thyroid hormone excess has been described to be associated with hypercalcemia, hypercalciuria, and osteoporosis. The mechanism of these findings is not entirely clear.

Currently, there is no compelling evidence that the calcitonin-like calcium-lowering hormones are critical regulators of calcium homeostasis in nonpregnant adult humans, perhaps because the low prevailing rate of bone turnover blunts the impact of the antiresorptive actions. However, calcitonin may have important calcitropic functions in pregnant and lactating women and in the fetus and neonate, and in other mammals, particularly rodents, whose bones are constantly growing. In human newborns the parafollicular C-cell population and serum calcitonin concentrations are much greater than in adults.

Perinatal Mineral Metabolism

During human pregnancy, approximately 30 g of calcium and more than 16 g of phosphorus are transferred transplacentally from the maternal circulation to the growing fetus during the third trimester, when fetal calcium accretion is approximately 140 to 150 mg/kg per day. In humans a doubling of maternal intestinal calcium absorption and a net increase of calcium accretion into bone compensate for the formidable demand on maternal calcium (Prentice, 2000). A midmolecule PTHrP hormone (Kovacs et al., 1996) expressed principally by the placenta regulates this transplacental calcium pump. TRPV6, a member of the transient receptor

potential channel superfamily, may be the primary calcium channel at the trophoblast apical membrane. Calcium flux across the placenta in *Trpv6*-null mice is reduced by approximately 40% (Suzuki et al., 2008).

Pregnancy

Pregnancy constitutes a unique hormonal milieu that promotes a state of “physiologic absorptive hypercalciuria” (Gertner et al., 1986). Maternal total serum calcium concentration declines slightly during pregnancy, reaches a nadir in the middle of the third trimester, and then increases slightly toward term. The maternal serum phosphorus and magnesium profiles are similar to that of calcium. Maternal serum 25(OH)₂D concentration varies seasonally and with vitamin D intake, but the vitamin D transport protein concentration increases during pregnancy. Serum 1,25(OH)₂D concentrations increase early in pregnancy and continue to rise throughout gestation (Seely et al., 1997). The calculated concentration of free 1,25(OH)₂D also rises. For many years it was believed that PTH levels also increased steadily throughout pregnancy. However, use of newer immunometric “sandwich” assays indicate that PTH concentration actually declines during pregnancy (Davis et al., 1988; Saggese et al., 1991; Seely et al., 1997). PTHrP levels, in contrast, may be higher in pregnant women than in nonpregnant women (Bertelloni et al., 1994). The role of circulating calcitonin in pregnancy is uncertain.

1,25(OH)₂D drives enhanced maternal intestinal mineral absorption (reviewed in Kovacs, 2008). After parturition, 1,25(OH)₂D concentrations and calcium absorption rates (Kent et al., 1991) fall to prepregnancy levels. The interplay of calcitropic and progestational hormones in pregnancy protects the maternal skeleton from demineralization. In contrast, during the relatively low estrogen state of lactation, calcium is mobilized from bone stores, possibly under the influence of PTHrP (Dobnig et al., 1995).

In the third trimester, fetal plasma total and ionized calcium and phosphorus levels are higher than maternal levels, producing a state of “physiologic fetal hypercalcemia” (Rubin et al., 1991). Fetal plasma PTH concentration is low, and calcitonin and PTHrP levels are relatively high. Even these low circulating PTH levels may be functionally important in fetal calcium and magnesium metabolism. There is also a close correlation between maternal and fetal serum 25(OH)₂D levels, consistent with transplacental transfer of this metabolite. Hypocalcemia is commonly found in infants born to women with low circulating 25(OH)₂D levels resulting from poor dietary intake of vitamin D and lack of sunlight exposure. Fetal plasma 1,25(OH)₂D concentration is also relatively low, despite robust renal 25(OH)₂D 1 α -hydroxylase activity, whereas the concentrations of 24,25(OH)₂D are high. The major function of the fetal kidneys in calcium homeostasis may be production of 1,25(OH)₂D rather than renal tubular regulation of calcium excretion. The high circulating concentrations of calcitonin may support this stimulated fetal 25(OH)₂D 1 α -hydroxylase activity. In contrast, the relatively low circulating fetal 1,25(OH)₂D concentrations are a consequence of enhanced placental clearance (Ross et al., 1989). Constitutively activated placental 24-hydroxylase activity (Rubin et al., 1993) also preferentially hydroxylates maternally derived 25(OH)₂D to 24,25(OH)₂D. This placental capacity to metabolize 25(OH)₂D and 1,25(OH)₂D accounts for the enhanced clearance of fetal 1,25(OH)₂D, limits access of placenta-synthesized 1,25(OH)₂D to the fetal and maternal circulations, and, in effect, partitions the maternal and fetal vitamin D pools.

The Neonate

Placental transfer of calcium ceases abruptly at birth. In healthy term newborns, total calcium concentration and Ca²⁺ concentration decline from nearly 11 mg/dL and 6 mg/dL, respectively, in umbilical cord blood to serum levels of 8 to 9 mg/dL and 5 mg/dL, respectively, by 24 to 48 hours. The nadir of Ca²⁺ concentration may range from 4.4 to 5.4 mg/dL. Concomitant rises in the concentrations of PTH and 1,25(OH)₂D stabilize serum calcium concentration as the newborn adapts to extrauterine mineral homeostasis and dietary calcium intake. In preterm infants, calcium absorption from the intestine is nonsaturable and may be vitamin D independent (Bronner et al., 1992). Serum calcitonin levels increase sharply during the first day and remain elevated compared with those in adults. In the mother, prolactin helps stimulate PTHrP expression in lactating breast tissue. PTHrP is secreted into milk at concentrations 10,000-fold higher than in serum. It is possible that the abundant milk PTHrP content ingested by the neonate is important for mineral regulation. By 2 weeks of life, serum calcium concentration rises to the mean values observed in older children and adults.

During the first week of life, urinary phosphate excretion is significantly higher in preterm newborns than in term newborns but then approximates that of term newborns, possibly owing to accelerated postnatal renal maturation. Calcium excretion is low during the first week, when the newborn must compensate for the postpartum fall in serum calcium concentration. After the first several days, calcium excretion increases with a magnitude inversely proportional to gestation. The high urinary calcium-to-creatinine ratio (UCa/Cr) of young infants then steadily declines with age (Sargent et al., 1993). However, in preterm breastfed infants who are more than 2 weeks old, the UCa/Cr can exceed 2.0 (Karlen et al., 1985). These changes may reflect the relative phosphate deficiency in many preterm infants, which results in an adaptively low urinary phosphate excretion, decreased bone mineralization, and, consequently, relatively high urinary calcium excretion.

Key points:

- Onset of neonatal hypocalcemia can present early or late during the neonatal period and depends on the underlying causes.
- Neonatal hypocalcemia may be asymptomatic if mild but may lead to severe symptoms such as hypotonia, seizures, or cardiac arrhythmia.
- Treatment of hypocalcemia includes intravenous or oral calcium supplement depending on the severity.

Neonatal Hypocalcemia

The definition of hypocalcemia depends on gestational age and birthweight. A precise definition of hypocalcemia, like hypoglycemia, in preterm infants is particularly difficult to formulate. Neonatal hypocalcemia has been defined as a serum calcium level below 8 mg/dL (2 mmol/L) in term infants or below 7 mg/dL (1.75 mmol/L) in preterm infants. It is also defined as a Ca²⁺ level less than 3.0 to 4.4 mg/dL (<0.75 to 1.10 mmol/L) (Jain et al., 2010). Under conditions of normal acid–base status and normal serum albumin concentration, serum total calcium and Ca²⁺ levels are linearly correlated, so total serum calcium measurements remain useful as a screening test. However, because Ca²⁺ is the physiologically active fraction, in sick infants it may be preferable to assay Ca²⁺ directly in freshly obtained blood samples. The causes of neonatal hypocalcemia are classified by the timing of onset. Early hypocalcemia occurs in the first 2 to 3 days of life. “Early” and

• BOX 95.1 Causes of Neonatal Hypocalcemia**Early-Onset Hypocalcemia (<48 Hours of Age)**

- Prematurity
- Perinatal distress/asphyxia
- Infants of diabetic mothers
- Intrauterine growth restriction

Late-Onset Hypocalcemia (First Week of Life)

- High phosphate load with or without hypoparathyroidism or vitamin D deficiency

Neonatal Hypoparathyroid Syndromes

- Parathyroid agenesis
- DiGeorge syndrome (22q11.2 deletions)
- Familial isolated hypoparathyroidism
- PTH* mutations

Autosomal Dominant Hypocalcemic Hypocalciuria

- Activating mutations of Ca^{2+} -sensing receptor

Neonatal Hypoparathyroidism Secondary to Maternal Hyperparathyroidism**Autoimmune Polyglandular Syndrome Type 1 (Autoimmune**

- Polyendocrinopathy–Candidiasis–Ectodermal Dystrophy)

Hypoparathyroidism Associated With Skeletal Dysplasias

- Kenny–Caffey syndrome
- Hypoparathyroidism–retardation–dysmorphism (Sanjad–Sakati) syndrome
- Osteogenesis imperfecta type II

Parathyroid Hormone Resistance (Transient Neonatal

- Pseudohypoparathyroidism)

Hypomagnesemia With or Without Distal Renal Tubular Acidosis

- Primary hypomagnesemia
- Renal tubular acidosis type 1

Abnormal Vitamin D (1,25-Dihydroxyvitamin D) Production or Action (“Hypocalcemic Rickets”)

- Vitamin D deficiency (secondary to maternal vitamin D deficiency)
- Acquired or inherited disorders of vitamin D metabolism
- Resistance to the actions of vitamin D

Hyperphosphatemia

- Excessive dietary phosphate
- Phosphate-containing enemas
- Rhabdomyolysis-induced acute renal failure
- Hyperphosphatemic renal insufficiency

“Hungry Bones Syndrome” (Mineralization Outpacing Osteoclastic Bone Resorption)**Other Causes**

- Metabolic or respiratory alkalosis
- Phototherapy
- Long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency
- Pancreatitis
- Sepsis, septic shock
- Rotavirus gastroenteritis
- Osteopetrosis and other skeletal dysplasias
- Pseudohypocalcemia (hypoalbuminemia)
- Medications
 - Bicarbonate
 - Rapid transfusion or plasmapheresis with citrated blood
 - Furosemide induced
 - Lipid infusions

“late” occurring hypocalcemia (Box 95.1) have different causes, usually occur in different clinical settings, and should prompt different approaches to evaluation and management.

Clinical Findings

Most infants with hypocalcemia are asymptomatic. Hypocalcemic signs in neonates are variable and may not correlate with the magnitude of the decline in calcium level. Calcium ions couple excitation and contraction in skeletal and cardiac muscle, so

increased neuromuscular excitability (tetany) is a cardinal feature of hypocalcemia. Such infants are jittery and hyperactive and frequently exhibit muscle jerks and twitches that are induced by environmental noise or other stimuli. Generalized or focal clonic seizures may occur. Other signs of neonatal tetany include poor feeding, hypotonia, apnea, tachycardia, tachypnea, high-pitched cry, and irritability (Thomas et al., 2012). Occasionally, respiratory or gastrointestinal rather than neurologic signs predominate. Rare presentations include inspiratory stridor caused by laryngospasm, wheezing caused by bronchospasm, or vomiting possibly resulting from pylorospasm, which may cause hematemesis or melena. At times the gastrointestinal signs are severe enough to mimic those of intestinal obstruction (Tohme and Bilezikian, 1993). Carpopedal spasm and Chvostek sign are not as reliably elicited in hypocalcemic newborns as in older children or adults. Hypocalcemia characteristically causes prolongation of the QT interval in the electrocardiogram (Benoit et al., 2005). Hypocalcemia prolongs phase 2 of the action potential and is associated with early afterrepolarizations and triggers dysrhythmias.

Early Neonatal Hypocalcemia

Hypocalcemia occurring during the first 3 days of life, usually between 24 and 48 hours postpartum, is termed *early neonatal hypocalcemia*. It is an exaggeration of the normal decline in circulating calcium concentration. Early neonatal hypocalcemia is typically encountered in any of four circumstances: prematurity, severe perinatal stress or asphyxia, maternal diabetes, or significant intrauterine growth restriction (IUGR).

In preterm infants there is a steeper and more rapid postnatal decline in serum calcium concentration. The magnitude of the depression is inversely proportional to gestational age. Approximately one-third of premature infants and most very low-birthweight (VLBW) infants have low total serum calcium level (<7.0 mg/dL) during the first 2 days after birth. However, the fall in Ca^{2+} concentration is not proportional to the fall in total calcium concentration, and the ratio of ionized to total calcium in these newborns is higher than at term. This “sparing” of Ca^{2+} may be related to the lower serum protein concentration and pH in prematurity. Multiple factors contribute to the fall in total serum calcium concentration, including low intake of milk, impaired response to PTH, and hypoalbuminemia, which does not lower the Ca^{2+} concentration. The sparing effect on Ca^{2+} concentration explains the frequent absence of hypocalcemic signs in preterm infants.

The neonatal parathyroid glands, regardless of the degree of prematurity, can mount an appropriate PTH response to hypocalcemia. Hypocalcemia in extremely preterm newborns (Rubin et al., 1991) or in infants undergoing cardiac bypass (Robertie et al., 1992) stimulates increases in serum PTH level at least as great as those seen in adults during citrate-induced hypocalcemia (Grant et al., 1990). PTH resistance plays an uncertain role in early neonatal hypocalcemia. A several-day delay in the phosphaturic and renal cAMP responses to PTH has inconsistently been reported, suggesting that there might be a maturational delay in renal responses to PTH. Calcitonin concentration usually peaks at 12 to 24 hours of life. The preterm infant’s exaggerated rise in calcitonin concentration may also promote hypocalcemia (Jain et al., 2010).

Early neonatal hypocalcemia with hyperphosphatemia is frequently observed in severely stressed or asphyxiated infants. Possible mechanisms include increased phosphate load caused by tissue catabolism, renal insufficiency, and acidosis. There is an exaggerated serum calcitonin response and elevated PTH levels. Low serum

Ca^{2+} and elevated serum magnesium levels have been correlated with the severity of hypoxic–ischemic encephalopathy and poor outcome (Ilves et al., 2000).

Hypocalcemia occurs in at least 10%–20% of infants of diabetic mothers (IDMs) (Rosenn et al., 1996). IDMs show an exaggerated postnatal drop in circulating calcium levels compared with gestational-age controls that typically occurs between 24 and 72 hours after birth and is often associated with hyperphosphatemia. The course is usually similar to that of early neonatal hypocalcemia in preterm infants, although hypocalcemia sometimes persists for several additional days. The greater bone mass and relative undermineralization typical of macrosomic IDMs may increase the neonatal demand for calcium, producing a deeper and prolonged decline in postnatal serum calcium levels. In addition, magnesium deficiency might play a significant role. Urinary magnesium losses occur with glycosuria, which may lead to significant maternal magnesium deficiency followed by fetal magnesium deficiency. Magnesium deficiency in these infants can lead to decreased PTH production and action (Banerjee et al., 2003; Rubin et al., 1991). Similar mechanisms may come into play in the transient hypocalcemia often observed in small-for-gestational-age infants. Hypercalcitonemia, hypoparathyroidism, abnormalities in vitamin D metabolism, and hyperphosphatemia have all been implicated, but none has been consistently found.

Neonatal hypocalcemia in IDMs has been associated with the severity and duration of maternal diabetes and inadequate glycemic control. Not surprisingly, preterm IDMs who have sustained IUGR and asphyxia as a result of uteroplacental insufficiency invariably become quite hypocalcemic. In recent years, improved metabolic control for pregnant diabetic women has markedly diminished the occurrence and severity of early neonatal hypocalcemia (Demarini et al., 1994). Healthy IDMs who are able to start milk feedings on the first day do not require serum calcium monitoring unless suspicious signs (e.g., jitteriness, stridor) are noted.

Hypocalcemia occurs with increased frequency in infants with IUGR. The mechanism is thought to involve decreased transfer of calcium across the placenta as a result of uteroplacental insufficiency.

Late Neonatal Hypocalcemia

Late neonatal hypocalcemia develops after 3 to 5 days of life and typically occurs at the end of the first week. It occurs more frequently in term than in preterm infants and is not usually associated with maternal diabetes, birth trauma, or asphyxia. Historically, late-onset hypocalcemia is associated with ingestion of cow's milk or formula with a high phosphate load (Institute of Medicine Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 1997). A previous review reported that excess phosphorus intake can disturb the absorption of calcium (Kim, 2007). The high phosphate level increases calcium deposition in bone and antagonizes PTH secretion and action, leading to hypocalcemia (Venkataraman et al., 1985). However, the widespread use of high-phosphorus formula in the United States and elsewhere contrasts with the rarity of this condition, which suggests that infants who develop phosphate-induced late neonatal hypocalcemia may have an otherwise undetected renal phosphate excretion problem.

The hyperphosphatemia may also result from various combinations of immature renal tubular phosphate excretion, transiently low levels of circulating PTH, hypomagnesemia, and inadequate maternal vitamin D intake. A relatively high dietary phosphate load coupled with a low GFR leads to an increase in serum phosphate levels and a reciprocal decline in serum calcium levels.

The physiologic response to hypocalcemia is an increase in PTH secretion, leading to increased urinary phosphate excretion and tubular calcium resorption. Serum calcium levels frequently increase when these infants are placed on a low-phosphate formula and supplemental calcium diet. After several days to weeks, serum PTH level usually increases, and the infants then can tolerate more dietary phosphate. The pathogenesis of this “transient hypoparathyroidism” in late neonatal hypocalcemia is not readily apparent. Some of these infants show a persistent or recurrent inability to mount an adequate PTH response to a hypocalcemic challenge, indicating partial hypoparathyroidism.

In other infants, maternal vitamin D deficiency can cause late (or occasionally “early”) neonatal hypocalcemia. This possibility is checked by measurement of maternal and neonatal serum 25(OH) D levels. Maternal vitamin D deficiency is implicated by the increased incidence of late neonatal hypocalcemia in winter due to inadequate sunlight exposure. The high prevalence of enamel hypoplasia of incisor teeth reported in affected infants indicates that the mineralization defect begins during the third trimester of pregnancy.

Hypocalcemia and hyperphosphatemia after the first 3 to 5 days always should prompt a thorough investigation for the underlying causes (Box 95.1). Hypocalcemia in this setting usually implies primary or secondary dysregulation of (1) the parathyroid–renal (PTH–1,25[OH]₂D) axis, (2) hypomagnesemia, or (3) renal insufficiency. The primary hormonal and end-organ disturbances that cause neonatal hypocalcemic syndromes are described later. As a cautionary note, observations of generally favorable neurologic outcomes in newborns with hypocalcemic or hypomagnesemic seizures may be valid for those who have a nutritional cause but are less relevant to patients with associated medical conditions. In this group, neurologic prognosis may be more closely related to the causative disorder (Lynch and Rust, 1994).

Hypocalcemia Caused by Hypoparathyroid Syndromes

The biochemical hallmarks of hypoparathyroidism are hypocalcemia and hyperphosphatemia in the presence of normal renal function. Serum PTH concentrations are inappropriately low or undetectable. Cytogenetic and molecular genetic diagnosis permits characterization of several types of congenital hypoparathyroidism. Isolated hypoparathyroidism is usually sporadic but may show X-linked, autosomal recessive, or autosomal dominant inheritance.

Congenital hypoparathyroidism is a common feature of DiGeorge syndrome (DGS). This disorder arises from a failure of migration of neural crest cells into the third and fourth pharyngeal pouches. Fully expressed DGS comprises hypoparathyroid hypocalcemia, thymic hypoplasia with defects in T-cell immunity, conotruncal cardiac defects, palatal insufficiency, dysmorphic facial features, and neurobehavioral and psychiatric features. DGS, velocardiofacial (Shprintzen) syndrome, and conotruncal face anomaly (Takao) syndrome commonly result from contiguous gene deletions in the same chromosomal region.

Most patients with a clinical diagnosis of DGS share a common 1.5- to 3-megabase deletion (monosomy or partial monosomy) of chromosome region 22q11.2, but there is molecular heterogeneity and rearrangement within this region (Shaikh et al., 2000). The size of the deletion does not correlate with the clinical phenotype. Haploinsufficiency of the TBX1 (T-box 1) transcription factor gene may be responsible for most features. Studies showed patients with point mutations in the gene encoding TBX1 manifested all major phenotypes of DGS (Yagi et al., 2003). Fluorescence in situ hybridization (FISH) using 22q11 probes had a higher detection

rate than high-resolution G-band karyotyping. However, the most sensitive (and preferred) laboratory assay is array-comparative genomic hybridization. Additionally, rarer identification of other cytogenetic abnormalities suggests that several distinct molecular defects (Rope et al., 2009) can lead to disturbed cranial neural crest cell migration and DGS phenotypes.

DiGeorge syndrome affects an estimated 1 in 4000 live births. It occurs sporadically or is transmitted as a variably penetrant autosomal dominant trait. Sporadic loss in the DGS chromosomal region is more common than parental transmission. In members of the same family, DGS may be associated with different phenotypic features. It often manifests itself in the first week of life with hypocalcemic tetany or seizures. Craniofacial features include microretrognathia, mandibular hypoplasia, submucous cleft palate, low-set and abnormal pinnae, telecanthus with short palpebral fissures, short philtrum, and a relatively small mouth. The presence of cardiac outflow tract or aortic arch abnormalities (especially pulmonary atresia/tetralogy of Fallot, type B interrupted aortic arch, truncus arteriosus, anomalies of aortic arch laterality, or abnormal branching of the brachiocephalic vessels) should prompt genetic investigation even in the absence of other DGS features. Parents of an infant with DGS should be screened for carrier status. These neonates require close anticipatory monitoring for the onset of hypocalcemia.

A normal serum PTH level obtained when an infant is relatively normocalcemic does not exclude the diagnosis of DGS. Absence of a thymic shadow on chest radiograph is not a reliable indicator. Infants with DGS may show resolution of hypoparathyroidism by early childhood, although PTH reserves may remain inadequate for defense against hypocalcemic stresses.

Hypoparathyroidism is a prominent feature of several rare skeletal dysplasias. Kenny–Caffey syndrome is a rare osteosclerotic bony dysplasia associated with hypocalcemia and ocular abnormalities (Tahseen et al., 1997). Autosomal recessive and autosomal dominant inheritance patterns have been described. Features include IUGR, transient neonatal hypoparathyroidism, short stature, macrocephaly, delayed fontanel closure, dysmorphic facies, and cortical thickening of tubular bones. The recessive form of Kenny–Caffey syndrome is the same disorder as hypoparathyroidism–retardation–dysmorphism (HRD) syndrome, which is also known as *Sanjad–Sakati syndrome*. HRD syndrome is an extremely rare disorder characterized by congenital hypoparathyroidism, growth retardation, characteristic facies (deep-set eyes, depressed nasal bridge, beaked nose, long philtrum, thin upper lip, large and floppy earlobes), small hands and feet, skeletal defects, and developmental delay (Sanjad et al., 1991). The loci for HRD syndrome and recessive Kenny–Caffey syndrome and HRD syndrome are allelic on chromosome band 1q42–q43. Parvari et al. (2002) have identified mutations in the gene encoding tubulin-specific chaperone E (*TBCE*) in both disorders. Hypoparathyroidism is also common in children with Kearns–Sayre syndrome (a mitochondrial myopathy associated with oculocraniosomatic disease) and Barakat syndrome (nerve deafness, refractory nephrosis, and renal failure). Knisely et al. (1988) showed that acute parathyroid hemorrhage (“parathyroid apoplexy”) is a common event in osteogenesis imperfecta type II and may contribute to early death.

The parathyroid glands are an infrequent target for autoimmunity, the exception being autoimmune polyglandular syndrome type 1 (APS 1), also known as *autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy* (Brown, 2009). APS 1 is a rare autosomal recessive disorder characterized by hypoparathyroidism, adrenal insufficiency, and chronic mucocutaneous candidiasis (Eisenbarth

and Gottlieb, 2004). It results from inheritance of mutations in an autoimmune regulator gene (*AIRE*). Candidiasis is usually the initial clinical manifestation, most often occurring in patients younger than 5 years. Hypoparathyroidism occurs next, usually in patients younger than 10 years. Hypoparathyroidism most often precedes development of hypoadrenocorticism. Affected individuals often eventually develop chronic hepatitis, malabsorption, juvenile-onset pernicious anemia, alopecia, and primary hypogonadism.

The association of several forms of autosomal dominant and autosomal recessive congenital hypoparathyroidism with allelic variants of the *preproPTH* and *CaSR* genes has advanced diagnosis and therapy for these disorders. Affected infants have subnormal or undetectable serum PTH levels but do not have congenital anomalies or developmental defects, DGS locus deletions, candidiasis or autoimmune polyglandular failure, or antiendocrine antibodies. Autosomal dominant and autosomal recessive forms of familial isolated hypoparathyroidism have been related to mutations in the *preproPTH* gene (Sunthornthevarakul et al., 1999). Activating mutations of *CaSR* can cause isolated hypoparathyroidism. Autosomal dominant hypocalcemia (ADH) is commonly caused by a gain-of-function mutation in the *CaSR* gene (Egbuna and Brown, 2008). The *CaSR* gene is located on chromosome band 3q13.3–q21, and it encodes a cell-surface protein that is expressed in the PTH-producing chief cells of the parathyroid glands and kidney tubules. Activation of the gene suppresses the secretion of PTH and renal calcium reabsorption from the cortical thick ascending limb (Lienhardt et al., 2001). The parathyroid and renal calcistat is reset downward, so hypocalcemia does not elicit normal compensatory PTH secretion or renal calcium reabsorption. Most patients with ADH are asymptomatic. Children, in particular, may become symptomatic with seizures and neuromuscular irritability during periods of stress such as febrile illness. Serum calcium level is usually in the range of 6 to 8 mg/dL (1.5 to 2.0 mmol/L), with normal or only slightly low serum PTH concentrations. Because de novo *CaSR* mutations producing *CaSR* hyperfunction are not uncommon (Egbuna and Brown, 2008), mutational analysis of the *CaSR* gene should be considered in the work-up of isolated hypoparathyroidism in infants, especially when hypocalcemia manifests itself with inappropriately normal urinary calcium excretion (relative hypercalciuria). Hypercalciuria and nephrocalcinosis can develop even when serum calcium concentration remains below the normal range. It is important to distinguish hypocalcemia due to an activating mutation of *CaSR* gene from isolated hypoparathyroidism because ADH patients can develop nephrocalcinosis and renal impairment during treatment with calcium and vitamin D. Therefore these patients require close monitoring of urinary calcium excretion for adjustment of therapy with 1,25(OH)₂D analogues.

Pseudohypoparathyroidism refers to a heterogeneous group of disorders that are usually inherited as an autosomal dominant trait characterized by hypocalcemia, hyperphosphatemia, and increased serum PTH concentrations due to PTH hormone resistance of peripheral tissues. Deletion of the PTH/PTHrP receptor is embryologically or perinatally lethal. Loss of one allele for *GNAS1*, which encodes G_sα, the G protein α subunit required for receptor-stimulated cAMP generation, produces pseudohypoparathyroidism type 1A (PHP1A) (Levine, 2012). In patients with PHP1A, there is the characteristic phenotype of Albright hereditary osteodystrophy (short stature, obesity, round face, brachymetacarpalism, and subcutaneous calcifications), and it is associated with multihormone resistance (e.g., thyroid-stimulating hormone, gonadotropins and

growth hormone). Isolated PTH resistance in the absence of a somatic phenotype is called *pseudohypoparathyroidism type 1B*. This disorder is an imprinting defect in which both *GNAS1* alleles have an unmethylated (paternal) pattern (Liu et al., 2000). Although most patients who eventually receive a diagnosis of PTH resistance syndrome are usually not hypocalcemic during the first month of life, transient neonatal pseudohypoparathyroidism has occasionally been reported (Minagawa et al., 1995). Individuals with pseudopseudohypoparathyroidism have the clinical phenotype of Albright hereditary osteodystrophy (AHO) but normal serum calcium, phosphorus, and PTH levels. Pseudohypoparathyroidism type 2 is characterized by hypocalcemia, hyperphosphatemia, and increased serum PTH level. However, individuals usually lack physical features associated with AHO (Mantovani, 2011).

Neonatal Hypocalcemia Associated With Maternal Hyperparathyroidism

Hypocalcemia is commonly observed in newborns of hyperparathyroid mothers (Cakir et al., 2013). These newborns may show increased neuromuscular irritability during the first 3 weeks of life but, occasionally, do so much later as limited PTH reserve and latent hypoparathyroidism emerge under stress or with time. The serum calcium levels usually range from 5.0 to 7.5 mg/dL, and the serum phosphate levels are often greater than 8.0 mg/dL. Hypocalcemic signs may be exacerbated by high-phosphate diets or maternal vitamin D deficiency. In some instances, signs of hypocalcemia can be quite severe and may be resistant to several weeks of calcium replacement therapy.

In maternal hyperparathyroidism the increased maternal serum calcium concentration facilitates transplacental calcium transport, producing fetal hypercalcemia greater than the moderate elevations of serum calcium concentration normally observed in the third trimester. As a result, fetal PTH secretion is suppressed (Jacobsen et al., 1978). The suppressed parathyroids are unable to maintain normal serum calcium levels postpartum. The reason for the hypomagnesemia observed in some infants born to hyperparathyroid mothers is uncertain, but this derangement may be due to (1) maternal magnesium depletion as a complication of hyperparathyroidism, (2) transient neonatal hypoparathyroidism, or (3) hyperphosphatemia, which may result from transient hypoparathyroidism or high dietary phosphate intake, or both.

Maternal serum calcium and phosphorus levels should be assayed whenever this diagnosis is suspected. Hypocalcemic tetany occurring in the infant may lead to a diagnosis of hyperparathyroidism in an asymptomatic mother. Maternal serum calcium levels in the upper normal range may be falsely reassuring if the samples were obtained during pregnancy, a time when serum calcium levels normally decline.

Neonatal Hypocalcemia Associated With Hypomagnesemia or Renal Tubular Acidosis

Hypomagnesemia causes hypocalcemia by interfering with the parathyroid cell CaSR-mediated release of PTH and by blunting end-organ PTH response. Hypomagnesemia with secondary hypocalcemia (HOMG1), can present in the first weeks of life as persistent hypocalcemia, tetany, and seizures uncontrolled by anticonvulsants or calcium therapy. Delay in establishing the diagnosis may lead to permanent neurologic impairment. This rare autosomal recessive disorder results from defective intestinal magnesium absorption and renal magnesium leak. Hypocalcemia is a secondary consequence of parathyroid failure and PTH resistance

as a result of severe magnesium deficiency. HOMG1 is caused by mutations in the *TRPM6* gene, a member of the transient receptor potential channel gene family expressed in intestinal epithelia and renal tubules (Schlingmann et al., 2005). Treatment includes immediate administration of magnesium, usually intravenously, followed by high-dose oral magnesium.

Several forms of primary renal hypomagnesemia have been described, including autosomal recessive familial hypomagnesemia with hypercalciuria and nephrocalcinosis caused by mutations in the claudin 16 (paracellin 1) gene (Simon et al., 1999) and a genetically heterogeneous autosomal dominant “isolated renal magnesium wasting.” The initial symptoms may include recurrent urinary tract infections, polyuria and polydipsia, moderate metabolic acidosis with an inappropriately high urine pH, muscle weakness, persistent tetany, failure to thrive, sensorineural hearing loss, and distal tubular acidosis. All patients exhibit hypercalciuria and nephrocalcinosis, and 50% of cases usually require renal replacement therapy in the second decade of life. The distal acidification defect is probably secondary to a medullary interstitial nephropathy. The serum magnesium level is frequently less than 0.8 mg/dL (normal range 1.6 to 2.8 mg/dL). High-dose enteral magnesium administration leads to increases in serum PTH and calcium levels and renal phosphate clearance. Kidney transplant normalizes serum magnesium and urinary calcium.

A transient hypomagnesemia in newborns often occurs in association with hypocalcemia. Less commonly, the serum calcium level may be normal. In transient hypomagnesemia the decrease in serum magnesium level typically is less severe (0.8 to 1.4 mg/dL) than in magnesium transport defects. In many infants with transient hypomagnesemia, the serum magnesium level increases spontaneously as the serum calcium level normalizes following the administration of calcium supplements. However, in other cases, hypocalcemia responds poorly to calcium therapy, but when magnesium salts are given, serum calcium and magnesium levels both rise.

Secondary hypomagnesemia from renal magnesium wasting can result from drug administration (e.g., loop diuretics, aminoglycosides, amphotericin B) or from urinary tract obstruction. It also may occur during the diuretic phase of acute renal failure. This disorder may be mistaken for neonatal hypoparathyroidism because of tetany and hypocalcemia or Bartter syndrome (hypokalemic alkalosis with hypercalciuria) because of secondary potassium wasting. An index of suspicion should be raised whenever hypomagnesemia occurs in one of these situations. The finding of low serum magnesium levels with inappropriately high urinary magnesium excretion confirms a diagnosis of renal magnesium wasting. Hypokalemia is a common laboratory feature of magnesium depletion. Attempts to replace the potassium deficit with potassium alone are usually unsuccessful unless magnesium is given concurrently.

Distal renal tubular acidosis (RTA1) is characterized by hypocalcemia, hypercalciuria, various degrees of hypomagnesemia, hyperchloremia, low serum bicarbonate level, and a fixed urinary specific gravity and urinary pH (about 5.0). The mineral excretion defect leads to nephrocalcinosis and metabolic bone disease. RTA1 sometimes manifests itself during early infancy, when hypocalcemia may precede the renal tubular acidosis.

Hypocalcemia Resulting From Vitamin D Disorders

Vitamin D increases the intestinal absorption of calcium. In older children and adults, disorders of vitamin D intake or metabolism rarely present as isolated hypocalcemia. Instead, most patients with

abnormalities in either production or action of $1,25(\text{OH})_2\text{D}$ have rickets or osteomalacia. In sharp contrast, young infants may exhibit hypocalcemic tetany before rachitic features become conspicuous. Abnormalities in vitamin D metabolism can be divided into three broad categories: vitamin D deficiency, acquired or inherited disorders of vitamin D metabolism, and resistance to vitamin D actions.

Maternal vitamin D deficiency is the major risk factor for neonatal vitamin D deficiency manifesting itself as hypocalcemia. Maternal vitamin D deficiency is becoming less common in countries where dairy products and other foods are supplemented with vitamin D. It is still a common and serious health problem of women of reproductive age and their infants in developing countries. Female immigrants from the Middle East or South Asia who wear traditional concealing dress, have inadequate dietary vitamin D intake, or are dark skinned are at particularly high risk (Dijkstra et al., 2007; Hobbs et al., 2009), especially during pregnancy. Breastfed infants of lactovegetarian mothers are also susceptible to early-onset hypocalcemic rickets. Nutritional rickets in newborns can be prevented by daily supplementation of 400 international units (IU) for infants and 400 IU daily for mothers during pregnancy and lactation or 1000 IU daily if supplementation is begun in the third trimester.

Intestinal absorption of fat-soluble vitamin D requires a functioning exocrine pancreas, biliary tract, and bowel mucosa. Consequently, pregnant women with malabsorption syndromes may be vitamin D deficient.

Anticonvulsant therapy (e.g., with phenobarbital or diphenylhydantoin) during pregnancy, which increases hepatic catabolism of $25(\text{OH})\text{D}$, can also induce maternal and fetal vitamin D deficiency. Pregnant women who take anticonvulsants should receive vitamin D supplementation (800 to 1000 IU per day).

Phosphate-Induced Hypocalcemia

Phosphate directly precipitates calcium in bone or soft tissues by inhibiting bone resorption and blocking renal synthesis of $1,25(\text{OH})_2\text{D}$. Conditions leading to phosphate-induced neonatal hypocalcemia include excessive phosphate intake, rhabdomyolysis-induced acute renal failure, and hyperphosphatemic renal insufficiency. Phosphate-containing enemas can produce significant phosphate absorption (Marraffa et al., 2004). Their use is hazardous and contraindicated for infants. Chronic renal failure is the primary cause of secondary hyperparathyroidism. Patients with mineral metabolism disorders commonly have low serum calcium levels, hyperphosphatemia, and calcitriol deficiency. In uremic conditions, however, the parathyroid glands become hyperplastic. In recent years fibroblast growth factor 23 (FGF-23) has been identified (Cozzolino et al., 2009). FGF23 is a bone-derived hormone that regulates systemic phosphate hemostasis and vitamin D metabolism. FGF23 inhibits renal tubular reabsorption of phosphate independently of PTH (Quarles, 2012).

Other Causes of Neonatal Hypocalcemia

It is important to recognize that hypocalcemia may occur whenever skeletal mineralization significantly outpaces the rate of osteoclastic bone resorption. Examples of this type of hypocalcemia occur with overzealous vitamin D replacement in infants with rickets or hypoparathyroidism (Thacher et al., 2006). Pancreatitis can cause hypocalcemia and tetany through the action of pancreatic lipase on retroperitoneal and omental fat to release free fatty acids (FFAs). FFAs avidly chelate calcium and remove it from the ECF. Pancreatitis may also result in release of pancreatic calcium-lowering factors

(Tomomura et al., 1995). Hypocalcemia is commonly seen in critically ill patients with sepsis. It is believed to be due to parathyroid gland suppression, failure to activate vitamin D, and calcium chelation (Kelly and Levine, 2013). Neonatal or infantile hypocalcemia and hypocalcemic seizures may accompany long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency and severe cases of rotavirus gastroenteritis (Foldenauer et al., 1998). Hypocalcemic jitteriness or seizures can be the presenting sign of infantile osteopetrosis (Chen et al., 2003). It is thought to arise from the failure of osteoclasts to resorb immature bone. Prompt recognition permits early referral for bone marrow or hematopoietic stem cell transplant.

Several common therapeutic interventions can induce hypocalcemia. Bicarbonate therapy, as well as any form of metabolic or respiratory alkalization, decrease both Ca^{2+} levels and bone turnover. Rapid blood transfusion or plasmapheresis can promote calcium complexes with the infused citrate, decreasing Ca^{2+} levels. Hypocalcemia after initiation of ECMO is related to the composition of the circuit-priming solution and to acute citrate loading and may lead to hemodynamic instability (Meliones et al., 1995). Large doses of ethylenediaminetetraacetic acid-containing contrast dyes have also been reported to cause hypocalcemia (Brown et al., 2006). Furosemide therapy, a therapy for hypercalcemia, promotes calciuresis and nephrolithiasis. Phototherapy for hyperbilirubinemia may be associated with mild hypocalcemia. This effect has been attributed to decreased melatonin secretion, which potentiates glucocorticoid actions on bone metabolism. Lipid infusions may elevate serum levels of FFAs, which form insoluble complexes with calcium. Most of these effects are transient, and cessation of therapy is followed by a return to normal serum calcium levels. The major exception is aggressive furosemide therapy, which, when prolonged, may lead to bone demineralization and renal dysfunction.

Treatment

The decision to treat hypocalcemia in an infant depends on the severity of the hypocalcemia and the presence of clinical signs and symptoms. The morbidity associated with calcium treatment must be weighed against the potential benefits. Hypocalcemic preterm infants who have no symptoms and are not ill from any other cause probably do not need specific treatment. Early neonatal hypocalcemia should resolve by day 3. Some clinicians begin treatment in preterm newborns once serum calcium levels have dropped to 6.0 to 6.5 mg/dL or after Ca^{2+} concentration has decreased to 2.5 to 3.0 mg/dL. Some advocate initiation of prophylactic calcium infusions (or calcium-containing parenteral nutrition) for all extremely low-birthweight infants (ELBW) within the first 24 hours. There is no role for prophylaxis or treatment with pharmacologic doses of vitamin D. For newborns who exhibit cardiovascular compromise (e.g., severe respiratory distress, pulmonary hypertension, asphyxia, sepsis) or who require cardiotoxic drugs or blood pressure support, monitoring of blood Ca^{2+} concentration is particularly helpful, with the aim of preventing the onset of significant hypocalcemia.

The mainstay of treatment for neonatal hypocalcemia is intravenous (IV) administration of calcium salts. Calcium gluconate is preferred over calcium chloride (which, in sufficient doses, produces hyperchloremic acidosis) or calcium lactate. A 10% solution of calcium gluconate contains 9.4 mg elemental calcium per mL. A constant infusion of approximately 45 to 75 mg/kg per day of elemental calcium usually produces a sustained increase in serum calcium level (7 to 8 mg/dL). Bolus infusions are hazardous and only transiently effective.

The risks associated with calcium infusions are minimized by attention to detail. Rapid IV infusion of calcium can cause a sudden elevation in serum calcium level, leading to bradyarrhythmias. Bolus infusion of calcium should be reserved for treatment of hypocalcemic tetany and seizures. Extravasation of calcium solutions into subcutaneous tissues may cause necrosis and subcutaneous calcification. Therefore meticulous care of peripheral IV catheter sites is particularly important when calcium-containing solutions are infused. Inadvertent intrahepatic infusion of calcium through an umbilical vein catheter (due to failure to reach the inferior vena cava) can cause hepatic necrosis. Rapid intraaortic infusion via an umbilical artery can cause arterial spasm and, at least experimentally, intestinal necrosis.

Hypocalcemic Crisis. Acute hypocalcemia constitutes an emergency that requires prompt attention. For emergency treatment of a hypocalcemic crisis with seizures or tetany, 1 to 2 mL/kg (100 to 200 mg/kg calcium gluconate) of a 10% solution of calcium gluconate should be given within 5 to 10 minutes. The dose can be repeated in 10 minutes if no response occurs. Calcium should not be administered rapidly intravenously because hypercalcemia may adversely affect cardiac conduction; monitoring of the electrocardiogram QT interval is useful in guiding therapy. Alternatively, calcium chloride (0.2 mL/kg) can be given. If necessary, IV calcium therapy may be repeated three or four times in 24 hours to help control acute symptoms. After short-term treatment, maintenance calcium gluconate should be added to IV fluid. Careful observation of the infant and infusion site is essential, and the infusion should be discontinued if there is bradycardia or when the desired clinical result is obtained. Serum calcium must be frequently monitored during infusion, and calcium should not be mixed with fluids containing phosphate or bicarbonate to avoid precipitation (Zhou and Markowitz, 2009).

Nonemergency Treatment. After acute symptoms have been controlled, calcium therapy should be continued as needed to maintain a serum calcium concentration above 7.0 mg/dL. In part, the level of serum calcium to be achieved depends on serum total protein, particularly albumin. In hypoalbuminemic infants, lower levels of total serum calcium are normally present. The dose varies with age. In preterm and sick infants for whom oral intake is limited, 5 to 8 mL/kg of 10% calcium gluconate (45 to 75 mg/kg elemental calcium) may be infused with IV fluids in a 24-hour period. For older infants and children, a starting dose of 200 mg/kg calcium gluconate (20 mg/kg elemental calcium) each 24 hours should be provided. The lower dose range is preferred whenever there is hyperphosphatemia. If oral feedings are tolerated, 10% calcium gluconate may be given in the 50- to 150-mg/kg per day range of elemental calcium in four to six divided doses. Alternatively, calcium glubionate (Neo-Calglucon), which contains 23.6 mg elemental calcium per mL, may be given in a dosage of 2 mL/kg per day divided into feedings. Orally administered calcium gluconate is better tolerated by young infants because the high sugar content and osmolality of calcium glubionate may cause gastrointestinal irritation or diarrhea. IV or oral calcium supplements should be continued until the serum calcium level stabilizes.

Dietary factors and hypoparathyroidism are important in the pathogenesis of late neonatal hypocalcemia. Therefore therapy is often directed at reducing the phosphate load and increasing the calcium-to-phosphorus ratio of feedings to 4:1. This can be accomplished by the use of low-phosphorus feedings such as human milk or Similac PM 60/40 (Abbott Nutrition) in conjunction with calcium supplements. These interventions inhibit intestinal

absorption of phosphorus. Phosphate binders are not generally necessary. Serum calcium and phosphorus levels should be monitored at least once to twice weekly, and the use of calcium supplements should be discontinued in a stepwise fashion after several weeks.

Magnesium Administration. When hypomagnesemia contributes to hypocalcemia, administration of magnesium salts is indicated. Magnesium may be given intramuscularly as a 50% solution of magnesium sulfate (50% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ contains 4 mEq/mL magnesium). The suggested intramuscular or IV dose of 50% magnesium sulfate is 0.1 to 0.2 mL/kg. IV infusions should be administered slowly with use of electrocardiographic monitoring to detect rhythm disturbances, which may include prolonged atrioventricular conduction time and sinoatrial or atrioventricular block. The magnesium dose may be repeated every 12 to 24 hours, depending on the clinical and serum magnesium response. Many infants with transient hypomagnesemia will respond sufficiently to one or two magnesium injections. Infants with primary hypomagnesemia have permanent magnesium wasting. The low serum magnesium levels may require lifelong treatment with magnesium supplements.

Vitamin D Treatment. Infants with normal intestinal absorption who develop late hypocalcemia with vitamin D–deficiency rickets usually respond within 4 weeks to 1000 to 2000 IU/day oral vitamin D. These infants should receive at least 40 mg/kg per day of elemental calcium to prevent hypocalcemia because the unmineralized osteoid may avidly incorporate calcium once vitamin D is provided (“hungry bones” syndrome). PTH-dependent renal production of $1,25(\text{OH})_2\text{D}$ is deficient in all hypoparathyroid states, renal failure, or vitamin D–dependent rickets. Vitamin D metabolites that do not require renal 1α -hydroxylation should be administered. Calcitriol ($1,25[\text{OH}]_2\text{D}$) is indicated, usually starting with 0.25–1 $\mu\text{g}/\text{d}$ in two divided doses. The dose may be adjusted at 2- to 4-week intervals. Most patients with hypoparathyroidism require lifelong calcium and vitamin D supplementation. The goals of therapy are to relieve symptoms and maintain the serum calcium level in the low normal range (e.g., 8.0 to 8.5 mg/dL [2.0 to 2.1 mmol/L]) to prevent hypercalciuria and nephrocalcinosis (Bilezikian et al., 2011). Assessment of urinary calcium excretion by urinary calcium-to-creatinine ratio should be performed within 2 weeks of initiation of therapy. The urinary calcium measurement reflects the effect of therapy and monitors the risk of nephrocalcinosis. The ratio of 0.2 mg/mg or greater usually defines hypercalciuria in older children, but the age-dependent 95th percentile can be as high as 0.7 mg/mg for infants (Singh et al., 2003; Habbig et al., 2011). Periodic renal sonograms are also recommended to detect nephrocalcinosis.

It is essential that patients with hypocalcemia be aware that their calcium level should be monitored more frequently during intercurrent illnesses that may affect calcium absorption to prevent the development of hypocalcemia or severe hypercalcemia.

Recombinant Parathyroid Hormone Analogue. Studies have suggested subcutaneous injection of recombinant PTH can be an alternative therapy for hypoparathyroidism. However, such treatment poses a number of challenges, particularly fluctuations in calcium level. Further studies are needed to assess the long-term effects (Rejnmark et al., 2015).

Neonatal Hypercalcemia

Hypercalcemia is usually defined as total serum calcium concentration greater than 11.0 mg/dL and Ca^{2+} concentration greater than 5.0 mg/dL. Hypercalcemia in neonates may be asymptomatic if there are only mild elevations in calcium levels, but as calcium

levels start to rise above 12.0 mg/dL, then symptoms may start to manifest themselves. These may include poor weight gain, poor feeding, weakness, constipation, and polyuria. As calcium levels get higher (>14 mg/dL), more severe symptoms may present, such as vomiting, respiratory distress, apnea, hypotonia, lethargy, and seizures. The polyuria seen in hypercalcemic states is due to impairment in the kidney's concentration ability, leading to a state of nephrogenic diabetes insipidus (Berl, 1987). This may be partly due to inhibition of sodium chloride reabsorption in the loop of Henle, which impairs the osmotic gradient needed for concentration, as well as decreased antidiuretic hormone-mediated water permeability in the collecting tubules (Hebert, 1996). Usually there are no physical examination findings associated with hypercalcemia unless findings are associated with genetic syndrome dysmorphisms or subcutaneous fat necrosis. Hypertension can be seen, most likely due to the effects of hypercalcemia on vasoconstriction as well as possible effects on catecholamine (Eiam-Ong et al., 2004). Shortened QT interval may also be seen on electrocardiogram (Ahmed and Hashiba, 1988). When hypercalcemia is chronic, calcifications can be seen in multiple tissues and organs, including kidney, skin, subcutaneous tissue, heart (valves, arteries, muscle), lung, brain, and gastric mucosa. Nephrocalcinosis, nephrolithiasis, and osteitis fibrosa cystica (loss of bone mass and replacement with fibrous tissue with formation of cystic lesions due to increased osteoclastic resorption) may also be seen in chronic, untreated hypercalcemia. In infants the most clinically apparent manifestation of chronic hypercalcemia is poor growth and failure to thrive.

Neonatal hypercalcemia is associated with several clinical entities (Box 95.2). Normally calcium concentrations are tightly regulated by interplays between PTH, CaSR, calcitonin, and vitamin D analytes affecting intestinal and renal absorption of calcium.

• BOX 95.2 Causes of Neonatal Hypercalcemia

Hyperparathyroidism

CaSR mutations

- Neonatal severe hyperparathyroidism
- Familial hypocalciuric hypercalcemia

Maternal hypocalcemia

Mucopolidosis type II

Iatrogenic

- Hypophosphatemia
- Vitamin D intoxication
- Calcium supplement excess
- Hypervitaminosis A
- Extracorporeal life support

Hypercalcemia Associated With Skeletal Dysplasias

- Infantile hypophosphatasia
- Jansen metaphyseal chondrodysplasia (activating mutations of parathyroid hormone/parathyroid hormone-related protein receptor)

Other Causes

- Williams syndrome
- Idiopathic infantile hypercalcemia/25-hydroxyvitamin D 24-hydroxylase deficiency
- Subcutaneous fat necrosis
- Blue diaper syndrome
- Tumor-associated hypercalcemia (parathyroid hormone-related protein secretion)
- Congenital lactase deficiency
- Congenital sucrase-isomaltase deficiency
- Immobilization

Hypercalcemia may occur when there is a disruption in this system and can be due to multiple causes, which can be categorized on the basis of abnormalities of PTH, CaSR, intake, or other causes.

Neonatal Hyperparathyroid Syndromes Associated With CaSR Mutations

Inactivating mutations of CaSR can lead to a spectrum of hypercalcemic diseases that may range from severe presentations such as neonatal severe hyperparathyroidism (NSHPT) to mild presentations as seen in FHH. These mutations cause a shift in the dose-response curve for calcium to the right, so there is a higher setpoint for the CaSR, leading to higher serum calcium levels and an elevated or inappropriately normal PTH level. Urinary calcium-to-creatinine clearance ratios will be less than 0.01, and there will often be a family history of hypercalcemia although de novo mutations can be present. FHH is usually due to a heterozygous mutation, while NSHPT is due to a homozygous mutation; however, there have been cases of mild homozygous mutations leading to an FHH presentation and severe heterozygous mutation presenting more like NSHPT (Iacobone et al., 2015; Mayr et al., 2016).

FHH is an autosomal dominant disorder characterized by mild hypercalcemia, low renal excretion of calcium leading to hypocalciuria, and normal or mildly elevated PTH level. Usually FHH is asymptomatic with normal phosphorus, magnesium, and vitamin D levels and bone density. Presentation can be masked if there is concomitant vitamin D deficiency (Szcawinska et al., 2014). There are three types of FHHs. FHH type 1 is the most common form and accounts for 65% of cases. It is due to a loss-of-function mutation of the *CaSR* gene on chromosome band 3q21.1. FHH type 2 and FHH type 3 account for the rest of the cases and are due to a loss-of-function mutation of *GNA11* on chromosome band 19p13.3 or *AP2S1* on chromosome band 19q13.2-q13.3, respectively (Iacobone et al., 2015). *AP2S1* encodes adaptor-related protein complex 2, which is involved in endocytosis of CaSR. *GNA11* encodes G protein subunit α_{11} , which is involved in CaSR signaling (Vargas-Poussou et al., 2016). FHH type 3 may have a more severe presentation with higher calcium levels. FHH generally does not require treatment unless there is evidence of symptoms or complications due to hypercalcemia. Parathyroid glands can be normal or hyperplastic, and surgical resection is usually not helpful (Iacobone et al., 2015). Individuals with FHH may have children with NSHPT.

NSHPT is a rare, life-threatening disorder associated with elevated PTH level, elevated serum calcium level, elevated magnesium level, and low urinary excretion of calcium. It is diagnosed within the first 6 months of life, but severe forms will present as a critically ill newborn with symptoms of poor feeding, dehydration, lethargy, and respiratory distress. Elevated PTH level may lead to bony changes, including demineralization, fractures, and rickets. Homozygous mutations of *CASR* usually lead to a more severe presentation, and both parents have FHH. Heterozygous mutations of the gene may result in lower calcium levels and may be inherited from one parent or occur from a de novo mutation (Hendy and Cole, 2013; Mayr et al., 2016). Typically, these patients have large, hyperplastic parathyroid glands that need to be surgically removed. However, before surgery, hypercalcemia needs to be medically managed. This includes the use of sodium-containing fluids and diuretics to induce sodium diuresis and therefore urine calcium excretion. Medications such as bisphosphonates and calcimimetics can decrease serum calcium concentration as well. Pamidronate at 0.5 to 1 mg/kg and cinacalcet at 0.4 to 2 mg/kg per day have been used successfully in reported cases to decrease serum calcium

levels as patients await total parathyroidectomy (Iacobone et al., 2015; Mayr et al., 2016).

Neonatal Hyperparathyroidism Not Associated With *CASR* mutations

Other forms of inherited hyperparathyroidism include those seen in syndromes such as multiple endocrine neoplasia (MEN) 1, MEN 2A, and hyperparathyroidism–jaw tumor syndrome, but these conditions would present with hyperparathyroidism later in life. Secondary neonatal hyperparathyroidism may also be seen as a consequence of maternal calcium–PTH abnormalities. Maternal hypocalcemia during pregnancy will lead to decreased placental transport of calcium, fetal hypocalcemia, and subsequent hyperplasia of the parathyroid glands. This condition is transient and normally resolves within the first few weeks of life. Hypercalcemia and hypophosphatemia are not seen universally in these cases but bony changes secondary to elevated PTH level are common (Loughhead et al., 1990). Another condition that may lead to secondary hyperparathyroidism is mucopolidosis type II. Such cases present with elevated PTH level, normal calcium level, and bony changes and resolve with vitamin D and calcium supplementation. The cause is hypothesized to be secondary to diminished transplacental calcium transport as this condition is associated with abnormalities of the placenta (Unger et al., 2005).

Jansen metaphyseal chondrodysplasia is a syndrome that has features of hyperparathyroidism but low levels of PTH. In this condition, mutations of *PTHr1* lead to constitutive, ligand-independent activation of the receptor, causing hypercalcemia despite low serum PTH levels. Such patients will also have irregularities of the metaphysis and rachitic changes seen on x-ray. Other distinguishing features include short-limbed dwarfism with postnatal growth failure, hypertelorism, and mandibular hypoplasia (Schipani et al., 1996; Hsu and Levine, 2004).

Williams Syndrome and Idiopathic Infantile Hypercalcemia

Williams syndrome (WS) (or Williams–Beuren syndrome) is a genetic disorder due to the deletion of the Williams–Beuren syndrome critical region (WBSCR) of chromosome band 7q11.23, which includes the elastin gene (*ELN*). The deletion can be detected by FISH or deletion/duplication testing. It is usually due to a de novo mutation but can be transmitted in an autosomal dominant fashion. It is characterized by distinctive facial features (broad forehead, stellate iris, short nose, broad nasal tip, malar flattening, long philtrum, wide mouth, small jaw, large earlobes), mild intellectual disability, a social personality, short stature, cardiovascular disease (elastin arteriopathy, peripheral pulmonary stenosis, supravalvular aortic stenosis, hypertension), hypotonia, joint hyperextensibility, and endocrine abnormalities (hypercalcemia, hypothyroidism, early puberty). In infancy, inguinal or umbilical hernias, hypotonia, poor feeding, failure to thrive, developmental delays, and rectal prolapse may also be present (Morris, 1999).

About 30% of patients have hypercalciuria and 15% have idiopathic hypercalcemia. The hypercalcemia is noted during infancy and typically resolves by 2 to 4 years of life, although rarely it can persist into adulthood. The cause of the hypercalcemia is not well understood. PTH level is normal or low, and there is no consistent pattern in vitamin D metabolism to explain the abnormality. Hypercalciuria is relatively common, and nephrocalcinosis has been reported (Pober et al., 1993). Therefore early screening for hypercalcemia and hypercalciuria is warranted in infancy. The severity of the calcium abnormalities can range from mild to severe. Treatment consists of limiting calcium and vitamin D intake until

the condition resolves. More severe cases have also been treated with short courses of calcitonin, glucocorticoids, and pamidronate (Morris, 1999; Cagle et al., 2004).

A similar calcium profile as seen in WS has been described in certain infants classified as having idiopathic infantile hypercalcemia (IIH). These infants may have failure to thrive, dehydration, and nephrocalcinosis. They do not have all the same clinical features or gene deletion as WS and can be differentiated from WS through genetic testing. In IIH, hypercalcemia also resolves in the first few years of life, and treatment is similar to that of children with WS (Martin et al., 1984). The cause of the hypercalcemia used to be a mystery; however, recently mutations of *CYP24A1*, which encodes the enzyme 25(OH)D 24-hydroxylase have been implicated as a factor in IIH. This enzyme plays an important role in the metabolism and deactivation of 1,25(OH)₂D. Therefore a loss-of-function mutation in this gene leads to elevated 1,25(OH)₂D levels as well as exaggerated increases of these levels and subsequent hypercalcemia if vitamin D supplementation is given. Biochemically, this leads to hypercalcemia, hypercalciuria, suppressed PTH levels, increased intestinal calcium absorption, and elevated 1,25(OH)₂D levels. An elevated 25(OH)D to 24,25(OH)₂D ratio can also be seen (Schlingmann et al., 2011; Cools et al., 2015). Treatment involves limitation of vitamin D and calcium intake.

Neonatal Hypercalcemia Associated With Subcutaneous Fat Necrosis

Subcutaneous fat necrosis (SFN) is a panniculitis that develops in term or postterm infants. The lesions are erythematous to violaceous, indurated subcutaneous nodules and plaques that appear in the first few days of life (mean 4 days) and are typically found on the back, head, and arms. The lesions may initially be painful and resolve within the first few months of life. Biopsy of the nodules shows granulomatous necrosis in the subcutis with crystal-like structures in adipocytes and giant cells. The risk factors for the development of SFN include birth trauma, asphyxia, meconium aspiration, infections, and cutaneous trauma. Maternal risk factors may include gestational diabetes, preeclampsia, cocaine or calcium blocker use during pregnancy, and high risk of thrombosis (Mahe et al., 2007). SFN has also been recognized as a complication of therapeutic hypothermia for neuroprotection after hypoxic–ischemic encephalopathy that may occur days to weeks after treatment, with lesions occurring in areas in contact with the cooling blanket (Scheans, 2012).

Complications of SFN include mild to severe hypercalcemia, dyslipidemia, hypoglycemia, thrombocytopenia, and eosinophilia. Hypercalcemia may occur 1 to 2 months after delivery; therefore in infants with SFN calcium levels should be monitored regularly. Some of the hypercalcemia may be asymptomatic despite high levels of calcium (Mahe et al., 2007; Shumer et al., 2014). Hypercalciuria is often present, and nephrocalcinosis may occur that can persist into early childhood but does not seem to result in adverse renal outcomes (Shumer et al., 2014). The cause of the hypercalcemia is thought to be due to the uncontrolled production of 1,25(OH)₂D by activated macrophages that infiltrate the necrotic fat. This leads to increased intestinal absorption of calcium in the gut. The necrotic fat also releases calcium and prostaglandin E (increases bone turnover), which may exacerbate the hypercalcemia (Hsu and Levine, 2004; Shumer et al., 2014). Treatment includes hydration, furosemide therapy, and a low-calcium/low–vitamin D diet. However, SFN is also highly responsive to glucocorticoids. Severe, refractory cases have also been treated with bisphosphonates (Shumer et al., 2014).

Hypercalcemia Due to Iatrogenic Causes

Neonatal hypercalcemia may also be due to increased intake of calcium or vitamin D or decreased intake of phosphorus. Hypercalcemia because of phosphate depletion is most often seen in VLBW infants who are fed unsupplemented human milk that is low in phosphorus or given parenteral nutrition with inadequate phosphate. There is a high consumption of phosphorus by cells in growing newborns. If inadequate phosphorus is given, then phosphorus will be released through bone resorption, which in turn also releases calcium. This may also be associated with high alkaline phosphatase (ALP) levels. Low phosphorus levels will also stimulate increased renal production of $1,25(\text{OH})_2\text{D}$ and increased absorption of calcium through the gut (Warner et al., 1998; Guellec et al., 2015). The condition is preventable by anticipatory monitoring of serum calcium and phosphorus levels in high-risk infants.

Excessive supplementation with vitamin D may cause neonatal hypercalcemia. The excess vitamin D may be from improperly made formula, ingestion of cow's milk fortified with vitamin D, or overdose of vitamin D supplements. Infants respond to discontinuation of vitamin D supplements. These occurrences have prompted vitamin reformulation of preterm nutritional products. Laboratory studies in hypervitaminosis D typically show elevated $25(\text{OH})\text{D}$ levels but not $1,25(\text{OH})_2\text{D}$ levels and suppressed serum PTH levels.

Hypervitaminosis A, caused by renal failure or ingestion of a large amount of vitamin A, has been associated with hypercalcemia. It is hypothesized that vitamin A inhibits osteoblasts and stimulates osteoclasts, leading to increased bone turnover (Clark and Smith, 1964; Bush and Dahms, 1984).

Hypercalcemia has also been reported in neonates receiving extracorporeal life support (ECLS). The mechanism is not well understood. High PTH level has been reported but does not seem to correlate well with the calcium and vitamin D levels. Hypercalcemia was associated with higher mortality and longer duration of ECLS (Fridriksson et al., 2001; Rambaud et al., 2015).

Other Causes of Neonatal Hypercalcemia

Tumor-associated hypercalcemia in neonates is extremely rare. Congenital mesoblastic nephroma has been associated with hypercalcemia in infancy and may be due to tumor secretion of PTHrP (Srivastava et al., 2011). Metabolic disorders, congenital lactase deficiency, and congenital sucrose–isomaltase deficiency are associated with hypercalcemia. Infants will also have symptoms of persistent diarrhea and failure to thrive. The hypercalcemia may be due to increased ileal absorption of calcium as well as effects of metabolic acidosis on release of calcium from bone (Saarela et al., 1995; Belmont et al., 2002). Immobility can also cause hypercalcemia because of increased bone turnover (Vyas et al., 2016).

Blue diaper syndrome is a rare familial disease in which hypercalcemia and nephrocalcinosis are associated with a defect in the intestinal transport of tryptophan (Drummond et al., 1964). Bacterial degradation of tryptophan in the intestine leads to excessive production of indole, which is converted to indican in the liver. Oxidative conjugation of indican in the urine forms the water-insoluble dye indigo blue (indigotin), with a consequent peculiar bluish discoloration of the diaper. The clinical course is characterized by failure to thrive, recurrent unexplained fever, infections, marked irritability, and constipation.

Hypophosphatasia is a rare autosomal recessive condition caused by mutations in the tissue nonspecific ALP because of mutations

in *ALPL*. Laboratory tests will show very low ALP levels. Depending on the age at diagnosis, six clinical forms are currently recognized: perinatal (lethal), perinatal benign, infantile, childhood, adult, and odontohypophosphatasia. Prominent features of the early-onset severe forms include respiratory complications, increased intracranial pressure, widespread undermineralization, rickets, and hypercalcemia (Mornet and Nunes, 2007).

Treatment

Treatment will depend on the cause and severity of hypercalcemia. If the hypercalcemia is mild to moderate, then restriction of calcium and vitamin D may be sufficient. In more severe cases, further steps must be taken. Part of the treatment is rehydration. Hydration with normal saline is recommended to help induce sodium diuresis and increase urine calcium excretion. Once adequate rehydration with adequate urine output has been established, a loop diuretic such as furosemide (1 mg/kg intravenously at 6- to 8-hour intervals) may be added as it inhibits calcium reabsorption. Hydrocortisone reduces calcium absorption in the intestine and can be given as 1 mg/kg every 6 hours. Calcitonin decreases skeletal reabsorption and inhibits renal reabsorption of calcium. Subcutaneous or intramuscular doses of 3 to 6 µg/kg every 6 hours can be given, but this is only effective for a few days as tachyphylaxis will occur. Bisphosphonates such as pamidronate have been used successfully in a number of hypercalcemic conditions. These agents inhibit osteoclasts and bone resorption. Pamidronate doses of 0.5 to 2 mg/kg have been reported. Cinacalcet is a calcimimetic that works to make CaSR more sensitive. It has been used in cases of *CaSR* mutations.

Neonatal Disorders of Serum Magnesium

Neonatal hypomagnesemia was discussed in Neonatal Hypocalcemia Associated With Hypomagnesemia or Renal Tubular Acidosis.

Neonatal hypermagnesemia is usually due to maternal magnesium sulfate administration or postnatal excess administration of magnesium-containing products including magnesium in neonatal parenteral nutrition solutions that exceeds magnesium clearance, use of magnesium hydroxide-containing antacids (e.g., milk of magnesia), or medication errors resulting in overadministration of magnesium. Magnesium inhibits acetylcholine release at neuromuscular junctions; therefore elevated levels will lead to symptoms of low tone in mild cases to paralysis in severe cases. The hypermagnesemic newborn may exhibit central nervous system depression (lethargy, hypotonia, poor suck ability), hyporeflexia, and hypotension in mild to moderate cases. Severe cases may result in muscle paralysis, hypoventilation, respiratory depression, coma, arrhythmias, complete heart block, and asystolic arrest. As hypermagnesemia decreases myocardial electrical conduction, cardiac features may include prolonged PR, QRS, and QT intervals, intraventricular conduction defects, bradycardia, and peaked T waves. Because of effects on increased bowel motility, increased fluid secretion and electrolyte abnormalities may be seen; however, delayed passage of meconium has also been reported. In addition, hypocalcemia caused by inhibition of PTH by high levels of magnesium may also be seen. Treatment is mostly supportive as eventually the magnesium will be cleared by the kidney. Any administration of magnesium (oral or IV) should be discontinued. Regular monitoring for cardiac, respiratory, and electrolyte abnormalities should be done, and any abnormalities should be treated accordingly. Feedings should be deferred until normalization of bowel function occurs. Calcium infusions may be helpful in severe, symptomatic cases as calcium is an antagonist of magnesium. Hemodialysis may be

considered in life-threatening situations in anuric patients (Sullivan and Berman, 2000; Narchi, 2001).

Metabolic Bone Disease in Newborns and Infants

Metabolic bone disease (MBD) is characterized by the reduction in the mineral content and protein matrix of bone, frequently manifesting itself with rachitic changes. MBD is common in premature infants and has also been referred to as *osteopenia* and *rickets of prematurity*. The forms of MBD manifesting themselves in infants and children are listed in Box 95.3.

The following are terms commonly used in describing MBD:

- *Osteopenia* is generally defined as diminished bone mineral density secondary to a reduction in the thickness of bone cortex and trabeculae.
- *Osteoporosis* is defined in pediatrics as a reduction in bone mineral density with clinically significant fractures (Gordon et al., 2014). Unlike in rickets and osteomalacia, in which mineralization defects predominate, the primary abnormality in osteoporosis is either a decrease in matrix formation or an increase in matrix and mineral resorption.
- *Rickets* results from undermineralization of the bone matrix, or osteoid, in growing bone; it involves both the growth plate (physis) and newly formed trabecular and cortical bone. In infancy, the most rapidly growing bones are the skull, upper limbs, and ribs. Early development of rickets therefore leads to craniotabes, characterized by softening of the skull with the “ping-pong ball” sign; widened cranial sutures; frontal bossing; swollen epiphyses of wrists; costochondral beading manifesting itself as a “rachitic rosary”; and Harrison sulcus, which is caused

by diaphragmatic depression of the lower thorax on inspiration. Manifestations of muscle weakness may include dilated cardiomyopathy and ventricular dysfunction, which respond to vitamin D therapy (Brown et al., 2009). Radiographic features in rickets result from expansion of the cartilaginous growth plate and delayed mineralization. They include lucency and widening of the gap between metaphysis and epiphysis, known as the *zone of provisional calcification*, as well as irregularity, cupping, or fraying of the metaphyseal margin. Serum phosphorus or calcium level or both are characteristically depressed, and serum ALP level is elevated. An exception is in the hyperphosphatemia of renal osteodystrophy.

- *Osteomalacia* is rickets that occurs in the absence of linear growth. This is the typical pattern in adults, but it may also occur in poorly nourished preterm infants. In osteomalacia, the radiographic features of rickets at the cartilage–shaft junction are generally absent.

Metabolic Bone Disease of Prematurity

MBD of prematurity is caused chiefly by deficiencies in dietary phosphate and calcium. Eighty percent of bone mineralization in the fetus occurs during the third trimester (Widdowson et al., 1951; Ellis et al., 1994), when fetal calcium and phosphorus requirements are 100 to 130 mg/kg per day and 60 to 75 mg/kg per day, respectively. Low mineral, especially phosphorus, containing diets do not meet the needs for bone mineralization of preterm infants and therefore predispose premature infants to this form of osteopathy. The greatest risks for phosphate deficiency result from (1) feeding unsupplemented human milk, (2) milk formulas not designed for use in preterm infants, or (3) prolonged parenteral nutrition. About 30% of preterm infants with birthweights less than 1500 g develop bone disease (Funke et al., 2006). The rates are thought to be higher still in infants with ELBW of less than 1000 g. Even close attention to nutrition support does not prevent a high prevalence of bone disease in ELBW infants (Mitchell et al., 2009).

Additional, nonnutritional risk factors for MBD in ill preterm infants include the early withdrawal of placental estradiol and progesterone, lack of mobility, and therapy with a variety of medications, including dexamethasone and methylxanthines (Box 95.4), which can increase urinary calcium excretion and contribute to serum mineral imbalance, nephrocalcinosis, and osteopenia (Aladangady et al., 2004). Glucocorticoids decrease bone formation by inhibiting osteoblast growth and increasing cell death of osteoblasts and osteocytes and, at least over several months, increasing osteoclastogenesis and bone resorption. Copper deficiency is a rare contributor to osteopenia in preterm infants (Olivares and Uauy, 1996).

• BOX 95.3 Forms of Metabolic Bone Disease Manifesting Themselves in Newborns and Infants

Phosphate Deficiency

- Osteopathy of prematurity
- X-linked hypophosphatemic rickets
- Fanconi syndrome
- Antacid-induced osteopathy (aluminum hydroxide)
- Tumor (including hemangioma)-associated rickets

Calcium Deficiency

- Osteopathy of prematurity
- Inadequate intake of dietary calcium after weaning
- Inadequate calcium in total parenteral nutrition solution

Vitamin D Deficiency

- Maternal vitamin D deficiency (congenital rickets)
- Inadequate intake of dietary vitamin D
- Lack of adequate sunlight exposure and dietary inadequacy

Vitamin D Malabsorption

- Hepatic disease (steatorrhea)
- Short bowel syndrome
- Pancreatic insufficiency

Vitamin D Metabolic Defects

- Hepatic rickets (inadequate vitamin D 25-hydroxylation)
- 1 α -Hydroxylase deficiency
- Renal insufficiency (renal osteodystrophy)
- Anticonvulsants (increased 25(OH)D metabolism)

Vitamin D Receptor Defects

- Hereditary vitamin D resistance

• BOX 95.4 Medications Associated With Metabolic Bone Disease in Preterm Infants

- Glucocorticoids
- Furosemide/loop diuretics
- Methylxanthines
- Anticonvulsants: phenytoin and phenobarbital
- Antacids: proton pump inhibitors and H₂ blockers
- Anticoagulants
- Cyclosporine

In preterm infants, osteopenia with or without rachitic changes at the cartilage–shaft junction usually appears between 6 and 12 weeks of age, and fractures are reported in up to 24% in VLBW infants (Eliakim and Nemet, 2005). The incidence and severity increase with decreasing gestational age and birthweight, and they are more common in preterm infants with a complicated medical course and delayed nutrition. In VLBW babies, postnatal bone mineralization significantly lags behind the expected intrauterine bone mineralization rate. The pathogenesis involves increased endosteal resorption more than decreased bone formation—that is, it is a high-turnover osteopathy (Beyers et al., 1994). On the other hand, osteopathy is usually not a problem for healthier, larger preterm infants.

Clinical Findings of Metabolic Bone Disease of Prematurity

The clinical findings in VLBW infants with severe osteopathy, as in older, term infants with rickets, include a widened anterior fontanel, craniotabes, bony expansion of wrists, costochondral beading, and rib or long-bone fractures. Rib undermineralization, softening, and fractures can lead to respiratory distress (especially tachypnea), atelectasis, or pneumonia. Long-term effects of osteopathy of prematurity may include delayed dental maturation (Seow, 1996) and reduced height and bone mineral density in adulthood (Hovi et al., 2009).

Mineral Deficiency in Metabolic Bone Disease

Phosphate depletion and osteopathy occur in rapidly growing preterm infants fed unsupplemented human milk, which has low phosphate content. Characteristically, these infants develop hypophosphatemia, hypophosphaturia, hypercalcemia, and hypercalciuria. Serum PTH level may be low or normal, 25(OH)D level is normal, and 1,25(OH)₂D level is elevated. The hypophosphatemia stimulates production of 1,25(OH)₂D, which in turn increases intestinal calcium absorption. However, in the presence of hypophosphatemia, only limited amounts of calcium can be deposited in bone, leading to hypercalcemia and hypercalciuria. The hypercalcemia inhibits PTH secretion.

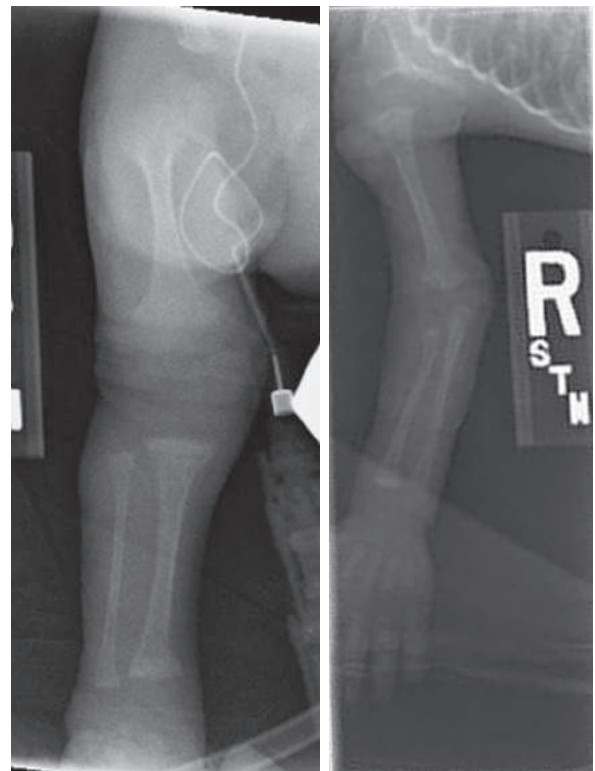
This form of osteopathy does not respond to vitamin D therapy unless vitamin D deficiency also is present. Vitamin D supplementation without prior correction of the underlying dietary phosphate deficiency may aggravate hypercalcemia and hypercalciuria by enhancing intestinal calcium absorption. The bone disease in these infants responds to increased dietary phosphate, accomplished by addition of a human milk supplement designed for preterm infants or by the switching to a preterm milk formula; both diets provide additional calcium as well as phosphorus. The recommendations for enteral nutrition for VLBW infants for phosphate and calcium range from 75 to 140 mg/kg per day and from 150 to 220 mg/kg per day, respectively (Abrams and Committee on Nutrition, 2013). The addition of 20 to 25 mg/kg per day of potassium phosphate may also be required for repletion of deficient serum phosphorus levels. However, because phosphate repletion promotes bone mineralization, serum calcium level may fall to subnormal levels (“hungry bones” syndrome) unless supplemental calcium (e.g., 30 mg/kg per day) also is provided. The recommended intakes of calcium and phosphorus (Demarini, 2005) have benefits of improved bone growth, less severe dolichocephaly, and avoidance of fractures. For infants with a history of osteopathy of prematurity, it is important that after hospital discharge a mineral-enriched diet

and serial laboratory monitoring be maintained for several weeks to months.

Most often, neither hyperparathyroidism nor vitamin D deficiency is present in phosphate-deficient osteopathy of prematurity. In contrast, the pathogenesis of calcium-deficiency rickets and that of vitamin D deficiency are similar in that hypocalcemia causes hyperparathyroidism. The elevated PTH level increases bone resorption and enhances renal 1,25(OH)₂D synthesis, which, in turn, increases intestinal calcium and phosphorus absorption. Individual preterm babies may have predominant phosphate depletion, but mixed phosphate and calcium deficiency is more common; isolated calcium deficiency is rare. In dual-mineral deficiency, laboratory tests may show low, normal, or slightly elevated serum calcium levels and low to low-normal phosphorus levels. In cases of severe or complicated bone disease, serum 25(OH)D is a useful screen for evaluation of sufficiency of vitamin D stores; levels less than 6 ng/mL indicate *severe* vitamin D deficiency (Misra et al., 2008).

Imaging for Metabolic Bone Disease

For evaluation of bone mineral status in preterm neonates, measurement of bone cortical thickness and visual inspection of the proximal part of the humerus on a chest radiograph is a common screen for bone disease. Additional images may be required to fully assess the neonate for MBD (Fig. 95.2). However, the appearance of “osteopenia” may not be appreciated on plain film until a 20%–40% decrease in mineralization is reached (Nelson and Koo, 1999). For that reason, assessment of bone mineral content by dual X-ray



• **Fig. 95.2** The long bones in this 6-week-old ex-26-week infant demonstrate severe generalized osteopenia. The zones of provisional calcification are frayed and very irregular. The metaphyses are irregular, showing cupping and fraying, particularly prominent at the knees and distal part of the femurs.

absorptiometry (DXA) may be used for its accuracy, reproducibility, and low radiation exposure. Normative data remain somewhat limited in preterm and former preterm infants, decreasing DXA's utility (Abrams and Committee on Nutrition, 2013). Longitudinal quantitative ultrasound measurement of the speed of sound in long bones, combined with measurement of serum bone markers, is a promising assessment tool (Ashmeade et al., 2007) although not one commonly used clinically at this time. Use of quantitative computed tomography to assess true volumetric bone density is limited by its significantly higher radiation exposure and lack of normative data in infants.

Laboratory Evaluation

Serum ALP, a marker of osteoblastic bone formation, is frequently used to monitor skeletal metabolism in preterm infants. However, the magnitude of elevations in ALP concentrations is not a good predictor of the extent of bone mineral deficits. As elevations of ALP concentrations and clinical metabolic bone disease are rare in the first 4 weeks of life, the American Academy of Pediatrics Committee on Nutrition recommends ALP and serum phosphorus concentrations be measured at 4 to 6 weeks after birth in VLBW infants and biweekly thereafter. The ALP concentration usually peaks at 400 to 800 IU/L and then decreases in VLBW infants who do not develop MBD (Abrams and Committee on Nutrition, 2013).

Use of PTH has been suggested (Moreira et al., 2014) as an early marker of MBD in preterm infants. Moreira et al. (2014) reported that a PTH level greater than 180 mg/dL at 3 weeks' chronologic age should alert clinicians to the possibility of MBD. Serial urinary biomarkers of bone metabolism (e.g., pyridinoline, deoxypyridinoline) have not yet been shown to be useful in predicting severe osteopathy because the levels are related to bone volume, and normative data for growing preterm infants are lacking.

Vitamin D–Deficiency Rickets

Vitamin D-deficiency rickets most often occurs in exclusively breastfed infants who also have little exposure to sunlight and are dark skinned. Unfortified human milk has a vitamin D activity ranging from 5 to 80 IU/L (Specker et al., 1985; Greer, 2004; Hollis et al., 2015), which may be insufficient for maintaining normal 25(OH)D levels in infants. Historically, a marked rise in the prevalence in nutritional rickets has accompanied industrialization and urban crowding. Clinical rickets often manifests itself at 3 months of age or later, but onset in early infancy is not uncommon. In developed countries, nutritional rickets has never been eradicated, and there is a resurgence of vitamin D deficiency in North America and Europe (Rovner and O'Brien, 2008). As reviewed, maternal vitamin D deficiency during pregnancy and lactation puts the newborn at high risk. There are long-term deleterious effects on musculoskeletal health (Nabulsi et al., 2008). The 2008 American Academy of Pediatrics statement on prevention of rickets and vitamin D deficiency (Wagner et al., 2008) doubles the previous American Academy of Pediatrics vitamin D intake recommendation. These guidelines state that breastfed and partially breastfed infants should receive supplementation with 400 IU vitamin D per day beginning in the first few days of life. All infant formulas available in the United States must contain at least 400 IU/L vitamin D. Consequently, the guidelines also state all nonbreastfed infants who are ingesting less than 1 L of formula per day should receive a vitamin D supplement of 400 IU/day.

In 2013 the American Academy of Pediatrics Committee on Nutrition recommended that VLBW infants receive 200 to 400 IU vitamin D per day. This can be increased to 400 IU/day when the weight exceeds 1500 to 2000 g and the infant is tolerating full enteral nutrition (Abrams and Committee on Nutrition, 2013). European guidelines suggest higher intakes of vitamin D of 800 to 1000 IU/day may be used for VLBW infants (Agostoni et al., 2010).

Higher doses of vitamin D may be necessary for infants with fat malabsorption or those taking anticonvulsant medications. Vitamin D status should be assessed with use of 25(OH)D and PTH concentrations and measures of bone mineral status. Stoss therapy, which occasionally is used to treat rickets, consists of a single large oral or intramuscular dose (150,000–500,000 IU) of vitamin D (Emel et al., 2012).

Nutritional rickets worldwide can be due to degrees of vitamin D deficiency or calcium deficiency. Calcium deficiency is the major cause of rickets in Africa and some parts of tropical Asia but is being recognized increasingly in other parts of the world (Thacher et al., 2006). As a consequence, in tropical populations, rickets may occur later than at higher latitudes, between 1 and 2 years of age, after weaning, and with introduction of a low-calcium diet. One study showed, for example, that in Turkey most rachitic children had vitamin D deficiency, whereas in Egypt they had mostly calcium insufficiency combined with vitamin D deficiency (Baroncelli et al., 2008). It is likely that relative deficiencies of calcium and vitamin D interact with genetic (e.g., vitamin D receptor genotypes) and/or environmental factors to stimulate the development of rickets. Therefore the current North American and European recommendations for vitamin D supplementation may need adjustments for other pediatric populations with limited calcium intake.

Congenital rickets should always prompt an investigation for maternal vitamin D deficiency. Rickets, hypercalciuria, and hypophosphatemia also occur in Fanconi syndrome. Prolonged treatment with aluminum-containing antacids can induce hypophosphatemia and rickets (Pattaragarn and Alon, 2001). These antacids should be avoided or used with caution during infancy.

Renal Osteodystrophy

Because normal renal function is essential for physiologic mineral and bone metabolism, renal insufficiency induces hyperphosphatemia and bone disease. Renal osteodystrophy can be predominantly high or low bone turnover, or the two types may alternate during the clinical course in an individual infant (Levin and Stevens, 2014). High bone turnover or osteitis fibrosa is a manifestation of secondary hyperparathyroidism. Parathyroid hyperfunction often occurs early in the course of renal failure. Contributing factors include phosphate retention, impaired renal 1,25(OH)₂D synthesis, hypocalcemia, parathyroid gland hyperplasia, and skeletal resistance to PTH actions. Low-turnover osteodystrophy (adynamic bone or osteomalacia) results from suppressed bone formation; it is a major concern in the treatment of dialyzed infants.

A principal goal of therapy is to lower serum phosphate level so as to prevent hypocalcemia and severe hyperparathyroidism (Dasgupta et al., 2013). Phosphate restriction is accomplished by the feeding of breast milk or Similac PM 60/40. Oral phosphate binders such as calcium carbonate may be needed to reduce the intestinal absorption of phosphate. Hypocalcemia and metabolic acidosis should be treated with appropriate supplements. If serum 1,25(OH)₂D level is low, 1,25(OH)₂D therapy will increase

intestinal calcium absorption, transcriptionally suppress PTH gene expression, and decrease parathyroid hyperplasia. Serum calcium, phosphorus, and PTH levels, as well as linear growth and bone radiographs, should be serially monitored (Kemper and van Husen, 2014). Management of severe renal osteodystrophy in neonates is particularly complicated by increased phosphate requirements for growth. Calcium supplementation and use of potent vitamin D metabolites may also produce an “oversuppression” of PTH, contributing to adynamic bone disease. As with any complex disorder, effective clinical management requires close monitoring and an integrated team approach.

Inherited Metabolic Bone Disease in Infancy

Several forms of MBD or rickets have been described that can present in newborns and infants (DiMeglio and Econs, 2001). 1α -Hydroxylase deficiency, formerly known as *vitamin D-dependent rickets type 1*, is caused by a mutation in the gene encoding 25(OH) D 1α -hydroxylase (*CYP27B1*) (Wang et al., 1998). This autosomal recessive disorder is associated with defective conversion of 25(OH) D to $1,25(\text{OH})_2\text{D}$ and is most common in French-Canadian kindreds. Muscle weakness with severe hypocalcemia and secondary hyperparathyroidism appear shortly after birth, and rickets presents within the first year of life. Treatment with 1α -hydroxylated vitamin D analogues induces remission.

Hereditary resistance to vitamin D, formerly known as *vitamin D-dependent rickets type 2*, is caused by mutations in the vitamin D receptor gene. This mutation leads to end-organ resistance to $1,25(\text{OH})_2\text{D}$. Affected infants show early-onset rickets, hypocalcemia, elevated serum $1,25(\text{OH})_2\text{D}$ levels, secondary hyperparathyroidism, and alopecia. Depending on the genotype, there is a variable response to supraphysiologic doses of $1,25(\text{OH})_2\text{D}$ analogues and calcium.

X-linked dominant hypophosphatemia (XLH), also known as *familial hypophosphatemic rickets*, is a disorder of phosphate homeostasis (Gattineni, 2014). Its prevalence is 1 in 20,000. XLH is characterized by hypophosphatemia, rickets, and poor linear growth and is associated with a low TmP and renal tubular phosphate leak. Defective regulation of vitamin D metabolism results in inappropriately normal $1,25(\text{OH})_2\text{D}$ concentrations in the face of hypophosphatemia. XLH is caused by mutations in the phosphate-regulating endopeptidase homologue, X-linked gene (*PHEX*). Treatment consists of supplemental phosphate and calcitriol, with the goal of normalizing the ALP level, keeping the PTH level normal, maximizing linear growth, and reducing rachitic changes. There is a similar condition of autosomal dominant hypophosphatemic rickets caused by an activating mutation in the fibroblast growth factor 23 gene (*FGF23*) on chromosome band 12p13. Treatment is similar to that for XLH. Autosomal recessive hypophosphatemic rickets type 1 and autosomal recessive hypophosphatemic rickets type 2 are caused, respectively, by mutations in the dentin matrix acidic phosphoprotein 1 gene (*DMP1*) and the ectonucleotide pyrophosphatase/phosphodiesterase 1 gene (*ENPP1*), which interfere with bone mineralization and renal phosphate handling.

Osteogenesis imperfecta (OI) is a heterogeneous group of disorders that cause various degrees of bone fragility and skeletal

deformity (Forlino and Marini, 2016). OI was originally identified as an autosomal dominantly inherited abnormality of type I collagen due to mutations of *COL1A1* and *COL1A2*. Four primary types were initially described. However, in the past several years, several other types have been identified, most of which have autosomal recessive inheritance. The most severe forms present with fractures at or shortly after birth. The affected babies may also have deformities of long bones, osteopenia of the skull, and early death due to respiratory deficiency. Administration of a bisphosphonate such as pamidronate may help reduce bone fractures and bone pain (Glorieux et al., 1998).

Hypophosphatasia is characterized by defective bone mineralization secondary to deficiency of the tissue-nonspecific isoenzyme of ALP. It is caused by a mutation of the *ALPL* gene on chromosome band 1p36. Perinatal lethal, infantile, childhood, and adult-onset forms have all been described, with the most severe forms being the perinatal and infantile forms. Clinical manifestations in infants include osteomalacia and rickets with respiratory insufficiency due to progressive chest deformity. Hypercalcemia and hyperphosphatemia can be seen in severely affected infants. Whyte et al. (2012) showed that enzyme replacement with recombinant ALP can improve skeletal findings as well as pulmonary and overall physical function in infants with the perinatal and infantile forms of hypophosphatasia.

Neonatal rickets with increased bone density rather than osteopenia can occur in infantile osteopetrosis, a rare autosomal recessive disorder of osteoclast formation. Diagnosis may be obscured by concurrent maternal vitamin D deficiency (Popp et al., 2000).

Suggested Readings

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96

Disorders of the Adrenal Gland

PATRICIA Y. FECHNER

KEY POINTS

- Infants born with ambiguous genitalia or nonpalpable testes need to be evaluated for congenital adrenal hyperplasia as it can be life threatening.
- Adrenal steroid levels vary with gestational age.
- Adrenal insufficiency should be treated with hydrocortisone to avoid adrenal crisis. High doses of hydrocortisone contain mineralocorticoid activity.

The Adrenal Gland

Embryology

Normal adrenal function is critically important for maintenance of intrauterine homeostasis, promotion of organ maturation, and adaptation to extrauterine life. The dual embryologic origin of the human adrenal gland results in an outer adrenal cortex and an inner adrenal medulla; each part secretes different vital hormones critical to fetal development. Embryologically, the adrenal cortex develops from the coelomic mesoderm of the urogenital ridge, whereas the medulla arises from neural crest tissue in the adjacent sympathetic ganglion at celiac plexus level. During the 5th week of fetal development, mesothelial cells from the posterior abdominal wall, between the root of the bowel mesentery and developing mesonephros, proliferate and form the primitive adrenal cortex. In the 6th week, a second wave of mesothelial cells surrounds the primitive cortex and later forms the adult or definitive cortex. By 8 weeks of gestation, the cortical mass separates from the rest of mesothelial tissue and becomes surrounded by connective tissue (Sadler, 2000). This separation divides adrenocortical and gonadal primordium (Mesiano and Jaffe, 1997). Chromaffin cells, which originate from neural crest, migrate toward the adrenal cortex around this time and gradually invade the medial aspect of the cortical tissue along its central vein to gain central position, forming the adrenal medulla. Fig. 96.1 summarizes the embryologic origin of the adrenal gland (Else and Hammer, 2005). By 8 to 9 weeks of gestation, the adrenal gland is encapsulated and contains an outer “definitive” zone where glucocorticoids and mineralocorticoids are synthesized and a larger inner “fetal” zone where dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) are produced and then subsequently converted to estriol by the placenta. Postnatally, the fetal or primitive zone of the adrenal gland rapidly involutes to disappear by approximately 6 months of age (Kempna and Fluck, 2008). Zonation of the cortex,

zona glomerulosa, and fasciculata is present at birth, but full differentiation into three separate zones occurs much later, at approximately 3 years of age, when zona reticularis development takes place (Barwick et al., 2005). Fig. 96.2 depicts these changes in the adrenal gland from 9 weeks of gestation through childhood. This chapter focuses on the development, function, and pathophysiology of the steroidogenic adrenal cortex.

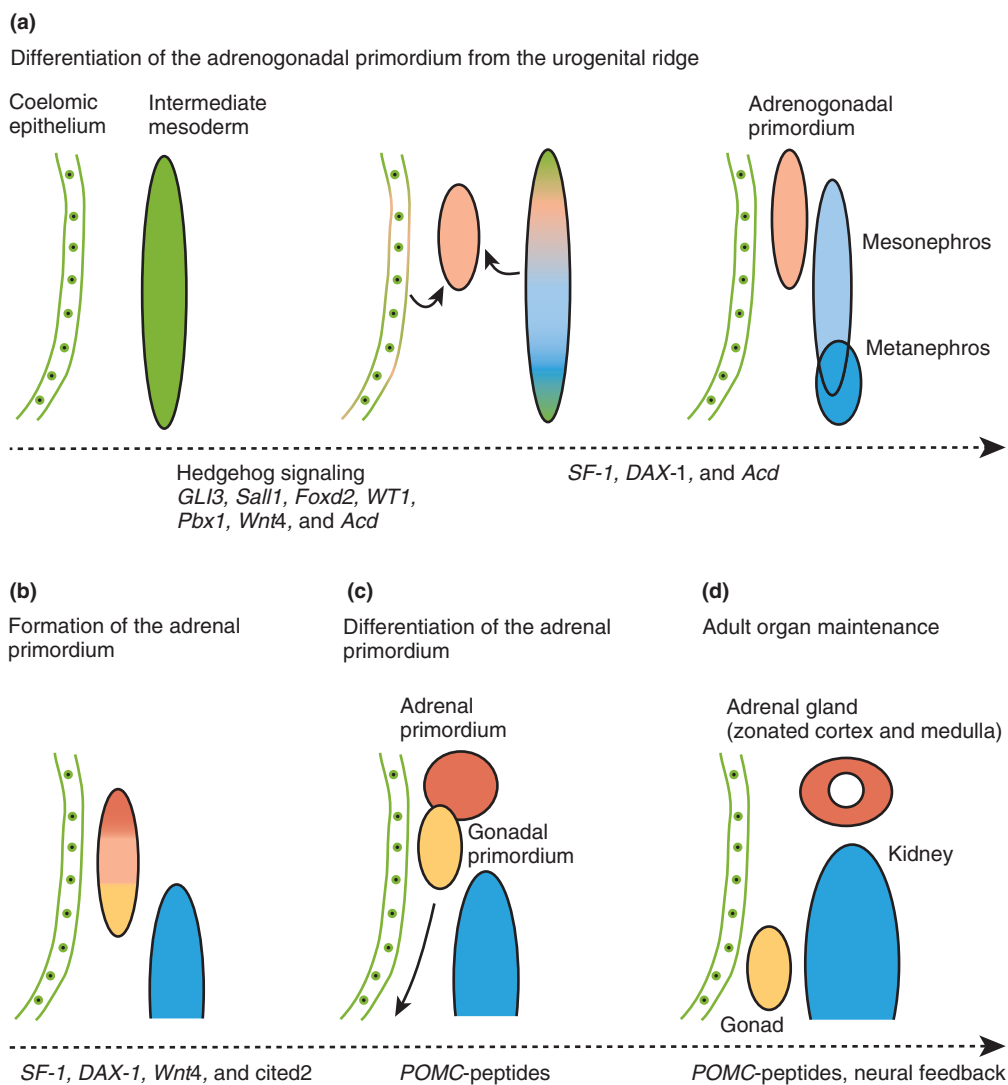
Morphology

The adrenal glands are bilateral structures, located above the kidneys in the retroperitoneum area. At birth, the adrenals are approximately one-third the size of neonatal kidneys, weighing 8 to 9 grams, and are 10- to 20-fold larger than the adult glands relative to body weight (0.4% vs 0.01%) (Moore and Persaud, 1998). In the third trimester and the first 3 months after birth, the glands predominantly consist of cortex, where active production of glucocorticoids, steroid precursors, estrogens, and progesterone takes place. Ultrasonographically, the neonatal adrenal gland characteristically has a thin reflective core surrounded by a thick transonic zone. The gland subsequently decreases in size as the active fetal cortex regresses to reach approximately 8% of the kidney size in adulthood (Barwick et al., 2005).

Histologically, the fetal adrenal cortex consists of a small outer definitive zone, which appears to produce few adrenal steroid hormones until late gestation, and a larger inner fetal zone that produces adrenal steroid hormones throughout gestation. In addition, there is a transitional zone where cortisol production takes place toward the end of fetal development (Mesiano and Jaffe, 1997). At birth, the large fetal zone of the fetal adrenal involutes and disappears by 6 months of age. Concurrently, the definitive zone together with the transitional zone develops into the fully differentiated zona glomerulosa and fasciculata by the age of 3 years. The zona reticularis begins to develop only after 4 years of age and may not be fully differentiated before the age of 15 years. In an adult adrenal gland, these three distinctive zones lie adjacent to one another. The zona glomerulosa is located immediately below the capsule, the zona fasciculata being in the middle, and the innermost zone next to the medulla is the zona reticularis.

Fetal and Adult Adrenal Functions

A cascade of adrenal steroidogenesis in the adult is shown in Fig. 96.3. Three major pathways of mineralocorticoid, glucocorticoid, and androgen synthesis take place mainly in the glomerulosa, fasciculata, and reticularis zones of the cortex, respectively. Aldosterone is the main mineralocorticoid regulating sodium and fluid



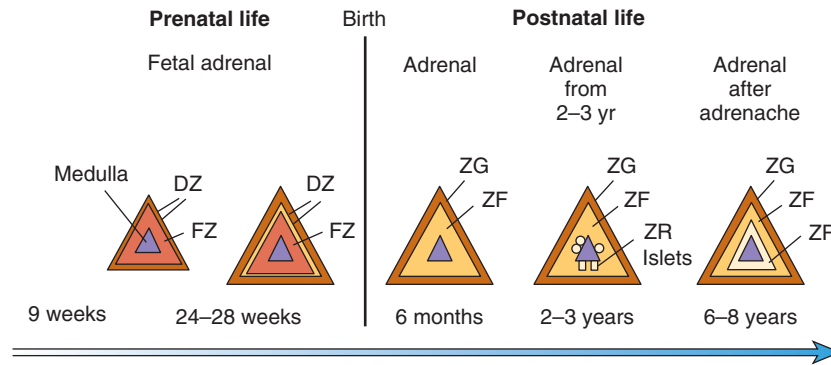
TRENDS in Endocrinology & Metabolism

• **Fig. 96.1** Adrenocortical Development From Urogenital Ridge to Adrenal Gland. *DAX-1*, Dosage-sensitive sex reversal-adrenal hypoplasia congenita critical region on the X chromosome gene-1; *POMC*, proopiomelanocortin; *SF-1*, steroidogenic factor-1. (From Else T, Hammer GD. Genetic analysis of adrenal absence, agenesis and aplasia. *Trends Endocrinol Metab.* 2005;16:458–468.)

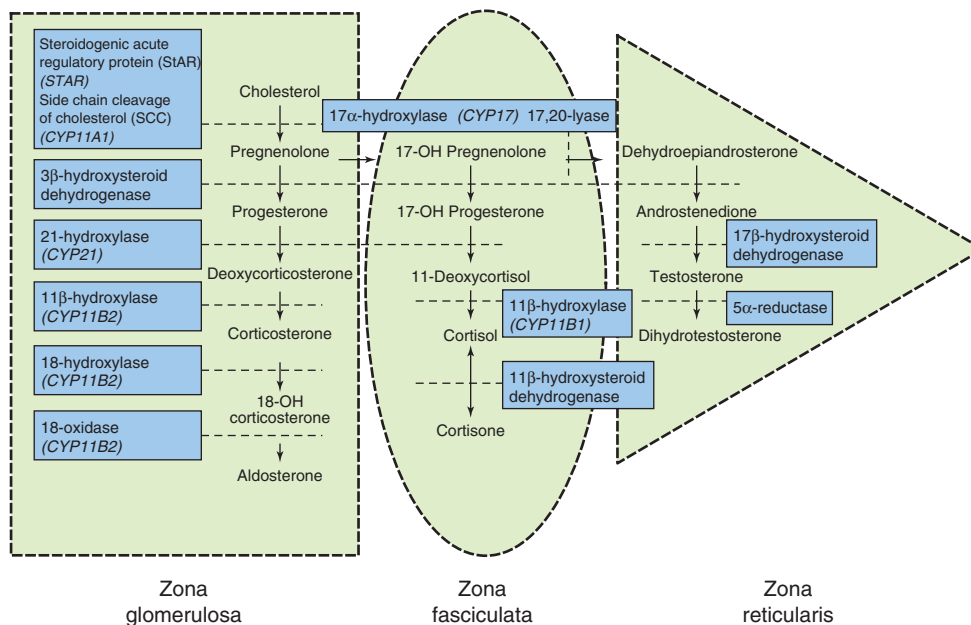
volume homeostasis; it is under the control of the rennin–angiotensin system and blood potassium concentrations (Kuhnle et al., 1981). The principal glucocorticoid in humans is cortisol and has a wide range of roles in regulating body functions, from carbohydrate metabolism, immune system, and acute and chronic stress response to musculoskeletal metabolism. Cortisol production is regulated through a negative feedback loop involving hypothalamic corticotropin-releasing hormone (CRH) and pituitary adrenocorticotrophic hormone (ACTH) (New and Wilson, 1999). Adrenal androgens have an age-specific secretion profile with an increase at adrenarche, which occurs 2 years before the time of puberty, and then a gradual decrease with aging until andropause (Orentreich et al., 1984). The regulatory mechanism behind normal adrenal androgen production is largely unknown but involves ACTH to some extent (Hanley and Arlt, 2006).

In the fetal adrenal gland, steroidogenic enzymes are found as early as 7 weeks' gestation (Goto et al., 2006; Hanley and

Arlt, 2006). At 8 weeks' gestation, the fetal adrenal gland produces cortisol under ACTH control. A transient expression of 3β -hydroxysteroid dehydrogenase type 2 (3β -HSD2) during this critical time from 7 to 12 weeks' gestation allows the fetal adrenal gland to produce cortisol. Activation of 3β -HSD2 serves principally to prevent virilization of the female genital anlage that would otherwise result from overwhelming amounts of DHEAS and its downstream androgen metabolites. It is believed that a transient peak of cortisol during this time suppresses the fetal hypothalamic–pituitary–adrenal (HPA) axis, keeping DHEAS production at a low level (Goto et al., 2006; White, 2006). By the end of the first trimester, cortisol secretion from the fetal adrenal gland begins to wane as a result of a decrease in 3β -HSD2 expression, thus decreasing HPA axis suppression with resultant increased DHEAS secretion. During the second and third trimesters, the fetal adrenal gland secretes abundant amounts of DHEA and its sulfated derivative DHEAS, earning



• **Fig. 96.2** Schematic Diagram of the Development of the Human Adrenal Cortex During Prenatal and Postnatal Life. DZ, Definitive zone; FZ, fetal zone; ZG, zona glomerulosa; ZR, zona reticularis; ZF, zona fasciculata. (Adapted from Stewart PM, Newell-Price JDC. The Adrenal Cortex. In: Melmed S, Polonsky KS, Larsen PR, Kronenberg HM (eds). *Williams Textbook of Endocrinology*. 13th ed. Philadelphia, PA: Elsevier, Inc.; 2016: 489–555.)



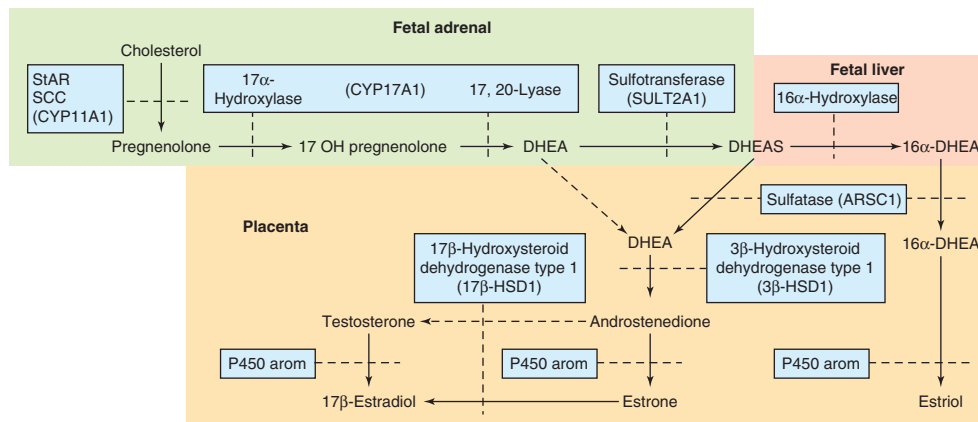
• **Fig. 96.3** Steroidogenesis of the Adult Adrenal Gland. Each of the three biosynthetic pathways takes place in different zones: aldosterone biosynthesis in the zona glomerulosa, cortisol biosynthesis in the zona fasciculata, and androgen production in the zona reticularis. The enzymes that catalyze the reactions are indicated in boxes.

the term *androgen factory* (Kempna and Fluck, 2008). The rate of steroid secretion by the fetal adrenal glands may be fivefold that of the adult adrenal glands at rest (Carr and Simpson, 1981). The placenta can also convert DHEAS back to DHEA by a sulfatase enzyme (i.e., arylsulfatase). These adrenal steroids serve as precursors for 17,20 lyase activity of the P450C17 enzyme for androgen production and subsequent estrogen production (Fig. 96.4). In addition, DHEAS is oxidized in the fetal liver to a 16 α -hydroxylated derivative, which is converted by the placenta to estriol by the same set of enzymes as in estradiol synthesis.

The physiology of human pregnancy involves a continuous supply of relatively increased amount of estrogens. In near-term human pregnancy, the rate of estrogen production increases strikingly, reaching concentrations 1000-fold greater than that of nonpregnant women (Carr and Simpson, 1981). During early gestation, the estradiol required to maintain pregnancy is provided

by the corpus luteum of the maternal ovary. But after 8 weeks' gestation, the fetoplacental unit synthesizes most of the estradiol required to maintain pregnancy (Simpson and MacDonald, 1981).

DHEAS is the main steroid secreted by the fetal adrenal cortex from midgestation onward. The activity of 3 β -HSD2 controls fetal cortisol synthesis. By the end of pregnancy, fetal cortisol is required in preparation for parturition (i.e., lung maturation or surfactant production) and could have a role in triggering parturition, as shown in other species (Gross et al., 2000). Maternal cortisol cannot normally reach the fetus because it is oxidized to cortisone, an inactive steroid, by placental 11 β -hydroxysteroid dehydrogenase type 2 (Wilson et al., 2001). When pregnancy approaches term, 3 β -HSD2 expression increases again and remains high, allowing increased cortisol secretion. In a child, 3 β -HSD2 secretion is high in the adrenal gland until adrenarche, when 3 β -HSD2 activity decreases again to allow an increase in DHEA (Gell et al., 1998).



• **Fig. 96.4** Steroidogenesis of the Fetal Adrenal Gland and Fetoplacental Unit. Predominant dehydroepiandrosterone and dehydroepiandrosterone sulfate production occurs after 12 weeks' gestation. DHEA, Dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; SCC, side chain cleavage of cholesterol; StAR, steroidogenic acute regulatory protein.

and its downstream androgen metabolite secretion, which gives rise to the development of pubic and axillary hair.

Control of Glucocorticoid and Mineralocorticoid Production

Two distinct regulatory circuits control adrenal glucocorticoid and mineralocorticoid secretion. The HPA axis determines the set point for circulating glucocorticoid (cortisol) concentration. The neuropeptide CRH (or factor) and arginine vasopressin (AVP) are synthesized in the hypothalamic paraventricular nucleus and released into the hypophyseal portal circulation at the median eminence in response to stress (Aguilera, 1994) and, beginning at approximately 6 months of age, to circadian cues (Onishi et al., 1983). These neuropeptides stimulate the release of ACTH from the anterior pituitary corticotrophs. CRH is the primary stimulator of ACTH while AVP amplifies the effect of CRH. ACTH released into the systemic circulation augments adrenocortical secretion of cortisol and DHEA by acting on the ACTH receptor (Clark and Cammas, 1996), a member of the melanocortin receptor family. The ACTH receptor is present on steroidogenic cells of the fetal zone and transitional zone of the fetal adrenal as well as the adult adrenal cortex. The resulting increase in plasma cortisol concentration limits further release of hypothalamic neuropeptides and ACTH by negative feedback through glucocorticoid receptors at the central nervous system and pituitary sites. As a corollary, if glucocorticoid production is impaired by intrinsic adrenal dysfunction, then neuropeptide CRH and ACTH release are augmented.

The components of the HPA axis are present early in human development (Mesiano and Jaffe, 1997). As detailed earlier, the fetal adrenal gland begins to develop at 4 weeks' gestation, when initial evagination of the pituitary primordium occurs (Conklin, 1968). ACTH-producing pituitary cells can be detected at 7 weeks' gestation, and an intact hypophyseal portal vascular system is present by 12 weeks' gestation (Baker and Jaffe, 1975; Thliveris and Currie, 1980). Nerve terminals containing CRH can be detected in the hypothalamus by approximately 16 weeks' gestation. Virilization caused by increased production of adrenal androgens in females with congenital adrenal hyperplasia (CAH) occurs before 12 weeks' gestation; therefore ACTH-producing corticotrophs must undergo cortisol-mediated feedback modulation at the initial stages of hypothalamic–pituitary development (White, 2006).

Mineralocorticoid (aldosterone) release by the zona glomerulosa of the adrenal cortex is determined by the renin-angiotensin system, with acute modulation to a lower extent by ACTH as well (Dzau, 1988). Decreases in vascular volume result in increased secretion of renin by the renal juxtaglomerular apparatus. Renin, a proteolytic enzyme, cleaves angiotensinogen to angiotensin I, a biologically inactive decapeptide. Angiotensin I is then cleaved and activated by angiotensin-converting enzyme in the lung and other peripheral sites to angiotensin II. Angiotensin II and its metabolite angiotensin III possess vasopressor and potent aldosterone secretory activity. Although angiotensin II receptors are present on cells of the definitive zone at 16 weeks' gestation, significant aldosterone production by the fetal adrenal gland does not occur until the third trimester of pregnancy (Mesiano and Jaffe, 1997).

Molecular Basis of Adrenal Development

Several transcription factors are critically important for normal adrenal development. Two related transcription factors have emerged as key regulators of adrenal development: the nuclear receptor DAX-1 (dosage-sensitive sex reversal-adrenal hypoplasia congenita [AHC] critical region on the X chromosome gene-1, encoded by *NROB1/AHC*) and steroidogenic factor-1 (SF-1, encoded by *NR5A1*, also known as AD4BP). SF-1 (*Nr5a1*) knockout mice lack adrenal glands and gonads, and subsequent identification of *NR5A1* mutations in humans with adrenal insufficiency confirms the essential role of SF-1 in development (Parker, 1998). SF-1 (*Nr5a1*) expression is found in the early urogenital ridge of the mouse in cells that give rise to both the bipotential gonad and adrenal cortex. Expression of SF-1 (*Nr5a1*) remains high throughout embryogenesis, the postnatal period, and adult life (Parker, 1998).

DAX-1 is an orphan nuclear receptor that colocalizes with SF-1 in the cells of adrenal glands, gonads, gonadotropes, and ventral–medial lateral nucleus of the hypothalamus. Deletion of *NROB1* results in AHC. Although the exact role of DAX-1 in adrenal development is not known, it has been shown to interact with SF-1 (Ikeda et al., 2001). Normally, DAX-1 recruits the nuclear corepressor N-CoR to SF-1 and represses SF-1 (Crawford et al., 1998).

Similarly, the Wilms tumor suppressor gene (*WT1*) protein has been shown to interact with SF-1. *WT1* encodes 24 different protein isoforms that act as transcription factors. *Wt1* is detected

in the urogenital ridge of the mouse embryo but is not detected in adult or fetal adrenals. Mutations in *Wt1* have resulted in abnormal development of the adrenal in the mouse but have not been clearly correlated with abnormal human adrenal development (Vidal and Schedl, 2000). *WT1* has been found though to be expressed in the fetal adrenal gland (Ambu et al., 2015).

Assessing Adrenal Function in the Newborn

Significant evolution of adrenal steroid production occurs over the first days and months of life as the adrenal cortex plays a major role in the newborn postnatal adaptation. During interpretation of the newborn adrenal steroidogenic function, special attention must be paid to age-related changes in adrenal steroid intermediates, circulating cortisol, and aldosterone concentrations that reflect ongoing adrenal maturation (Sippell et al., 1978, 1980; Lashansky et al., 1991). For example, until at least 1 month postnatally, a large proportion of cortisol and its metabolites is excreted as sulfate esters. This sulfation may serve to inactivate a number of circulating cortisol metabolites during fetal and neonatal life (Ducharme et al., 1970).

Immediately after birth the third trimester fetal zone that once was the predominant component of the adrenal cortex in the fetus and preferentially produced DHEA and DHEAS starts to reduce in size. This rapid loss of the fetal zone during this period results in a dramatic fall of the circulating DHEA concentration over the 1st week to 1 month postnatally. The variable pattern of decline in the ensuing weeks probably reflects variation in remodeling of the fetal zone and emergence of the zona fasciculata of the definitive zone, the latter being a feature of an adult cortex. In addition to diminished 3β -HSD2 activity, preterm infants have sustained elevations in 17-hydroxyprogesterone and the 17-hydroxyprogesterone-to-cortisol ratio, suggesting a reduction in 21-hydroxylase activity (Lee et al., 1989; al Saedi et al., 1995). Because blood-spot 17-hydroxyprogesterone concentration is used for newborn screening of CAH in many states (White, 2009), many preterm infants initially have an abnormal test result. Subsequent follow-up testing is then required to determine whether CAH is present. Plasma aldosterone concentrations tend to be higher in preterm infants than in term infants, both of which in turn are higher than in older children and adults (Kotchen et al., 1972; Doerr et al., 1988).

Cortisol has a critical role in maintaining homeostasis in response to stress. Relative adrenal insufficiency occurs when the HPA axis produces less than adequate cortisol for the degree of illness or stress. Immaturity of the adrenal gland and the HPA axis of the premature newborn infant suggest a rationale for why preterm infants are at increased risk of cortisol insufficiency (Fernandez and Watterberg, 2009). Clinicians are commonly faced with critically ill infants who have cardiovascular insufficiency with hypotension, a condition that has been associated with adverse consequences. The question often arises as to whether these manifestations reflect underlying glucocorticoid insufficiency. There is increasing evidence that relative adrenal insufficiency may be a cause of hemodynamic instability and hypotension in the critically ill newborn, but there is definitely a paucity of data in this population.

Random plasma cortisol measurement is often inadequate to answer this question, because the majority of critically ill newborns have low cortisol and ACTH values, without the expected increase in response to critical illness. Infants also do not exhibit diurnal variation of ACTH and cortisol until 6 months of age. Thus obtaining two or more samples of cortisol may be informative.

Response to exogenous ACTH (cosyntropin) is usually normal, suggesting that the inadequate response to critical illness in these newborns does not result from adrenal dysfunction but arises from some other components of the HPA axis (Fernandez et al., 2008). Interestingly, in extremely low birthweight infants (500 to 999 g) low cortisol concentrations were not predictive of adverse short-term mortality and morbidity. In contrast, high basal cortisol levels were associated with severe intraventricular hemorrhage, and extremely elevated values were associated with morbidity and death (Aucott et al., 2008).

Data associating treatment of adrenal insufficiency with outcomes in the term newborn are limited, and there have been no studies on outcomes beyond the immediate neonatal period. Nonetheless, no adverse events have been attributed to glucocorticoid treatment based on a relatively small number of study subjects. Currently there is insufficient evidence to support the routine use of glucocorticoids in critically ill newborns. On encountering an infant with vasopressor-resistant hypotension, accompanied by signs of cardiac hypofunction, the clinician must consider the risk-to-benefit ratio before arriving at the appropriate management. Therapeutic trials with hydrocortisone at the dose of 1 mg per kilogram of body weight have been suggested (Fernandez and Watterberg, 2009) and can be discontinued if there is no clinical improvement or if the pretreatment cortisol level is later observed to be greater than 15 μ g/dL. A metaanalysis of the use of hydrocortisone for hypotension and vasopressor dependence in preterm infants showed improvement in blood pressure and less need for vasopressor, but the clinical benefit is unknown, and long-term effects of hydrocortisone use are not known (Higgins et al., 2010). Special attention should be paid to the premature newborn concurrently receiving indomethacin, because the combination of hydrocortisone and indomethacin is associated with spontaneous gastrointestinal perforation (Peltoniemi et al., 2005).

Primary Adrenal Disorders

Steroidogenic Defects Caused by Adrenal Enzyme Deficiency

CAH refers to a family of inherited disorders in which defects occur in one of the enzymatic steps required to synthesize cortisol from cholesterol in the adrenal gland; therefore impaired cortisol synthesis is the cornerstone shared by all forms of CAH. The pathway of steroidogenesis in the adrenal cortex is illustrated in Fig. 96.3. Five forms of CAH with autosomal recessive mode of inheritance are summarized in this section.

Disorders That Lead to Virilization in Females

21-Hydroxylase Deficiency

Pathophysiology. In 21-hydroxylase deficiency (21-OHD), which is responsible for 90%–95% of all CAH cases, the conversion of 17-hydroxyprogesterone (17-OHP), the main substrate of the 21-hydroxylase enzyme, to 11-deoxycortisol in the pathway of cortisol synthesis is impaired, and precursors are shunted through the androgen pathway. The enzyme defect also impairs the conversion of progesterone to aldosterone, causing abnormal salt loss (New et al., 1966; New and Seaman, 1970). There are two forms of classic 21-OHD: simple virilizing and salt-wasting, distinguished by the adrenal gland's ability to produce adequate aldosterone. In both forms, severe 21-OHD results in elevated levels of adrenal androgens that cause ambiguous genitalia in the genetic female fetus. Diagnosis is made by the detection of extremely high

TABLE 96.1 Adrenal Steroid Levels in Response to Adrenocorticotrophic Hormone for Various Ages

| Steroid | | Premature 26–28 Weeks' Gestation | Premature 34–36 Weeks' Gestation | 1–6 Months | <1 Year |
|---------------------------|------------|----------------------------------|----------------------------------|------------|---------|
| Androstenedione ng/dL | Baseline | 92–892 | 90–837 | 6–78 | 6–78 |
| | Stimulated | 145–1248 | 183–1367 | 21–114 | 21–139 |
| | Change | 40–718 | 13–1084 | 9–76 | 10–75 |
| Cortisol mcg/dl | Baseline | 1–11 | 3–34 | 3–22 | 3–23 |
| | Stimulated | 6–52 | 16–76 | 27–50 | 32–60 |
| | Change | 4–41 | 6–44 | 19–41 | 17–40 |
| Deoxycorticosterone ng/dl | Baseline | 20–105 | 28–78 | 7–48 | 7–57 |
| | Stimulated | 44–320 | 28–95 | 40–158 | 20–157 |
| | Change | 17–215 | 1–67 | 13–144 | 26–110 |
| 11-Deoxycortisol ng/dL | Baseline | 110–1376 | 70–455 | 10–200 | 10–200 |
| | Stimulated | 206–2504 | 81–645 | 101–392 | 80–390 |
| | Change | 15–1128 | 40–190 | 5–366 | 5–350 |
| 17-OH progesterone ng/dL | Baseline | 124–841 | 186–472 | 13–173 | 11–173 |
| | Stimulated | 285–1310 | 334–1725 | 85–250 | 85–466 |
| | Change | 50–596 | 18–1253 | 52–193 | 50–275 |

Data was obtained by extraction, chromatography, and RIA (Radioimmunoassay) method and may not be applicable to other methods. These values should be treated as approximate and not exact cutoffs. In the preterm infants, testing was done on postnatal days 2–4. Adapted with permission from Nakamoto JM, Mason PW (eds). *The Quest Diagnostics Manual. Endocrinology*. 5th ed. Madison New Jersey: Quest Diagnostics Incorporated; 2012.

concentrations of basal and stimulated 17-OHP after performing an ACTH stimulation test. Table 96.1 lists steroid levels at baseline and following 250 mcg of ACTH given as an intravenous (IV) bolus in infants at various ages. The diagnosis is confirmed by molecular genetic analysis of the *CYP21* gene.

Clinical Signs and Symptoms. Females with simple virilizing CAH can be diagnosed at birth immediately because of the apparent genital ambiguity (Prader, 1958). For newborn males, however, differentiation of the external genitalia is not affected, because the main source of testosterone is the testes and not the adrenal gland. Postnatally, genitalia may continue to virilize because of an excess of adrenal androgens, and pseudoprecocious puberty can occur. In affected females, signs of hyperandrogenism include facial, axillary, and pubic hair, adult body odor, temporal balding, severe acne, irregular menses, and reduced fertility. Poor control of adrenal androgens in males has been associated with small testes, infertility, and short stature. Infertility occurs because the excess androgens are aromatized peripherally to estrogens, which suppress pituitary gonadotropins and function of the gonads, and due to the development of testicular adrenal rest tumors. The high estrogens also advance bone age. The high levels of androgens can also accelerate growth in early childhood, producing an unusually tall and muscular child. Thus the patients are tall as children but short as adults. The salt-wasting phenotype, which occurs in approximately 75% of CAH cases (New and White, 1995), is biochemically distinct from the simple virilizing form because of a deficiency of aldosterone, the salt-retaining hormone (New et al., 1966; Nimkarn et al., 2007). Resulting hyponatremia, hyperkalemia, high plasma renin activity, and fluid volume depletion that occur at day 5 to 15 of life are potentially fatal.

Epidemiology. Newborn screening worldwide of almost 6.5 million babies has demonstrated an overall incidence of 1:15,000 live births for the classic form of 21-OHD (Pang et al., 1988; Pang and Clark, 1990, 1993). The incidence of classic CAH in

either homogeneous or heterogeneous general populations is as high as 1 case per 7500 live births (Speiser et al., 1985).

Molecular Genetics. Hormonally and clinically defined forms of 21-OHD CAH are associated with distinct genotypes characterized by varying enzyme activity demonstrated by in vitro expression studies. The gene encoding 21-hydroxylase is a microsomal cytochrome P450, family 21, subfamily A, polypeptide 21 (*CYP21A2*) located on the short arm of chromosome 6, within the human leukocyte antigen complex (Dupont et al., 1977; Nebert et al., 1991). *CYP21A2* and its homolog pseudogene *CYP21A1P* alternate with two genes, *C4B* and *C4A*, which encode two isoforms of the fourth component of the serum complement system (White et al., 1986). More than 100 mutations have been described to date. These mutations include point mutations, small deletions, small insertions, and complex rearrangements of the gene (Stenson et al., 2003). The most common mutations appear to be the result of two types of meiotic recombination between *CYP21A2* and *CYP21A1P*: (1) misalignment and unequal crossing over, resulting in large-scale DNA deletions, and (2) apparent gene conversion events that result in the transfer to *CYP21A2* of smaller-scale deleterious mutations present in *CYP21A1P*. It is not always possible to accurately predict the phenotype on the basis of the genotype; such predictions have been shown to be 79%–88% accurate (Speiser et al., 1992; Wedell et al., 1992; Wilson et al., 1995) with some nonconcordance. Studies have demonstrated that there is often a divergence in phenotypes within mutation-identical groups; the reason for this requires further investigation (Krone et al., 2000; Chemaitilly et al., 2005).

Management Issues. Hormone replacement therapy with corticosteroids is used to correct the deficiency in cortisol secretion, which will in turn suppress ACTH overproduction and subsequent stimulation of the androgen pathway (New and Wilson, 1999). Thus further virilization is prevented, allowing normal growth and onset of puberty. The dose of hydrocortisone

required is usually 15 to 20 mg/m² per day divided into three doses per day (Clayton et al., 2002; New et al., 2006) but may be even higher in the newborn. The dose can then be decreased to 10 to 15 mg/m² per day after there has been initial suppression of the ACTH–adrenal axis. It is important that the appropriate balance be maintained to avoid hypercortisolism, which can result in Cushing syndrome and suppression of linear growth. Attempts to suppress 17-OHP levels to normal will inevitably result in iatrogenic Cushing syndrome. Hormonal control can be difficult to achieve in some cases, and adrenalectomy may be offered as an extreme alternative therapeutic option in select patients (New, 1996; Van Wyk et al., 1996; Gmyrek et al., 2002). Patients with salt-losing CAH have elevated plasma renin activity and require mineralocorticoid replacement with 0.05 to 0.4 mg per day of 9 α -fludrocortisone. Neonates have mineralocorticoid resistance as evidenced by higher levels of aldosterone present in normal infants and thus the required dose of 9 α -fludrocortisone is higher in infants than in adults. In infancy, patients also require oral salt supplement as in other forms of primary adrenal insufficiency. There is no parenteral formulation for mineralocorticoid. Therefore if 9 α -fludrocortisone cannot be given orally, hydrocortisone at higher doses can be given IV along with sodium chloride. A dose of 20 mg of hydrocortisone will provide the equivalent of 0.1 mg 9 α -fludrocortisone. With the advent of newborn screening, infants are diagnosed with CAH prior to a salt-wasting crisis, and thus it is not possible to distinguish the infant with the 75% chance of having salt-wasting CAH without genetic testing. Because females with classic 21-OHD CAH are born with ambiguous genitalia caused by the production of excess androgens in utero, corrective surgery is contemplated. However, before surgical correction is considered as a form of treatment in patients with ambiguous genitalia, consultation with the patient's parents, psychologist, pediatric endocrinologist, and pediatric urologist is essential.

11 β -Hydroxylase Deficiency

Pathophysiology. The 11 β -hydroxylase deficiency (11 β -OHD) form of CAH represents 5%–8% of all cases in the general population (White et al., 1994). Deficiency of this enzyme results in an accumulation of 11-deoxysteroid precursors, which are shunted into the androgen pathway. Excess adrenal androgen secretion results in ambiguous genitalia in the affected female fetus. Hypertension in patients with this disorder is commonly attributed to deoxycorticosterone (DOC)-induced sodium retention (Nimkarn and New, 2008b). The hallmark serum abnormality in patients with 11 β -OHD is normal or suppressed renin, because hypokalemia is not uniformly present in all cases. Infants may also present with hyponatremia due to the physiologic newborn resistance to mineralocorticoids. 17-OHP may also be elevated due to the high 11-deoxycortisol levels and be detected by the newborn screen for 21-OHD CAH. Diagnosis is made by the determination of extremely high basal and stimulated levels of DOC and 11-deoxycortisol after performing an ACTH stimulation test. The diagnosis can be confirmed by molecular genetic analysis of the *CYP11B1* gene.

Clinical Signs and Symptoms. Hypertension occurs in approximately two-thirds of patients with 11 β -OHD and distinguishes 11 β -OHD from the more common 21-OHD in cases of virilizing CAH (Rosler et al., 1982; Nimkarn and New, 2008b). However, hypertension correlates variably with the presence of hypokalemia or with the extent of virilization (Rosler et al., 1982). Patients can present with or without hypokalemic alkalosis. It is usually not

identified until later in childhood or in adolescence, although its appearance in early childhood has been documented. A patient was positively identified by the newborn screening program aiming for 21-OHD CAH (Peter et al., 2008).

Epidemiology. Classic 11 β -OHD CAH occurs in approximately 1 in 100,000 births in the general white population (Zachmann et al., 1983). A large number of cases have been reported in Israel, where the incidence was estimated to be 1 in 5000 to 1 in 7000 births, with a gene frequency of 1 in 71 to 1 in 83. A subsequent study showed that 11 β -OHD CAH occurred in a lower frequency (Paperna et al., 2005) yet remains more common in this population than in others. This unexpected clustering of cases was traced to Jewish families of North African origin, particularly from Morocco and Tunisia (Rosler et al., 1992).

Molecular Genetics. Two 11 β -hydroxylase genes have been identified within the human adrenal cortex, each encoding for a different enzyme with distinct enzymatic ability. The two genes *CYP11B1* and *CYP11B2* are located 30 to 40 kilobases apart on chromosome 8q (Chua et al., 1987; Taymans et al., 1998). Although gene conversions occur between *CYP11B1* and *CYP11B2* (Merke et al., 1998; Mulatero et al., 1998), the majority of the mutations found in *CYP11B1* are random point mutations (Rosler et al., 1982; Zachmann et al., 1983; White et al., 1991; Curnow et al., 1993), unlike as found in 21-OHD CAH. By 2003, approximately 41 mutations in *CYP11B1* from individuals of diverse ethnic backgrounds had been identified (Stenson et al., 2003).

Management Issues. Similar to 21-OHD CAH, glucocorticoid therapy is the most effective means of regaining hormonal control in patients with 11 β -OHD. Corticosteroids at the same dose range as for 21-OHD CAH provide feedback inhibition of ACTH, reduce stimulation of the androgen pathway, and allow normal growth and the onset of puberty. Treatment with corticosteroids also contributes to the reduction of DOC and thus controls hypertension. Through careful clinical monitoring, doses can be continuously adjusted to match patients' needs while avoiding suppression of linear growth caused by overdosing. In addition to hormonal therapy, reduced salt intake is often used to reduce fluid volume and hypertension. Maintaining fluid balance in children, however, is often difficult and poses an ongoing challenge to treatment. Affected females suffer from genital ambiguity and may require genital reconstructive surgery after multidisciplinary consultations. To prevent prenatal virilization, a similar protocol to 21-OHD CAH for prenatal diagnosis and treatment can be performed (Cerame et al., 1999).

Disorders That Lead to Males With Undervirilization

17 α -Hydroxylase/17,20-Lyase Deficiency

Pathophysiology. This form of CAH involves an enzyme that catalyzes more than one reaction—namely both the 17 α -hydroxylation and 17,20-lyase reactions—with both reactions commonly being impaired in the disorder. Affected individuals cannot produce cortisol but synthesize large amounts of corticosterone (a weak glucocorticoid that mitigates the adrenal insufficiency) and deoxycorticosterone, which causes hypertension and hypokalemia. Deficiency of 17,20-lyase impairs the ability to synthesize androgens and estrogens and causes male 46,XY disorder of sex development (DSD) at birth and results in failure to virilize at puberty. Affected females have primary amenorrhea and clinical hypogonadism (Yanase et al., 1991; Auchus, 2001).

Clinical Features. The typical features of complete deficiency include hypertension and hypokalemia with delayed puberty in genetic females and undervirilization in genetic males. Nevertheless,

there is considerable variability in the clinical and biochemical features, including a few mutations that cause isolated 17,20-lyase deficiency (Auchus, 2001). The age of onset of hypertension and the severity of hypokalemia are highly variable, even among individuals with the same mutations (Costa-Santos et al., 2004) and occur due to increased ACTH stimulation of the mineralocorticoid pathway.

Epidemiology. This disorder has an estimated frequency in most countries of approximately 1 case per 50,000 newborns and accounts worldwide for approximately 1% of all cases of CAH (Yanase et al., 1991). However, 17-hydroxylase (CYP17) deficiency is the second most common cause of CAH in Brazil (Santos et al., 1998; Costa-Santos et al., 2004), and this remarkable frequency is the result of two founder effects in areas with high coefficients of consanguinity such that two mutations account for more than 80% of cases in that country (Costa-Santos et al., 2004).

Molecular Genetics. Since the cloning of the gene (Picado-Leonard and Miller, 1987) nearly 50 different mutations have been described in *CYP17* (Krawczak and Cooper, 1997). Founder effects probably explain the high incidence of the disease in other patient populations in the Netherlands and Japan (Costa-Santos et al., 2004). Severity of disease tends to be milder with mutations that retain partial catalytic activity, but the nature of the variability in hypertension and hypokalemia is unclear.

Management Issues. Adequate glucocorticoid administration suppresses ACTH and the excessive mineralocorticoid secretion and generally normalizes the blood pressure. Adult females and males reared as females require estrogen therapy. Abdominal testes should be relocated or removed because of the risk of malignancy. Adult genetic males reared as males need surgical correction of the external genitalia and androgen replacement.

3 β -Hydroxysteroid Dehydrogenase Type 2 Deficiency

Pathophysiology. 3 β -Hydroxysteroid dehydrogenase converts 3 β -hydroxy $^5\Delta$ steroids (pregnenolone, 17-hydroxypregnenolone, and DHEA) to 3-keto $^4\Delta$ steroids (progesterone, 17-OHP, and androstenedione) and is essential for the biosynthesis of mineralocorticoids, glucocorticoids, and sex steroids (Mebarki et al., 1995). Two forms of the enzyme have been described in humans: type 1 enzyme expressed in placenta and skin and type 2 expressed in adrenal glands and gonads. The type 1 and 2 genes are closely linked on chromosomal region 1p13.1. The two forms are closely related in structure and substrate specificity, although the type 1 isoenzyme has higher substrate affinity and a fivefold greater enzymatic activity than type 2 (Simard et al., 2005). The type 1 isoenzyme can lead to elevated 17-OHP and androstenedione levels in addition to the expected elevated levels of pregnenolone, 17-hydroxypregnenolone, and DHEA.

Clinical Signs and Symptoms. 3 β -HSD2 isoenzyme is essential for the formation of progesterone, the precursor for aldosterone, 17-OHP, the precursor for cortisol in the adrenal cortex, androstenedione, testosterone, and estrogen. Simultaneous 3 β -HSD2 deficiency in both gonads and adrenal glands results in incomplete virilization of the external genitalia in males. Male patients with 3 β -HSD2 deficiency present with ambiguous external genitalia, characterized by micropenis, perineal hypospadias, bifid scrotum, and blind vaginal pouch (Simard et al., 1994) with or without salt loss (Mebarki et al., 1995). Gynecomastia is common at pubertal stage in affected males. In females, virilization of external genitalia occurs as a result of the androgen effect from the peripheral conversion of circulating $^5\Delta$ precursors to active $^4\Delta$ steroids; therefore genital ambiguity can result in both sexes. Newborns can present

with adrenal insufficiency due to deficiency of glucocorticoids, mineralocorticoids, and sex steroids. Later clinical presentations also include salt-wasting crisis, premature pubic hair development, hirsutism, and menstrual disorders (Lutfallah et al., 2002).

Epidemiology. The exact frequency of this rare disorder remains unknown.

Molecular Genetics. The disorder has an autosomal recessive inheritance. *HSD3B2* is the gene responsible for 3 β -HSD2 deficiency CAH. There are approximately 40 mutations in the *HSD3B2* gene already described (Stenson et al., 2003). Mutations that lead to the abolition of 3 β -HSD2 activity lead to the salt-wasting form (Rheume et al., 1992; Chang et al., 1993; Alos et al., 2000; Lutfallah et al., 2002). Mutations that reduce but do not abolish type II activity lead to CAH with mild or no salt loss, which in males is associated with 46,XY DSD as a result of the reduction in androgen synthesis (Lutfallah et al., 2002). Mild mutations were also associated with hyperandrogenic symptoms of premature pubic hair development and hirsutism (Pang et al., 2002; Mermejo et al., 2005).

Management Issues. Similar to other forms of CAH, corticosteroid is the mainstay therapy. Salt-wasting phenotypes in some patients can be managed the same way as the salt-wasting form of 21-OHD CAH. Male patients with 3 β -HSD2 deficiency have ambiguous external genitalia. Although most males are raised as males and retain the male social sex at puberty, gender identity is an important management issue. In one Brazilian family, two cousins with 46,XY DSD caused by 3 β -HSD2 deficiency were reared as females; one of them had bilateral orchiectomy in childhood and retained the female social sex; the other retained their testes during childhood and changed to male social sex at puberty (Mendonca et al., 1987).

Male patients may require testosterone replacement therapy during puberty and adulthood. The aim of the surgical treatment after consultation with a DSD team in this condition is to allow development of adequate external genitalia. Only skilled surgeons with specific training in the surgery of DSD should perform these procedures (Hughes et al., 2006).

Lipoid Congenital Adrenal Hyperplasia

Lipoid CAH is a severe form of congenital adrenal insufficiency. Affected patients exhibit glucocorticoid and mineralocorticoid deficiencies early in life, and males exhibit undervirilization. The reduced synthesis of steroids in patients with lipoid CAH results from an inability to transfer cholesterol to the inner mitochondrial membrane where the cholesterol side-chain cleavage complex is located (Lin et al., 1995). It is characterized by lipid droplet accumulation in the cytoplasm of the adrenocortical cells. Most cases of lipoid CAH are caused by autosomal recessive mutations in the gene encoding steroidogenic acute regulatory protein (StAR). The *STAR* locus is in the 8p11.2 region and encodes a protein with an essential role in cholesterol transfer from the outer to the inner mitochondrial membrane, thus providing the substrate for steroid hormone biosynthesis (Bose et al., 1996). Once in the mitochondria, cholesterol is converted to pregnenolone by the cytochrome P450 side-chain cleavage (CYP11A1) enzyme, and then steroid biosynthesis is initiated. Karyotypic 46,XY persons are phenotypically female because of Leydig cell destruction and impaired testosterone production. Sertoli cell function though is intact so that anti-müllerian hormone is secreted and thus müllerian ducts regress leading to absence of uterus and fallopian tubes. The ovary in XX subjects is initially spared damage because steroidogenesis is delayed until the time of puberty, after which

stimulation of steroidogenesis by the tropic hormones (i.e., luteinizing and follicle-stimulating hormones) causes progressive damage to the ovary (Fujieda et al., 1997). *StAR* mutations have been described most frequently in Japanese and Palestinian populations, in part because certain mutations occur repeatedly, probably reflecting a founder effect (Bose et al., 1996; Nakae et al., 1997). Although less common than mutations in *StAR*, mutations in *CYP11A1* can also cause lipid CAH (Katsumata et al., 2002; Hiort et al., 2005).

Rare Form of Congenital Adrenal Hyperplasia With Variable Phenotypes

Cytochrome P450 oxidoreductase (POR) deficiency is an autosomal recessive disorder of steroidogenesis involving P450c17, P450c21, and P450-aro with a phenotypic spectrum ranging from cortisol deficiency at the milder end to classic Antley–Bixler syndrome (ABS) at the severe end, and the phenotype of cortisol deficiency can range from clinically insignificant to life threatening (Fluck et al., 2004). Manifestations can include ambiguous genitalia in males and females, primary amenorrhea and enlarged cystic ovaries in females, poor masculinization during puberty in males, and maternal virilization and low estriol levels during pregnancy with an affected fetus carrying certain POR mutations. Manifestations of ABS include craniosynostosis, hydrocephalus, distinctive facies, choanal stenosis or atresia, low-set, dysplastic ears with stenotic external auditory canals, skeletal anomalies (radiohumeral synostosis, neonatal fractures, congenital bowing of the long bones, joint contractures, arachnodactyly, clubfeet), renal anomalies (ectopic kidneys, duplication of kidneys, renal hypoplasia, horseshoe kidney, hydronephrosis), and reduction of cognitive function and developmental delay. The skeletal differences have been hypothesized to be due to decreased activity of CYP26B1, a POR-dependent enzyme that degrades retinoic acid (Laue et al., 2011). In moderate POR deficiency, craniofacial and skeletal anomalies are less severe than in ABS (Scott and Miller, 2008). Infants with ABS with normal steroidogenesis and without ambiguous genitalia have a mutation in the fibroblast growth factor receptor 2 and not in the *POR* gene (Huang et al., 2005). As more and more patients are identified with POR deficiency, this newly discovered form of CAH may not be as rare as formerly thought.

Familial Glucocorticoid Deficiency

Familial glucocorticoid deficiency (FGD) is an autosomal recessive disorder resulting from defects in the action of ACTH to stimulate glucocorticoid synthesis in the adrenal leading to ACTH resistance. It is also known as isolated glucocorticoid deficiency or hereditary unresponsiveness to ACTH. The majority of patients with FGD have episodes of hypoglycemia in the neonatal period. These episodes will often respond quickly to more frequent feeding regimens. In a few cases, excessive skin pigmentation is recognized at this early stage. Biochemically, patients with FGD have low or undetectable cortisol levels and—because of the failure of the negative feedback loop to the pituitary and hypothalamus—grossly elevated ACTH levels are found. Mineralocorticoid deficiency usually is not a presentation; therefore aldosterone levels, plasma renin measurements, and serum electrolytes are normal. A clinical feature sometimes observed in patients with FGD is tall stature that is identified later in life (Elias et al., 2000). Approximately half of all cases result from mutations in the ACTH receptor (*MC2R*, melanocortin 2 receptor) FGD type

1 or from mutations in the melanocortin 2 receptor accessory protein (*MRAP*), FGD type 2, but other genetic causes of this potentially lethal disorder remain to be discovered (Clark et al., 2009).

Triple A Syndrome

Triple A or Allgrove syndrome is a similar disorder to FGD, with additional features of alacrima and achalasia. Alacrima is the first symptom, followed by achalasia and then adrenal insufficiency. Presenting in the first decade of life, it is frequently associated with progressive neurologic dysfunction, polyneuropathy, deafness, mental retardation, and hyperkeratosis of palms and soles (Houlden et al., 2002). Some of these families have a defect in the alacrima–achalasia–adrenal insufficiency neurologic disorder (*AAAS*) gene encoding a protein named ALADIN (Tullio-Pelet et al., 2000). ALADIN participates in the nuclear translocation of the ferritin heavy chain protein. Mutations in *AAAS* prevent ferritin heavy chain protein from entering into the nucleus, which is necessary to prevent oxidative damage of the nucleus (Storr et al., 2009). ALADIN belongs to a WD-repeat family of regulatory proteins that shares a common motif made up of highly conserved repeating units usually ending with tryptophan-aspartate (WD) (Neer et al., 1994).

Neonatal Adrenoleukodystrophy

Neonatal adrenoleukodystrophy (NALD) is a fatal rare autosomal recessive disease of impaired peroxisome biogenesis. NALD belongs to a class of disorders involving peroxisomal biogenesis that includes Zellweger syndrome and infantile Refsum disease. NALD is the only one of the three diseases that often involves adrenal insufficiency. Mutations in seven different peroxisome biogenesis factor genes have been shown to cause NALD (Moser, 1999). Mutations in peroxisome biogenesis factor 1 (*PEX1*) are the most common cause of NALD (Tamura et al., 2001). As in X-linked adrenoleukodystrophy (X-ALD), patients with NALD accumulate very long chain fatty acids and develop degenerative changes of the white matter of the nervous system and adrenal atrophy. Infants with NALD characteristically demonstrate dolichocephaly, prominent and high forehead, esotropia, epicanthic folds, broad nasal bridge, high-arched palate, low-set ears, and anteverted nostrils. Affected patients usually die in early childhood (Walter et al., 2001). X-ALD is a recessively inherited X-linked defect of the adrenoleukodystrophy protein (ALDP) (Lightenberg et al., 1995). It is encoded by the *ABCD1* gene on Xq28. It is also a peroxisomal defect that usually results in adrenal insufficiency and central nervous system deterioration. X-ALD can present in early childhood, and at birth there is already elevation of very long chain fatty acids. Because early diagnosis can improve outcome through the use of hematopoietic stem cell transplantation or gene therapy (research), some state newborn screening programs have added X-ALD to their newborn screening panel. X-ALD can also manifest later in adulthood (Moser et al., 1984).

Defective Cholesterol Metabolism: Smith–Lemli–Opitz Syndrome

The clinical picture of adrenal insufficiency and 46,XY gonadal dysgenesis may be caused by a deficiency of 7-dehydrocholesterol C-7 reductase enzyme that catalyzes the final step in cholesterol biosynthesis leading to primary adrenal insufficiency. The syndrome

results from mutations in the sterol Δ -7-reductase gene (*DHCR7*) located at 11q12-q13. Smith–Lemli–Opitz (SLO) syndrome can manifest with typical facial appearance, mental retardation, microcephaly, proximally placed thumbs, congenital cardiac abnormalities, syndactyly of the second and third toes, incomplete development of the male genitalia, and photosensitivity. The biochemical abnormalities of SLO syndrome include low cholesterol and high 7-dehydrocholesterol (Tint et al., 1994). In utero, the primary defect in fetal adrenal glands results in a combination of low maternal estriol levels, undervirilization of the male fetus, and large adrenal glands in the fetus with SLO syndrome. Preliminary studies suggested that cholesterol supplementation may be of benefit to patients with SLO syndrome (Andersson et al., 1999). Unfortunately replacement of cholesterol has not been as beneficial as once hoped. Cholesterol does not cross the blood–brain barrier and has not been shown to reduce developmental delay (Sikora et al., 2004). A more recent placebo-controlled trial using simvastatin found that the medication crossed the blood–brain barrier and was relatively safe. There was improvement in serum dehydrocholesterol-to-total sterol ratio and improvement of irritability symptoms (Wassif et al., 2017). The birth prevalence of SLO syndrome is estimated to be approximately 1:20,000 to 1:40,000 live births (Tint et al., 1994). Among persons of northern or central European ancestry, it has been estimated to range from 1:10,000 to 1:60,000 (Porter, 2000). SLO syndrome is less common in those of Asian or African ancestry. As it is an autosomal recessive disorder, the recurrence risk is 25%. Thus it is important to consider the diagnosis as an elevated 7-dehydroxycholesterol level will confirm the diagnosis.

Adrenal Insufficiency Associated With Other Syndromic Disorders

Lysosomal Storage Disorders

Complete deficiency of lysosomal esterase can also result in adrenal insufficiency in Wolman disease, a rare autosomal recessive disease with an incidence of 1 in 350,000 infants. Wolman disease usually is fatal in the 1st year of life. Affected infants exhibit mild mental retardation, hepatosplenomegaly, vomiting, diarrhea, growth failure, and adrenal calcifications. Calcifications that delineate the outline of both adrenals are pathognomonic of this condition (Wolman, 1995).

Mitochondrial Disorders

Adrenal insufficiency can result from mitochondrial disorders, characterized by chronic lactic acidosis, myopathy, cataracts, and nerve deafness (Nicolino et al., 1997; Bruno et al., 1998). Cases with the Kearns–Sayre syndrome form of mitochondrial myopathy and deafness, with large-scale deletions in mitochondrial DNA, are often associated with endocrine dysfunction, particularly short stature, hypogonadism, diabetes, hypoparathyroidism, hypothyroidism, and adrenal insufficiency (Artuch et al., 1998; Boles et al., 1998).

Intrauterine Growth Retardation, Metaphyseal Dysplasia, Adrenal Hypoplasia Congenita, and Genital Anomalies

Three patients with AHC and additional findings that represent a new syndrome known as *IMAGe* (i.e., intrauterine growth retardation, metaphyseal dysplasia, AHC, and genital anomalies) have been reported. Genital abnormality was described as bilateral cryptorchidism, small penis, and hypogonadotropic hypogonadism.

The patients also had hypercalciuria with or without hypercalcemia resulting in abnormal calcium deposits in vital organs (Vilain et al., 1999). As of 2016, 28 individuals in 16 families have been reported (Bennett et al., 2016). Mutations in the proliferating cell nuclear antigen (PCNA) binding domain of the maternally expressed cyclin-dependent kinase inhibitor (*CDKN1C*) gene on the short arm of chromosome 11, which results in the loss of PCNA binding, have been identified as the etiology of *IMAGe* syndrome. These mutations serve as a gain of function mutation (Arboleda et al., 2012). *CDKN1C* is a tumor suppressor gene, and it encodes an inhibitor of cell cycle progression. Beckwith–Wiedemann syndrome, an overgrowth syndrome, also has mutations in the *CDKN1C* gene but at a different location, resulting in a loss of function.

Adrenal Hypoplasia Congenita

AHC, a familial condition in which the adrenal cortex has arrested development, occurs in approximately 1 in 12,500 births (Laverty et al., 1973; Jones et al., 1995). The disorder can manifest as four clinical forms of primary adrenal insufficiency: (1) a sporadic form associated with pituitary hypoplasia; (2) an autosomal recessive form with a distinct miniature adult adrenal morphology, characterized by small glands with a permanent cortical zone but a diminished fetal zone (the genetic basis of the recessive form of AHC is unknown); (3) an X-linked cytomegalic form associated with hypogonadotropic hypogonadism; and (4) an X-linked form associated with glycerol kinase deficiency and Duchenne muscular dystrophy (Bartley et al., 1982; Ten et al., 2001). Mutations in the *NROB1* gene encoding DAX-1 are responsible for both X-linked forms.

The X-linked or cytomegalic form of AHC is characterized by the absence or near absence of the permanent or adult zone of the adrenal cortex and by structural disorganization of the fetal cortex with abnormally large cells. It differs from the autosomal recessive miniature adult form of AHC, in which the adrenal cortex has the normal adult structure but is small. X-linked AHC results in severe primary adrenal insufficiency involving glucocorticoids and mineralocorticoids and failure to respond to elevated levels of ACTH with usual age at onset in the neonatal period or during infancy. However, in some patients, age of onset is later, up to several years of age and presumably caused by residual functional cortex (McCabe, 2000; Achermann et al., 2001). The secretion of other pituitary hormones is not impaired. Hypogonadotropic hypogonadism can manifest with cryptorchidism or delayed puberty (Golden et al., 1977). Whereas presentation of adrenal insufficiency can occur from birth, there is great variability of presentations. Isolated adrenal insufficiency in infancy, isolated adrenal insufficiency later in life, isolated hypogonadotropic hypogonadism, adrenal insufficiency and hypogonadotropic hypogonadism, delayed-onset adrenal insufficiency from 2 to 9 years of age with incomplete hypogonadotropic hypogonadism, and delayed puberty in females all may result (Ten et al., 2001). The phenotypic variation does not correlate well with genotype.

Adrenal Hypoplasia as Part of Contiguous Gene Deletion Syndrome

An X-linked form of adrenal insufficiency, associated with glycerol kinase deficiency, is characterized by psychomotor retardation, muscular dystrophy, characteristic facies with hypertelorism, alternating strabismus, and drooping mouth. Additional phenotypic features can include testicular abnormalities (anorchia or cryptorchidism), short stature, and osteoporosis. Time of presentation

can vary from birth through childhood. Nearly all patients reported were male. The genetic locus was mapped to Xp21.3-21.2, and variants of contiguous gene deletion syndrome (glycerol kinase deficiency, Duchenne muscular dystrophy, ornithine transcarbamylase deficiency, and mental retardation) can be seen.

Abnormalities of Development: DAX-1 and Steroidogenic Factor-1 Deficiency

The nuclear receptors DAX-1 and SF-1 (Phelan and McCabe, 2001) have an important role in adrenal development and function, and mutations in the genes that encode these transcription factors have been found in patients with adrenal hypoplasia (see Molecular Basis of Adrenal Development, earlier). Both SF-1 and DAX-1 belong to the family of nuclear hormone receptors. DAX-1 protein is expressed in the developing urogenital ridge, ovary, testis, all zones of the fetal adrenal cortex, hypothalamus, and anterior pituitary gland—sites in which it colocalizes with SF-1 (Guo et al., 1995; Parker et al., 2002). SF-1 is essential for the development of the adrenal cortex, gonads, and ventromedial nucleus of the hypothalamus because it interacts with the promoter of the *NROB1* gene, anti-müllerian hormone gene, and the genes for the α -subunits of the pituitary glycoprotein hormones (Kawabe et al., 1999). Furthermore, SF-1 is a transcription factor that regulates gene expression of the CYP steroid hydroxylases (21-hydroxylase, the aldosterone synthase isoenzyme of steroid 11 β -hydroxylase, CYP11A), 3 β -hydroxysteroid dehydrogenase, aromatase, and StAR in the adrenal gland; therefore it is essential for development of the adrenal cortex.

In one large study of this relatively rare disease, *NROB1* mutations were found in 58% of 46,XY phenotypic boys referred with adrenal hypoplasia and in all boys with hypogonadotropic hypogonadism and a family history suggestive of adrenal failure in males. *NR5A1* (SF-1) mutations causing adrenal failure were found in only two patients with 46,XY gonadal dysgenesis. No *NROB1* or *NR5A1* mutations were identified in the adult-onset group (Lin et al., 2006).

Human mutations in *NR5A1* are even less common and have been described in a few patients with primary adrenal failure. Two individuals with a 46,XY genotype, female phenotype, and müllerian structures harbored missense mutations that affected DNA binding (Achermann et al., 1999, 2002), whereas a 46,XX girl with an *NR5A1* mutation had primary adrenal failure and apparently normal ovarian development (Biaison-Laubert and Schoenle, 2000). In addition, it is now emerging that heterozygous nonsense or frameshift mutations associated with haploinsufficiency of *NR5A1* can cause 46,XY gonadal dysgenesis in patients with normal adrenal function (Correa et al., 2004; Hasegawa et al., 2004; Mallet et al., 2004). Therefore it is possible that a range of different endocrine phenotypes are associated with mutations in different domains of *NR5A1*.

Adrenal Hemorrhage

Adrenal hemorrhage is not uncommon at birth. At birth, adrenal hemorrhage from anoxia or sepsis is most common, and adrenal insufficiency usually manifests in neonates. The incidence in the neonate is reported to be 1.7 cases per 1000 autopsied infants and as many as 3% of infants screened by abdominal ultrasound examination. The etiology of neonatal adrenal hemorrhage is largely unknown, but it has been associated with birth trauma related to difficult deliveries, sepsis, coagulopathies, traumatic shock, and ischemic disorders. Infants with minimal hemorrhage may be asymptomatic and be discovered incidentally to have adrenal

calcifications, indicating an earlier hemorrhage. Major adrenal hemorrhage can manifest as an abdominal mass, anemia from blood loss, or jaundice from reabsorption of the hematoma. Hemorrhage can also lead to adrenal insufficiency, which can manifest as neonatal hypoglycemia, hypotension, hypothermia, apnea, or shock. Because of the location of the right adrenal gland between the liver and spine, it is the one most often affected by hemorrhage (Velaphi and Perlman, 2001).

In meningococcal septicemias, hemorrhage into the adrenal glands can complicate the clinical picture, leading to circulatory collapse (Waterhouse–Friderichsen syndrome or adrenal hemorrhage in association with fulminant septicemia; Enriquez et al., 1990). Other infections in the neonate that have been associated with adrenal hemorrhage and insufficiency include those caused by herpes virus, *Pseudomonas aeruginosa*, *Bacteroides* spp., herpes simplex virus type 6, and echovirus types 11 and 6 (Margaretten et al., 1963; Ohta et al., 1978; Bekdash and Slim, 1981; Jain et al., 1996; Schmitt et al., 1996). Septic shock in newborns, especially in those who are small for their age, can result in adrenal hemorrhage with rhabdomyolysis and renal insufficiency (Ten et al., 2001).

Secondary and Tertiary Adrenal Insufficiency

Iatrogenic Adrenal Insufficiency

Secondary and tertiary forms of adrenal insufficiency result from defects in pituitary corticotroph and hypothalamic function, respectively. Supraphysiologic doses of glucocorticoids are often used for the treatment of bronchopulmonary dysplasia. With prolonged use of supraphysiologic doses of glucocorticoids, these neonates are at risk for iatrogenic suppression of corticotroph ACTH release, with secondary adrenocortical atrophy and adrenal insufficiency (Axelrod, 1992). Evidently, even a single course of prenatal betamethasone treatment induces a suppression of stress reactivity in healthy newborns (Schaffer et al., 2009). The duration of recovery of corticotroph function from adrenal suppression, once administration of glucocorticoids is discontinued, is highly variable with evidence of suppression of the HPA axis evident in some patients for more than 1 year (Livanou et al., 1967). Even in preterm infants, the HPA axis behaves in a similar manner as in adult subjects, and the pituitary function recovers earlier than that of the hypothalamus and the adrenals (Ng et al., 2008).

Developmental Adrenal Insufficiency

Secondary or tertiary adrenal insufficiency in the neonate often is a consequence of abnormalities in development of the hypothalamus and pituitary associated with adrenal insufficiency, including de Morsier syndrome (septo-optic dysplasia; De Morsier, 1956), hydranencephaly or anencephaly, and pituitary hypoplasia or aplasia. If these infants have concomitant diabetes insipidus, they have an increased risk of sudden death during childhood (Dattani et al., 1998; Kelberman et al., 2006). Patients with developmental abnormalities of the pituitary or hypothalamus often have deficiencies of other hormones. ACTH deficiency can be part of a multiple pituitary hormone deficiency syndrome caused by abnormal expression of *HESX1*, *LHX4*, *SOX3*, or *PROPI*, which encode transcription factors (Mullis, 2001). Isolated ACTH insufficiency is a rare condition that can be caused by mutations in *TPIT*, a T-box factor that controls transcription of the proopiomelanocortin gene in corticotrophs only, thereby resulting in an adrenal-only phenotype (Vallette-Kasic et al., 2005). However, approximately

50% of patients do not carry mutations in *TPIT*, suggesting that other unknown factors exist (Metherell et al., 2004; Vallette-Kasic et al., 2004). Septo-optic dysplasia can be caused by mutations in *HESX1* and *SOX2* (Dattani et al., 1998; Kelberman et al., 2006). Signs of hypopituitarism in a neonate include hypoglycemia, prolonged jaundice, shock, and microphallus in males. Trauma to the hypothalamus, pituitary, or hypophyseal portal circulation from significant head injury, cerebrovascular accident, Sheehan syndrome, or hydrocephalus may be a cause of central adrenal insufficiency. Historical factors associated with increased risk for central adrenal insufficiency include maternal drug use and traumatic delivery.

There have been rare case reports of families with inherited abnormalities of neuropeptides involved in HPA axis regulation. Adrenal insufficiency, pigmentary abnormalities, and obesity have been described in families with a defect in proopiomelanocortin (POMC) (Krude et al., 1998). One kindred has been reported with Arnold–Chiari type I malformation and suspected *CRH* deficiency. The mutation in this kindred is linked to the *CRH* locus; however, a specific mutation in the *CRH* gene has not yet been defined (Kyllo et al., 1996). Mutations in *TPIT*, which encodes a highly restricted transcription factor involved in the expression of the *POMC* gene, have been found in eight individuals with congenital isolated ACTH deficiency. It is an autosomal recessive disorder (Pulichino et al., 2003).

Management

Adrenal crisis is a potentially life-threatening disorder that can manifest with a salt-losing crisis or profound hypoglycemia in infancy or childhood and requires immediate resuscitation and appropriate steroid replacement. Determining the exact cause of this condition can be challenging once the child has started treatment, but defining a precise etiology has important implications for long-term management, for identifying associated features, and for appropriate counseling regarding inheritance and the risks of other family members being affected (Lin et al., 2006). Detailed questioning about family history that could reveal any insight into possible adrenal disease is important.

Initial Management

In the event of a suspected adrenal crisis, blood for determination of electrolytes, aldosterone, plasma renin activity, cortisol, and ACTH should be drawn and treatment started before the results are obtained. Fluid resuscitation with normal saline containing 5% or 10% dextrose should be given to restore cardiovascular stability. Plasma sodium should be monitored closely, as rapid correction of hyponatremia with sodium repletion of more than 0.5 to 1 milliequivalent (mEq)/L per hour increases the risk of central pontine myelinolysis. The sodium deficit may be calculated by subtracting the infant's sodium from a customarily normal sodium of 140 mEq/L and then multiplying this value by $0.6 \times \text{weight (in kg)}$. The rate of replacement should occur over an initial rate such that the sodium increase does not exceed 0.5 mEq/L per hour. Hydrocortisone IV should be given initially at 100 mg/m² and then continued at 100 mg/m² per day, divided every 6 to 8 hours, until the infant's condition is stable.

Maintenance Therapy

Corticosteroid and mineralocorticoid replacement therapies should suppress the excessive secretion of CRH, ACTH, and resting renin

levels. The normal daily cortisol production rate has been shown to be 6 to 7 mg/m² per day in children and adolescents (Linder et al., 1990; Kerrigan et al., 1993). This rate translates to approximately 10 to 12 mg/m² per day of oral hydrocortisone, to allow for step-down losses from absorption, hepatic processing, and metabolic bioavailability. Because the bioavailability of oral steroids varies from person to person (Bright and Darmaun, 1995), infants should be monitored closely for signs of either inadequate cortisol replacement or cortisol excess (Heazelwood et al., 1984). Although adults and older children may be able to take hydrocortisone twice daily, most infants should be dosed three times daily to avoid hypoglycemia associated with low cortisol on a twice-daily regimen (Groves et al., 1988; DeVile and Stanhope, 1997). Hydrocortisone is the preferred steroid for treatment of infants because it has fewer growth-suppressive effects than synthetic steroids (Allen, 1996; Allen et al., 1998; Punthakee et al., 2003). The United States Food and Drug Administration withdrew oral hydrocortisone suspension from the market because of poor absorption and undertreatment of children (Ten et al., 2001). The smallest tablet for hydrocortisone is 5 mg, and it can be cut into quarters for a dose of 1.25 mg.

In primary adrenal insufficiency, aldosterone production is usually decreased. Physiologic doses of hydrocortisone do not provide enough mineralocorticoid activity to prevent salt wasting (New et al., 1966); therefore these infants often require 0.05 to 0.4 mg/day of fludrocortisone acetate (Florinef, 9 α -fluorocortisol) and added salt. Because after the 1st month of life aldosterone production does not vary, the dose of fludrocortisone does not increase with growth and aging (Weldon et al., 1967; Sippell et al., 1978). Infants with mineralocorticoid deficiency require 1 to 2 g of sodium chloride (1 g contains 17 mEq of sodium) added to their diet, because formula and breast milk are low in sodium content (approximately 8 mEq/L) (Mullis et al., 1990).

Stress Replacement

The normal response to surgery, trauma, or critical illness is to increase plasma ACTH and cortisol levels (Lamberts et al., 1997). The secretion rate of cortisol has been found to be proportional to the degree of stress and ranges from 60 to 167 mg/day in adults after surgery (Hume et al., 1962; Chernow et al., 1987). Based on data from adults, it is recommended that infants with adrenal insufficiency receive 30 to 100 mg/m² per day of hydrocortisone when stressed, divided every 6 to 8 hours. Stress doses of hydrocortisone should be given with the onset of fever and gastrointestinal or other significant illness and continued for 24 hours after the symptoms resolve (Nimkarn and New, 2008a). Usually this treatment translates to the routine steroid dose being tripled and administered over three divided daily doses. If oral steroids are not tolerated, an intramuscular or IV dose should be given. For surgery, infants should be given 30 to 100 mg/m² of IV hydrocortisone on call to the operating room before the administration of anesthesia. Alternatively the 30 to 100 mg/m² of hydrocortisone can be given continuously during the surgery in the IV fluids. Stress dosing of hydrocortisone (30 to 100 mg/m² per day divided every 6 to 8 hours) should be continued postoperatively for the next 24 to 48 hours. It is unnecessary to give mineralocorticoids over such periods if the patient begins the operative period in adequate salt balance.

The patients and their families should have instructions for such instances. Every patient should wear a medical identification (e.g., MedicAlert) bracelet or necklace and carry the emergency medical information card that is supplied with it. Both should

Case Study

A 3-week-old phenotypic male newborn presented with lethargy, poor oral intake, and progressive vomiting for 1 week. He refused to eat over the previous 7 hours. There was no history of fever. Prenatal history was unremarkable. Birth history: normal vaginal delivery, Apgar scores of 8 and 9 with a birthweight of 8 pounds, 14 ounces (4.03 kg). The newborn examination performed in the nursery identified perineal hypospadias, but the newborn was discharged home on day 2 of life. Over the first 2 weeks of life, weight gain was poor, and appetite was markedly decreased over the 1 to 2 days before evaluation in the emergency department. Family history revealed that he was the second child of the family, from a nonconsanguineous marriage of Chinese background. (The first boy died at 1 month of age in China because of gastroenteritis.) On examination, vital signs were body temperature, 36.5°C; blood pressure, 52/32 mmHg; pulse, 160 beats/min. He was severely dehydrated. There was no hyperpigmentation. The physical examination was notable for a weight of 3.1 kg and a phallus with a length of 2.5 cm, a midshaft diameter of 1.5 cm with hypospadias near the perineum, a hyperpigmented and rugated shawl scrotum, and no palpable testes. Initial blood glucose level was 40 mg/dL. The infant's mental status was improved after fluid and dextrose administration via an intraosseous line. Resuscitation was continued with dextrose containing normal saline. Initial laboratory values showed sodium of 107 mmol/L, potassium of 7.1 mmol/L, chloride of 87 mmol/L, and total CO₂ of 5.0 mmol/L. Blood urea nitrogen was 40.5 mg/dL, and creatinine was 0.6 mg/dL. After the critical blood sample

was obtained, the newborn was given 100 mg/m² of IV hydrocortisone. Additional evaluation revealed 46,XX chromosomes and enlarged adrenals, as well as a small uterus on ultrasound examination. The clinical course was complicated by necrotizing enterocolitis that led to a stay in the hospital for 3 weeks with conservative management including administration of antibiotics. Initial laboratory results noted 17-OHP of 111,000 ng/dL, ACTH of 4755 pg/mL, and cortisol 3.9 µg/dL before hydrocortisone treatment (no ACTH stimulation performed). Aldosterone and renin analyses were not performed because of the difficulty in obtaining adequate blood. *CYP21A2* analysis revealed a genotype as follows: exon1, intron2, and exon3/intron2 mutations (<1% of enzyme activity in vitro for both mutations). This case is a classic presentation of the salt-wasting form of 21-OHD CAH.

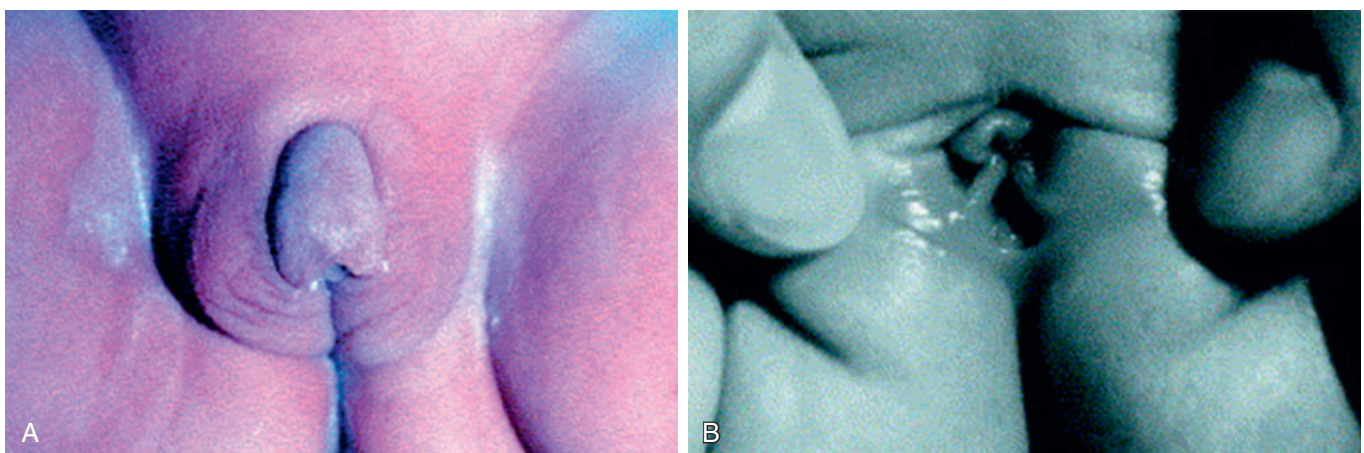
Comment

The lesson learned from this case is that in a phenotypic male without palpable testes, the genitourinary anomalies should be considered ambiguous genitalia. Further work-up is merited in the immediate perinatal period. Family history suggests a severe recessive disorder. Genetic analysis is available and can be used to confirm the diagnosis before lifetime therapy and is of value in genetic counseling. In addition, the institution of newborn screening for elevated 17α-hydroxyprogesterone allows earlier detection of salt-wasting 21-hydroxylase deficiency, before the occurrence of a life-threatening event.

CONTROVERSY BOX

Prevention of prenatal virilization in affected females is possible with a proper prenatal diagnosis and treatment program. In families where the risk of having a baby girl with congenital adrenal hyperplasia (CAH) is one in eight, the disease can be diagnosed prenatally through molecular genetic analysis of fetal DNA. Appropriate prenatal treatment by dexamethasone administration to the pregnant mother carrying an at-risk fetus before 6 weeks post conception is effective in reducing virilization in the genetic female, making postnatal genitoplasty unnecessary and thereby avoiding potential impairment of sexual function (Fig. 96.5A–B). At 7–10 weeks, cell-free DNA testing may allow for discontinuation of the dexamethasone if an XY fetus is identified. After diagnosis is made by fetal DNA analysis obtained from chorionic villus sampling at 10 to 12 weeks' gestation, therapy in unaffected or male fetuses is discontinued (Nimkarn and New, 2006). Some believe there are accurate, compelling data from the largest human studies (Forest et al., 1989; Mercado et al., 1995; New et al., 2001) indicating the benefit of prenatal treatment and that it is safe in the short

term for both the fetus and the mother. Some preliminary data from long-term studies also support these results (Nimkarn and New, 2009), although long-term follow-up studies are still under way. Studies from Sweden, however, found that children without CAH treated prenatally with dexamethasone had decreased verbal working memory and decreased self-perception of scholastic ability. Those with CAH had decreased verbal processing speed that normalized though when adjusted for intelligence quotient (Hirvikoski et al., 2007; Lajic S et al., 2011). The ethics of prenatal dexamethasone therapy remains controversial because of unknown long-term outcomes and because seven fetuses were treated unnecessarily. Dexamethasone therapy also incurs maternal risk. Consequently, multiple endocrine societies consider prenatal dexamethasone therapy to be experimental and that it should only be performed in a research environment with long-term outcome studies, as the risk for long-term effect is not known (Speiser et al., 2010).



• **Fig. 96.5** Two Sisters With Congenital Adrenal Hyperplasia. (A) Female infant with congenital adrenal hyperplasia. There is enlargement of the clitoris with fusion and rugation of labial scrotal folds. (B) Sister of the female infant in (A) who also has congenital adrenal hyperplasia but whose mother received dexamethasone therapy prenatally to prevent virilization of external genitalia. (From Forest MG, Morel Y, David M. Prenatal treatment of congenital adrenal hyperplasia. *Trends Endocrinol Metab.* 1998;9:284–289.)

indicate the diagnosis, the daily medications and doses, and the physician to call in the event of an emergency.

Secondary or Tertiary Adrenal Insufficiency

Cortisol replacement for patients with secondary or tertiary adrenal insufficiency is the same as described for patients with primary adrenal insufficiency. To minimize growth suppression, these children can be treated with doses of hydrocortisone that are slightly less than physiologic replacement doses. Furthermore, because mineralocorticoid production is under the control of the renin-angiotensin system, patients with secondary or tertiary adrenal insufficiency do not require mineralocorticoid replacement. However, these infants require evaluation for deficiencies of other pituitary hormones.

Suggested Readings

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Disorders of Sexual Differentiation

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KEY POINTS

- Differences in sex development (DSD) are due to many different causes that have important consequences for long-term outcome and require the expertise of the pediatric endocrinologist, pediatric urologist, geneticist, and child psychologist as well as the adolescent gynecologist, cytogeneticist, radiologist, and ethicist in some cases to aid in the diagnosis and treatment of infants with DSD.
- Understanding normal sex development males and females is critical to determining the cause of DSD.
- Genetic testing has advanced our understanding of the origins of sex development, and specific testing may be driven by results of hormonal testing. In other situations the differential diagnosis for a given condition such as complete gonadal dysgenesis is so large that the use of a DSD panel is necessary. Knowing the genetic diagnosis provides closure for families and identifies other risks associated with the diagnosis, such as recurrence, renal disease, or tumor risk. A genetic diagnosis may also predict outcome at puberty.

“Is it a boy or a girl?” is often the first question asked when a baby is born. For parents of a newborn with genitalia that are not clearly male or female, the ambiguity is a distressing matter that needs to be addressed with sensitivity and some urgency. There are four general classifications that can cause disorders of or differences in sex development (DSD): (1) virilization of the 46,XX individual, (2) incomplete virilization of the 46,XY individual, (3) sex gonadal differentiation and chromosomal disorders, and (4) syndromes associated with incomplete genital development ([Box 97.1](#)). The first category, 46,XX DSD, comprises the majority of definable cases of ambiguous genitalia, whereas in the second category, 46,XY DSD, a genetic diagnosis is feasible in less than 50% of cases.

This chapter presents an overview of the pathophysiology of DSD, including a discussion of the practical aspects of diagnosis and management. An overview of the traditional surgical methods used in the treatment of infants with DSD, including the risks and benefits of these procedures, is included at the end of the chapter.

General Considerations in the Approach to the Newborn With Ambiguous Genitalia

The medical evaluation of a newborn with ambiguous genitalia is necessarily time consuming. Open and honest discussions with

the parents are invaluable in allaying anxiety and establishing a trusting relationship. Full disclosure of available information is essential in this regard.

Care must be taken to avoid premature sex assignment for the infant. Proper evaluation of the infant with ambiguous genitalia requires a multidisciplinary team that should include the primary care physician, neonatologist, pediatric endocrinologist, psychologist, pediatric urologist, pediatric geneticist, and pediatric radiologist ([Moshiri et al., 2012](#)). Psychologic assessment and support of the family are essential in the newborn period, along with long-term psychologic follow-up evaluation ([Slijper et al., 1998](#); [Hines, 2004](#); [Guerra-Junior and Maciel-Guerra, 2007](#)). Decisions regarding the sex of rearing should be made collaboratively between the multidisciplinary team and the parents, with the recognition that cultural and psychosocial factors play a role ([Grumbach and Conte, 1998](#); [Kuhnle and Krahle, 2002](#)).

In the past, sex assignment was based largely on phallus size, relative ease of surgical reconstruction, or the potential for fertility. This approach has come under criticism as dissatisfaction with sex assignment based on these criteria has been reported in several case studies ([Diamond and Sigmundson, 1997a](#); [Reiner, 1997](#); [Phornphutkul et al., 2000](#); [Hughes et al., 2007](#)). The importance of prenatal androgen imprinting has been implicated as an important variable in some of these cases ([Reiner, 1997](#)). Studies in undervirilized 46,XY males indicate that a small phallus can be associated with a satisfying adult sex life ([Reilly and Woodhouse, 1989](#)). Other studies have found that gender role tends to increasingly correspond with assigned sex as individuals with DSD proceed into adulthood ([Pappas et al., 2008](#)); therefore female sex assignment might not be necessarily warranted for intermediately undervirilized 46,XY males.

The degree of genital virilization is still an important determinant of sex assignment in the infant with ambiguous genitalia; however, other, incompletely understood factors appear to be involved. The formation of a healthy gender identity seems to involve a complex interplay between psychobiologic and environmental factors ([Money and Ehrhardt, 1972](#); [Meyer-Bahlburg et al., 1996](#); [Slijper et al., 1998](#); [Meyer-Bahlburg et al., 2006](#); [Singh et al., 2010](#)).

Embryology of Sex Determination and Differentiation

Normal and abnormal sex determination and differentiation constitute superb examples of how an understanding of embryology is critical to the approach and management of a group of complex

• BOX 97.1 Differential Diagnosis for Ambiguous Genitalia

46,XX Virilized Female

- Congenital adrenal hyperplasia
 - 21-Hydroxylase deficiency
 - 11-Hydroxylase deficiency
 - 3 β -Hydroxysteroid dehydrogenase deficiency
- Aromatase deficiency (fetal and maternal virilization)
- Virilizing maternal conditions
- Ovotesticular disorder of sex development
- Adrenal/ovarian tumors/luteoma of pregnancy
- Maternal ingestion of progestins, androgens

46,XY Undervirilized Male

- Androgen insensitivity
 - Partial
 - Complete
- 5 α -Reductase type 2 deficiency
- Testosterone biosynthetic defects
 - 17 β -Hydroxysteroid hydrogenase type 3 deficiency
 - 3 β -Hydroxysteroid dehydrogenase deficiency
 - 17 α -Hydroxylase/17,20-lyase deficiency
 - Congenital lipoid adrenal hyperplasia
- Leydig cell hypoplasia
- Idiopathic, undetermined
- Drug ingestion: progestins, spironolactone, cimetidine, phenytoin
- Persistent müllerian duct syndrome

Gonadal Differentiation and Chromosomal Disorders

- 46,XY gonadal dysgenesis
 - Complete (Swyer syndrome)
 - Partial
 - Mixed (45,X/46,XY)
- Ovotesticular disorder of sex development
 - 46,XX, 46,XY, 45X/46XY, 46,XX/46,XY

Syndromes Associated With Ambiguous Genitalia

- Gonadal dysgenesis
 - 46,XY partial gonadal dysgenesis (Turner syndrome features)
 - Camptomelic dysplasia
- Renal degenerative diseases and gonadal dysgenesis
 - Denys–Drash syndrome
 - Frasier syndrome
 - WAGR (Wilms tumor, aniridia, genitourinary abnormalities, mental retardation) syndrome
- Smith–Lemli–Opitz syndrome (7-dehydrocholesterol reductase deficiency)
- Robinow syndrome

and intriguing clinical disorders. Development of the testis and ovary is unique compared with the development of other organs in that they both derive from the same bipotential gonad. The establishment of a testis or an ovary depends on which pathway is taken on the basis of the genetic background. In the past the ovarian pathway was thought to be the default pathway. However, from studies of individuals with DSD, it is now known that ovarian development is an active process, and certain genes must be present for the ovary to develop.

Sex determination and differentiation are sequential processes that can be divided into three stages. Jost et al. (1970) established the sequence as follows: chromosomal sex is determined at fertilization and dictates the differentiation of the bipotential gonad, which in turn dictates the phenotypic sex, or the differentiation of the internal ductal system and external genitalia on the basis of which

hormones are produced by the gonad (Grumbach and Conte, 1998).

Chromosomal sex is determined at the moment of conception by the sex chromosome complement of the fertilizing sperm. If this sperm carries an X chromosome, a 46,XX (normal female) complement results. If the sex chromosome is Y, a 46,XY (normal male) genotype results. The *SRY* (sex-determining region of the Y chromosome) gene is necessary but not sufficient for testicular differentiation as other autosomal and X chromosomal genes are necessary for male sex development. *SRY* though is the only gene on the Y chromosome involved in testicular determination and differentiation. *SRY* through a number of steps instructs the medullary region of the bipotential gonad to develop into Sertoli cells and later into testicular cords and seminiferous tubules. *SRY* continues to be expressed at low levels in Sertoli cells until adulthood.

In addition to X chromosome genes, autosomal genes influence sex differentiation, insofar as mutations of these genes result in disorders of sex differentiation. Some of these genes include *WT1* (Wilms tumor gene 1), associated with Denys–Drash syndrome and WAGR (Wilms tumor, aniridia, genitourinary abnormalities, mental retardation) syndrome; *NR5A1* (nuclear receptor subfamily 5, group A, member 1), which encodes steroidogenic factor 1; and *SOX9*, which has been associated with camptomelic dysplasia and testicular dysgenesis in 75% of XY individuals (Foster et al., 1994). *SOX 9* expression is upregulated by *SRY*. The *DMRT1* and *DMRT3* genes on chromosome arm 9p when deleted are associated with female development in an XY individual. More recently, a de novo missense mutation in *DMRT1* was identified in a female with 46,XY karyotype, providing evidence that *DMRT1* is the critical gene for testicular differentiation (Murphy et al., 2015). The *FOXL2* gene is expressed in fetal and adult ovarian follicular cells and is critical to ovarian development, fertility, and maintenance of the ovary. It is also expressed in eyelids, with mutations leading to blepharophimosis–ptosis–epicanthus inversus syndrome, and is located within the homologous region in the human at 3q23 (Crisponi et al., 2001). The *DAX1* gene is located on Xp22 and appears to be necessary for correct testis determination and, in the mouse at least, necessary for the upregulation of *Sox9* expression (Bouma et al., 2005). When the *DAX1* gene is duplicated, 46,XY individuals develop gonadal dysgenesis with a female phenotype (Bardoni et al., 1994). The *WNT4* gene is critical for normal ovarian and female sexual development. A mutation in *WNT4* leads to müllerian duct regression and virilization in a 46,XX female (MacLaughlin and Donahoe, 2004), whereas duplication of the locus containing *WNT4* leads to overexpression of *DAX1* and thus a 46,XY female phenotype (Jordan et al., 2001). The desert hedgehog gene (*DHH*) is a member of a family of signaling genes with an important role in regulating morphogenesis. The follistatin gene (*Fst*) and the bone morphogenetic protein 2 gene (*Bmp2*) appear to be important for ovary organogenesis (Menke et al., 2003; Yao et al., 2004). Mutations in *Gata4* or *Fog2* can cause sex reversal in mice (Viger et al., 1998; Ketola et al., 2000; Tevosian et al., 2002). In humans, *GATA4* is a transcription factor necessary for genital ridge formation, testicular and ovarian differentiation, and male and female fertility. *GATA4* interacts with multiple genes, such as *SRY*, *SOX9*, and *AMH* as well as others involved in hormonal synthesis. *GATA4* acts synergistically with *NR5A1* to activate the *AMH* promoter, and a mutation in *GATA4* led to the lack of this synergy with *NR5A1* as well as inability for *FOG2* to bind to *GATA4*, resulting in familial 46,XY DSD and congenital heart defects (Lourenço et al., 2010). *FOG2* modifies

The diagram illustrates the genetic pathways for Ovary and Testis differentiation, branching from the **Genital ridge** and progressing over **TIME** from **Foetal** to **Adult** stages.

Ovary Pathway:

- Genital ridge** leads to **WNT4** and **RSPO1**, which activate β -catenin.
- β -catenin inhibits **SOX9**.
- NR5A1** (indicated with an orange arrow and a question mark) promotes **Steroidogenesis**.
- Steroidogenesis** leads to the formation of the **OVARY**.

Testis Pathway:

- Genital ridge** leads to **GATA4/FOG2/WT1**, which activates **SRY**.
- DMRT1** (indicated with an orange arrow and a question mark) also promotes **SRY**.
- NR5A1** (indicated with an orange arrow and a question mark) promotes **Sox8/SOX9**.
- FGF9** and **PTGDS** also promote **Sox8/SOX9**.
- NR5A1, GATA4, WT1, and SOX8** collectively promote **AMH**.
- SRY** promotes **DHH**, which leads to **Leydig cell** formation.
- Leydig cell** produces **NR5A1**, which promotes **Steroidogenesis**.
- Steroidogenesis** leads to the formation of the **TESTIS**.

Adult Stage and Mutual Inhibition:

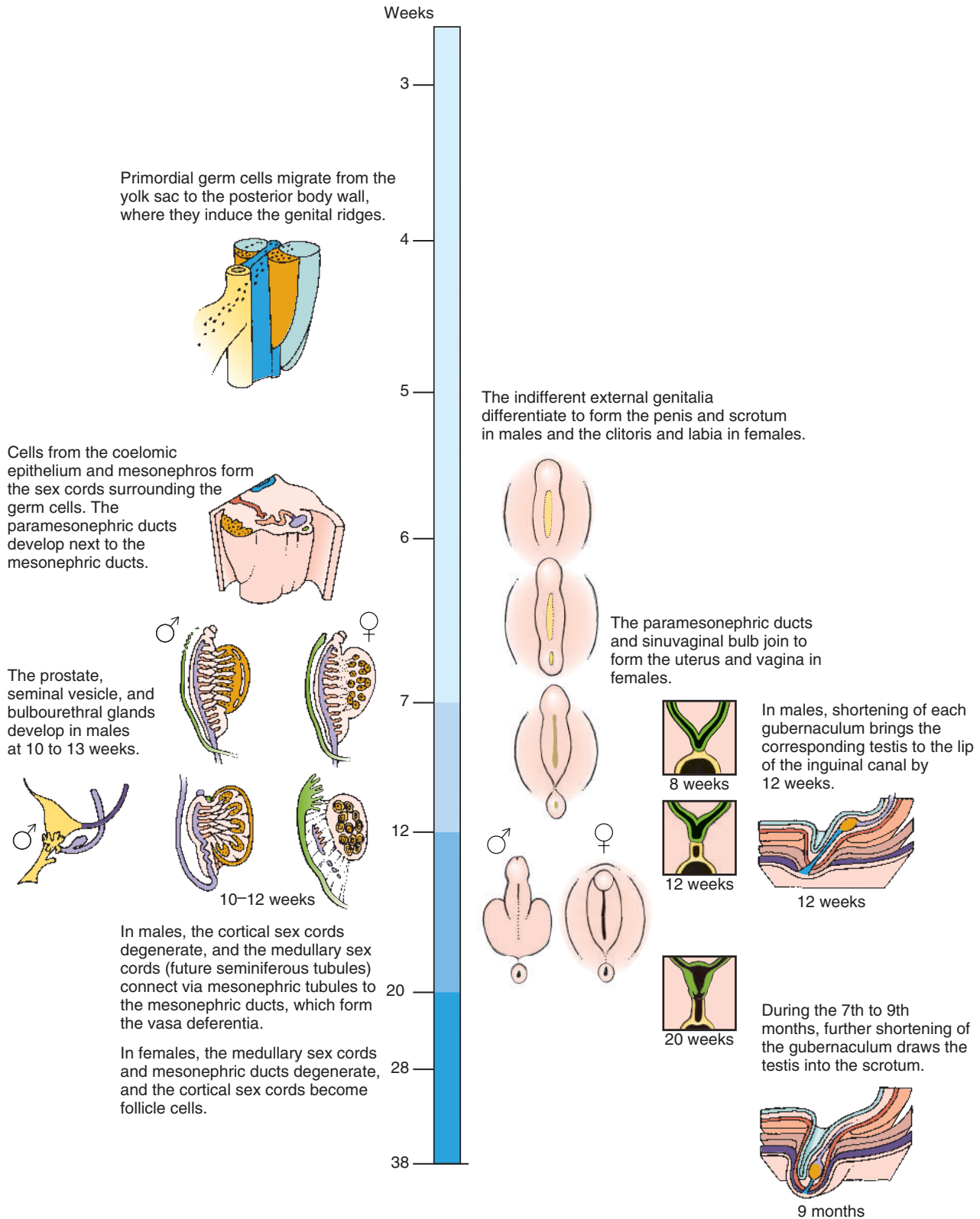
- In the **Adult** stage, **DMRT1** (indicated with an orange arrow and a question mark) promotes **FOXL2** in the ovary.
- FOXL2** inhibits **SOX9** in the ovary.
- In the testis, **DMRT1** inhibits **FOXL2**, and **FOXL2** inhibits **SOX9**.
- A large orange double-headed arrow indicates a mutual inhibitory relationship between **SOX9** in the ovary and **FOXL2** in the testis.

GATA4 activity by binding to GATA4's zinc finger (Zaytouni et al., 2011). Missense mutations in *FOG2* have been identified in two individuals with 46,XY gonadal dysgenesis (Bashamboo et al., 2014). The current pathways to testicular and ovarian development are illustrated in Fig. 97.1.

Gonadal sex is established by 7 weeks' gestation. At this stage the fetus contains two internal ductal systems—wolffian and müllerian—and undifferentiated external genital primordia. The wolffian, or mesonephric, duct is a tubular structure that connects the capillary network of the mesonephros to the urogenital sinus. Evagination of the coelomic epithelium leads to formation of a second tubular structure adjacent to the mesonephric duct – the paramesonephric, or müllerian, duct. The distal ends of these two ducts are joined. That portion of the urogenital sinus distal to the

Phenotypic sexual differentiation is predicated on establishing gonadal sex. If an ovary develops, the wolffian ducts involute because of a lack of local testosterone exposure, and only the terminal portion persists as a Gartner duct. The müllerian ducts develop into the proximal portion of the vagina, uterus, and fallopian tubes (see [Fig. 97.2](#)). The unfused cephalic portions of the müllerian ducts form the fallopian tubes, whereas the caudal ends fuse to form the ureterovaginal canal (see [Fig. 97.2](#)). The union of the fused caudal ends of the müllerian ducts and urogenital sinus forms the vagina. The proximal two-thirds of the vagina is of müllerian duct origin, and the distal third is of urogenital sinus origin. There is no fusion of the labioscrotal folds and no increase in clitoral-phallic structure in the absence of elevated levels of circulating androgens.

Male phenotypic differentiation is the result of the elaboration of two distinct testicular hormones: testosterone and antimüllerian hormone (AMH). These factors are produced and secreted by the 8-week stage of development, and they are essential for normal male differentiation. Involution of the müllerian ducts is caused by AMH, which is a glycoprotein secreted by the fetal Sertoli cells. The remnants of the müllerian ducts persist caudally as the prostatic



• **Fig. 97.2** Embryologic timeline for gonadal development and development of internal and external genitalia. (From Larsen WJ. *Human Embryology*. New York, NY: Churchill Livingstone; 1993:237.)

utricle and cephalically as the appendix testis. AMH exerts its action unilaterally and locally (exocrine secretion) rather than bilaterally via the systemic circulation.

Immediately after müllerian duct regression, the wolffian ducts develop under the local influence of testosterone secreted by the fetal Leydig cells. The Leydig cells, like the Sertoli cells, differentiate from the mesenchymal cells within the gonadal ridges; this occurs at 9 to 10 weeks' gestation. Under the influence of testosterone, the wolffian ducts evolve into the epididymis, vas deferens, and seminal vesicles (see Fig. 97.2). The mesonephric tubules develop into the ductuli efferentes, which will provide continuity between the seminiferous tubules and rete testis to the vas deferens. This process occurs as a direct action of testosterone on the ductal structures.

Virilization of the male external genitalia, fusion of labioscrotal folds, and movement of the urethral opening, starts at approximately 8 weeks' gestation (see Fig. 97.2) and relies on the ability of the tissues involved to convert testosterone into a more potent androgen: dihydrotestosterone (DHT). The target cells possess the enzyme 5 α -reductase type 2, which is necessary for this conversion. By 12 to 14 weeks' gestation, formation of the male external genitalia is nearly complete. Androgen exposure after this time results in further phallic enlargement. Testicular descent occurs in two stages. The initial transabdominal descent occurs at 8 to 15 weeks and depends on insulin-like 3 and its G protein-coupled receptor. Other testicular factors likely also play a role as most dysgenetic gonads are intra-abdominal. Descent into the inguinoscrotal region occurs at 25 to 35 weeks and is androgen dependent.

Clinical Assessment of Differences of Sex Development

History

A detailed family history is important in the evaluation of ambiguous genitalia. Information on early neonatal deaths, consanguinity, or urogenital anomalies should be obtained. A family history of female infertility, amenorrhea, or müllerian duct abnormalities can be suggestive of a DSD. In one study of androgen insensitivity disorders that are X-linked, a positive family history of a sex differentiation disorder was often overlooked (Viner et al., 1977).

The presence of maternal virilization is suggestive of a variety of disorders that can affect fetal masculinization. Features of maternal virilization include hirsutism, severe acne, deepening of the voice, and clitoromegaly on examination.

The ingestion of any recreational drugs, alcohol, or medications by the mother during pregnancy should be noted. Particular attention to medications with androgenic or progestational activity is indicated. Medications that affect fetal genital development include cimetidine, spironolactone, hydantoin, and progestational agents (Grumbach and Conte, 1998). Progestational agents are used in assisted reproductive technology to support the pregnancy in the first trimester; therefore asking if the pregnancy was conceived spontaneously or through the use of assisted reproductive technology is important.

Physical Examination

There is significant overlap of the genital anatomy among the various sex differentiation disorders. The physical examination, however, can provide the first clues to the underlying disorder. In addition, the physical examination will provide important

information about the degree of virilization of the external genitalia and the presence or absence of palpable gonads. Prader stage (Fig. 97.3A) is used to describe the virilization of a female, and the external masculinization score (see Fig. 97.3B) developed by Ahmed et al. (2000) is used to describe the undervirilization of a male. The physical examination will also inform the examiner as to when the DSD occurred in fetal life.

Clitoris

Significant clitoral enlargement deserves careful evaluation. A point to keep in mind is that premature infants have relatively underdeveloped labia majora, so the clitoris may appear enlarged. A truly enlarged clitoris can be distinguished from a large clitoral hood by the presence of palpable corporal or erectile tissue.

Penis (Phallus)

Measurements of the phallic stretch length and middle shaft diameter are important in determining the degree of virilization. The phallus should be stretched and measured from the pubic ramus to the tip of the glans. Gestational age-corrected phallic stretch lengths are shown in Fig. 97.4. The presence of a chordee structure on the ventral surface of the phallus can impair measurement of the true phallic length (Feldman and Smith, 1975; Fig. 97.5). A chordee is residual urethral tissue that tethers the phallus to the perineum. Measurement of the middle shaft diameter is particularly useful in this circumstance. For term male infants, a normal middle shaft diameter is approximately 1 cm (Feldman and Smith, 1975).

A microphallus warrants careful evaluation for the presence of hypopituitarism or growth hormone deficiency, particularly in the presence of hypoglycemia or unexplained jaundice. Microphallus and undescended testes may occasionally be the presenting phenotype for a DSD.

Labioscrotal Folds

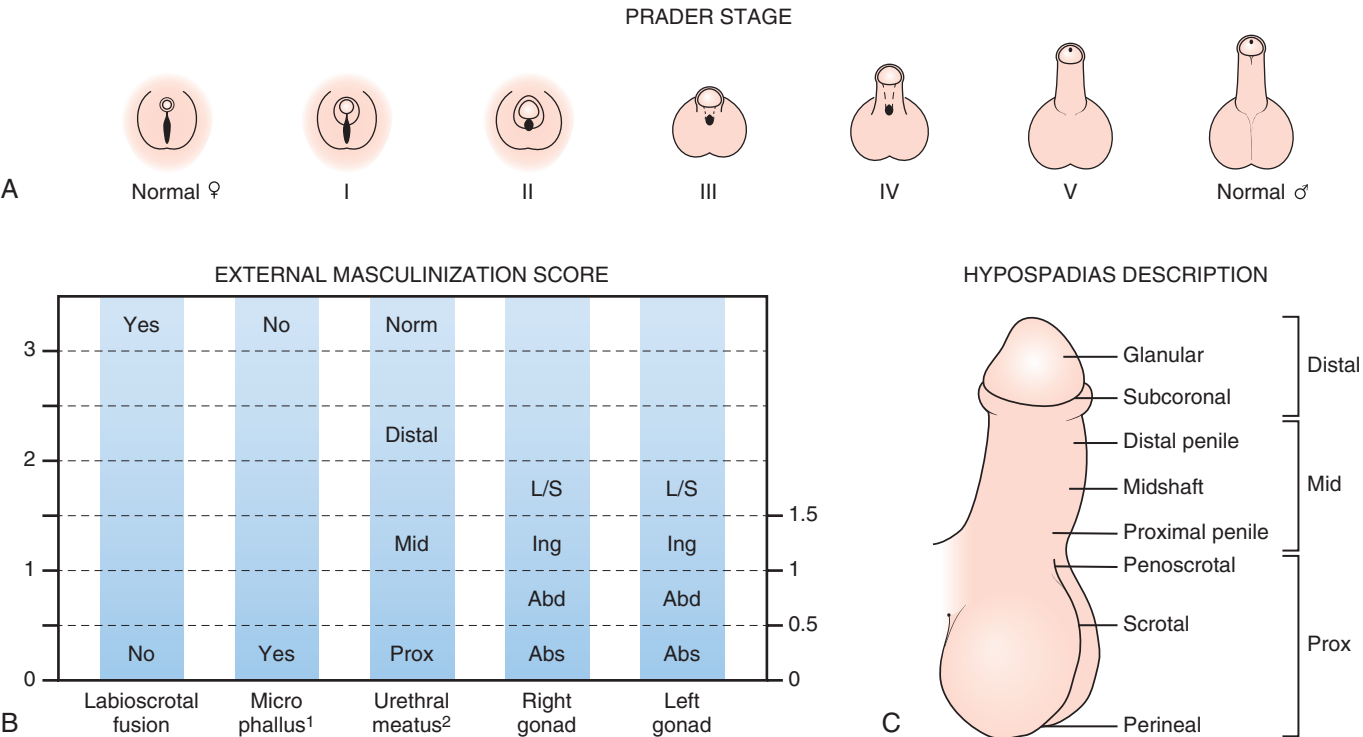
Assessment of the degree of fusion of the labioscrotal folds should be performed. When the infant is exposed to androgens during embryogenesis, fusion of the labioscrotal folds progresses from a posterior to an anterior direction. The spectrum of labial fusion can range from mild posterior fusion to complete labial fusion (Fig. 97.6). Fusion of the labioscrotal folds occurs in the first trimester. The examiner should note whether the folds are rugated or hyperpigmented. Is the phallus positioned in the normal superior position relative to the scrotum, or is there a shawl scrotum (penoscrotal transposition)? Is the scrotum fused normally in the midline or is the scrotum bifid (see Fig. 97.5)?

Gonads

Careful examination for the presence of gonads should be performed in all infants with ambiguous genitalia. The presence of bilateral gonads in the labial folds is highly suggestive of an undermasculinized genetic male (see Fig. 97.5). A unilaterally palpable gonad is often seen in infants with mixed gonad dysgenesis or ovotesticular DSD (Fig. 97.7), although other disorders such as androgen insensitivity can manifest themselves similarly. When cryptorchidism and hypospadias occur simultaneously, there is a greater than 25% chance of a DSD (Rajfer and Walsh, 1976; Albers et al., 1997).

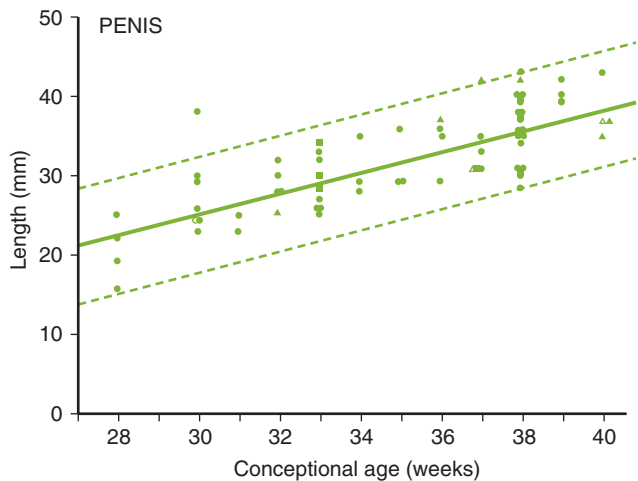
Hypospadias, Perineum

The severity of hypospadias can differ, with the condition ranging from mild granular hypospadias to penoscrotal hypospadias (see

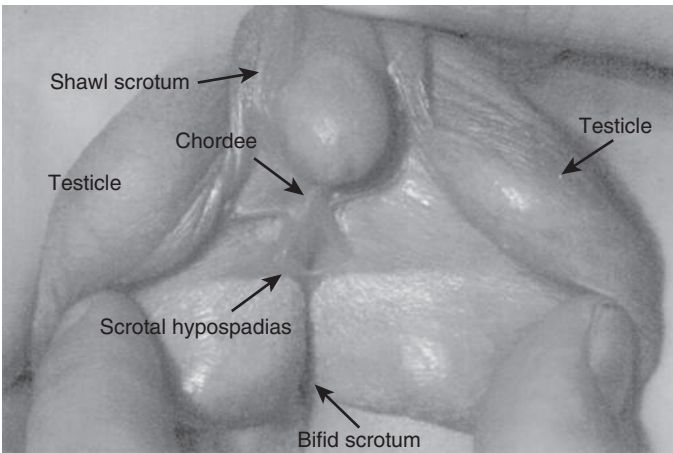


¹Microphallus is a phallic length below the male reference range.
²The location of the urethral meatus is based on the location described in C.

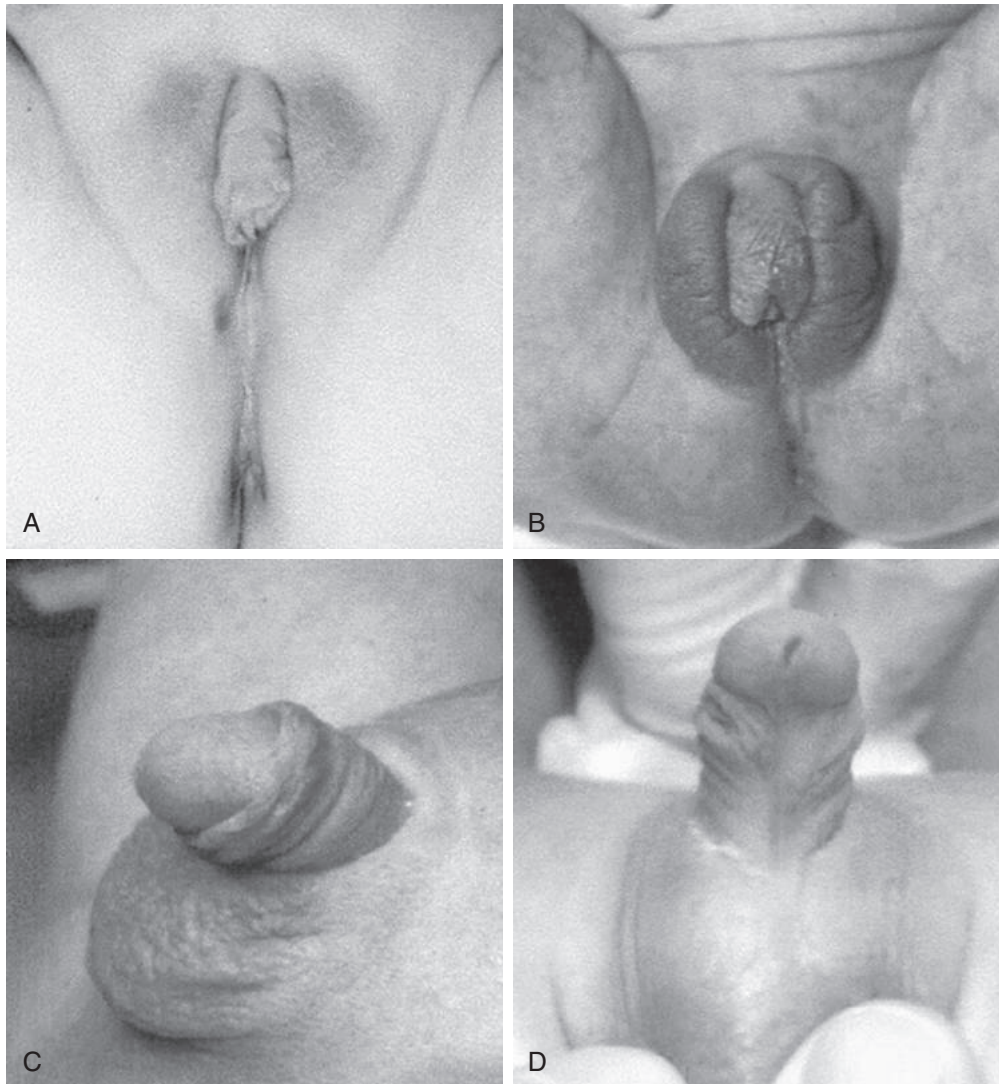
• **Fig. 97.3** (A) The stages of virilization of the female with congenital adrenal hyperplasia as developed by Prader. Stage I indicates mild clitoromegaly only; stage V indicates complete masculinization. (B) External masculinization score. A score of 0 to 3 is given for four aspects of male external genitalia. The sum of the four values is the external masculinization score. A normal male has a value of 12. (C) Hypospadias description. *Abd*, Abdomen; *Abs*, absent; *Ing*, inguinal; *L/S*, labioscrotal; *norm*, normal; *prox*, proximal. ([A] Reproduced with permission from Migeon CJ, Berkovitz G, Brown T. Sexual differentiation and ambiguity. In: Kappy MS, Blizzard RM, Migeon CJ, eds. *Wilkins' Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence*. 4th ed. Springfield, IL: Charles C. Thomas; 1994:573. [B–C] Reproduced with permission from Ahmed SF, Rodie M. Investigation and initial management of ambiguous genitalia. *Best Pract Res Clin Endocrinol Metab*. 2010;24:197–218.)



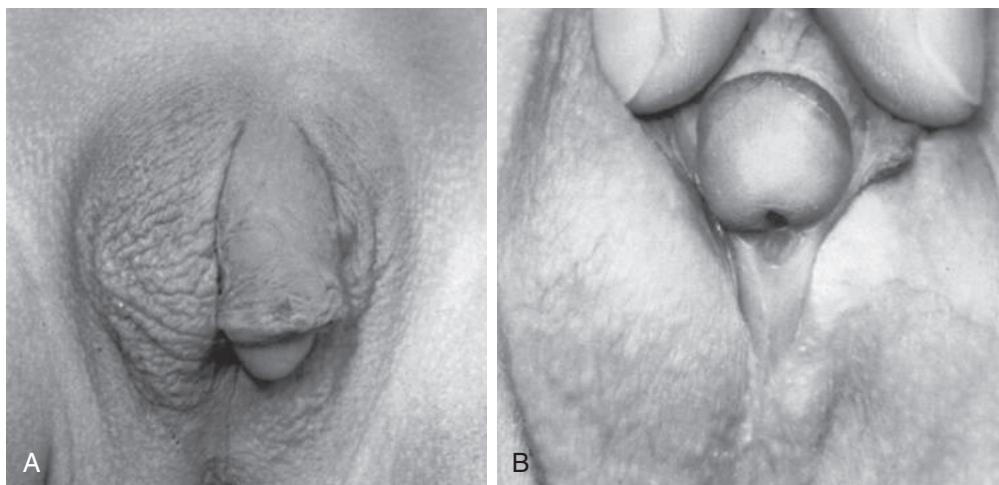
• **Fig. 97.4** Penis stretch length in 63 normal premature and full-term male infants (*circles*), showing lines of mean \pm two standard deviations. Superimposed are data for two small-for-gestational-age infants (*open triangles*), seven large-for-gestational-age infants (*closed triangles*), and twins (*squares*). (Reproduced with permission from Feldman KW, Smith DW. Fetal phallic growth and penile standards for newborn male infants. *J Pediatr*. 1975;86:395–398.)



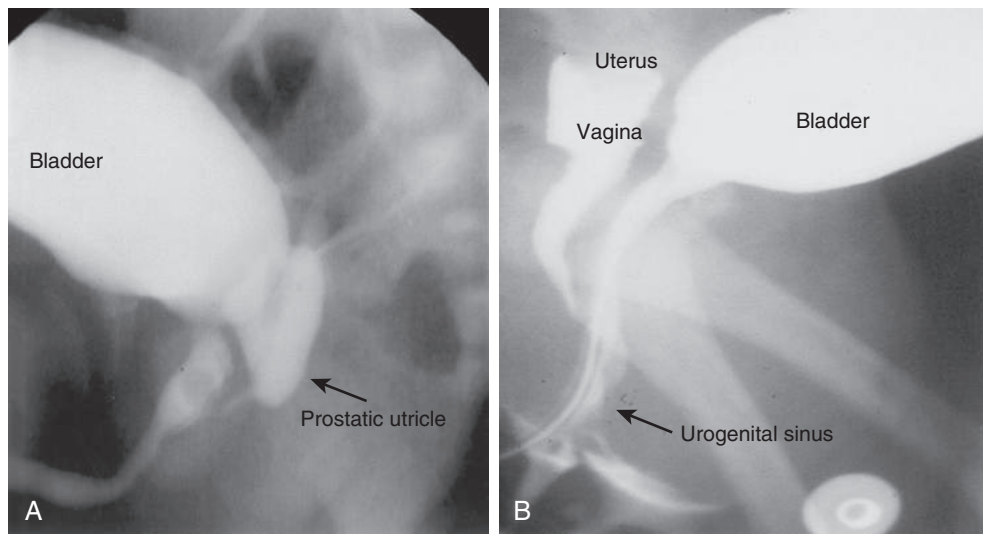
• **Fig. 97.5** Undervirilized male demonstrating bifid scrotum, scrotal hypospadias, chordee, and bilateral descended testes.



• **Fig. 97.6** Virilization of external genitalia in 46,XX congenital adrenal hyperplasia (21-hydroxylase deficiency). (A) There is a mild to moderate degree of virilization, with primarily clitoral hypertrophy and significant fusion of the labia. (B) Virilization is moderate, with clitoromegaly, labial fusion, and rugation of labial folds. (C–D) Complete masculinization is evident.



• **Fig. 97.7** Asymmetric external genitalia with left unilateral descended testis (A), penoscrotal hypospadias, and chordee (B). Asymmetric external genital development or gonadal descent would be characteristic of mixed gonadal dysgenesis or ovotesticular disorder of sexual development.



• **Fig. 97.8** Genitourethrogram. (A) Genitogram from a 46,XY infant with microphallus and undescended testes with a clearly delineated prostatic utricle (see [Case Study 2](#)). (B) Genitogram from a 46,XX infant with congenital adrenal hyperplasia and severe masculinization of external genitals. The confluence of the vagina with the urogenital sinus is of intermediate severity.

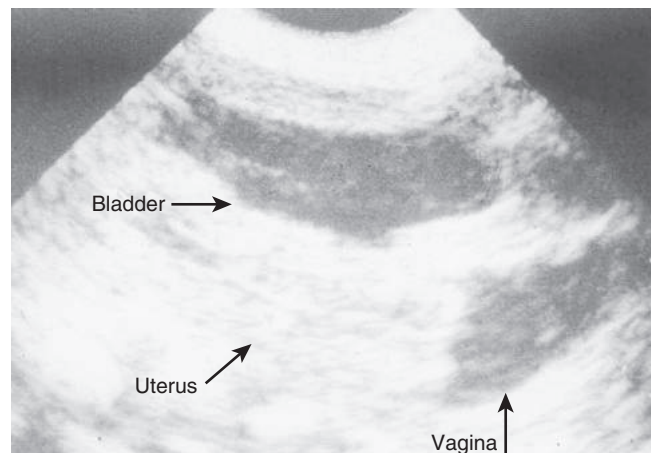
[Fig. 97.3C](#)), although most disorders of sexual differentiation manifest themselves with severe penoscrotal or scrotal hypospadias (see [Fig. 97.5](#)). Examination for the presence of separate urethral and vaginal openings versus a single perineal opening (urogenital sinus) conveys important anatomic information. The vagina may be blind ending or completely formed. A urogenital sinus results from failure of the urologic and genital tracts to differentiate completely. In virilized females the level at which the vagina enters the sinus (low-level versus high-level vaginal entry) has important implications for determining the ease of subsequent surgical exteriorization of the vagina ([Fig. 97.8](#)). In addition, when the urethra enters a urogenital sinus, there is potential for urinary stasis and therefore urinary tract infections. Excessive pigmentation of the genitals or signs of dehydration should alert the examiner to the possibility of congenital adrenal hyperplasia (CAH).

Dysmorphic features suggestive of Turner syndrome indicate the possibility of gonadal dysgenesis or mixed gonadal dysgenesis. Such abnormalities or multiple congenital anomalies could indicate any of a variety of syndromes associated with ambiguous genitalia ([Box 97.1](#)).

Radiologic Investigations

Pelvic Ultrasonography

Pelvic ultrasonography reveals vital information in the evaluation of DSDs. The presence or absence of a uterus is a critically important determinant in the initial evaluation ([Figs. 97.9–97.11](#)). The newborn period is a time when the uterus, ovaries, and adrenal glands are optimally visualized ([Wright et al., 1995](#)). The presence of a well-developed uterus will direct the differential diagnosis toward virilization of a genetic female, 46,XY ovotesticular DSD, 46,XY complete gonadal dysgenesis, or persistent müllerian duct syndrome (PMDS); however, a rudimentary uterus may be seen in 46,XY gonadal dysgenesis or ovotesticular DSD. The inability to identify a uterus though is not conclusive for lack of a uterus. Ultrasonography can locate undescended testes and determine gonadal size or irregularity, such as an oblong ovotestis. Gonads in the newborn are not always well visualized by ultrasound

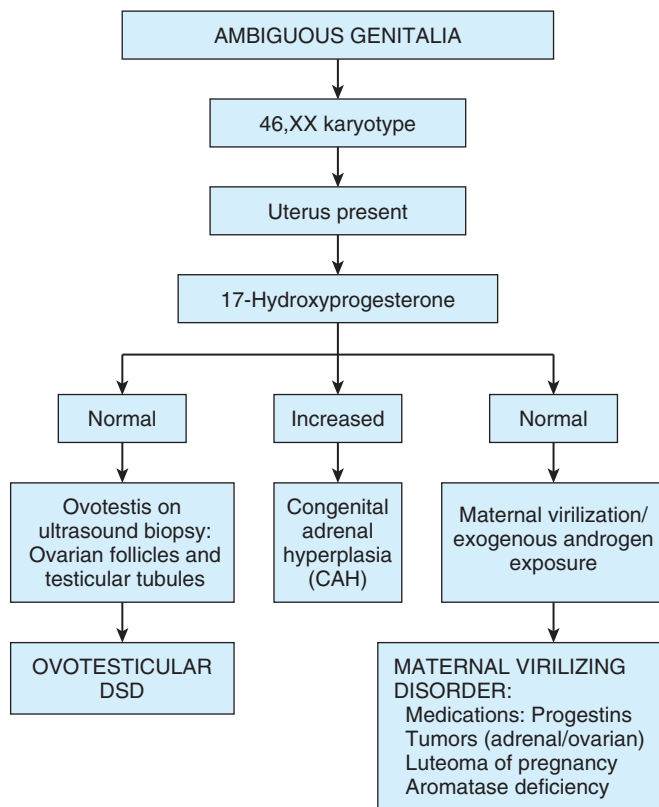


• **Fig. 97.9** Ultrasound image from a 46,XX infant with ambiguous genitalia. Note the presence of a well-developed uterus with an endometrial stripe.

examination, but that does not necessarily mean they are absent or abnormal. In ovotesticular DSD, loss of the uniform testicular echotexture suggests the presence of an ovotestis. Ultrasound examination can determine whether the adrenal glands appear enlarged, as in CAH; however, normal adrenal size does not rule out CAH.

Genitourethrogram

A genitourethrogram is a fluoroscopically guided genital dye study that can provide important information on the urethra and internal genital ducts ([Wright et al., 1995](#)). An experienced radiologist should perform this study. It is important to ensure that all perineal orifices are examined. The main features to be noted are the presence or absence of a vagina (or prostatic utricle) and the relationship between the vagina and the urethra (see [Fig. 97.8](#)). Demonstration of the level at which the vagina opens into the urogenital sinus and its relationship to the external sphincter has important surgical



• **Fig. 97.10** Algorithm for evaluation of the 46,XX infant with ambiguous genitalia. The presence of a uterus would be determined radiographically by ultrasound examination, genitourethrogram, or magnetic resonance imaging.

implications. Recognition of male or female urethral configurations may also be possible during genitourethrography.

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) has been used to assess the internal genitalia of a limited number of infants and children with genital differentiation disorders. The strength of MRI lies in its ability to image large areas in multiple planes and characterize soft tissues. Detailed information about müllerian and wolffian structures and the position of the gonads can be obtained; however, thin sections (3 to 5 mm) are required for an adequate study. Streak gonads remain difficult to visualize. MRI has the capability to differentiate between an enlarged clitoris and a penis, because the bulbospongiosus muscle and transverse perineal muscle are absent or poorly visualized in the virilized female (Wright et al., 1995). MRI is a promising modality for the evaluation of ambiguous genitalia; however, further study is needed to demonstrate efficacy over other imaging modalities.

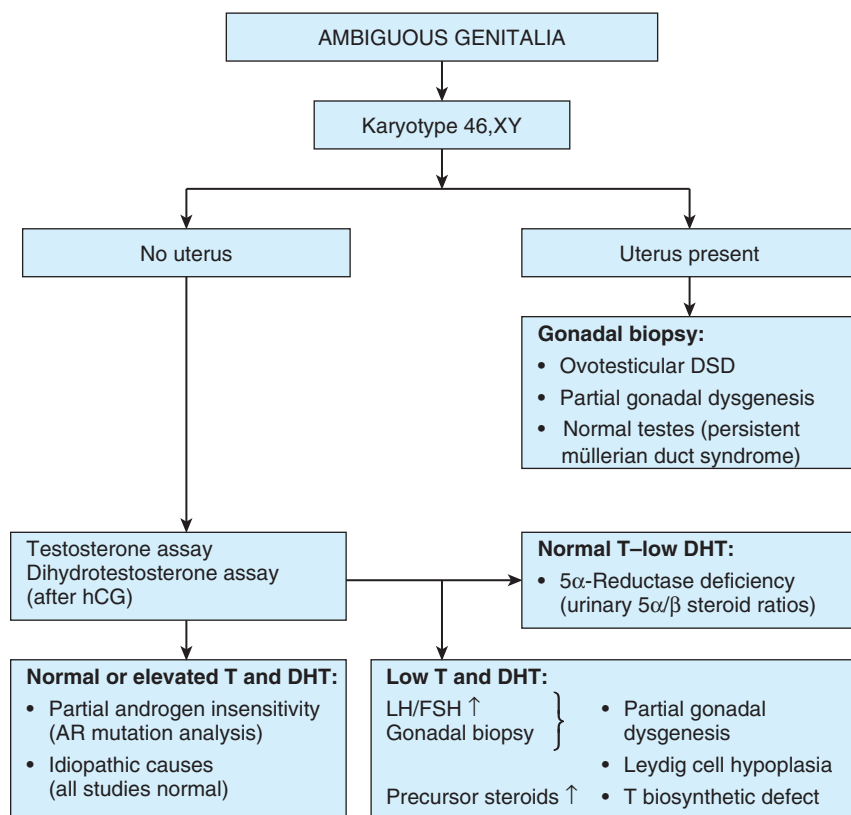
Laboratory Investigations

Endocrine and genetic laboratory studies are germane in the evaluation of ambiguous genitalia in the newborn. Day 1 of life is an ideal time to obtain serum testosterone and dihydrotestosterone (DHT) levels by liquid chromatography—tandem mass spectrometry (LC–MS/MS), as testosterone levels fall rapidly in the first several days of life (Forest et al., 1980). It is important to use LC–MS/MS assays as other assays measure interfering substances that will falsely elevate the testosterone level (Fuqua et al., 1995).

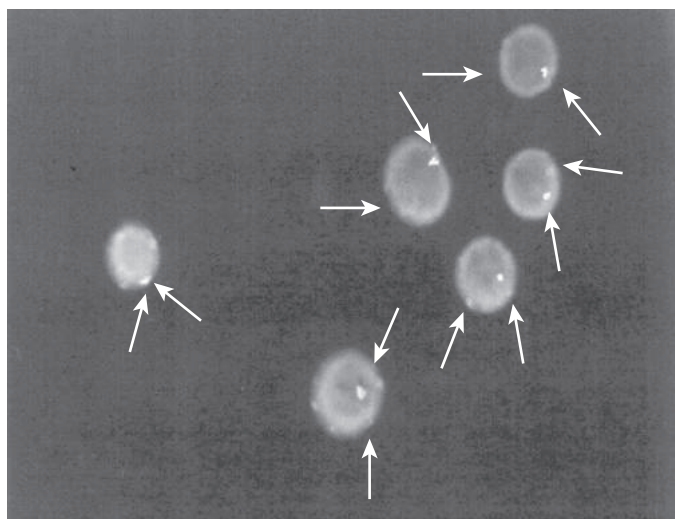
Chromosomal studies should optimally be performed on day 1, as a preliminary star karyotype with five cells can be available by 24 to 48 hours with use of the fetal nucleated red blood cells. Fluorescence in situ hybridization (FISH) for *SRY* should also be performed. On day 2 or day 3 of life, determinations of serum 17-hydroxyprogesterone (17-OHP), 17-hydroxypregnenolone, 11-deoxycortisol, dehydroepiandrosterone (DHEA), androstenedione, and plasma renin activity are performed if CAH is suspected. Some clinicians perform an adrenocorticotrophic hormone (ACTH) stimulation test at this time to more clearly demonstrate a block in steroid biosynthesis. Results of these studies should be sent immediately to the appropriate reference laboratory for analysis. Alerting the reference laboratory to the urgent nature of the studies performed will facilitate rapid processing. 17-OHP levels are physiologically elevated on the first day of life, and screening for CAH should preferably not be done at this time. Serum gonadotropins are often suppressed in the immediate newborn period, so they should be measured after 1 week of life. Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels are helpful in assessing newborns for androgen insensitivity, gonadal dysgenesis, and LH receptor abnormalities. Repeated LH, FSH, testosterone, and DHT testing should be done between 2 and 8 weeks of life in the evaluation of undervirilized males. This period coincides with the physiologic testosterone surge (mini-puberty) seen in healthy male infants (Forest et al., 1980). When CAH is suggested, it is important to check sodium and potassium levels on a daily basis to prevent a salt-wasting crisis.

A human chorionic gonadotropin (hCG) test can be useful in the evaluation of suspected incomplete masculinization of the genetic male. However, if LH level is elevated, then hCG testing is not useful as it will not provide any additional information. hCG binds the LH receptor and stimulates the testes to synthesize sex steroids. A testosterone response greater than 200 ng/dL rules out a testosterone biosynthetic defect and is considered a normal response to hCG (a value less than 200 ng/dL may be normal in some assays). A normal testosterone-to-DHT ratio of less than 8:1 argues against 5 α -reductase type 2 deficiency, with the caveat that the testosterone level must be high enough to adequately test the enzyme function. The gold standard for 5 α -reductase type 2 deficiency is genetic testing as the testosterone-to-DHT ratio can be misleading. In addition, the hCG test can result in phallic enlargement if there is a good testosterone response. Almaguer et al. (1993) reported an increase in phallic length of 0.25 to 0.75 cm in six 46,XY males with idiopathic micropallus within 5 days of beginning injections. A bolus of 1500 international units (IU) of hCG was given intramuscularly on three consecutive days, with steroids and phallic length measured on the fifth day. Growth of the phallus in response to hCG suggests that the phallus will further virilize at puberty, although no longitudinal study has documented this assumption.

FISH can rapidly determine the sex chromosome complement of the newborn by use of X chromosome- and Y chromosome-specific centromeric probes (Schwartz et al., 1997; Fig. 97.12). In addition, this method allows the detection of low levels of chromosomal mosaicism, because hundreds of cells can be analyzed rapidly. FISH is also useful in identifying an *SRY* gene translocated to an X chromosome (Wang et al., 2009). FISH analysis for determination of sex chromosome constitution has been shown to be highly reliable, although this method has not been used extensively in the evaluation of ambiguous genitalia of the newborn. As a result, results should be interpreted with some degree of caution until confirmation by karyotypic analysis is available.



• **Fig. 97.11** Algorithm for evaluation of the infant with ambiguous genitalia and 46,XY karyotype. The presence or absence of a uterus would be determined by radiographic imaging, including ultrasound examination, genitourethrogram, or magnetic resonance imaging, as appropriate. Testosterone (T) and dihydrotestosterone (DHT) levels ideally should be obtained after human chorionic gonadotropin (hCG) stimulation or between 2 weeks and 3 months during the mini-puberty. AR, Androgen receptor; FSH, follicle-stimulating hormone; LH, luteinizing hormone.



• **Fig. 97.12** Fluorescence in situ hybridization technique demonstrating the sex chromosome constitution of peripheral blood leukocytes. Arrows indicate sex chromosomes in each cell.

In a small percentage of ambiguous genitalia cases, laparoscopy with gonadal biopsy is necessary to confirm the diagnosis of ovotesticular DSD, gonadal dysgenesis, or Leydig cell aplasia. Obtaining a karyotype from gonadal tissue may be helpful when sex chromosome mosaicism is suggested.

46,XX Differences in Sex Development

Virilization of the Female

Virilization of an XX infant is most commonly caused by CAH, although other virilizing conditions can be involved (see Fig. 97.10). CAH encompasses a group of disorders of adrenal steroid hormone biosynthesis, of which more than 90% to 95% are due to 21-hydroxylase deficiency (OHD) (New, 1992; Fig. 97.13).

Occasionally, the presence of excess androgens of maternal origin can result in virilization of the female fetus (Grumbach and Conte, 1998). A unique cause of both maternal and fetal masculinization is placental aromatase deficiency (Conte et al., 1994). 46,XX testicular DSD, formally referred to as 46,XX maleness, can occur when the SRY gene is translocated from the Y chromosome to an X chromosome. Most patients with SRY-positive 46,XX testicular DSD have normal male external genitalia, but occasionally the genitalia are ambiguous (Fechner et al., 1993; Ergun-Longmire et al., 2005; Wang et al., 2009).

Congenital Adrenal Hyperplasia

21-OHD is the most common cause of ambiguous genitalia in the newborn female (New, 1992). It has a population frequency of approximately 1 in 15,000, and the disorder is inherited in an autosomal recessive fashion. Males and females are equally affected; however, in classic cases, females are virilized at birth, resulting in the clinical presentation of ambiguous genitalia.

Assessment for the less common forms of CAH resulting in ambiguous genitalia—11 β -OHD and 3 β -hydroxysteroid dehydrogenase (3 β -HSD) deficiency—includes measurement of 11-deoxycortisol, deoxycorticosterone (DOC), and 17-hydroxypregnenolone. Use of a comprehensive steroid panel from a reliable reference laboratory will limit the amount of blood required to 3 to 5 mL.

11 β -OHD accounts for approximately 5% of CAH cases worldwide, and this disorder will result in masculinization of the female fetus (see Fig. 97.13). However, the presence of volume overload and hypertension distinguishes this disorder from 21-OHD (New, 1992). Presumably the hypertension is caused by the excess mineralocorticoid activity of the DOC metabolite. Typically, 11-deoxycortisol level is elevated and plasma renin activity is suppressed in this disorder. However, hypertension may not present in the young infant, and thus absence of hypertension does not exclude 11 β -OHD.

3 β -HSD may result in ambiguous genitalia in the newborn period (see Fig. 97.13). Unlike 21-hydroxylase, this enzyme is present in the gonads as well as the adrenal glands, and deficiency of 3 β -HSD can result in undermasculinization of the male infant or mild masculinization of the female infant. 3 β -HSD is needed for testosterone biosynthesis; therefore the male fetus may be inadequately masculinized. This enzyme deficiency results in excess production of the steroid DHEA, which can be converted to more potent androgens peripherally. The female infant with this disorder can have clitoromegaly, although such children are often phenotypically normal. A marked increase in the ratios of 17-hydroxypregnenolone to 17-OHP and of DHEA to androstenedione is diagnostic in mutation-positive forms of this disorder (Sakkal-Alkaddour et al., 1996).

Treatment

Treatment of CAH in the newborn period consists of glucocorticoid and mineralocorticoid replacement. Hydrocortisone is the most physiologic form of synthetic glucocorticoids and is less likely to result in unwanted side effects. Hydrocortisone is administered orally initially at a dosage of approximately 25 mg/m² per day (divided into three doses) in the newborn period. No liquid preparation of hydrocortisone is currently available, so tablets must be crushed and administered carefully with formula or food. Mineralocorticoid replacement (fludrocortisone acetate [Florinef]) at a starting dose of 0.1 to 0.2 mg/d in the newborn is recommended but often needs to be increased. Sodium chloride supplements (approximately 1 to 2 g/d) are useful adjunctive therapy in salt-losing CAH, because formula and human milk have low salt contents. Once therapy has been initiated, careful monitoring of the infant's growth and determination of serum electrolytes, 17-OHP, androstenedione, testosterone, and plasma renin activity are recommended.

Salt-losing crisis and acute adrenal insufficiency should be treated with stress dosages of hydrocortisone (100 mg/m² per day), which can be given either continuously as a drip or divided into equal doses every 6 hours. Intravenous fluids with ample sodium chloride (normal saline for boluses and 0.5 N saline for maintenance fluids) are an essential component of treating salt-losing crisis. Care should be taken not to overhydrate or underhydrate the infant. Hyperkalemia often abates after sodium chloride and hydrocortisone are provided intravenously, although severe cases of hyperkalemia may require additional therapies.

Sex assignment for 46,XX infants with 21-OHD has traditionally been female (Donahoe, 1991; New, 1992; Migeon et al., 1994; American Academy of Pediatrics Committee on Genetics et al., 2000; Forest, 2001). Gender identity of 46,XX adults with CAH

is typically female, with various degrees of masculinized behavior (Money and Ehrhardt, 1972; Reiner, 1997). Prenatal androgenization affects gender-related behavior, but not gender identity, in girls with CAH aged 5 to 12 years (Meyer-Bahlburg et al., 2004a). Cases of gender identity disorder have been reported in treated females with CAH (Slijper et al., 1998), and cases of gender reassignment from female to male have been reported (Meyer-Bahlburg et al., 1996). Individuals with undiagnosed 46,XX CAH who are profoundly virilized have functioned successfully as males (Blizzard, 1999; Meyer-Bahlburg, 2009). Diamond and Sigmundson (1997b) suggested male sex assignment in profoundly virilized 46,XX individuals, although female sex assignment is likely to prevail in current practice until evidence is obtained to indicate otherwise (Lee, 2001; Crouch et al., 2008).

In addition to medical therapies for CAH, efforts to normalize the appearance of the external genitalia may be pursued. It should be kept in mind that hypertrophy of the clitoris will gradually lessen after medical therapy is instituted; however, complete normalization in the more virilized cases is not likely to occur. In severe cases of clitoral enlargement, clitoral recession surgery is a treatment option, although suboptimal cosmetic results have been reported in long-term outcome studies. Atrophy or loss of the clitoris or excessive regrowth of clitoral tissue has been described in examinations of adolescent and adult patients who underwent genital surgery in early childhood (Alizai et al., 1999; Creighton et al., 2001). If CAH is not well controlled with hormonal suppression of the adrenal gland, there will be clitoral enlargement. The risk of surgery needs to be balanced against the potential detrimental effects of masculinized genitalia on the development of a poor body image (Meyer-Bahlburg et al., 1996) and of social stigmatization by family or community members (Money et al., 1986). Nerve-sparing ventral clitoroplasty in virilized females has been shown to preserve dorsal nerves for better sensitivity after surgery (Poppas et al., 2007).

Surgical correction of the vagina in CAH is performed to exteriorize the vagina and to enlarge the vaginal opening so that successful intercourse can occur later in life (see [Overview of the Surgical Management of Disorders of Sexual Differentiation](#)). There is considerable debate about when to perform vaginal exteriorization surgery. A number of studies have demonstrated the development of vaginal stenosis when vaginoplasties are performed in the prepubertal period (Alizai et al., 1999; Krege et al., 2000; Creighton et al., 2001). The investigators advocate delaying vaginoplasty until puberty or later, when manual dilation can be undertaken by the patient, and estrogenization of the vaginal mucosa can help to prevent stricture formation. Others recommend that vaginoplasties be undertaken early in life, because the procedure is technically easier in the first several years of life, and the emotional trauma of a major surgery in adolescence is avoided (Donahoe, 1991; Schnitzer and Donahoe, 2001). Reports of long-term outcome of genitoplasty by patient advocate groups such as the Accord Alliance and others have called for a general moratorium on all nonessential genital surgery in infancy until affected individuals are old enough to express their wishes and give consent (Diamond and Sigmundson, 1997b). Problems with loss of sexual sensation and pleasure have been reported in adult patients as a consequence of genital surgery in early life. However, in cases of severe discordance between assigned sex and genital appearance, the psychosocial consequences of uncorrected genital anomalies can be damaging (Money and Ehrhardt, 1972; Money et al., 1986). Participation of the parents in the decision to pursue genital surgery after they have been fully informed of the benefits and risks is perhaps the most judicious

approach at this time (Reiner, 1997; Daaboul and Frader, 2001; Lee, 2001).

Significant reductions in fertility have been reported in women with salt-losing CAH (Mulaikal et al., 1987; Kuhnle et al., 1995). Suggested explanations for reduced fertility included increased anovulatory cycles, low rates of heterosexual activity, inadequate vaginal introitus, and poor adherence to medical treatment (Mulaikal et al., 1987). Problems with a disturbed body image and feeling less feminine were associated with a lower rate of success among women with CAH in terms of the ability to establish a partnership or marry (Kuhnle et al., 1995). Overall quality of life for female patients with CAH was comparable with that for controls, suggesting that affected women may develop coping strategies and cognitive appraisals that enable them to accept their life and view it as satisfying (Kuhnle et al., 1995).

CASE STUDY 1

History

- A 3.5-kg term infant born after an uncomplicated pregnancy.
- Cryptorchidism and hypospadias noted, with the infant discharged home as a male with a plan for outpatient surgical evaluation.
- Infant fed poorly at home, with intake of approximately 4 oz/day, no vomiting.
- On day 5 of life, notification of abnormal newborn screen for congenital adrenal hyperplasia by health department; referred to emergency department.

Evaluation

- On presentation to emergency department: infant was well appearing, weight 2.9 kg (representing a loss of 0.6 kg).
- Genital examination: 2.3 cm \times 1.1 cm phallus, severe hypospadias, marked labial fusion, no palpable gonads, marked hyperpigmentation of genitalia.
- Electrolytes: sodium 140 mEq/L, potassium 5.8 mEq/L, chloride 107 mEq/L, CO₂ 19 mEq/L, blood urea nitrogen 15 mg/dL, creatinine 1.0 mg/dL.
- Glucose 94 mg/dL.
- Ultrasound examination: uterus present, enlarged adrenals, no gonads seen.
- Karyotype 46,XX.
- 17-Hydroxyprogesterone 20,000 ng/dL (normal <200 ng/dL – unstimulated).
- Plasma renin activity 201 ng/mL per hour (normal <26 ng/mL per hour).

Management and Outcome

- Sex was reassigned female.
- Treatment with hydrocortisone (Cortef) and fludrocortisone acetate (Florinef) was started along with sodium chloride supplements.
- On day 10 of life, weight 3.2 kg, feeding 4 oz every 4 hours.
- By 4 months of age, significant reduction in clitoral and labial enlargement as well as resolution of hyperpigmentation.

Prenatal Diagnosis and Treatment

Diagnosis

Since the report by Jeffcoate et al. (1965) of the successful identification of an affected fetus by elevated concentrations of 17-ketosteroids and pregnanetriol in the amniotic fluid, investigators have performed prenatal diagnosis for CAH by measurements of hormone levels in pregnancy (Jeffcoate et al., 1965; New and Levine, 1973; Levine, 1986). The most specific hormonal diagnostic test for 21-OHD is elevated 17-OHP level in the amniotic fluid (Frasier et al., 1974;

Nagamani et al., 1978; Hughes and Laurence, 1979; Pang et al., 1980; Hughes and Laurence, 1982); Δ^4 -androstenedione level can be used as an adjunctive diagnostic assay (Pang et al., 1980). Amniotic fluid testosterone levels might not be outside the normal range in an affected male (Wilson et al., 1995). Measurement of hormone levels in amniotic fluid detects only severe cases, and for the most part it has been replaced by molecular diagnosis.

Molecular analysis of the 21-hydroxylase locus of amniotic fluid cells can make the diagnosis. It is important to know parental genotype so that the correct phase (*cis* versus *trans*) can be established. With the advent of chorionic villus sampling, evaluation of the at-risk fetus is currently possible in the first trimester at 10 to 12 weeks' gestation. The fetal DNA is used for specific amplification of the *CYP21A2* gene using polymerase chain reaction and Southern blotting, which has the advantage of requiring small amounts of DNA (Speiser et al., 1990). In the future, DNA analysis using cell-free DNA obtained from maternal blood may be possible. Because of improved accuracy and earlier diagnosis, molecular analysis of fetal DNA is now the method of choice for prenatal diagnosis. See Chapter 96 for more details.

Treatment

Treatment with dexamethasone can be used in pregnancies at risk of 21-OHD (Evans et al., 1985; Dorr et al., 1986; Speiser et al., 1990; Forest and David, 1991; Mercado et al., 1995) but is controversial and is still considered experimental. When properly administered, dexamethasone is effective in preventing ambiguous genitalia in the affected female. The current dose of dexamethasone is 20 μ g/kg divided into two or three doses daily (Mercado et al., 1995).

Adrenocortical steroidogenesis begins at approximately 6 weeks' gestation, and differentiation of the external genitalia and urogenital sinus begins at approximately 9 weeks' gestation (New et al., 1989). The institution of dexamethasone therapy is recommended as soon as pregnancy is confirmed and no later than 9 weeks after the last menstrual period; this will effectively suppress adrenal androgen production, allow normal separation of the vaginal and urethral orifices, and prevent clitoromegaly. The need to initiate therapy at such an early date means that treatment is blind to the sex of the fetus. If the fetus is determined to be a male on karyotyping or an unaffected female on DNA analysis, treatment is discontinued. Otherwise, treatment is continued to term.

Between 1978 and 2002, prenatal diagnosis and treatment of CAH caused by 21-OHD were performed in 595 pregnancies at the NewYork-Presbyterian Hospital-Weill Medical College of Cornell University, among which 108 infants had classic 21-OHD. Of these, 64 were females, 51 of whom were treated prenatally with dexamethasone. Dexamethasone administered at or before 9 weeks' gestation was effective in reducing virilization, thus avoiding postnatal genitoplasty (Carlson et al., 1999; New et al., 2001). No significant or enduring side effects were noted in the mothers (other than greater weight gain and a higher incidence of striae and edema than in untreated mothers) or the fetuses. There was no statistically significant difference in hypertension or gestational diabetes between treated and untreated mothers. All mothers who received partial or full treatment stated they would take dexamethasone again in the event of a future pregnancy. In this report and in others, no cases have been reported of cleft palate, placental degeneration, or fetal death, which have been observed in a rodent model of in utero exposure to high-dose glucocorticoids (Goldman et al., 1978; Nimkarn, 2005). In contrast, another study noted some significant maternal side effects, including excessive weight gain, cushingoid facial features, severe striae resulting in permanent

scarring, and hyperglycemic response to oral glucose administration (Pang et al., 1992).

A long-term follow-up study of 44 children treated prenatally in Scandinavia demonstrated normal prenatal and postnatal growth compared with matched controls (Lajic et al., 1998). Prenatally treated newborns also did not differ in weight, length, or head circumference from untreated, unaffected newborns (Carlson et al., 1999; New et al., 2001). Moreover, a survey of 174 children aged 1 month to 12 years and exposed prenatally to dexamethasone (including 48 with CAH) compared with 313 unexposed children (including 195 with CAH) found no differences in cognitive or motor development between the two groups (Meyer-Bahlburg et al., 2004b). Maria New believes proper prenatal treatment of fetuses at risk of CAH can be considered effective and safe. Long-term studies on the psychologic development of patients treated prenatally are currently under way. This therapy is controversial in other centers because of concerns of unknown long-term adverse effects and the fact that seven fetuses are treated for the benefit of one fetus. The current recommendations by the Endocrine Society and other societies are that dexamethasone therapy should be undertaken only as part of a controlled study with long-term outcomes (Speiser et al., 2010).

The efficacy of prenatal treatment in 11 β -OHD CAH has been shown as well. In 1999 Cerame et al. (1999) reported the first prenatal diagnosis and treatment of an affected female with 11 β -OHD CAH. As an indication of the treatment's success, the prenatally treated newborn had normal female external genitalia.

Increased Levels of Maternal Androgens and Progestins

Masculinization of the female fetus has been reported in pregnant mothers taking various progestational agents to prevent miscarriage. These agents include norethindrone, ethisterone, and medroxyprogesterone. Danazol, which has been used in the treatment of endometriosis, has been associated with fetal masculinization (Grumbach and Conte, 1998). Masculinization of the female fetus because of maternal virilizing ovarian or adrenal tumors or luteomas of pregnancy has been reported. In such cases, virilization beyond the time of birth does not occur, and the prognosis is good (Grumbach and Conte, 1998).

Placental Aromatase Deficiency

Placental aromatase deficiency has been associated with masculinization of the female fetus. Aromatase is a cytochrome P450 enzyme that is responsible for the conversion of testosterone to estradiol and of androstenedione to estrone. Autosomal recessive inheritance of aromatase deficiency causes virilization of the female because of a failure to metabolize the large amounts of androstenedione and testosterone produced by the placenta. This disorder also will cause significant virilization of the mother. The affected infant will be virilized, with normal müllerian structures. The levels of gonadotropins are elevated in infancy, and ovarian cysts may develop. At puberty, females have hypergonadotropic hypogonadism with failure to feminize and progressive virilization. Plasma androstenedione and testosterone levels are elevated, whereas estrone and estradiol levels are low. Postpubertal patients have delayed bone maturation, tall stature, and osteopenia (Conte et al., 1994).

46,XY Differences in Sex Development

Incomplete masculinization refers to absence of or incomplete masculinization of the external and internal genitalia in a person with a 46,XY karyotype and normal testes. Disorders leading to

incomplete masculinization of the male include androgen receptor defects, testosterone biosynthetic defects, 5 α -reductase deficiency, Leydig cell hypoplasia, and effects of maternal medications (see Fig. 97.11).

In 25%–50% of undermasculinized males, a specific cause cannot be found (Eil et al., 1984; Al-Agha et al., 2001; Baxter et al., 2015). Other factors such as medications and placental insufficiency may potentially interfere with genital masculinization. Placental insufficiency may be related to genital underdevelopment through the presumed mechanism of inadequate hCG production. Placental hCG is required for early fetal testosterone production and therefore early fetal genital development.

Medications such as cimetidine, spironolactone, phenytoin (Dilantin), phenobarbital, medroxyprogesterone, and cyproterone acetate have been associated with altered androgen action or metabolism. Their use during pregnancy may be detrimental to male genital development (Donahoe, 1991; Grumbach and Conte, 1998). Furthermore, various xenobiotics can bind the androgen receptor; therefore there has been speculation regarding the role of environmental factors in abnormal sex differentiation (Danzol, 1998).

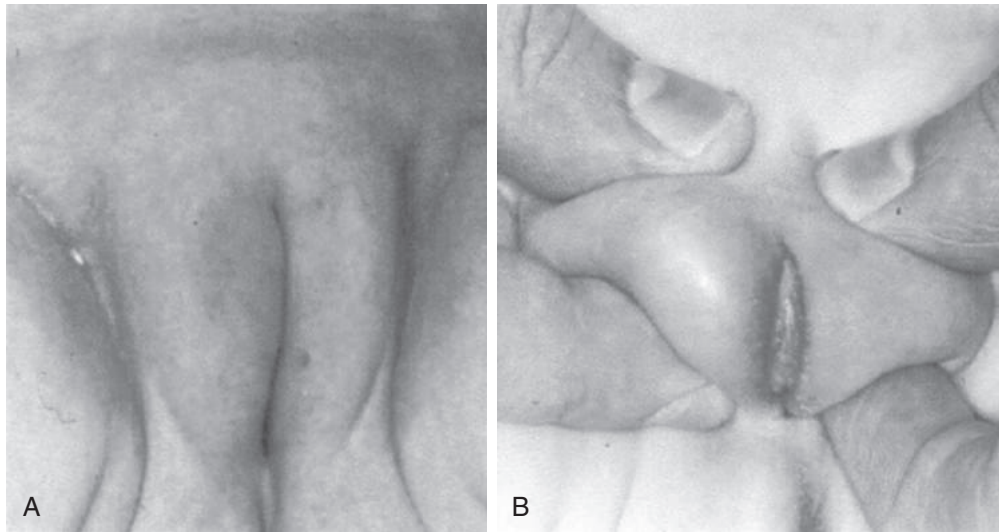
Androgen Receptor Defects (Androgen Insensitivity)

Disorders of the androgen receptor are the most common definable cause of incomplete masculinization of the genetic male (Quigley et al., 1995). Disorders of the androgen receptor can be divided into complete androgen insensitivity syndrome (CAIS) and partial androgen insensitivity (PAIS) syndrome. The gene for the androgen receptor (*AR*) is located on the X chromosome, and more than 400 germline mutations have been found. Information regarding the *AR* mutations can be found in the McGill Androgen Receptor Gene Mutation Database (<http://www.androgendb.mcgill.ca>). Mutations have been found throughout the *AR* gene, with most mutations occurring in the DNA- or steroid-binding domains. Despite extensive characterization of the molecular genetics of *AR* mutations, no genotype–phenotype correlation has been found (Quigley et al., 1995).

The sex-differentiating actions of testosterone and DHT are mediated by the androgen receptor. DHT is important in the differentiation of the male external genitalia and prostate, whereas testosterone is important in the differentiation of internal wolffian ducts to epididymis, vas deferens, and seminal vesicles.

Complete Androgen Insensitivity

CAIS in a 46,XY individual is characterized by phenotypically normal female external genitalia (Fig. 97.14). Affected girls may have an inguinal hernia before puberty or primary amenorrhea after puberty onset. Robust breast development occurs at puberty that is due to peripheral aromatization of testosterone to estrogen. There is usually scant or absent pubic and axillary hair, with some vulvar hair. The vagina is short and blind ending, and müllerian structures (cervix, uterus, fallopian tubes) are absent because of testicular production of AMH. There are only vestigial or no wolffian duct–derived internal structures. The testes are located in the abdomen or inguinal canal or in the labia majora. Even though there is testosterone production at puberty, in the newborn there is absence of testosterone production during the mini puberty (Bouvattier et al., 2002). Gender identity and role behaviors are typically female. Removal of the testes is controversial because of low risk of development of germ cell tumors in childhood and



• **Fig. 97.14** (A) Appearance of external genitalia of a 46,XY infant with complete androgen insensitivity syndrome. (B) Note presence of bilateral palpable gonads.

during puberty (Hannema et al., 2006), and there is no consensus on the timing of orchidectomy (Migeon et al., 1994; Forest, 2001; Deans et al., 2012).

Partial Androgen Insensitivity

PAIS is characterized by various degrees of ambiguity of the external genitalia. The term *Reifenstein syndrome* was formerly used to describe partial androgen insensitivity with intermediate degrees of masculinization. Affected infants have a small phallus and a ventral chordee that tethers the phallus to the perineum. There is often a penoscrotal hypospadias and a bifid scrotum, which may or may not contain gonads (see Fig. 97.5). Cryptorchidism is a common finding. Müllerian structures are absent, and the wolffian duct-derived structures are absent or poorly developed. A genitourethrogram may demonstrate a urogenital sinus.

The diagnosis of partial androgen insensitivity is complex. A family history of ambiguous genitalia in male relatives on the maternal side would be suggestive of this diagnosis since it is X-linked. However, Viner et al. (1977) reported a family history in only 25% of patients with PAIS. 5α -Reductase deficiency and testosterone biosynthetic defects should be ruled out by appropriate steroid and/or genetic analysis. This can be accomplished by measurement of intermediates in testosterone biosynthesis, especially androstenedione, to exclude 17β -hydroxysteroid dehydrogenase (17β -HSD) type 3 deficiency and by measurement of the ratio of testosterone to DHT to exclude 5α -reductase deficiency. Abnormal testosterone-to-DHT ratios can be seen in PAIS (Ahmed et al., 1999); this could be caused by poor development of tissues, which express 5α -reductase type 2 in PAIS (Griffin et al., 2001). Genetic testing is the gold standard, and with the availability of DSD panels, sequencing in parallel rather than series is now possible.

Androgen levels in normal newborns are highest at birth and then decline rapidly during the first week. A second testosterone surge occurs between 15 and 60 days of life (Forest et al., 1980), which is often referred to as *mini puberty*. Androgen and LH levels should be obtained at these peak production times. Alternatively, an hCG stimulation test may be performed. There are many protocols for hCG stimulation testing. One hCG test is as follows: a dose of 3000 IU/m² of hCG is given intramuscularly daily for 3 days; androgen and gonadotropin levels are measured at the baseline and

then 24 hours after the third dose. Abnormally elevated levels of LH, testosterone, or both in the first several months of life are suggestive of partial androgen insensitivity. A recent report of five neonatal cases of PAIS showed testosterone values in the high-normal range on days 2 to 7 of life (mean 107 ± 27 ng/dL) and on day 30 (mean 411 ± 154 ng/dL; Bouvattier et al., 2002). LH levels were elevated in comparison with those of historic controls (mean LH level on days 7 to 15 of life was 5.2 IU/L ± 4.0 ng/dL; on day 30 of life the mean level was 8.7 IU/L ± 2.5 ng/dL). Testosterone response to hCG and LH response to gonadotropin-releasing hormone were exaggerated (Bouvattier et al., 2002).

The androgen receptor binding assay used to be considered standard for defining this disorder. However, normal ligand binding does not rule out androgen insensitivity, because there may be mutations in domains of the androgen receptor not involved in ligand binding. Direct sequencing of the androgen receptor for mutation analysis is commercially available (GeneTest) and can detect up to 95% of mutations associated with complete androgen insensitivity. Androgen receptor mutations, however, are not always found in cases of possible partial androgen insensitivity, and it is speculated that defects in androgen receptor-interacting proteins may be involved (Adachi et al., 2000) or the child may have another disorder. In the past, individuals were often labeled as having PAIS when in reality they had another disorder such as 17β -HSD deficiency. This has serious implications as at puberty this individual will virilize as other isoenzymes convert the androstenedione to testosterone.

Determining the sex of rearing in partial androgen insensitivity is a difficult task, and multiple factors must be considered. If there is a significant degree of virilization (Prader stages IV and V; see Fig. 97.3A), then male sex assignment is made (Diamond and Sigmundson, 1997b; Reiner, 1997). If masculinization is severely limited (Prader stages I and II), then female sex assignment is recommended. In intermediate forms (Prader stage III), responsiveness to exogenously administered hCG or testosterone may be of help in the decision-making process (Grumbach and Conte, 1998; Daaboul and Frader, 2001). However, adult males with a small phallus have reported satisfactory sex lives (Reilly and Woodhouse, 1989). These cases deemphasize the importance of phallic size in male sex assignment. Slijper et al. (1998) reported the absence of serious gender identity disorder in five undervirilized 46,XY males,

although these boys were “more fearful and bothered about the smallness of their penises.” Money and Ehrhardt (1972) cautioned about the devastating psychosocial effects of male sex assignment when the phallus is only slightly larger than a clitoris and does not respond to testosterone.

In a study of 32 undervirilized males who were assigned female gender for rearing, significant gender transposition (i.e., gender change and homosexual orientation) correlated with the presence of childhood stigmatization and a relatively late age at feminizing surgery (Money et al., 1986). Stigmatization in the home was reflected by the child being treated differently from other children, including elaborate efforts at maintaining the privacy of the genital anomaly, keeping the child at home and forbidding play with neighborhood children, and refusal of open communication within the family about the medical condition. Stigmatization in the community was reflected by teasing, such as about the genital anomaly, body habitus, and mannerisms. Stigmatization by parents or the community was also found to be associated with gender transposition in undervirilized males who were assigned male gender for rearing; cosmetic inadequacy of the external genitalia was less important (Money and Norman, 1987).

These studies indicate the importance of a nurturing, supportive environment for a successful long-term outcome for these children. More comprehensive long-term outcome studies of partial androgen insensitivity are greatly needed.

5 α -Reductase Type 2 Deficiency

5 α -Reductase type 2 deficiency is an autosomal recessive disorder that results in an inability to convert testosterone to DHT. DHT is required for the development of the male external genitalia.

At birth, these 46,XY infants typically have a very small phallus that appears to be a normal or slightly enlarged clitoris. However, more significant virilization of the phallus may occur, and the affected child will then be identified as a male with hypospadias. There is usually severe penoscrotal hypospadias. The scrotum is bifid, with slight posterior fusion. The testes are usually in the inguinal canals or labial folds. Approximately half of these patients have a penoscrotal urethra with a separate blind-ending vagina; a smaller percentage have a single urogenital sinus opening on the perineum (Griffin et al., 2001). A more recent study identified isolated microphallus (36%), microphallus with hypospadias (56%), and female external genitalia with clitoromegaly (9%) as the presenting phenotype (Cheng et al., 2015). Another study, of 55 patients with genetically confirmed 5 α -reductase type 2 deficiency, found the following phenotypes: clitoromegaly (49.1%), microphallus with hypospadias (32.7%), female external genitalia (7.35%), and isolated microphallus (3.6%) (Maimoun et al., 2011). Thus with the advent of genetic testing, the phenotypic range for 5 α -reductase type 2 deficiency is much broader than originally described. Müllerian structures are absent, and wolffian duct–derived structures (vas deferens, epididymis, seminal vesicles) are well developed. The prostate is poorly developed.

At the time of puberty, individuals with this disorder will characteristically virilize. The phallus will typically increase to a length of 4 to 8 cm (Migeon et al., 1994). In affected individuals who were raised as females, a change to the male gender role after puberty is commonly seen (Imperato-McGinley, 1997; Griffin et al., 2001). Unlike in partial androgen insensitivity, gynecomastia does not occur in the pubertal period.

In the past, 5 α -reductase type 2 deficiency was most often diagnosed in the postpubertal period, although diagnosis in the

newborn period has been reported (Imperato-McGinley et al., 1986; Odame et al., 1992). The disorder is diagnosed by assessment of the ratio of testosterone to DHT in blood (Peterson et al., 1977). The normal testosterone-to-DHT ratio in the newborn period is 4:1, whereas the ratio in infants and children with this disorder is often greater than 14:1. An hCG stimulation test is usually needed to obtain a more definitive diagnosis in the prepubertal period. A positive response to hCG rules out Leydig cell aplasia or a testosterone biosynthetic defect. Measurement of normal androstenedione levels will rule out the testosterone biosynthetic defect—deficiency of 17 β -HSD type 3. This enzyme is responsible for the conversion of androstenedione to testosterone in the testes, and the level of androstenedione is elevated compared with that of testosterone when 17 β -HSD deficiency is present. The clinical presentation of 17 β -HSD deficiency in infancy can be similar to that of 5 α -reductase deficiency or PAIS.

In addition, abnormal ratios of 5 β -urinary steroids to 5 α -urinary steroids can establish a definitive diagnosis of 5 α -reductase type 2 deficiency (Imperato-McGinley et al., 1986) but it can be challenging to perform the test. Distinguishing 5 α -reductase deficiency from partial androgen insensitivity is important because androgen insensitivity can cause a secondary DHT deficiency owing to the incomplete development of tissues that express 5 α -reductase activity (Griffin et al., 2001). Measurement of urinary 5 β -glucocorticoids and 5 α -glucocorticoids will help to make this distinction, because only 5 α -reductase deficiency will also affect glucocorticoid metabolism. Finally, analysis for mutations in the *SRD5A2* gene is diagnostic and is the gold standard. This gene test is available commercially as a separate test or as part of the DSD panel.

Sex assignment in cases of 5 α -reductase deficiency is a complicated issue, but long-term outcome information is available. As in androgen insensitivity disorders, sex assignment is often significantly influenced by the degree of masculinization at birth, and because infants with this disorder are usually markedly undervirilized, female sex assignment has been advocated (Migeon et al., 1994; Grumbach and Conte, 1998). However, if the disorder is diagnosed early, topical DHT treatment, which is not available in the United States, has been shown to enlarge the phallus (Odame et al., 1992). In addition, the natural history of the disorder is for masculinization of the phallus to occur at puberty. Testicular histopathologic analysis in males with this disorder has shown that, unlike in isolated bilateral cryptorchidism, their testes display type Ad (i.e., dark) spermatogonia and a normal germ cell count. These patients are largely infertile because of defective transformation of spermatogonia into spermatocytes (Hadziselimovic and Dessouky, 2008). However, there have been case reports of men fathering children (Katz et al., 1997; Kang et al., 2014). 46,XX females with 5 α -reductase deficiency do not have a known phenotype.

There are frequent reports of reversal from female to male gender behavior after puberty (Wilson et al., 1993; Wilson, 2001). The accumulated evidence supports male sex assignment in this disorder (Imperato-McGinley, 1997), although female sex assignment is likely in the newborn period if the diagnosis is overlooked.

Testosterone Biosynthetic Defects

Five enzymes are necessary for the synthesis of testosterone. A defect at any step will result in inadequate testosterone synthesis (see Fig. 97.13). Defects in the first three enzymes of the testosterone synthesis pathway will also affect adrenal steroid production, resulting in both an undervirilized male and CAH. Because

testosterone production is impaired in these disorders, wolffian duct structures are likely to be underdeveloped, whereas müllerian structures are absent because of normal testicular AMH production. These enzyme deficiencies are rare; therefore these disorders are discussed only briefly.

17 β -Hydroxysteroid Dehydrogenase Type 3 Deficiency (17-Ketosteroid Reductase Deficiency)

The final step in testosterone biosynthesis involves the conversion of androstenedione to testosterone by the enzyme 17 β -HSD type 3 in the testis. Mutations that impair the function of 17 β -HSD are the cause of this relatively rare autosomal recessive disorder (Wilson, 2001).

The clinical presentation externally is that of a female at birth with perhaps a mild degree of clitoral enlargement. The phenotype is female; therefore these infants are typically raised as females unless the diagnosis is made at birth. At puberty, there is progressive masculinization, with enlargement of the phallus to 4 to 6 cm, with labial enlargement and rugation. By late puberty, the testes are found at the lower ring of the inguinal canal, and they are of normal size and consistency. Internal wolffian duct–derived structures are found. In addition, a male body habitus develops, with deepening of the voice and appearance of male body hair, including a mustache and beard (Migeon et al., 1994).

In the past patients received a diagnosis at puberty or as adults. Now with the advent of genetic testing, this diagnosis is made more frequently in infancy. Endocrine studies reveal markedly elevated androstenedione levels, whereas testosterone levels are in the low-normal range (Mendonca et al., 2000). Plasma LH levels are consistently high. In infancy or childhood, the presence of inguinal hernias may bring the child to medical attention. Androstenedione levels in the prepubertal patient may be normal, and the hCG stimulation test is required to elucidate the defect outside the mini puberty.

Sex of rearing is often influenced by the cultural context. In societies in which a high priority is given to the male, sex reassignment at puberty has been successful (Forest, 2001). Mendonca et al. (2000) observed changes in gender role (female to male) in 3 of 10 affected individuals. Despite virilization in some affected individuals, the female gender role was maintained.

Congenital Lipoid Adrenal Hyperplasia

Lipid accumulation in both the adrenal glands and the gonads is characteristic of this disorder pathologically—hence the name *congenital lipoid adrenal hyperplasia*. Because all adrenal and gonadal steroid synthesis is affected by this disorder, infants are likely to exhibit complete adrenal insufficiency, characterized by vomiting, weight loss, and hypotension. The phenotype is likely to be female, although there is clinical variability. In the 35 cases reported in the medical literature, only 11 patients have survived beyond infancy (Forest, 2001).

Endocrine findings include elevation of ACTH level and plasma renin activity but low or immeasurable levels of all steroid hormones. The main consideration in the differential diagnosis is another form of CAH. The presence of markedly enlarged lipid-laden adrenals on ultrasound, computed tomography, or MRI studies is highly suggestive of the disorder. Successful treatment has occurred, and requires replacement of both glucocorticoids and mineralocorticoids. All individuals with this diagnosis have been raised as females (Grumbach and Conte, 1998).

In vitro studies performed on either adrenal or testicular tissue demonstrated an inability to convert cholesterol to pregnenolone

in these patients. A defect in the first step of adrenal and gonadal steroid biosynthesis mediated by the cytochrome P450 side-chain cleavage enzyme was suspected. However, subsequent molecular studies demonstrated mutations in the steroidogenic acute regulatory protein (StAR) (Grumbach and Conte, 1998). StAR acts to promote sterol translocation to the cytochrome P450 side-chain cleavage enzyme in mitochondria (see Fig. 97.13 and Case Study 2).

CASE STUDY 2

History

- The infant was born at 41 weeks' gestation, weighing 3.2 kg.
- Pregnancy was complicated by preterm contractions from 29 to 34 weeks requiring bed rest.
- Triple screen results were abnormal and amniocentesis was performed, revealing a 46,XY karyotype with no mosaicism. The parents did not want to know the karyotype because they did not wish to know the sex of the baby.
- At birth the newborn was thought to have normal female genitalia and was assigned to the female sex.
- On careful physical examination, the newborn was found to have mildly virilized genitalia.

Physical Examination and Laboratory Studies

- Genital examination: mild clitoromegaly, mild posterior labial fusion, a single urogenital sinus, and bilateral inguinal hernias with gonads present.
- Ultrasound examination: testicle-like structures in the upper portion of the inguinal canal without evidence of a uterus or ovaries.
- Karyotype and FISH: confirmed 46,XY, no mosaicism.
- Endocrine studies.

Six Weeks of Age

- Testosterone, 20 ng/dL; DHT, 11 ng/dL.
- FSH, 4.3 mIU/mL; LH, 9.0 mIU/mL.
- No mutations identified in the androgen receptor.

Three Months of Age

- Patient underwent a bilateral orchiectomy, inguinal hernia repair, and vaginoplasty.
- Testicular disease was consistent with prepubertal seminiferous tubules, spermatic cord with vas deferens, epididymis, and hernia sac.

Further Clinical Course: 6 Months of Age

- The patient had a 2-week history of irritability and several days of intermittent vomiting and was admitted to the hospital with dehydration and in adrenal crisis.
- Serum sodium level was 124 mEq/mL.
- Serum potassium level was 7.7 mEq/mL.
- There were low levels of all adrenal hormones, including testosterone, with no response to ACTH stimulation; baseline cortisol level was 10 μ g/dL, stimulated to only 12 μ g/dL 60 minutes after ACTH was administered.
- Plasma renin activity was 14,345 ng/dL per h.
- Baseline ACTH level was 4781 pg/mL.
- Genetic analysis revealed c. 178+1G>C intron 2 homozygous splicing mutation of the *STAR* gene.

Diagnosis

- Congenital lipoid adrenal hyperplasia owing to *STAR* mutation.

Management and Outcome

- Glucocorticoid supplementation with hydrocortisone and mineralocorticoid supplementation with fludrocortisone initiated.
- Female sex assignment maintained.

3 β -Hydroxysteroid Dehydrogenase Deficiency 2

3 β -HSD deficiency was first reported by Bongiovanni (1962). 3 β -HSD is an important enzyme required for the conversion of Δ^5 -steroids to Δ^4 -steroids in the adrenal glands and gonads (see Fig. 97.13). There is marked heterogeneity in clinical presentation, and both sexes are affected (Forest, 2001). With severe deficiency of 3 β -HSD, salt-losing crisis can occur. Male infants may have ambiguous or completely feminine external genitalia, whereas female infants may be mildly virilized. Severely undervirilized males may have normal mineralocorticoid activity; fully masculinized males may display salt loss.

Diagnosis of this disorder in the newborn period can be difficult because of relatively high levels of Δ^5 -steroids physiologically. The diagnosis is based on the elevated ratio of 17-hydroxypregnenolone to 17 OHP and of DHEA to androstenedione in the basal and stimulated states (Sakkal-Alkaddour et al., 1996).

17 α -Hydroxylase/17,20-Lyase Deficiency

A single enzyme encoded on the cytochrome P450c17gene (*CYP17A1*) mediates the 17-hydroxylation of pregnenolone and progesterone and the conversion of 17-hydroxypregnenolone and 17-OHP to DHEA and androstenedione. Specific mutations can cause partial loss of 17 α -hydroxylase/17,20-lyase activities or dissociation between the 17 α -hydroxylase and 17,20-lyase function (Dhir et al., 2009). Clinical disorders of this enzyme affect primarily either the hydroxylation or the lyase reaction, although there have been reports of combined 17 α -hydroxylase and 17,20-lyase deficiency (Sahakitrungruang et al., 2009). Cases of primarily 17-OHD should be considered in undervirilized males or females with low renin hypertension and hypokalemic alkalosis. The hypertension is presumably due to elevated levels of DOC and corticosterone (see Fig. 97.13). Most cases are diagnosed in the pubertal period because the 46,XY phenotype is largely female. Although cortisol synthesis is blocked, the overproduction of DOC and corticosterone is protective against adrenal insufficiency. This disorder is treated with glucocorticoid, which suppresses ACTH overproduction and subsequently suppresses DOC and corticosterone overproduction (Grumbach and Conte, 1998). Sex steroid replacement is needed at puberty.

17,20-Lyase deficiency results in various degrees of undermasculinization of the 46,XY infant. The phenotype ranges from complete female external genitalia to ambiguous genitalia to a mildly undervirilized male. 46,XX females will fail to enter puberty. The levels of gonadotropins will be elevated, and there will be impaired formation of DHEA and androstenedione (see Fig. 97.13). DOC and corticosterone levels are normal in this form of the disorder. ACTH and hCG stimulation tests may be helpful to more fully reveal the steroid biosynthetic block.

Leydig Cell Hypoplasia

Failure of the testes to produce testosterone in response to hCG is characteristic of this disorder. Histologic examination of the testes reveals absent or low numbers of Leydig cells, normal-appearing Sertoli cells, and seminiferous tubules with spermatogenic arrest (Grumbach and Conte, 1998). LH receptor mutations have been described in this disorder (Leung et al., 2006). Phenotypically, the external genitalia range from those of a normal female to those of a male with micropallus. Müllerian-derived structures are absent in all patients, whereas wolffian structures may be present. LH and FSH levels are elevated in postpubertal patients, and LH levels

decrease after testosterone administration. In less severe forms of the disorder, testosterone therapy augments phallic growth. In severe forms of testicular unresponsiveness to hCG/LH, sex assignment has been female. The gonads are removed, and estrogen replacement therapy is instituted at the time of expected puberty (Grumbach and Conte, 1998).

Persistent Müllerian Duct Syndrome

The diagnosis of PMDS is often made in otherwise phenotypically normal 46,XY males at the time of surgery for an inguinal hernia or orchidopexy. In the case of hernia repair, a fallopian tube and uterus are often found along with a partially descended testis. In other cases, testes, uterus, and fallopian tubes are found in the pelvis. Inheritance of PMDS is autosomal recessive, although the female phenotype is completely normal. The disorder has been found to be due to a mutation in AMH or its receptor in 85% of cases and unknown causes in 15% of cases (Josso et al., 2005). Therapy involves orchidopexy and partial hysterectomy, with care taken to avoid injuring the vas deferens, which is embedded in the uterine wall.

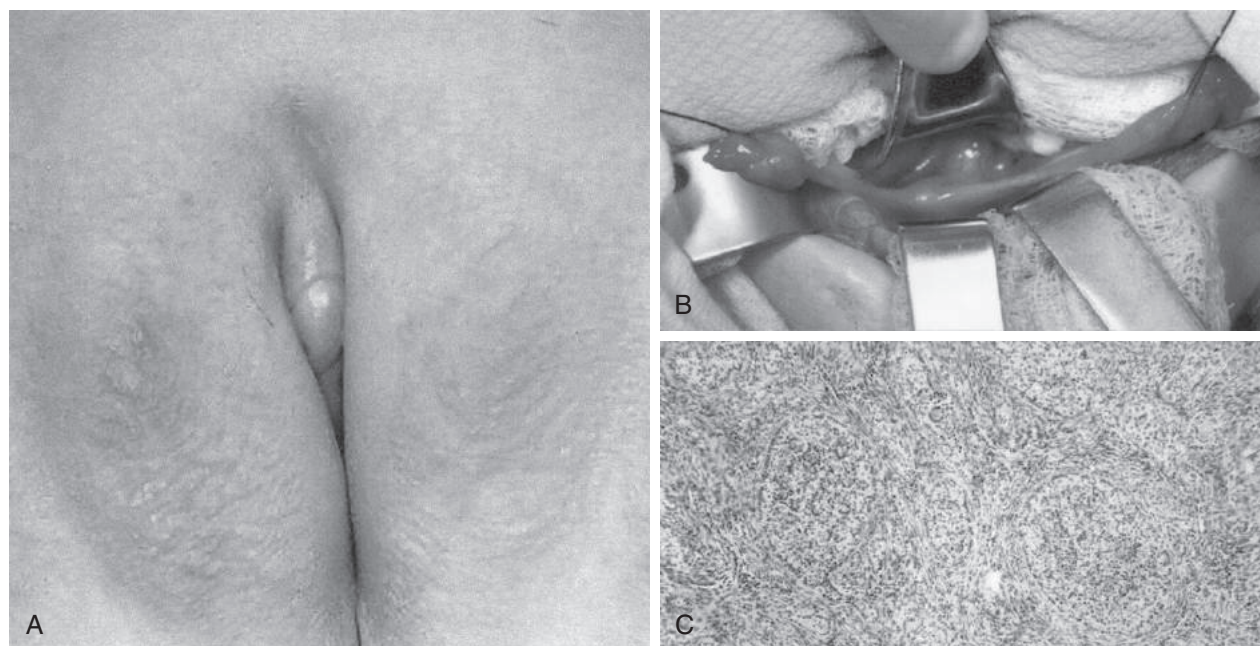
Gonadal Differentiation and Chromosomal Disorders

46,XY Complete Gonadal Dysgenesis

46,XY complete gonadal dysgenesis was first described by Swyer in 1955 (Grumbach and Conte, 1998). The phenotype of the external genitalia is female, with normal development of müllerian-derived internal structures in a 46,XY individual. The gonads did not develop into testes but rather formed streak gonads. Streak gonads are completely nonfunctional; they do not produce AMH or testosterone. Therefore müllerian structures do not regress, and wolffian structures are poorly developed or absent (Fig. 97.15). Most affected individuals are seen in the teenage years with lack of pubertal development. Serum gonadotropin levels are elevated. Mutations in the *SRY* gene account for only a small proportion of these cases (Forest, 2001). Fig. 97.1 depicts other genes that when mutated can lead to complete gonadal dysgenesis. Familial cases have been reported. There has been a report of a 46,XY mother who developed as a normal woman and gave birth to a 46,XY daughter with complete gonadal dysgenesis in a family with multiple disorders of sexual development, suggesting an unidentified sex-determining gene (Dumic et al., 2008). In up to 30% of affected individuals, gonadoblastomas will develop in the streak gonad; therefore removal of the streak gonad is recommended (Migeon et al., 1994; Looijenga et al., 2007; Pleskacova et al., 2010; see Fig. 97.15). Females with gonadoblastoma may present with breast development as the gonadoblastoma produces estrogen. Estrogen replacement therapy will provide appropriate feminization, and the subsequent addition of progesterone will lead to menses. Women with complete gonadal dysgenesis can successfully carry a pregnancy through the use of donor oocytes and appropriate hormonal supplementation.

46,XY Partial Gonadal Dysgenesis

Patients with 46,XY partial gonadal dysgenesis will typically be seen in the newborn period for evaluation of ambiguous genitalia (Berkovitz et al., 1991). The extent of masculinization of the external genitalia depends on the extent of testicular differentiation.



• **Fig. 97.15** (A) Appearance of external genitalia in an infant with 46,XY complete gonadal dysgenesis. (B) Uterus and fallopian tubes were found at surgery, along with a gonadoblastoma, at 1 year of age. (C) Micrograph of tumor cells.

Gonadal tissue is usually intra-abdominal, but testes can be found in the scrotum. One-quarter of these patients will have phenotypic features of Turner syndrome (Migeon et al., 1994). Testosterone response to hCG is variable but usually low. In most cases there is a mix of müllerian and wolffian structures. The presence of müllerian structures on genitourethrogram or ultrasound examination increases the index of suspicion for this disorder. The diagnosis is confirmed by gonadal biopsy. Some affected children are found to have one dysgenetic gonad on one side and a streak gonad on the other; others have bilateral dysgenetic gonads. Dysgenetic gonads are histologically defined by poorly formed and disorganized seminiferous tubules surrounded by wavy ovarian stroma. In many cases the dysgenetic gonads resemble ovotestes, except that primordial ovarian follicles are lacking (Berkovitz et al., 1991).

Mixed Gonadal Dysgenesis

Mixed gonadal dysgenesis refers to asymmetric gonadal dysgenesis with ambiguous genitalia (see Fig. 97.7) and a mosaic karyotype with an XY cell line. The most common karyotype is 45,X/46,XY. There is a wide spectrum of phenotypes, ranging from a female with clitoral enlargement to a male with hypospadias. Asymmetric external and internal genital development has been classically described in this syndrome (Forest, 2001). Considerable phallic development was reported in most patients in one study, and there was often penoscrotal hypospadias (Davidoff and Federman, 1973). The phenotypic features of Turner syndrome are described in a significant percentage of patients, although these features may not be readily apparent in the newborn period. An incompletely formed uterus is found in almost all patients. Fallopian tubes are always found on the side of the streak gonad and often on the side with the dysgenetic gonad. Wolffian structures may be developed on the side with the dysgenetic gonad.

A genitourethrogram is likely to demonstrate internal müllerian structures that can be confirmed at laparoscopy. Demonstration of abnormal gonadal histopathologic features will confirm the diagnosis. Mixed gonadal dysgenesis shares many features with partial gonadal dysgenesis, and some authors view these disorders as representing a continuum of gonadal dysgenesis (Berkovitz et al., 1991). Histologic analysis will also differentiate this disorder from ovotesticular DSD. Although the characteristic karyotype is 45,X/46,XY, this genotype has been associated with normal male differentiation in most cases diagnosed by prenatal amniocentesis (Wheeler et al., 1988). While these males may have normal external genitalia, they may also have short stature and cardiac features associated with Turner syndrome.

Some authors advocate female sex assignment in mixed gonadal dysgenesis, because surgical repair of the vagina is usually easy and a uterus or hemiuterus is present. In addition, the dysgenetic gonad is at risk of development of a tumor and should be removed, particularly if the gonad cannot be brought down into the scrotum (Forest, 2001). However, sex assignment is likely to be guided by the degree of virilization, with the more virilized cases being assigned as males. The capacity for near-normal androgen production in this disorder has been described (Davidoff and Federman, 1973). In all cases the streak gonads should be removed because of the risk of malignancy.

Testicular Regression Syndrome

Testicular regression syndrome refers to the spectrum of disorders affecting individuals with 46,XY karyotype who demonstrate evidence of prior testicular function, followed by usually symmetric gonadal regression (Marcantonio et al., 1994). Loss of testicular function between weeks 8 and 10 of gestation would result in ambiguous genitalia and variable internal genitalia. Loss of testicular function after 12 to 14 weeks' gestation would result in normal

male genital differentiation with a small phallus. When the male external and internal ducts are completely normal, the term *vanishing testis syndrome* is used by some authors. Presumably the testes were lost during the second half of pregnancy. Testicular torsion or loss of blood supply has been invoked as a possible explanation in this syndrome.

FSH levels are elevated in infancy, and an exaggerated response to gonadotropin-releasing hormone in the prepubertal period is typically seen (Grumbach and Conte, 1998). AMH levels in infancy and childhood are very low in anorchia and intermediately low with abnormal testes (Lee, 2001).

Ovotesticular Disorder of Sex Differentiation

Ovotesticular DSD is defined as the presence of both testicular tissue with distinct seminiferous tubules and ovarian tissue containing mature graafian follicles in a single individual. Both testicular and ovarian elements may be found in the same gonad, or one testis and one ovary may be found in the same individual. In most cases the karyotype is 46,XX; 46,XX/46,XY chimerism and 46,XY karyotypes can also be found. Clinically the external genitalia are often ambiguous, but predominantly male or female phenotypes have been described (Hadjiathanasiou et al., 1994; Grumbach and Conte, 1998). In ambiguous cases a relatively marked degree of virilization can be found. Almost all have some degree of hypospadias and incomplete labioscrotal fold fusion. The labioscrotal folds are asymmetric, with an appearance of a hemiscrotum on one side and labium majus on the other being seen in 10 of 22 cases (Hadjiathanasiou et al., 1994). At least one gonad is usually palpable. A vagina and a uterus are present in most patients, and a genitourethrogram may provide further elucidation. Internal duct development is consistent with the associated gonad, although müllerian ducts predominate with an ovotestis. Breast development is common during puberty, and menses can occur in up to 50% of individuals.

The diagnosis should be suggested in 46,XX/46,XY individuals with ambiguous genitalia. Palpation of a polarized gonad should also lead the clinician to suggest the diagnosis. In one study, 11 of 12 individuals with 46,XX ovotesticular DSD examined before 6 months of age had baseline testosterone levels greater than 40 ng/dL; normal levels for females of this age would be less than 15 ng/dL (Hadjiathanasiou et al., 1994).

Sex assignment depends on the degree of masculinization, the capacity of testicular tissue to secrete testosterone, and the presence or absence of a uterus and tubes. In general, a female sex assignment is favored because of the presence of ovarian tissue and external genitalia that can more easily be reconstructed as female. Male sex assignment is more likely if there is significant virilization. Removal of ductal or gonadal structures not consonant with the sex of rearing is controversial as gender identity is not known in infancy. Testes are usually dysgenetic, which carries an increased risk of gonadoblastoma formation; therefore careful follow-up evaluation is indicated. As in all cases of DSD, gender identity and behavior issues, along with general psychologic well-being, should be evaluated longitudinally.

Syndromes Associated With Ambiguous Genitalia

Denys–Drash syndrome is a rare syndrome consisting of the classic triad of congenital nephrotic syndrome leading to end-stage

renal failure, XY ambiguous genitalia, and Wilms tumor. The external genitalia of 46,XY individuals are either ambiguous or female. Gonadal development encompasses a spectrum from streak gonads to dysgenetic testes. Nephropathy and proteinuria are noted at an early age, and renal biopsy will demonstrate mesangial sclerosis. More than 90% of cases will have a mutation in the *WT1* gene, which is a critical gene for the development of the normal genital tract. *WT1* is also associated with WAGR (Wilms tumor, aniridia, genitourinary anomalies, retardation) syndrome. Large deletions of chromosome band 11p13 that encompass the *WT1* gene are responsible for this disorder. Frasier syndrome also involves the *WT1* gene. This syndrome manifests itself in 46,XY females with gonadal dysgenesis and progressive glomerulopathy. The risk of gonadal malignancy in Frasier syndrome is reported as 60% and in Denys–Drash with a Y chromosome as 40% (Looijenga et al., 2007).

Camptomelic dysplasia is a rare autosomal dominant disorder associated with often lethal skeletal dysplasia, in which 75% of affected 46,XY males have dysgenetic testes associated with undervirilization (Foster et al., 1994). Manifestations of this disorder include bowing of the femora and tibiae, hypoplastic scapulae, 11 rib pairs, pelvic malformations, bilateral clubfoot, cleft palate, macrocephaly, micrognathia, hypertelorism, and a variety of cardiac and renal defects. The disorder is caused by heterozygous mutations in the *SOX9* gene (Wagner et al., 1994). Most patients die of respiratory distress in the neonatal period.

Smith–Lemli–Opitz syndrome is an autosomal recessive disorder with an estimated frequency of 1 in 20,000 to 1 in 40,000. The disorder is caused by deficiency of 7-dehydrocholesterol reductase (Forest, 2001). This enzyme catalyzes the final step in cholesterol biosynthesis; therefore the combination of low serum cholesterol level and a high serum 7-dehydrocholesterol level is suggestive of the diagnosis. Growth and severe developmental delay and multiple congenital anomalies characterize this disorder. Genital anomalies may include hypospadias, cryptorchidism, micropenis, and hypoplastic scrotum. Craniofacial abnormalities may include microcephaly, narrow bifrontal diameter, broad maxillary ridges, ptosis of the eyelids, micrognathia, and anteverted nostrils (Jones, 1997). Syndactyly of the second or third toes is a common feature. Adrenal insufficiency has been reported in this condition. Treatment with cholesterol has been used to try to improve growth and neurodevelopmental status. Unfortunately cholesterol replacement has not been as beneficial as once hoped as it does not cross the blood–brain barrier and has not been shown to reduce developmental delay (Sikora et al., 2004). A more recent placebo-controlled trial using simvastatin (non–FDA-approved indication) found that the medication crossed the blood–brain barrier and was relatively safe. There was improvement in serum dehydrocholesterol-to-total sterol ratio and improvement of irritability symptoms (Wassif et al., 2017).

Robinow syndrome is an autosomal dominant disorder characterized by a flat facial profile, short forearms, and hypoplastic genitals (Jones, 1997). Sporadic cases have been reported. Microphallus may be severe in males, although normal virilization at the time of puberty has been reported (Jones, 1997). Undescended testes have been reported in 65% of affected boys. Females have characteristic hypoplastic labia and clitoris. Other features include small size at birth, macrocephaly, frontal bossing, hypertelorism, prominent eyes, small upturned nose, micrognathia, and posteriorly rotated ears. Short forearms are seen in 100% of described cases. Other skeletal abnormalities include thoracic hemivertebrae, fusion or absence of ribs, and scoliosis. The abnormal facial features become

less pronounced as the child grows, and cognitive performance has been normal in most affected individuals.

Other Disorders of Genital Differentiation

Hypospadias is one of the most common anomalies of male genital development, with an estimated incidence of 4 to 8 cases per 1000 male births (Grumbach and Conte, 1998). Hypospadias can be classified as glanular, subcoronal, distal penile, midshaft, proximal penile, penoscrotal, scrotal, and perineal (see Fig. 97.3C). Typically, the more severe forms of hypospadias have been associated with DSD, although the phenotypic spectrum of DSD is wide. In a study of 33 patients with severe (scrotal or penoscrotal) hypospadias, 12 were found to have a DSD, which included Denys–Drash syndrome (in 3 of the 12), partial androgen insensitivity (in 2), ovotesticular DSD (in 2), chromosomal abnormality (in 1), AMH abnormality (in 1), gonadal dysgenesis (in 1), 5 α -reductase deficiency (in 1), and 46,XX *SRY*-positive karyotype (in 1) (Albers et al., 1997). The testes were undescended in 11 of the 12 patients. Aarskog (1970) found an approximately 15% prevalence of DSD in association with hypospadias, in addition to a significant role for maternal progestins. Intrauterine growth retardation and prematurity also have a higher association with hypospadias and undescended testes thought to be due to placental insufficiency, with lower hCG levels leading to decreased androgen production early in the pregnancy (Sekaran et al., 2013).

Thus severe cases of hypospadias require a thorough evaluation, including karyotype and evaluation of testosterone biosynthesis, along with examination of the genitourinary tract, particularly if accompanied by undescended testes.

Overview of the Surgical Management of Disorders of Sexual Differentiation

Surgery for DSD conditions has been criticized recently. The criticism has focused not only on the timing of surgery but also on whether reconstructive surgery should be done at all. Some authorities have advised that surgery be postponed until the affected person is of an age to make his or her own decision regarding the advisability of surgical correction (Diamond and Sigmundson, 1997b). Others have found that delay in surgery may be associated with problematic outcomes (Money et al., 1986; Meyer-Bahlburg et al., 1996; Reiner, 1997). It is imperative that these divergent viewpoints be discussed with the parents of an infant with a DSD condition.

These issues must be kept in mind in any decision regarding surgery for management of DSD. Current surgical techniques that are available for correction of ambiguous genitalia and DSD are presented next.

Feminizing Genitoplasty

Feminizing genitoplasty is one of the more common procedures done for correction of ambiguous genitalia. Feminizing genitoplasty is indicated in the genetic female who is externally virilized, most commonly as the result of CAH. The degree of virilization can be highly variable (see Fig. 97.6) and will have a significant influence on the type of procedure done, especially the vaginoplasty portion of the operation. Reconstruction in this group of patients has three components: clitoral reduction, vaginoplasty, and labial reconstruction. The timing of surgical correction also has undergone some

changes over the years. The current thinking is that once a decision is made to proceed with genital reconstruction, performance of this type of surgery at a younger age will have distinct advantages, including easier mobilization of the urogenital sinus and a more benign postoperative course.

Clitoral Reduction

Attempts at managing the enlarged clitoris in genetic females with clitoral hypertrophy started with total clitorectomy. Young (1937) originally advocated this procedure. Later, Lattimer (1961) suggested a recession rather than a resection of the clitoris, and he hoped to be able to preserve the arousal function of the clitoris. This led to cases in which painful clitoral erections occurred later in life; therefore further modification was needed. Spence and Allen (1973) advocated the preservation of the glans with reduction in the size of the clitoris. Since then, several reports have examined preservation of the neurovascular bundle using a clitoral reduction and recession type of approach. Kogan et al. (1987) and Snyder et al. (1983) separately described a similar approach in which the erectile tissue of the clitoris is removed, but preservation of the neurovascular bundle and the glands is afforded to preserve the neurologic and arousal functions of the clitoris. If the glans is unusually large, then a reduction of the glans size may be indicated as well.

Vaginoplasty

Reconstruction of the vagina in cases of virilization in females requires an understanding of the anatomy. One may consider the anatomic abnormality an embryologic arrest of maturation with a persistence of an early embryologic stage. The anatomic issue that is important to the surgical management of the common urogenital sinus is the site of confluence of the genital tract and urethra. This site varies considerably but is somewhat predictable from the appearance of the degree of external virilization. Children with severe degrees of external virilization are more likely to have a higher confluence of the urethral and vaginal channels, leading to a longer urogenital sinus or a more masculinized urogenital sinus. In the classic article on urogenital sinus abnormalities, Hendren and Crawford (1969) described the variable anatomy that can be seen in these children and noted that the operative procedures needed to be tailored toward the location of the confluence of the urinary and genital tracts. One may describe the confluence anatomically as it relates to the external sphincter, with confluences distal to the external sphincter being considered *low* and those proximal to the external sphincter being referred to as *high*. One also may describe the variable anatomy according to the length of the urethra from the bladder neck to the point of confluence. If that length of urethra were long, then one would consider this a low confluence. Conversely, if the length of the urethra were short and therefore close to the bladder neck, then a high confluence would be present (see Fig. 97.8B).

The low confluence cases can generally be repaired either by a cutback procedure on the fused labioscrotal folds or by a flap vaginoplasty. A cutback procedure would be indicated in cases with a minor degree of fusion of the labioscrotal folds. The middle to high vaginal confluence, however, generally requires either a pull-through vaginoplasty or a total urogenital mobilization to bring the vagina down to the perineum.

Flap Vaginoplasty

Flap vaginoplasty should be used for a low confluence of the urogenital sinus. The procedure entails mobilization of a

perineum-based flap with its apex at the meatus of the urogenital sinus. Dissection then proceeds along the posterior wall of the urogenital sinus until the vaginal opening is identified. The perineum-based flap is then inserted into the posterior wall of the vagina, thereby exteriorizing the vagina to the perineum.

Total Urethral Mobilization

Total urogenital mobilization can be used for the high urogenital sinus, which has been advocated by [Pena \(1997\)](#) and subsequently substantiated by a report from [Rink et al. \(1997\)](#). This approach has been shown to have a superior cosmetic result, compared with that obtained with a flap vaginoplasty, for a middle to high confluence. In addition, there has been a reduced incidence of urethral vaginal fistula and vaginal stenosis. The mobilization occurs in a plane both anterior to the urogenital sinus and up to the bladder neck under the pubic symphysis and posteriorly along the urogenital sinus, and then along the posterior wall of the vagina.

Pull-Through Vaginoplasty

Pull-through vaginoplasty is reserved for use in severely masculinized genetic females, whose surgical treatment continues to present a major challenge. Initially, the approach was a combined perineal and abdominal approach with complete mobilization of the vagina and uterus and separation of the vagina from the urethra at the confluence. The abdominal mobilization will then allow the vagina to be brought down to the perineum. A modification of this approach was described by [Passerini-Glazet \(1989\)](#) in which the more distal urogenital sinus tissue was used to provide an anterior vaginal wall flap, which will then connect to the true vagina and allow a complete perineal approach to the procedure.

Vaginal Agenesis

Vaginal replacement has a role in certain DSD or structural abnormalities of the genital urinary tract. The DSD in which vaginal replacement may be indicated are 46,XX vaginal agenesis, 46,XY male karyotype with severely inadequate virilization or CAIS, and structural urogenital defects such as cloacal exstrophy or persistent cloaca or after a pelvic exenteration for malignancy. A variety of tissues and techniques are used for vaginal reconstruction: skin grafting, progressive perineal indentation, and split-thickness or fold thickness tissue grafts with expanders, myocutaneous flaps, and bowel segment. The critical point in creating a vagina is to maintain an adequate perineal opening, an adequate-length tunnel, and good fixation to pelvic structures. This area is highly controversial in terms of the best management. Overall, the most popular tissue for vaginal plate replacement has been the split-thickness skin graft as described by [McIndoe \(1950\)](#). The major disadvantage of this technique has proved to be the need for long-term dilatation to maintain patency and to avoid vaginal stenosis. Recently, buccal mucosa grafts have been used rather than skin grafts for vaginal replacement as an alternative to the McIndoe graft. The use of bowel segments for vaginal replacement was first described by [Baldwin \(1904\)](#). Because of an extraordinarily high mortality rate associated with this approach, earlier attempts using this technique were abandoned. Since then, this approach has been adopted by many groups and has been shown to be highly successful, with minimal complication rates. Early on, intestinal mucus production can be a problem, but this lessens over time, and mucus may act as a natural lubricant. Minimal perineal scarring is associated with this approach as well, and it can be done at a very young age ([Hensle and Dean, 1992](#)). Because of the risks and complications with any surgical approach, vaginal dilation has

been recommended, when feasible, as first-line therapy for patients with vaginal agenesis who are candidates for a nonsurgical approach.

Undervirilized Males

Inadequate virilization results in hypospadias with or without cryptorchidism. In more severe cases, such as a complete form of androgen insensitivity, these children may appear as phenotypically normal females and present at the time of puberty with primary amenorrhea. Vaginoplasty is frequently needed after puberty in that population if vaginal dilation is unsuccessful. Children with varying degrees of hypospadias, with or without cryptorchidism, will usually require a repair following the usual principles of hypospadias and cryptorchidism repair. These repairs are generally done at 6 months of age and are tolerated well as outpatient procedures.

Gonadectomy

Gonadectomy is considered under two circumstances in DSD: when the assigned sex is different from the gonadal sex, mostly in the 46,XY DSD and 45,X/46,XY DSD, and when there is a risk of tumor related to the testicular tissue. This is most commonly seen in androgen insensitivity syndrome (46,XY karyotype) in which normal testes are present but a female sex assignment has been made. Conservative management of the gonads in CAIS until puberty is recommended, since tumors in these gonads do not develop until puberty and the risk is less than 1%. Alternatives to gonadectomy include bringing the gonads down (orchiopexy), monitoring with clinical examinations, imaging studies, and biopsies, although there are limited data regarding safe and effective long-term surveillance. In DSD where there is a risk of virilization at puberty, hormonal suppression may be an alternative to surgical management until the patient is able to consent. Gonadal malignancy is a significant risk in DSD in which dysgenetic gonads and a Y chromosome are present. An example is shown in [Fig. 97.15](#), in which a 1-year-old infant with complete XY gonadal dysgenesis was found to have a gonadoblastoma on gonadectomy. Other syndromes in which gonadal malignancy is a concern include mixed gonadal dysgenesis and the presence of a dysplastic testis in a dysgenetic XY male.

Removal of Müllerian Remnants

Removal of müllerian remnants in male-assigned patients is not recommended unless they are associated with symptoms, such as dysuria, urinary tract infections, pain, bleeding, herniation, and stone formation. Removal of the müllerian remnant can be performed laparoscopically. In most cases, müllerian remnants are asymptomatic, and cancers have rarely been reported ([Josso et al., 2005](#); [Farikullah et al., 2012](#)).

Conclusions

Significant advances have been made in our understanding of the pathophysiology and molecular genetics of DSD. A systematic diagnostic approach to the infant with ambiguous genitalia should be undertaken to identify the underlying disorder. Establishment of the diagnosis will provide a better understanding of the natural history of the disorder and enable better counseling of the family. Decisions regarding sex of rearing, need and urgency for gonadectomy, and recurrence risk are also enhanced if a specific diagnosis can be made.

CASE STUDY 3

History

- A 3-week-old 46,XY male infant with penoscrotal hypospadias presented with history of weight loss during the previous 2 weeks. He had a normal cortisol level of 50 µg/dL after ACTH stimulation testing at 1 week of age.
- The infant had decreased breastfeeding and had taken only 2 oz by bottle during the previous 24 hours.
- No emesis. No lethargy. No fever.

Evaluation

- On presentation to the emergency department the infant had a loss of 0.4 kg since his visit to the DSD clinic 2 weeks previously.
- Genital examination: 3 cm × 1.2 cm phallus, severe chordee, penile scrotal hypospadias, bilateral nonpalpable gonads, and bifid scrotum.
- Electrolytes: sodium 119 mEq/L, potassium 6.7 mEq/L, chloride 90 mEq/L, CO₂ 14 mEq/L.
- Urinalysis: leukocyte esterase and protein 3+, blood 2+, white blood cells 50 to 100/µL, moderate levels of bacteria.
- Urine culture positive for *Escherichia coli*.
- Aldosterone 875 ng/dL (normal for term infant <217 ng/dL).
- Ultrasound examination revealed presence of a utricle.

Management and Outcome

- Infant with pseudohypoaldosteronism secondary to urinary tract infection (Riepe, 2013).
- Infant treated with antibiotics and normal saline with resolution of hyponatremia and urinary tract infection.
- Urology follow-up to discuss urinary tract infection prophylaxis and need for removal of utricle.

The need for surgical intervention in DSD should be assessed on a case-by-case basis. The parents need to be informed about the risks and benefits of surgery. Surgery on the external genitalia should perhaps be reserved for individuals with significant discord between the sex of rearing and the appearance of the external genitalia.

The infant with a DSD and the family will have to cope with difficult psychosocial challenges throughout life. Physicians caring for these patients should ensure the integration of well-trained mental health professionals into the longitudinal care of these complex infants and children.

CONTROVERSY BOX

Gonadectomy in a 46,XY individual with ambiguous genitalia raised as a female is controversial. The risk of gonadal tumor is low if the testis is not dysgenetic. But the gonad can produce testosterone or DHT at puberty via alternative pathways or isoenzymes. For example, individuals raised as a female with 5α-reductase type 2 deficiency or 17β-HSD type 3 deficiency may virilize at puberty if the testes are left in place. Will the retention of testes in 46,XY DSD females promote male gender identity or cause irreversible virilization? Should a gonadectomy be done before a child can give assent/consent? Is it permissible to remove gonads so that a child raised as a female has perhaps a greater chance of having a female gender identity? Endocrinologists can suppress gonadal production of testosterone through the use of long-acting gonadotropin-releasing hormone analogues once the gonad becomes active in puberty. This may then permit the child or adolescent to make the decision as to whether or not to pursue gonadectomy at a later age.

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Disorders of the Thyroid Gland

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KEY POINTS

- An understanding of thyroid embryogenesis and the physiology of the thyroid gland in the perinatal period is important for proper interpretation of abnormal laboratory results and initiation of appropriate treatment.
- Appropriate thyroid hormone function is essential for normal neurodevelopment in infancy and childhood. Hypothyroidism in the first year of life can result in significant deleterious effects on growth and neurologic injury.
- Delay in treatment of congenital hypothyroidism (CH) is the most common preventable cause of mental retardation.
- Neonatal screening can provide early diagnosis and can prevent delays in treatment. Newborn screening methods differ and may possibly miss rare forms of congenital hypothyroidism.
- Eighty-five percent of cases of permanent CH are associated with abnormal development of the thyroid gland.
- In preterm newborns, thyroid hormone levels may fall because of immaturity of the thyroid gland, but these changes may be exacerbated by complications of prematurity.
- Thyroid metabolism can be affected by exogenous sources of iodine, dopamine infusions, blood transfusion, and glucocorticoid treatment.
- The clinical manifestations of Graves disease in the newborn include irritability, flushing, diarrhea, vomiting, tachycardia, hypertension, poor weight gain, thyroid enlargement, and exophthalmos.
- There is concern for neurologic damage in infants with hemangiomas when hypothyroidism is occult and untreated.

Thyroid hormone is essential for normal fetal brain development and for growth and regulation of energy metabolism throughout infancy and childhood. The interactions of the hypothalamus and pituitary gland with the thyroid are integral to the maintenance of normal thyroid function. An understanding of thyroid physiology and embryogenesis of the thyroid gland in the perinatal period is important for proper interpretation of abnormal laboratory results and initiation of appropriate treatment. Additionally, the interplay of the different thyroid hormones maintains the appropriate balance where needed.

Regulation of Thyroid Function

The hypothalamic–pituitary–thyroid (HPT) axis functions as a typical feedback loop (Fig. 98.1). Triiodothyronine (T_3) levels in the pituitary gland direct secretion of thyroid-stimulating hormone (TSH). Also, an inverse relationship exists between thyroid hormone

formation and iodide level in the thyroid. Subsequently, the rate of hormone production is not affected by rapidly changing levels of iodide, and the reservoir of thyroid hormone balances against quick changes in hormone synthesis.

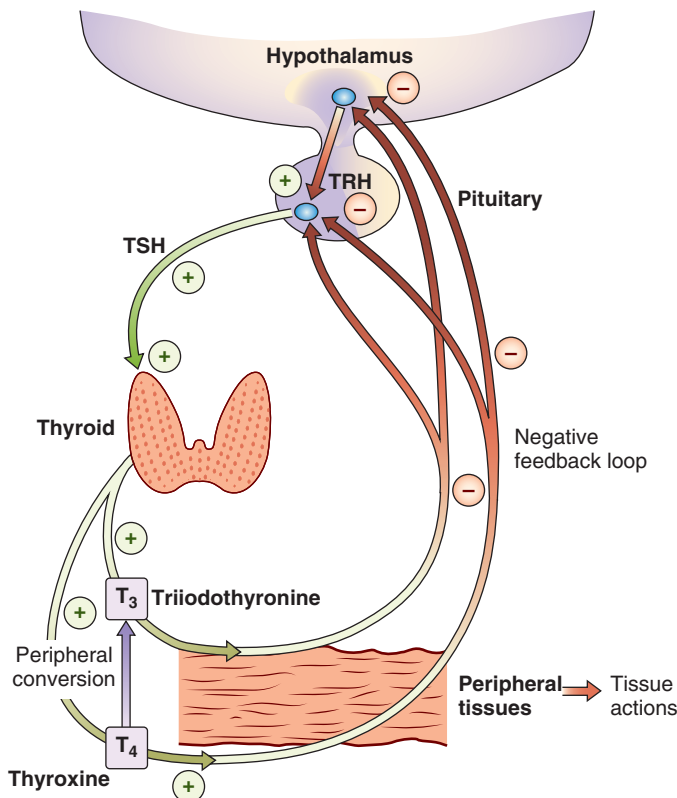
TSH, produced in the anterior pituitary, is the chief player in thyroid gland function and morphology. TSH provides negative feedback by decreasing synthesis of thyrotropin-releasing hormone (TRH) from the hypothalamus, as well as blocking the action of TRH that stimulates TSH release (Melmed et al., 2016). Release of TSH fuels uptake of iodide by the thyroid gland and accelerates many steps in thyroid hormone synthesis. It also drives growth and vascularization of the thyroid gland itself. Both T_3 and thyroxine (T_4) provide negative feedback to TSH secretion, while TRH determines the setpoint. Given the logarithmic relationship between free T_4 (fT_4) and TSH, TSH levels are a sensitive indicator of thyroid hormone status.

Thyroid Hormone Synthesis

Synthesis of thyroid hormone is initiated by entry of iodide molecules into the follicular cell of the thyroid gland via the sodium–iodide transporter; these are then transported into the colloid. Once they are in the thyroid gland, oxidation of iodide occurs, after which it is quickly associated with thyroglobulin (TG). Organification of the iodide forms tyrosine residues, which are then coupled to form the two active thyroid hormones: T_4 and T_3 . This complexed TG, which resides in the colloid of the thyroid gland, serves as the reservoir for production of T_3 and T_4 . Thyroid hormones are then moved via endocytosis into the follicular cell. This vesicle fuses with lysosomes, after which T_4 and T_3 are dissociated from TG via hydrolysis and eventually released into the bloodstream. Residual monoiodothyronine (MIT) and diiodothyronine (DIT) are deiodinated and enter the synthesis cycle again along with iodide (I^-) and tyrosine. Important steps in thyroid hormone synthesis are summarized in Fig. 98.2.

Serum Protein Binding and Transport

In the circulation, T_3 and T_4 are transported by attachment to binding proteins that include T_4 -binding globulin (TBG), T_4 -binding prealbumin (TBPA), and albumin. These binding transport proteins are produced in the liver. Production of these proteins increases through the latter half of gestation and is stimulated by estrogen. Free thyroid hormone levels appear to remain constant despite the increase in total hormone concentrations because of an increase in binding protein production.



• **Fig. 98.1** The hypothalamic–pituitary–thyroid axis. Thyroid stimulating hormone (TSH) is secreted in response to TSH-releasing hormone (TRH) and stimulates secretion of thyroxine (T₄) and triiodothyronine (T₃) from the thyroid. T₃ and T₄ have actions in peripheral tissues and exert negative feedback on the hypothalamus and the pituitary.

The serum concentration of T₄ is vastly greater than that of T₃, by a factor of 50–100. The primary transport protein (~75% of T₃ and T₄) is TBG. The remaining 25% of thyroid hormone is equally distributed between TBPA and albumin. The fT₄ concentration more precisely reflects the metabolic status, in comparison with total T₄ or T₃. This is because only unbound hormone can enter cells to exert its action (Benvenega, 2013).

Embryogenesis of Hypothalamic–Pituitary–Thyroid Axis

The thyroid gland is the first endocrine organ to develop in the fetus. The development of the thyroid gland and the HPT axis occurs in two phases. The first phase is characterized by the embryogenesis of the structures involved, and the second phase is characterized by the maturation of the HPT axis, which will be described later (Polak and Luton, 2014). By about 3 weeks of gestation, the rudimentary beginnings of the hypothalamus have developed. Subsequently, anlagen from the floor of the forebrain and the Rathke pouch converge to form the pituitary gland. By 14 to 15 weeks of gestation, this embryologic structure has become the mature pituitary gland (Van Vliet and Deladoey, 2014). Detection of TSH has been observed as early as 10 to 12 weeks of gestation (Kratzsch and Pulzer, 2008).

A programmed sequence of transcription and homeobox factors (e.g., transcription termination factor 2 [TTF-2], forkhead box

E1 [FOXE-1], paired box 8 [PAX8]) directs thyroid embryogenesis. Thyroid gland development begins with convergence of three structures: the median anlage from the floor of the pharyngeal pouch (caudal end of the foregut) and two lateral anlagen from the fourth pharyngobranchial pouch. The median anlage is visible by day 22 after conception. The lateral anlagen develop into a pair of ultimobranchial bodies from which the calcitonin-producing parafollicular C cells arise. Between day 32 and day 48 after conception, this primordial thyroid structure breaks off from the pharyngeal pouch and completes its migration to the pretracheal region. Little is known about the molecular factors involved in this migration; however, FOXE-1 has been implicated in cases of thyroid ectopy (Szinnai, 2014).

By 4 weeks after this descent is complete, functional and structural changes are mature, and the thyroid is capable of producing thyroid hormone. Follicles fill with colloid. By 12 weeks of gestation, the thyroid gland is now able to take up iodide, synthesize TBG, and produce hydrogen peroxide (H₂O₂) at the apical membrane—all steps required for thyroid hormone production. T₄ has been detected in human fetal blood as early as 11 to 12 weeks of gestation (Calvo et al., 2002). This accrual of the ability to produce thyroid hormone coincides with maturation of the hypothalamus and pituitary gland with production of TRH and TSH.

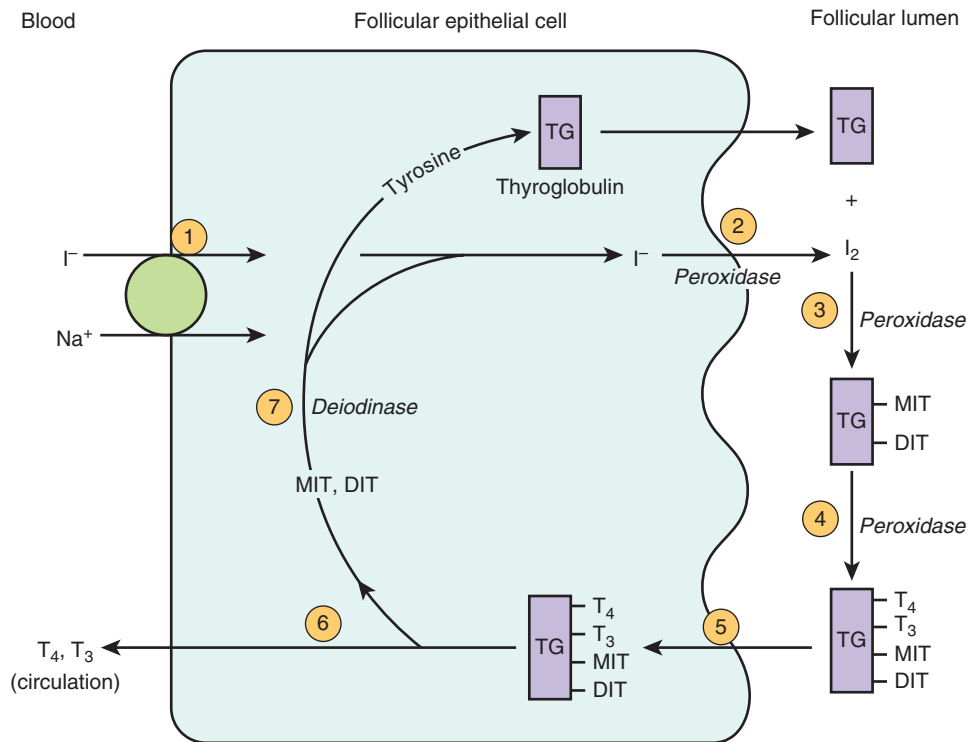
Fetal–Placental–Maternal Thyroid Interaction

The placenta is of utmost importance in fetal thyroid physiology as it regulates the passage of maternal thyroid hormone to the fetus (Fig. 98.3). Thyroid hormone is paramount for normal neurodevelopment. Maternal-to-fetal transfer of T₄ is essential, especially in the first trimester, when the fetal thyroid axis has yet to mature.

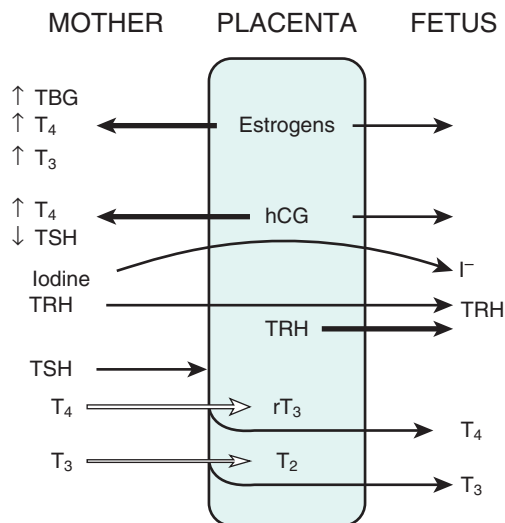
Multiple clinical studies have shown the deleterious effects of inadequate maternal thyroid hormone levels (Haddow et al., 1999; Pop et al., 2003; Li et al., 2010). However, the placenta is not able to address maternal hypothyroidism, as fetal T₄ levels reflect maternal levels (Chan et al., 2009). With the landmark study by Vulsma et al. (1989), it was shown that T₄ does cross the placenta, contradicting previous concepts: placental transfer of T₄ in athyreotic fetuses resulted in levels that were 25%–50% of those of normal term newborns. Thus the hypothyroid fetus in a euthyroid mother has some protection; accordingly, a hypothyroid fetus in a hypothyroid mother has the most substantial deficits (Glinioer, 2001).

TSH does not cross the placenta; however, TRH does. During pregnancy, maternal thyroid axis changes result in increases in fT₄ levels early and maintenance of higher levels until birth (Fig. 98.4). This is a result of human chorionic gonadotropin produced by the placenta, which is homologous to TSH with some TSH-like activity (Moleti et al., 2014).

Although most of the transfer of the maternal thyroid hormone is dependent on maternal thyroid hormone concentrations, plasma membrane thyroid hormone transporters and metabolism via deiodinases (DIs) also play a role. Thyroid hormone transporters are found in the apical and basolateral membrane of the placenta. Several transporters have been discovered in the placenta (monocarboxylate transporters, L-type amino acid transporters, and organic anion–transporting polypeptides) (Fujiwara et al., 2001; Visser et al., 2008). The placenta also has abundant type 3 DI, which is involved in metabolism of thyroid hormone in this milieu. T₃ is



• **Fig. 98.2** Steps involved in the synthesis of thyroid hormones: (1) iodide transport; (2) oxidation of I^- to I_2 ; (3) organization of I_2 into monoiodothyronine (MIT) and diiodothyronine (DIT); (4) coupling reaction of MIT and DIT to form triiodothyronine (T_3) and thyroxine (T_4); (5) endocytosis of thyroglobulin (TG); (6) hydrolysis of T_4 and T_3 ; T_4 and T_3 enter circulation; (7) deiodination of residual MIT and DIT, recycling of I^- and tyrosine.



• **Fig. 98.3** The placental role in thyroid metabolism. The placenta produces estrogens and human chorionic gonadotropin (hCG), which increase maternal thyroxine (T_4)-binding globulin (TBG) levels and stimulate maternal thyroid hormone production, respectively. Both activities tend to increase maternal T_4 and triiodothyronine (T_3) concentrations and inhibit maternal thyroid stimulating hormone (TSH) secretion. Iodide and TSH-releasing hormone (TRH) readily cross the placenta. In addition, the placenta synthesizes TRH. The placenta is impermeable to TSH and only partially permeable to T_4 and T_3 . rT_3 , Reverse triiodothyronine.

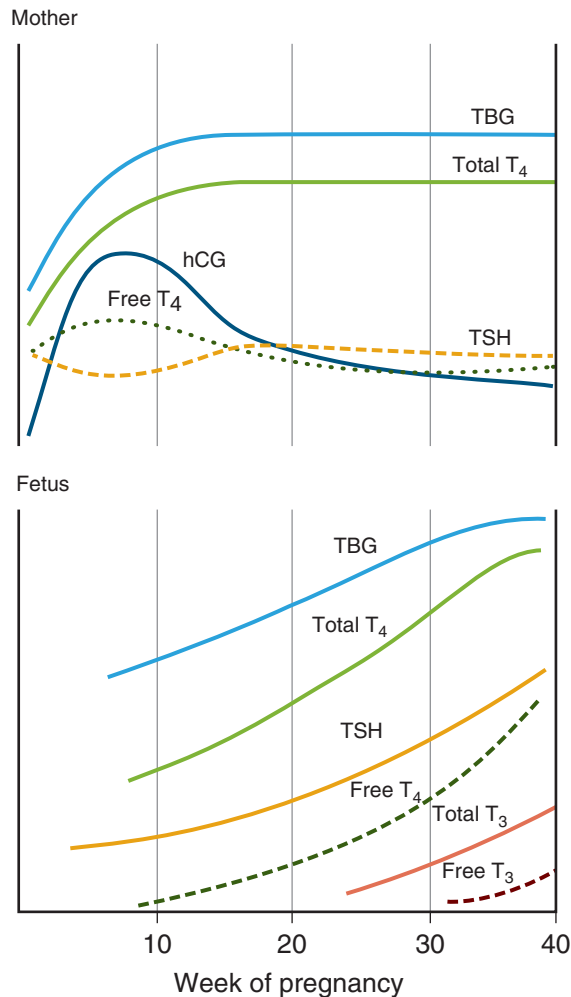
thought not to cross the placenta; however, this is not clear (Mitchell et al., 1992).

Iodide is taken up by the thyroid gland for thyroid hormone synthesis. There is free passage of iodides between the mother and the fetus. Large-quantity ingestion of iodine will decrease synthesis of T_4 transiently. This is due to decreased uptake via the sodium-iodide symporter. Typically, there is escape from this phenomenon in about 48 hours. However, cases of goiter and hypothyroidism have been reported with excessive iodine intake (Overcash et al., 2016). An adequate amount of iodine is still recommended for pregnant women, around 150 μ g per day according to the American Thyroid Association (Becker et al., 2006).

Miscellaneous other compounds can cross the placenta, affecting fetal thyroid function. TSH receptor-blocking antibodies (TRBAs), also known as TSH Binding Inhibitory Immunoglobulins, have been implicated in cases of transient neonatal hypothyroidism (Evans et al., 2011). In mothers with a history of Graves disease, thyroid-stimulating immunoglobulin (TSI) can lead to thyrotoxicosis in the neonate (Levy-Shraga et al., 2014). Antithyroid medications ingested by the mother can result in fetal goiter with or without hypothyroidism (Rosenfeld et al., 2009).

Thyroid System Maturation

The fetal thyroid gland is able to synthesize and secrete thyroid hormone after 12 weeks of gestation. As the pregnancy continues, the levels of f T_4 and T_4 continue to rise in step with the gestational age of the fetus. T_3 levels, however, remain low until about 30 weeks of gestation, after which they increase slowly until birth. This delayed rise in T_3 levels is due to low rates of conversion of T_4 to T_3 by type 1 DI and high activity of type 3 DI metabolizing T_3 .



• **Fig. 98.4** Relative changes in maternal and fetal thyroid function during pregnancy. The effects of pregnancy on the mother include a marked and early increase in hepatic production of thyroxine (T_4)-binding globulin (TBG) and placental production of human chorionic gonadotropin (hCG). The increase in serum TBG level, in turn, increases serum T_4 concentrations; hCG has thyroid stimulating hormone (TSH)-like activity and stimulates T_4 secretion. The transient hCG induces increase in serum T_4 and inhibits maternal secretion of TSH.

to diiodothyronine (DIT) (Van Vliet and Deladoey, 2014). With an increased amount of type 1 DI produced by the liver after 30 weeks, there is increased conversion of T_4 to T_3 in the liver and decreased metabolism of T_3 by placental type 3 DI, resulting in the steady rise until delivery. TSH has also been detected at 12 weeks of gestation, with subsequent increases in its level in parallel with increases in fT_4 level (LaFranchi, 1999a). The level of reverse T_3 (rT_3) is quite high early in the third trimester because of placental type 3 DI activity converting T_4 to rT_3 and T_3 to DIT (Fig. 98.5).

Fetal Thyroid Hormone Metabolism

The thyroid gland is the body's only source of T_4 . Conversion of T_4 to T_3 peripherally contributes most of the circulating T_3 . This conversion is accomplished by a group of three DIs, types 1, 2, and 3 (see Fig. 98.5). T_3 , with its greater affinity for the thyroid hormone receptor, is the active form of thyroid hormone. T_3 is

derived from deiodination of the outer ring of T_4 . Conversely, deiodination of T_4 at the inner ring produces the inactive rT_3 (Kuiper et al., 2005).

Type 2 and type 3 DIs are selective in their action, performing deiodination on only the outer ring and inner ring, respectively. Type 1 DI is nonselective, with deiodination at the outer ring converting T_4 to T_3 and deiodination at the inner ring producing rT_3 from T_4 . Type 1 DI is produced in the liver, kidney, and thyroid. Type 1 DI is inhibited by propylthiouracil and rT_3 and stimulated by thyroid hormone. Type 2 DI is primarily found in brain, pituitary, placenta, skeletal muscle, heart, thyroid, and brown adipose tissue and is not sensitive to propylthiouracil and is inhibited by thyroid hormone. It converts T_4 to T_3 . Type 3 DI is found primarily in fetal tissues, including the placenta, brain, and skin. Type 3 DI converts T_4 to rT_3 and T_3 to DIT (Huang, 2005).

During pregnancy, fetal DI levels vary with time and are reflective of thyroid hormone concentrations. Type 1 DI in hepatic tissues remains quiescent until late in gestation, resulting in a late rise of fetal T_3 levels. Type 3 DI activity is responsible for high rT_3 levels in the fetus. The interplay of these enzymes is critical to normal brain development. Given that both type 1 and type 2 DI are sensitive to thyroid hormone levels, a hypothyroid fetus will have increasing type 2 DI activity in the brain and decreasing type 1 hepatic activity, resulting in more T_4 in the brain, at which point it is converted to T_3 by type 2 DI (Fisher et al., 1994).

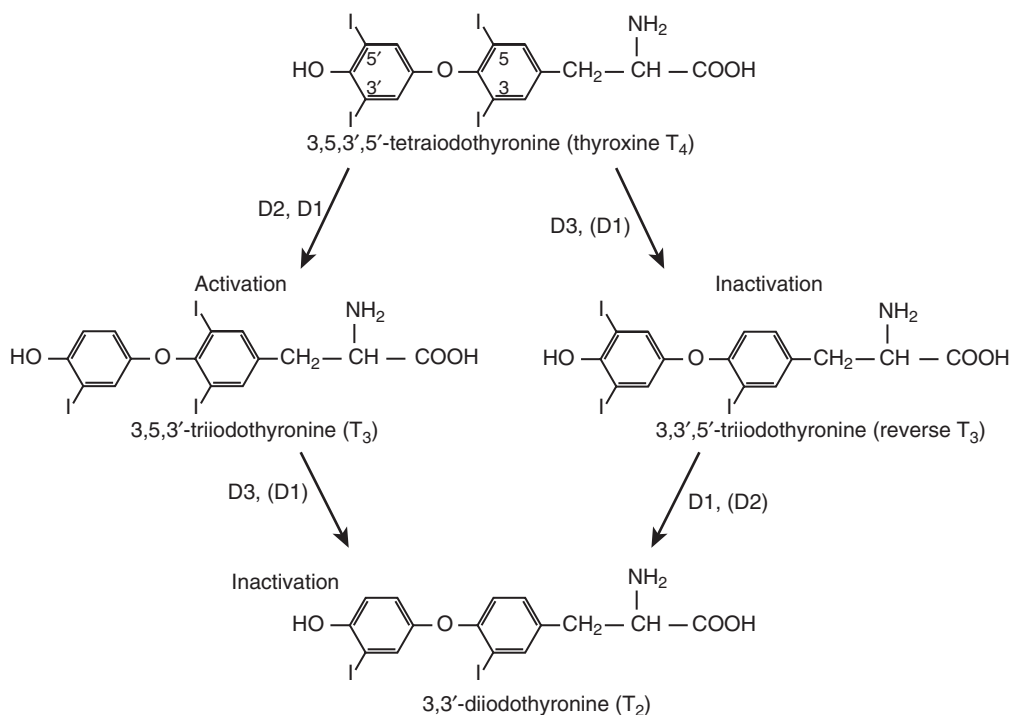
Extrauterine Thyroid Adaptation

At birth, fT_4 levels in the newborn are slightly below that of the maternal concentration, and the T_3 level is quite low in umbilical cord blood. Also, at this time, partly in response to cold and stress, there is a TSH surge that peaks at about 2–4 hours of life and then returns to its initial value within about 48 hours (Fig. 98.6). T_3 and T_4 levels also rise within a few hours of birth and reach their peak by the end of the 24 hours. TSH, as well as extrathyroidal conversion of T_3 to T_4 by type 1 and type 2 DIs, contributes to this surge of thyroid hormone (Salvatore et al., 2016). During the next 4 to 5 weeks of life, T_4 and T_3 levels progressively decrease to levels just above the normal range of children. By 1 month of age, TSH level has declined to about 5 mU/L (LaFranchi, 1979) and declines further to a near-adult range of 0.5–4 mU/L by the second month (Baloch et al., 2003).

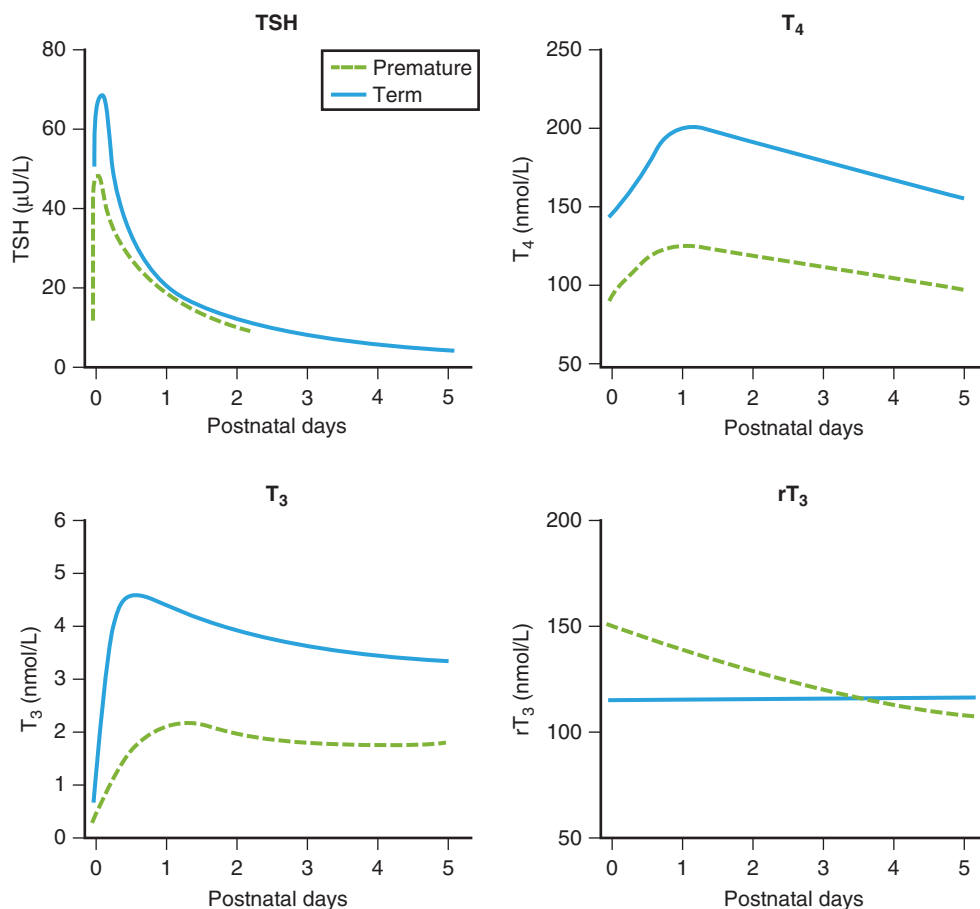
Infants born prematurely appear to have an attenuated TSH surge. This is due to an immature HPT axis. T_3 and T_4 levels also have a blunted rise in comparison with the levels in term newborns. TSH level can peak at about 40 mU/L by 30 minutes (LaFranchi, 1999b). Many factors need to be taken into account when one is assessing thyroid function in preterm infants. As many preterm newborns have other complications, such as respiratory distress syndrome or nutritional problems, serum T_4 , and especially T_3 , concentration may fall to low levels as a result of immaturity of the thyroid gland, as well as reduced TBG production. Illness additionally causes suppression of the hypothalamic-pituitary-thyroid axis, impairs conversion of T_4 to T_3 , and increases type 3 DI activity (Salvatore et al., 2016).

Congenital Hypothyroidism

Congenital hypothyroidism (CH) is a common cause of mental retardation if not treated promptly. The overall incidence is 1 in 2500 to 1 in 3000 newborns (Waller et al., 2000; Harris and Pass, 2007). Higher incidence rates have been reported in certain



• **Fig. 98.5** Enzymatic conversion of iodothyronines. $D1$, Type 1 deiodinase; $D2$, type 2 deiodinase; $D3$, type 3 deiodinase.



• **Fig. 98.6** Postnatal thyroid stimulating hormone (TSH), thyroxine (T_4), triiodothyronine (T_3), and reverse T_3 (rT_3) secretion in the term and premature infant in the first week of life. (Adapted from www.thyroidmanager.org, the free online thyroid textbook. In Brown R, Larsen PR, De Groot LJ, eds: *Thyroid gland development and disease in infants and children*, South Dartmouth, MA, 2009, Endocrine Education, Inc, Chapter 15, Figure 15-4, p 9.)

populations. The prevalence rates of CH can differ with sex, ethnicity, and birthweight. Twice as many female as male infants are affected. Compared with whites, the prevalence of CH is higher in Asians and Hispanics and lower in blacks. A study noted an increased risk of CH in macrosomic (>4500 g) and low birthweight (<2000 g) infants (Waller et al., 2000). There is increased risk in infants with Down syndrome.

In most cases the disorder is permanent and results from an abnormality in thyroid gland development (dysgenesis or agenesis) or a defect in thyroid hormonogenesis. Less commonly, the altered thyroid function is transient, attributable to transplacental passage of maternal medication, maternal blocking antibodies, or iodine deficiency or excess. In rare cases, CH may result from a pituitary or hypothalamic abnormality (Rose et al., 2006). Potential causes are listed in Table 98.1. Regardless of the duration of the thyroid dysfunction, immediate treatment with levothyroxine (LT₄) in the newborn period is needed to prevent cognitive or neurodevelopmental decline. The developing fetal brain is protected in utero by an adequate source of T₃, supplied by local deiodination of maternal T₄ in the fetal brain.

TABLE 98.1 Thyroid Disorders and Their Approximate Incidence in the Neonatal Period

| Disorder | Incidence |
|--|-----------------------------|
| Thyroid Dysgenesis | 1 in 2500 to 1 in 4000 |
| Agenesis | |
| Hypogenesis | |
| Ectopia | |
| Thyroid Dyshormonogenesis | 1 in 30,000 |
| TSH receptor defect | |
| Iodide-trapping defect | |
| Thyroid peroxidase defect | |
| H ₂ O ₂ generation defect | |
| Defect in thyroglobulin | |
| Pendred syndrome | |
| Dehalogenase defect | |
| TSH signaling defect: Albright hereditary osteodystrophy | |
| Transient Hypothyroidism | 1 in 12,000 to 1 in 30,000 |
| Drug induced | |
| Maternal antibody induced | |
| Idiopathic | |
| Hypothalamic–Pituitary Hypothyroidism | 1 in 80,000 to 1 in 100,000 |
| Hypothalamic–pituitary anomaly | |
| Panhypopituitarism | |
| Isolated TSH deficiency | |

H₂O₂, Hydrogen peroxide; TSH, thyroid stimulating hormone.

Clinical Manifestations

The diagnosis of hypothyroidism should be considered in any infant with prolonged jaundice, transient hypothermia, an enlarged (>1 cm) posterior fontanel, failure to feed properly, or respiratory distress with feeding (Pezzuti et al., 2009). Approximately one-third of maternal T₄ crosses to the fetus at term. With a half-life of 6 days, the maternal T₄ will be metabolized and excreted by 3 to 4 weeks of age (LaFranchi, 2011). The classic signs evolve during the first few weeks after birth. Fetal growth is normal; however, there is delay in bone maturation and a rapid reduction in growth rate after birth (Van Vliet, 2005), with progressively worsening myxedema in subcutaneous tissues and tongue. The thickened tongue becomes protuberant, and increasing difficulty in nursing and handling salivary secretions is noted. The cry is hoarse because of myxedema of the vocal cords. Additional signs and symptoms include marked muscular hypotonia; constipation; thick, dry, cold skin; long and abundant coarse hair; large tongue; abdominal distention; umbilical hernia; hyporeflexia; bradycardia; hypotension with narrow pulse pressure; anemia; and widely patent cranial sutures. Goiter can be present. The typical facies are characterized by a depressed nasal bridge, a relatively narrow forehead, and puffy eyelids (Foley, 1994). The cardiac silhouette may be enlarged, and the electrocardiogram shows low voltage and a prolonged conduction time. Some of the signs and symptoms are present by 6 to 12 weeks postnatally, especially lethargy, constipation, and umbilical hernia. The cretinoid facies and growth retardation become increasingly obvious during the first several months of life.

Nonspecific symptoms and signs associated with hypothyroidism are listed in Table 98.2. Because clinical manifestations of hypothyroidism may not appear until weeks after birth, even in athyreotic infants, newborn screening has enabled pediatricians to identify newborns with low thyroid hormone production and to initiate

TABLE 98.2 Clinical Signs and Symptoms of Congenital Hypothyroidism in Infancy

| Age/Manifestation | Frequency (%) |
|----------------------------------|---------------|
| 0–7 Days | |
| Prolonged jaundice >3 days | 73 |
| Birthweight >4 kg | 40 |
| Poor feeding | 40 |
| Transient hypothermia | 38 |
| Large posterior fontanel (>5 mm) | 32 |
| 1–4 Weeks | |
| Failure to gain weight | 45 |
| Constipation | 35 |
| Hypoactivity | 33 |
| 1–3 Months | |
| Failure to thrive | 90 |
| Umbilical hernia | 49 |
| Macroglossia | 43 |
| Myxedema | 40 |
| Hoarse cry | 30 |

therapy within the first 2 weeks of life, before the development of signs and symptoms (Rose et al., 2006).

Neonatal Screening for Hypothyroidism

Newborn screening programs for CH avoid delay in its diagnosis, because signs and symptoms of CH may not manifest themselves for several weeks. The screening programs are designed to detect elevated serum TSH levels in blood samples collected on filter paper. Some programs measure TSH directly, and others measure TSH in those samples with low or low-normal T_4 concentrations. Screening programs have been established in industrialized countries: in the United States, western Europe, parts of eastern Europe, Japan, Australia, and parts of Asia, South America, and Central America. However, many countries still do not have a nationwide program for neonatal thyroid screening.

Both methods of screening—primary TSH/backup T_4 method and primary T_4 /backup TSH method—appear to be capable of detecting almost all infants with primary CH. In addition, an increasing number of programs use a combined TSH plus T_4 approach.

Primary CH is associated with a low serum T_4 and fT_4 concentration and a high TSH concentration in umbilical cord blood or neonatal blood samples. It is estimated that 5%–8% of affected infants can escape detection by newborn screening because of a delayed elevation in serum TSH concentration or because of errors in sample collection or laboratory routine. In addition, infants with TSH deficiency who have normal TSH levels are not detected, because most newborn screening programs report only those infants with elevated TSH levels. Infants with central hypothyroidism may have normal or low TSH. Normal TSH would be inappropriate in the setting of low thyroid hormone levels. Newborn screens that use elevated TSH to detect congenital hypothyroidism will miss diagnosing these infants. Infants with signs or symptoms suggestive of thyroid dysfunction (Table 98.2) should be investigated regardless of previous screening results. Determination of serum fT_4 , T_4 , and TSH values is necessary in any infant with suspicious clinical or laboratory findings.

Every infant should be tested before discharge from the nursery, optimally by 2 to 4 days of age. The practice of early hospital discharge (before 48 hours of age) has led to an increased frequency of false-positive results because of the normal physiologic TSH surge that occurs after birth. Infants screened before 48 hours of age require the newborn screen to be rechecked by the primary care physician at 2 weeks of life. False-negative results may occur by the screening of a very sick newborn or after transfusion.

Accurate screening results depend on good-quality blood samples. Blood samples should be collected on approved forms of filter paper, dried at room temperature, and not subject to excessive heat. Capillary blood samples are placed in the circular areas to fill and saturate them, applied to one side only. Filter paper should not be handled or placed on wet surfaces (Rose et al., 2006).

Thyroid Function Tests

When the diagnosis of CH is suspected, thyroid function tests should be performed. It is advisable to assess fT_4 , T_4 , TSH, and TG levels. Measurements of T_3 , rT_3 , free T_3 , and T_3 resin uptake (T3U) are not indicated (Rose et al., 2006).

Elevated serum TSH value is the most sensitive and specific test to confirm the diagnosis of primary hypothyroidism. Typical laboratory findings for primary hypothyroidism include elevated

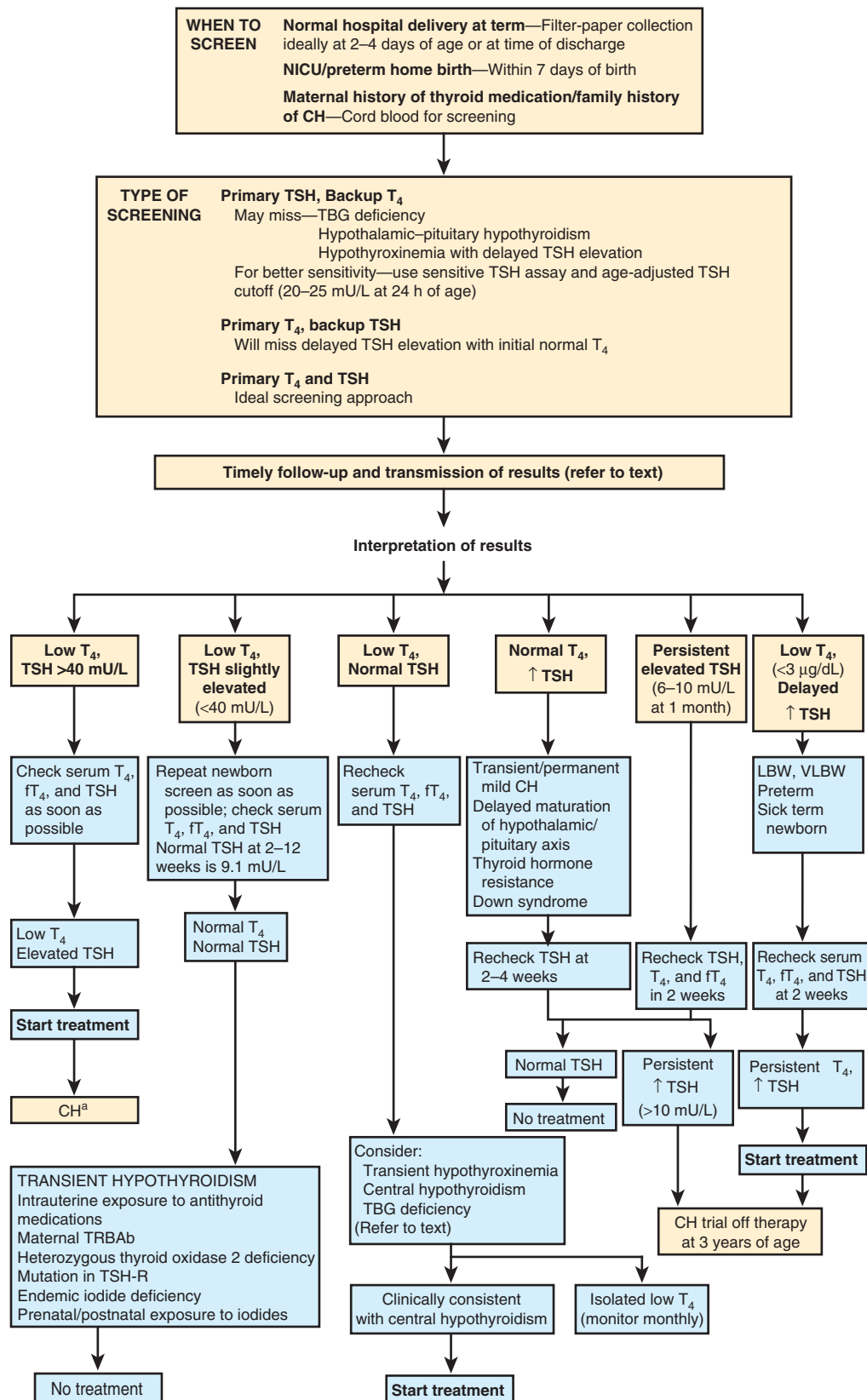
TSH levels, with T_4 and fT_4 values in the low or low-normal range. A low or undetectable TG concentration (Sobrero et al., 2007) with TSH level elevation confirms a dysgenetic or absent thyroid gland (Djemli et al., 2004), whereas a high TG concentration with TSH level elevation suggests an organification defect (Vulsma et al., 1991). In central hypothyroidism, TSH level is usually normal, T_4 level is low normal, and fT_4 level is in the lowest third of normal (Van Tijn et al., 2008).

Interpreting Thyroid Function Tests

When interpreting thyroid function tests, it is critically important to take into account the day of life on which the samples were drawn. The recommended period for newborn screening of thyroid function is at 24 hours of life. If screening is done at less than 24 hours of life, it must be redone, regardless of the results. After 24 hours of life, a TSH value greater than 40 μ U/L is highly suggestive of hypothyroidism, but confirmatory testing is required. A TSH value above the normal limit for the hours of life and gestational age but below 40 mU/L is indeterminate. Therefore repeated testing is necessary, and the primary care physician is informed of the need to obtain a second screen (Fig. 98.7). The practice of early hospital discharge (<48 hours of age) has led to a higher rate of indeterminate results.

Eight percent to 10% of infants with CH have TSH values between 20 and 40 mU/L. One in 12 to 1 in 24 hypothyroid infants (1 in 50,000 to 1 in 100,000 newborns) will have a screening TSH level of less than 20 mU/L, with a delayed postnatal increase to hypothyroid levels (Hyman et al., 2007). Therefore any infant with suspicious screening or sampling results requires confirmatory testing with measurement of serum fT_4 , T_4 , and TSH concentrations (Rose et al., 2006).

The biochemical profile of hypothalamic–pituitary hypothyroidism comprises low serum T_4 concentration with a normal TSH value (1 in 80,000 to 1 in 100,000). A similar biochemical profile characterizes TBG deficiency (1 in 5000). However, the two states can be differentiated by further assessment of TBG and fT_4 levels. A low serum TBG concentration and a normal fT_4 level identify patients with TBG deficiency. In contrast, a low or low-normal fT_4 level and a normal TBG level identify a patient with hypothalamic–pituitary hypothyroidism. An infant with a low fT_4 concentration needs to be carefully examined for evidence of hypothyroidism, and other tests of pituitary function should be conducted. TRH stimulation testing can be used to differentiate between primary and central hypothyroidism. TRH, however, is not available in the United States. A subnormal TSH response to TRH or a normal but delayed and prolonged TSH response to TRH is indicative of central hypothyroidism (Van Tijn et al., 2008). TSH deficiency may be isolated or associated with other pituitary hormone deficiencies. Frequently it is not possible to discriminate between secondary and tertiary hypothyroidism, so the most useful diagnostic term is *central hypothyroidism*. The presence of midline facial abnormalities, hypoglycemia (growth hormone and/or adrenocorticotrophic hormone deficiencies), microphallus (gonadotropin and growth hormone deficiencies), nystagmus or blindness, or polyuria (antidiuretic hormone deficiency) should suggest the possibility of a hypothalamic abnormality. Septo-optic dysplasia, often associated with pituitary hormone deficiencies, can manifest itself as CH. Mutations in *HESX1* have been described in septo-optic dysplasia. Alternatively, multiple pituitary hormone deficiencies suggest a genetic defect in the cascade leading to fetal pituitary formation, such as in *PROP1*, *LHX3*, *LHX4*, and *POU1F1*.



• **Fig. 98.7** Algorithm for evaluating congenital hypothyroidism (CH) on the basis of newborn screening results. fT_4 , free thyroxine; LBW, low birthweight; NICU, neonatal intensive care unit; T_4 , thyroxine; TBG, thyroxine-binding globulin; TRBAbs, thyroid stimulating hormone receptor–blocking antibody; TSH, thyroid-stimulating hormone; TSH-R, thyroid stimulating hormone receptor; VLBW, very low birthweight. (Modified from Rose SR, Brown RS, Foley T, et al. Update of newborn screening and therapy for congenital hypothyroidism. *Pediatrics*. 2006;117:2290–2303.)

Isolated TRH deficiency may cause low-normal T_4 and low-normal TSH levels. Mutations have been identified in the TRH gene, TRH receptor gene, and the gene encoding the β subunit of TSH.

Newborns who are born before term, of low birthweight (<2500 g) or very low birthweight (<1500 g), and ill are found among those with this set of laboratory values. In neonates/infants, inhibition of TSH causing low T_4 concentration can result from dopamine infusions or high-dose glucocorticoids (Rose et al., 2006).

A strategy of second screening should be considered for the following conditions: preterm neonates; low birthweight or very low birthweight neonates; ill newborns admitted to the neonatal intensive care unit; specimen collection within the first 24 hours of life; and multiple births.

Thyroid Dysgenesis

Eighty-five percent of cases of permanent CH are associated with abnormal development of the thyroid gland, which includes agenesis, ectopy, or hypoplasia of the gland. Thyroid ectopy accounts for approximately two-thirds of cases worldwide (LaFranchi, 1999a). There is female predominance associated with ectopic glands. Ectopic locations of the thyroid gland include lingual, neck, and substernal locations. Most cases are sporadic; however, more recent studies show evidence of genetic factors being involved in the pathogenesis.

A unique combination of transcription factors control the embryonal development of the thyroid gland. Mutations in the genes encoding these transcription factors can lead to varying degrees of abnormalities, from athyreosis to normal glands. Other clinical features can be associated as noted in Table 98.3. Thyroid ectopy, the most common form of thyroid dysgenesis, remains unexplained.

TABLE 98.3 Genes and Thyroid Development

| Gene | Thyroid Phenotype | Other Features |
|---------------------|----------------------------|---|
| <i>TTF-2/FOXE-1</i> | Athyreosis | Cleft palate, choanal atresia, kinky hair, bifid epiglottis |
| <i>TTF-1/NKX2.1</i> | Athyreosis to normal gland | Respiratory distress syndrome, developmental delays/hypotonia, ataxia/choreoathetosis |
| <i>PAX-8</i> | Athyreosis to normal gland | Cysts within thyroid remnants, kidney and urinary tract malformations |
| <i>GLIS3</i> | Athyreosis to normal gland | Congenital glaucoma, deafness, liver/kidney and pancreatic abnormalities |
| <i>TSHR</i> | Athyreosis to normal gland | None |
| <i>NKX2.5</i> | Athyreosis, ectopy | Cardiac defects |

FOXE-1, Forkhead box E1; *GLIS3*, GLIS family zinc finger 3; *PAX-8*, paired box 8; *TSHR*, thyroid stimulating hormone receptor; *TTF-1*, transcription termination factor 1; *TTF-2*, transcription termination factor 2.

Mutation in *NKX2-5*, which encodes a transcription factor involved in heart morphogenesis, has also been reported to be associated with thyroid dysgenesis (Dentice et al., 2006).

Extrathyroidal abnormalities occur at a higher frequency in children with CH. The most frequent malformations associated with thyroid dysgenesis are cardiac, largely septation defects (Devos et al., 1999). Other relatively common malformations include anomalies of the gastrointestinal tract, nervous system, and eyes (Olivieri et al., 2002; Kreisner et al., 2005; Gu et al., 2009).

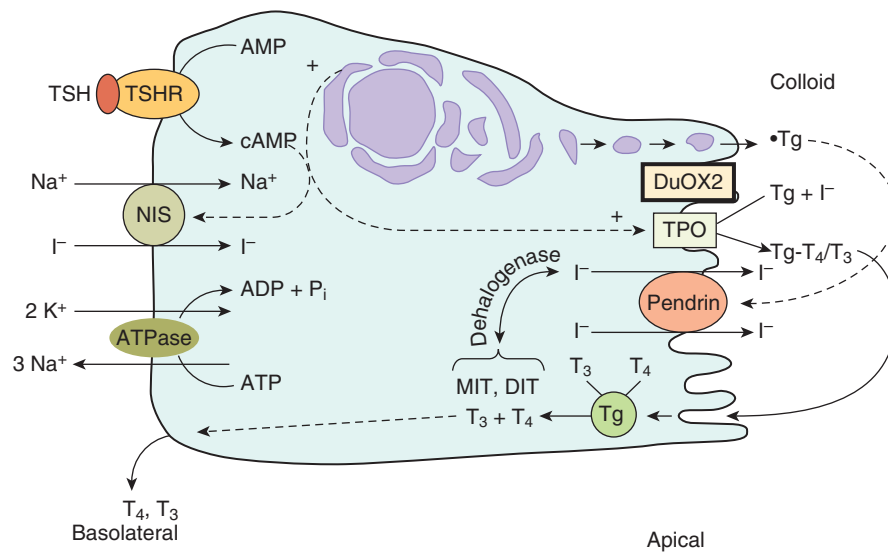
The biochemical picture can differ depending on the amount of thyroid tissue remaining. The presence of residual thyroid tissue is evident by detectable TG levels and normal or near-normal T_3 levels with low T_4 and elevated TSH values. The presence of residual thyroid tissue can be confirmed with a thyroid scan.

Thyroid Dyshormonogenesis

The remaining 10%–15% of cases of CH involve defects in synthesis of thyroid hormone. Hereditary defects in virtually all of the steps of thyroid hormone synthesis, secretion, and action have been described (Fig. 98.8). These patients often have a normally located and normal-sized gland. Thyroid enlargement can present at birth or even before, but more commonly goiter develops later in life. The inheritance pattern is autosomal recessive (Topaloglu, 2006).

Defects associated with trapping of iodide or in the oxidation or organification of iodide can result in dyshormonogenesis. These mutations include:

1. Sodium–iodine symporter defect. The gene encoding the sodium–iodine symporter is *SLC5A5* on chromosome 19. Hypothyroidism presents most frequently in the neonatal or infantile period. Patients have goiter on physical examination or ultrasound imaging, contrasting with limited or absent uptake on scintigraphy.
2. Thyroid peroxidase (TPO) defect. TPO is the enzyme responsible for iodide oxidation, organification, and iodothyrosine coupling. Mutations in the thyroid peroxidase gene are the most common cause of thyroid dyshormonogenesis. The enzyme is located at the apical membrane of the follicular cell. The clinical presentation is permanent goitrous, CH, high uptake on thyroid scintigraphy, and a high serum TG level.
3. Defects in H_2O_2 generation. Dual oxidase 1 and dual oxidase 2 are enzymes that generate H_2O_2 at the apical membrane.
4. TG defect. TG is an iodinated glycoprotein. The TG gene is located on chromosome 8. TG serves as the matrix for the synthesis of T_4 and T_3 and the storage of thyroid hormone and iodine. It is the essential substrate for organification and the major protein component of the colloid in the follicular lumen. Goiter and hypothyroidism manifest themselves at birth or before, and patients have low or undetectable TG levels.
5. Pendred syndrome is an autosomal recessive disorder associated with a partial defect in iodine organification, goiter, and sensorineural deafness. It is caused by mutations in the *SLC26A4* gene, on chromosome 7, which encodes the chloride–iodide transporter pendrin. The gene is expressed in the cochlea, thyroid, and kidney. Pendrin functions to maintain the endocochlear potential and is involved in the apical efflux of iodide in the thyroid follicular cells (Kopp et al., 2008). The thyroid phenotype is usually mild and is seldom identified by neonatal TSH screening. Goiter development and hypothyroidism are variable and depend on nutritional iodine intake.
6. Dehalogenase defects. MIT and DIT, the main iodinated by-products of thyroid hormonogenesis, are deiodinated in the



• **Fig. 98.8** A thyroid follicular cell, indicating areas of possible defects in thyroid hormone synthesis (e.g., thyroid dysmorphogenesis). *ADP*, Adenosine diphosphate; *AMP*, adenosine monophosphate; *cAMP*, cyclic AMP; *ATPase*, adenosine triphosphatase; *DIT*, diiodothyronine; *DuOX2*, dual oxidase 2; *K⁺*, potassium ion; *MIT*, moniodothyronine; *Na⁺*, sodium ion; *NIS*, sodium-iodide symporter; *P_i*, inorganic phosphate; *T₃*, triiodothyronine; *T₄*, thyroxine; *Tg*, thyroglobulin; *TPO*, thyroid peroxidase; *TSH*, thyroid stimulating hormone; *TSHR*, thyroid stimulating hormone receptor. (Modified from Van Vliet G, Deladoey J. Disorders of the thyroid in the newborn and infant. In: Sperling MA, ed. *Pediatric Endocrinology*. 4th ed. Philadelphia, PA: Saunders; 186–208.)

thyroid cell by dehalogenase. In this way iodine is conserved within the thyroid gland for another cycle of synthesis of thyroid hormone (Topaloglu, 2006). Patients with iodothyronine dehalogenation defects have goiter and CH. The diagnostic hallmark is the presence of MIT and DIT in large amounts in the urine.

Central Hypothyroidism

Congenital central hypothyroidism is uncommon, and isolated TRH/TRH receptor/TSH β subunit deficiency is exceedingly rare. In the United States the combined incidence of secondary and tertiary hypothyroidism is about 1 in 80,000 to 1 in 100,000 births. Studies in the Netherlands suggest a higher incidence of 1 in 21,000 (Van Tijn et al., 2005; Kempers et al., 2006). Congenital central hypothyroidism is often associated with mutations in the gene that encodes transcription factors involved with pituitary development, including *Pit-1*, *LHX3*, *LHX4*, *POU1F1*, and *HESX1*, and can be associated with other hormone deficiencies.

Other midline facial, cranial, or intracranial defects should suggest the possibility of hypopituitarism, including altered functioning of the HPT axis. Septo-optic dysplasia, often associated with pituitary hormone deficiencies, can be manifested as secondary or (more commonly) tertiary hypothyroidism. Clinical symptoms of hypopituitarism, such as neonatal hypoglycemia (from growth hormone and adrenocorticotrophic hormone deficiencies), polyuria (from antidiuretic hormone deficiency), or a small phallus in boys or undescended testicles (from gonadotropin deficiency), whether or not accompanied by the presence of blindness, congenital nystagmus, or midline defects of the brain, should alert the physician to suspect septo-optic dysplasia. Prolonged jaundice, with unconjugated hyperbilirubinemia in CH and conjugated hyperbilirubinemia in adrenocorticotrophic hormone or cortisol deficiency can also be seen.

The consequences of central hypothyroidism are as neurologically devastating as those of primary hypothyroidism and therefore also require prompt treatment (Van Tijn et al., 2005). However, many newborn screening programs that use elevated TSH values to recognize thyroid dysfunction are inadequate in identifying newborns with central hypothyroidism.

Central hypothyroidism may be acquired during difficult delivery from traumatic brain injury; however, the fT₄ level may not become abnormal until 2 weeks of age.

The biochemical picture comprises normal or low TSH levels with low or low-normal T₄ levels and low fT₄ levels. Other laboratory evidence includes an abnormal TSH surge of less than 50% and a subnormal TSH response to a TRH stimulation test. Attention to other clinical features such as hypoglycemia, microphallus, and midline facial defects can lead to earlier identification of central hypothyroidism.

Down Syndrome and the Thyroid

The association of thyroid abnormalities in individuals with Down syndrome has been well known for several decades, with early case reports of hypothyroidism in individuals with trisomy 21 dating back to the 1960s (Daniels and Simon, 1968; Aarskog, 1969). Examination of fetal thyroid glands showed small thyroid follicles and that TSH concentration was consistently in the highest percentiles. This may suggest that the cause of thyroid problems in Down syndrome may be thyroidal in nature (Luton et al., 2012). Thyroid disorders seen in neonates and infants include overt CH (elevated TSH level with low T₄ level occurring at birth often detected by neonatal screening), subclinical hypothyroidism (elevated TSH level with normal T₄ levels), which can be congenital or acquired, acquired primary hypothyroidism (autoimmune or nonautoimmune), and rarely hyperthyroidism. Compared with the general population, CH is seen

more commonly in Down syndrome, with prevalence estimates ranging from 1.5% to 6.1% (King et al., 2014). Hypoplasia of the thyroid gland appears to be the commonest occurrence, whereas thyroid agenesis, dysgenesis, and ectopic thyroid are rare causes of CH (Kariyawasam et al., 2015). When the diagnosis of CH is clear with hyperthyrotropinemia and low T_4 level, treatment should be initiated and guidelines followed as for neonates without Down syndrome. What is more difficult to determine is the natural history and benefit of treatment in those with subclinical hypothyroidism. In one study, Claret et al. (2013) found that subclinical hypothyroidism resolved in 73.6% of young children with Down syndrome. The rate of remission was more significant in those without positive thyroid autoantibodies and without an enlarged thyroid gland. Evidence to support treatment of subclinical hypothyroidism includes improved growth (Sharav et al., 1988; Karlsson et al., 1998) and better cognitive and developmental outcomes (Van Trotsenburg et al., 2005). Also, there is little in the way of adverse effects of LT_4 treatment when thyroid function is being normalized. However, proponents of not treating subclinical hypothyroidism cite studies such as that of Claret et al. in which a large majority of those with subclinical hypothyroidism progress back to normal thyroid function. Hyperthyroidism is exceedingly rare in the newborn period, with onset most common in the adolescent years. Prevalence estimates for hyperthyroidism in the Down syndrome population range from 0% to 3% (King et al., 2014).

Consumptive Hypothyroidism

In infants with hepatic hemangiomas and in adults with hemangioendothelioma and malignant fibrous tumors, consumptive hypothyroidism may occur (Huang et al., 2000; Guven et al., 2005; Ruppe et al., 2005). During infancy, hemangiomas occur at a high frequency. Rapid growth during the first year of life can be observed, followed by involution and gradual regression by adolescence. The first year of life is a critical time for development (Huang et al., 2000). There is concern for neurologic damage in infants with hemangiomas when hypothyroidism is occult and untreated (Huang et al., 2000).

Hypothyroidism in hemangiomas involves increased expression of type 3 DI, and this action accelerates the rate of inactivation of thyroid hormone (Huang et al., 2000; Bessho et al., 2010). Type 3 DI catalyzes the conversion of T_4 to rT_3 and T_3 to DIT. rT_3 and DIT are inactive metabolites. Type 3 DI is particularly expressed in placenta, uterine endometrium, and neurons of the central nervous system (Larsen et al., 2008). Because of the high rate of thyroid hormone degradation, the hypothyroidism can be refractory to thyroid hormone treatment (Huang et al., 2000). These patients required very high doses of LT_4 and liothyronine to reduce serum TSH concentrations to normal (Huang et al., 2000). The presenting thyroid function tests showed low T_3 and T_4 levels and elevated TSH and serum rT_3 levels. With regression of the hemangioma, the thyroid hormone levels normalize (Huang et al., 2000).

Transient Primary Hypothyroidism

Transient CH is associated with excess maternal iodine ingestion or deficiency, maternal ingestion of antithyroid drugs, maternal TRBAs, heterozygous mutations of *DUOX2*, and large congenital hepatic hemangiomas (increased type 3 DI activity) (LaFranchi, 2011).

Hypothyroidism in the newborn period can be transient in 10% of cases (Rose et al., 2006). Studies in the Netherlands reported an incidence of 1 in 12,000 (Kempers et al., 2006). The newborn screen is initially abnormal, with a low T_4 level and a slightly elevated TSH level. However, repeated laboratory evaluation may demonstrate normalization of thyroid function test results, indicating a transient state of thyroid dysfunction. Because normalization may not occur for several months, thyroid hormone therapy should be initiated for protection of the infant's brain development if the TSH level remains elevated at 2 weeks of life. Therapy should be continued until age 3 years, when the child should be reevaluated. Transient hypothyroidism is more common in preterm infants.

Transplacental passage of maternal TRBAs (also known as *TSH-binding inhibitory immunoglobulins*) is estimated to cause transient thyroid dysfunction in 2% of newborns with CH (Brown et al., 1996). This transient thyroid dysfunction is often difficult to differentiate from permanent forms of hypothyroidism in the newborn period. These babies should be treated but will not require lifelong treatment.

Although many women with autoimmune disease are receiving thyroid replacement therapy, some may be clinically euthyroid and not receiving medications. Some of these women actually have hyperthyroidism or a history of Graves disease. Therefore diagnosis of thyroid dysfunction may be delayed until the postpartum period (Brown et al., 1996).

Maternal autoimmune thyroid disease may be associated with the production of TRBAs, which are a type of immunoglobulin G. TRBAs, like other immunoglobulins G, do not cross the placenta until after 16 weeks of gestation. TRBAs can cross the placenta and block TSH receptor in neonatal thyroid. Therefore the TRBAs do not affect thyroid embryogenesis, and infants do not develop permanent abnormalities in thyroid function (McKenzie and Zakarija, 1992). The severity and duration of the hypothyroid state in these newborns correlate with the initial titer of the blocking antibody and the duration of its presence in the infant's blood (Brown et al., 1996). Subsequent offspring remain at risk, because the antibodies can persist for up to 7 years in maternal sera (Brown et al., 1996).

The biochemical profile is that of CH, comprising low T_4 levels with elevated TSH levels that subsequently normalize. A thyroid scan is not helpful because the TRBAs are sufficiently potent to block TSH-induced uptake, which can be misleading and suggestive of thyroid agenesis. The distinguishing feature is the presence of TRBAs in newborn and maternal sera. However, routine screening for TRBAs is not currently indicated. This diagnosis should be suspected in any infant with CH born to a woman who has a history of autoimmune disease or if her previous offspring had thyroid disease (Brown et al., 1996).

Euthyroid Sick Syndrome

In acutely ill patients, thyroid function changes occur. It is hypothesized that alterations in thyroid hormone occur as an adaptive response to decreased basal metabolic rates in severely ill patients. The syndrome has been observed in sick infants and children (Carrascosa et al., 2008). These patients may have acute or chronic nonthyroidal illnesses. There is a decrease in T_3 activation in the periphery. The consistent finding is an abnormally low serum T_3 level, an increase in the rT_3 level, and reduction in TSH secretion. Normal TSH secretion can also be observed during mild or moderate illnesses. However, the TSH level is inappropriately low in the context of low serum T_3 levels (Larsen et al., 2008). T_4

level may be low or normal and fT_4 level may be normal, depending on the metabolic clearance rate of T_4 .

In preterm infants, T_4 , fT_4 , and T_3 levels are naturally lower than those in term infants, and rT_3 level is high (Fisher, 2007). Therefore thyroid tests in preterm infants may be hard to interpret especially when infants are sick from nonthyroidal diseases. The neonates most commonly with euthyroid sick syndrome are preterm infants with respiratory distress syndrome (Fisher, 2007; Tanaka et al., 2007). In the pediatric population, nonthyroidal illnesses associated with this syndrome include severe gastroenteritis, acute leukemia, anorexia nervosa, renal disease, burns, and surgical stress.

Euthyroid sick syndrome is found in patients with metabolic stress (e.g., diabetic ketoacidosis) and in pediatric patients who undergo cardiac surgery. There is a sharp rise in rT_3 level and a less dramatic fall in T_3 level by 2 hours after cardiac surgery. The rT_3 level returns to normal before the T_3 level. The changes in thyroid function test results are a continuum (Larsen et al., 2008). There is an inverse relationship between the severity of illness and the T_3 level. In euthyroid sick syndrome, abnormal thyroid function gradually reverts to normal function as the patient's primary illness resolves (Foley, 1994). During recovery, TSH level may be transiently elevated (up to 15 mU/L). Treatment with thyroid hormone is not indicated in these patients.

Transient Hypothyroxinemia of Prematurity

In preterm infants, postnatal thyroid hormone concentrations may differ from those in term infants. The thyroid hormone concentrations vary depending on the degree of prematurity. There are multiple causes, such as loss of maternal transfer of T_4 (Morreale de Escobar and Ares, 1998), immaturity of the HPT axis (Murphy et al., 2004), peripheral metabolism of iodothyronines (Pavelka et al., 1997), iodine deficiency (Ares et al., 1997), nonthyroidal illness (Fisher, 2007), and decreased TBG concentrations secondary to undernutrition or hepatic dysfunction (Klein et al., 1997). Thyroid metabolism can be affected by other factors: exogenous sources of iodine, dopamine infusions, blood transfusion, and glucocorticoid treatment.

In preterm infants, HPT immaturity is characterized by a limited neonatal TSH surge, decline in T_4 level in the first week of life, limited TSH response to hypothroxinemia, and a prolonged TSH response to TRH. Impairment of conversion of T_4 to T_3 and increases in type 3 DI activity may also influence thyroid function test results (LaFranchi, 1999b). Only in the third trimester does the hypothalamic TRH level begin to increase markedly (Fisher and Polk, 1989; LaFranchi, 1999b).

In transient hypothyroxinemia in premature infants (before 30 to 32 weeks of gestation), thyroid function tests show low T_4 and fT_4 levels with normal or low TSH levels. Thyroid hormone replacement has not been consistently effective in improving neurologic outcomes or reducing morbidity (Osborn, 2001; Valerio et al., 2004). Research efforts continue to investigate this question. At the current time, therapy is recommended only when a low T_4 level is accompanied by TSH level elevation (Osborn, 2001; Fisher, 2007).

Abnormalities in thyroid levels in preterm infants can be difficult to interpret when the infants are ill from nonthyroidal diseases. Preterm infants at risk should be monitored by serial determinations of fT_4 and TSH. LT4 treatment should be initiated if the illness state is expected to be persistent and TSH level remains elevated for 1 month or longer. A dose of 4–5 μ g of orally administered LT4 per kilogram was suggested in earlier dose studies (Amino and Hidaka, 2006).

Low Triiodothyronine Syndrome in Premature Infants

Changes in thyroid function test results during neonatal adaptation are qualitatively similar to those in term infants but occur at lower concentrations in premature infants. The neonatal surge and T_4 and T_3 peak responses diminish with decreasing gestational age (LaFranchi, 1999b). Premature infants have an increased susceptibility to neonatal morbidity, including birth trauma, acidosis, hypoxia, hypoglycemia, hypocalcemia, and infection, all superimposed on feeding disorders and relative malnutrition. All of these factors tend to inhibit peripheral T_4 to T_3 conversion. This action leads to the characteristic low T_3 state seen in premature infants. Serum T_3 values may remain low in these infants for 1 to 2 months. Other features of low T_3 syndrome in premature infants are variable but usually include elevated serum rT_3 levels and normal or low total serum T_4 concentrations. The fT_4 levels are usually in the range of those of healthy premature infants or of infants of matched gestational age and weight (LaFranchi, 1999b). TSH values are low in these infants. Treatment with thyroid hormone is not warranted (Osborn, 2001; Valerio et al., 2004).

Iodine Deficiency

Severe iodine deficiency is associated with cretinism. Iodine deficiency is a rare cause of transient hypothyroidism in North America but may occur in women who are dieting and thus not eating bread or using salt. Many countries have initiated salt iodination.

Iodine is a critical component of thyroid hormone synthesis; therefore even mild to moderate forms of iodine deficiency can result in an adverse outcome for the fetus. Although the mother with iodine deficiency may be clinically euthyroid with normal T_3 levels, maternal T_4 concentration is low in iodine deficiency. An increase in type 2 DI activity is detected in the fetus in response to iodine deficiency. Because normal development of the fetal neocortex is dependent on maternal T_4 , which is the primary source of cerebral T_3 , low levels of maternal T_4 place the infants at risk of neurologic cretinism. The fetus can experience several neurologic manifestations, such as deafness, motor deficits (spasticity, trunk rigidity, flexion dystonia, and muscle wasting), and mental retardation. Therefore it is imperative for the pregnant mother to receive an adequate supply of iodine early in pregnancy to avoid brain damage in the fetus (Bernal, 1999; Abalovich et al., 2007). Vitamin supplements that contain at least 250 μ g iodine should be used daily (Abalovich et al., 2007).

Preterm infants are at increased risk of iodine deficiency. The premature separation from the maternal supply of iodine and thyroid hormone prevents the preterm infant from accumulating adequate amounts of intrathyroidal hormone. Therefore the infant is unable to keep up with postnatal thyroid hormone demands. The biochemical profile of iodine deficiency in the newborn comprises low T_4 and elevated TSH levels. Iodine deficiency can lead to a transient state of thyroid dysfunction. An infant is sensitive to maternal iodine nutrition during fetal development.

The thyroid is also protected against iodide excess that might otherwise lead to hyperthyroidism. The sources of excess iodide are pharmaceutical: amiodarone, povidone–iodine, and radiographic dyes. The quantity of iodine organified to TG displays a biphasic response to increasing doses of iodide, at first increasing and then decreasing. The decreasing yield is termed the *Wolff–Chaikoff effect*.

The inhibition of iodothyronine formation is reduced over time, the escape phenomenon. This does not occur in the third-trimester fetus, so long-term high iodine intake must be avoided because it will cause fetal hypothyroidism.

Disorders of Thyroid Hormone Carrier Protein

Thyroid hormone in the circulation travels bound to transport proteins (TBG, TBPA, and albumin). As discussed previously, these carrier proteins are produced by the liver. The gene for TBG, the primary transport protein, is located on the long arm of the X chromosome; thus TBG defects are inherited in an X-linked manner. Given that TBG is the major transport protein, TBG deficiency causes significant thyroid changes in total thyroid hormone concentration. But given that free concentrations remain normal, these individuals are euthyroid. Treatment is not required. Primary T_4 newborn screening methods may be misleading, and confirmation with fT_4 levels is necessary before initiation of treatment.

Thyroxine-Binding Globulin Deficiency

The prevalence of TBG deficiency ranges from 1 in 2500 to 1 in 12,000 newborns (Mandel et al., 1993; Bhatkar et al., 2004; Larsen et al., 2008). The frequency in males is much higher, 1 in 2400 to 1 in 2800 males because it is an X-linked trait (Mandel et al., 1993; Abalovich et al., 2007). The transmission of this trait is usually from affected males to female offspring (Larsen et al., 2008). TBG deficiency has no clinical importance but leads to abnormal laboratory test results. Serum TBG levels are very low in affected males and are approximately half of normal in carrier females. In about half of the families with this trait, the TBG level shown by radioimmunoassay is very low. In the other half, the defect is partial; serum T_4 levels vary similarly. Affected individuals are euthyroid, with normal serum TSH responses to exogenous TRH. Treatment is not indicated (Bhatkar et al., 2004).

As many as 26 different mutations have been reported in the TBG gene. These mutations have included a single amino acid substitution or deletion leading to abnormal posttranslational processing (Larsen et al., 2008). Two novel mutations in the TBG gene identified most recently include a thymine (T) insertion at the beginning of intron 1 between nucleotides 2 and 3 and a T deletion in exon 1 leading to a truncated protein. Both mutations fail to produce a functional TBG molecule (Mannavola et al., 2006). In partial TBG deficiency, the defects are associated with altered TBG binding of T_4 (Larsen et al., 2008).

Females tend to have partial TBG deficiency whereas males generally have complete TBG deficiency. A partial deficiency comprises reduced TBG levels and normal TSH and fT_4 levels, with T_4 values at the lower limit of normal. A complete deficiency comprises undetectable TBG levels with normal TSH levels, normal fT_4 levels, and low levels of T_4 . Another useful laboratory tool is T_3RU , which is elevated in TBG deficiency (Noguchi et al., 1993). However, this is an indirect measure of TBG levels.

Thyroxine-Binding Globulin Excess

The prevalence of TBG excess is estimated to be 1 in 15,000 to 1 in 25,000 individuals. TBG excess is inherited as an X-linked

trait (Bhatkar et al., 2004). Individuals with increased levels of TBG have increased total serum T_4 concentrations, with normal TSH and fT_4 levels. Serum T_3 level is modestly increased. These individuals are euthyroid. In these individuals, TBG production rates and serum levels are correlated, suggesting that the mechanism for high TBG levels is increased production, presumably by the liver. TBG levels are increased fourfold to fivefold in affected individuals. Carrier females have intermediate serum TBG values (Larsen et al., 2008).

Familial Dysalbuminemic Hyperthyroxinemia

Familial dysalbuminemic hyperthyroxinemia is characterized by almost a 60-fold increase in the affinity of albumin for T_4 but not for T_3 . Mutations in the *ALB* gene (which encodes albumin) are inherited in an autosomal dominant manner (Larsen et al., 2008). The biochemical profile demonstrates increased serum T_4 concentrations but normal fT_4 , total serum T_3 , and TSH levels. Although binding of T_4 to albumin is increased, T_3 is less avidly bound, accounting for the preferential increase in serum T_4 concentration. Patients with this disorder are euthyroid with normal thyroid hormone production rates (Cartwright et al., 2009).

Diagnosis is confirmed by protein electrophoresis of serum containing labeled T_4 . The fraction of T_4 label associated with TBG, thyroid hormone binding by human serum prealbumin (TBPA), or albumin is measured. The amount of albumin-bound T_4 can be calculated and related to normal values. Measurement of TBG and TBPA concentrations is useful; antithyroid therapy is not necessary, and these measurements help avoid the misdiagnosis of hyperthyroidism (Stockigt et al., 1986).

Treatment of Hypothyroidism

Treatment of hypothyroidism relies on replacement with exogenous thyroid hormone. LT4 is the drug of choice because of its uniform potency and reliable absorption (Rose et al., 2006). Appropriate doses of synthetic T_4 produce normal serum levels of T_3 via peripheral conversion. The best guide to adequacy of therapy is periodic measurement of circulating levels of T_4 , fT_4 , and TSH. Treatment with LT4 should be started as soon as possible and no later than the first 2 weeks of life. History and physical examination are important in the follow-up evaluation, but mild hypothyroidism or hyperthyroidism cannot always be excluded on clinical grounds.

The usual starting dosage of thyroid hormone for hypothyroid infants is 10–15 $\mu\text{g}/\text{kg}$ per day, which approximates to 100 $\mu\text{g}/\text{m}^2$ per day. Infants with severe disease, as defined by a very low T_4/fT_4 concentration, should be treated with the highest initial dose, and those with mild to moderate hypothyroidism should be treated with a lower dose. If intravenous treatment is necessary, the dose should be no more than 80% of the oral dose. The aim of treatment is to keep the T_4 level in the upper half of the normal range, approximately 10–16 $\mu\text{g}/\text{dL}$ or the fT_4 level in the 1.4–2.3 ng/dL range, with the TSH level on the lower half of the normal range (0.5–2.0 mU/L), during the first 3 years of life. Recent studies have reported neurodevelopmental benefits of quick normalization of T_4 and fT_4 levels within 3 days of initiation of thyroid hormone therapy with high-dose treatment. Thyroid hormone requirements, however, quickly drop after 2 weeks, and therefore close monitoring of growth and development along with thyroid function tests is

necessary (Salerno et al., 2002; Selva et al., 2002). The treatment of each patient must be individualized. The adequate dosage of thyroid hormone in the first year usually ranges between 25 and 50 μg daily. Only LT₄ tablets should be used; currently there are no liquid formulations licensed by the US Food and Drug Administration. LT₄ suspensions prepared by individual pharmacists may lead to an unreliable dosage (Rose et al., 2006). The tablet is crushed and given orally in a small amount of liquid. Care should be taken to avoid concomitant administration of soy, fiber, calcium, or iron. The thyroid hormone–pituitary feedback setpoint is altered in rare infants with CH, and in such infants, serum TSH concentration remains elevated in the face of a normal or even elevated serum T₄ level (Fisher et al., 2000).

Infants with presumably transient hypothyroidism resulting from maternal goitrogenic drugs need not be treated unless the low serum T₄ and elevated TSH levels persist beyond 2 weeks of age. Infants with TRBAb-induced hypothyroidism may require treatment for as long as 6 months.

When infants with severe myxedema associated with fluid retention are being treated, potential complications should be kept in mind. Cardiac insufficiency caused by overtaxing of the myxedematous heart, through too rapid a mobilization of the myxedema fluid into the circulation, is well known in the adult. This complication in older children and adults is prevented by administration of a small dose of thyroid hormone at first, followed by gradual increase of the dose. However, infants generally tolerate a rapid restoration to the euthyroid state better than adults, and a prompt restoration of T₄ concentration to a normal value is important for the recovery of brain development and maturation. Nevertheless, excessive thyroid hormone therapy must be avoided, and the dose must be adjusted judiciously if there is evidence of severe myxedema, particularly of the heart.

After initiation of therapy with LT₄, the growth rate should accelerate. Any growth deficit is commonly restored within a few months. Bone age is a sensitive index of thyroid deficiency; however, radiographs are not routinely obtained in the newborn period. Overtreatment can induce tachycardia, excessive nervousness, disturbed sleep patterns, and other problems suggesting thyrotoxicosis. Excessive thyroid hormone administered over a long period can produce premature synostosis of cranial sutures and advancement of bone age.

During the first few years of life, patients should be monitored frequently: the first follow-up examination should occur 2 to 4 weeks after the start of LT₄ treatment. Subsequent evaluations should occur at least every 1 to 2 months during the first 6 months of life, every 3 months between 6 months and 3 years, and then twice a year until growth is completed (Box 98.1; LaFranchi, 2011). Evaluations should occur at more frequent intervals when adherence is questioned, abnormal values are obtained, or the dose of medication has been changed. Clinical observation should be supplemented with monitoring of the growth curve and T₄, fT₄, and TSH levels. Because poor adherence and nonadherence have major sequelae, the initial and ongoing counseling of parents is of great importance.

Patients with permanent CH (e.g., dysgenesis, dyshormonogenesis) require lifetime substitution therapy. After age 3 years, if there is uncertainty about whether the disease is permanent or transient or if the dose of LT₄ has not required an increase, discontinuation of LT₄ therapy for 4 to 6 weeks, with close monitoring of the TSH level, should distinguish transient from permanent CH. Recent reports indicate that treatment of CH is discontinued within 3 years in more than one-third of children with CH. This

•BOX 98.1 Management of Congenital Hypothyroidism

Initial Work-Up

Detailed history and physical examination
Referral to pediatric endocrinologist
Recheck serum TSH and FT₄ levels
Thyroid ultrasonography and/or thyroid scan (see text for recommendations)

Medications

LT₄: 10–15 $\mu\text{g}/\text{kg}$ by mouth once daily

Monitoring

Recheck serum FT₄ (or total T₄) and TSH
Two to 4 weeks after initial treatment has begun
Every 1–2 months in the first 6 months
Every 3 months between 6 months and 3 years of age
Every 6–12 months from 3 years of age to end of growth
Four weeks after a change in LT₄ dosage

Goal of Therapy

Normalize TSH and maintain T₄ and FT₄ in the upper half of the reference range

FT₄, Free thyroxine; LT₄, levothyroxine; T₄, thyroxine; TSH, thyroid-stimulating hormone.

is inconsistent with current guidelines; however, it remains unknown how many of the children in whom treatment was discontinued prematurely experience adverse effects or require continued treatment.

Hyperthyroidism

The overall incidence of neonatal hyperthyroidism is low. It occurs more commonly in pregnancies complicated by Graves disease. One percent of the offspring born to women with Graves disease have hyperthyroidism (Polak, 1998). The thyroid dysfunction in the fetus and newborn is associated with transplacental passage of TSH receptor–stimulating antibodies/TSIs. The mother may have active or inactive Graves disease. The hyperthyroidism is not related to transfer of maternal thyroid hormone. The antibodies stimulate the fetal and neonatal thyroid gland. It is generally a transient state that clinically resolves by 4 months of age with the clearance of maternal antibodies from the infant's circulation (Polak, 1998). Rarely, neonatal hyperthyroidism is caused by mutations in the genes that encode the TSH receptor or its mediators. These causes include activating mutations of the gene encoding the stimulatory G protein, as in McCune–Albright syndrome, or an activating mutation of the gene encoding the TSH receptor (Guerin et al., 2004; Chester et al., 2008).

In pregnancies complicated by maternal Graves disease, the fetal levels of TSI approximate those of the mother at 30 weeks of gestation. Therefore fetal thyrotoxicosis generally manifests itself in the third trimester as fetal tachycardia, fetal goiter, and intra-uterine growth retardation. Fetuses of mothers with TSI levels greater than 250% of the upper limit are at increased risk of thyrotoxicosis. Therefore these fetuses need to be monitored more closely (Zimmerman, 1999).

The clinical manifestations of Graves disease in the newborn include irritability, flushing, diarrhea, vomiting, tachycardia, hypertension, poor weight gain, thyroid enlargement, and exophthalmos. Thrombocytopenia, hepatosplenomegaly, jaundice, and

hyperviscosity syndrome have also been reported. If thyrotoxicity is severe and treatment is inadequate, arrhythmias, congestive heart failure, and death may occur. In some infants the onset of symptoms and signs may be delayed for as long as 8 to 14 days. Late onset of neonatal disease can occur for at least two reasons: (1) postnatal depletion of transplacentally acquired blocking doses of maternal antithyroid drugs and the abrupt increase in conversion of T_4 to active T_3 shortly after birth in the newborn and (2) the presence of maternal TRBAs, which can block the effect of TSH receptor–stimulating antibodies for several weeks (Zimmerman, 1999).

The diagnosis is confirmed by measurement of high levels of T_4 , fT_4 , and T_3 in postnatal blood. Umbilical cord blood values may be normal or near normal, whereas the levels at 2 to 5 days may be markedly increased; the serum TSH concentration is suppressed below normal levels. Neonatal Graves disease resolves spontaneously as maternal TSH receptor–stimulating antibodies in the newborn are degraded. The usual clinical course of neonatal Graves disease is 3–12 weeks.

Fetal thyrotoxicosis is treated by administration of antithyroid agents to the mother. Propylthiouracil (PTU) is recommended during pregnancy, because it is associated with a lower rate of fetal malformations than methimazole (MTZ). PTU crosses the placenta to inhibit the fetal production of excess thyroid hormone (Aslam and Inayat, 2008). Since the concentration of the drug in breast milk is very low, hyperthyroid mothers receiving antithyroid drugs may breastfeed their infants. Medical treatment of hyperthyroidism in the newborn period depends on the severity of the illness. The medications include iodide or antithyroid agents. Antithyroid agents inhibit thyroid hormone synthesis. Iodide rapidly inhibits hormone release.

Lugol's solution (5% iodine and 10% potassium iodide, containing iodine at 126 mg/mL) is given in a dose of one drop (8 mg iodine) three times daily. Antithyroid agents such as MTZ or carbimazole are administered in dosages of 0.5–1 mg/kg per day, divided, at 8-hour intervals. PTU is no longer recommended for use in children because of increasing rates of PTU-induced liver failure (Rivkees and Mattison, 2009). Propranolol can be given in a dosage of 2 mg/kg per day to decrease β -adrenergic symptoms and inhibit deiodination of T_4 to T_3 . A therapeutic response should be observed within 24 to 36 hours. If a satisfactory response is not observed, the dose of antithyroid drug and iodide can be increased by 50%.

More severe cases may require corticosteroids. Steroids suppress deiodination of T_4 to T_3 . Digoxin treatment can be used if cardiac failure is present. If hyperthyroidism persists (as in cases with a strong family history of Graves disease, an activating mutation of

the gene encoding the stimulatory G protein, or an activating mutation of the gene for the TSH receptor), ablative therapy such as thyroidectomy must be performed (Chester et al., 2008).

The treatment goal is to achieve a euthyroid state while avoiding hypothyroidism in the infant. Frequent monitoring to adjust MTZ doses is indicated. Another method to treat hyperthyroidism using antithyroid medication is to completely block the synthesis of thyroid hormone while replacing thyroid hormone.

Suggested Readings

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Disorders of Carbohydrate Metabolism

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KEY POINTS

- Neonatal hypoglycemia requires diagnostic consideration and urgent management to prevent recurrent hypoglycemia and neurologic injury.
- Neonatal metabolism in the first few days of life reflects a transition from the passive glucose consumption of the fetus to the active regulation of glucose in a fasting neonate.
- Diagnosing the cause of hypoglycemia requires an evaluation of the hormonal and metabolic response to hypoglycemia. Patients with hyperinsulinemic hypoglycemia should be assessed for diazoxide responsiveness, and nonresponsive patients should have an evaluation to determine if the process is focal or diffuse.
- Diabetes diagnosed before 6 months of age is very likely to have a genetic cause.
- Patients with neonatal diabetes because of mutations in the ATP-sensitive potassium channel genes can be treated with oral sulfonylurea therapy in place of insulin therapy.

Glucose is the primary metabolic fuel for the neonatal brain, and maintenance of normal glucose levels in the serum and across the blood–brain barrier is essential for normal neurologic function and development. Hypoglycemia in the neonate therefore requires thoughtful diagnostic evaluation and urgent treatment to prevent injury to the central nervous system. The mechanisms underlying neonatal hypoglycemia are best understood as inadequate hormonal and metabolic responses to hypoglycemia occurring in the context of the necessary shift from fetal to neonatal glucose metabolism in the first few days of life. These pathways and disorders are the focus of the initial portion of this chapter.

Hyperglycemia in the neonate most commonly occurs in the context of a physiologic stressor such as sepsis with cortisol and catecholamine release, intravenous (IV) glucose infusion, and exogenous glucocorticoid administration combining to cause hyperglycemia. Rarely, genetic causes of hyperglycemia result in transient neonatal diabetes mellitus (TNDM) or permanent neonatal diabetes mellitus (PNDM), which is the focus of the latter portion of this chapter.

Fetal to Neonatal Transition and Energy Metabolism

Fetal glucose supply is dependent on maternal plasma levels and its diffusion across the placenta, with no evidence for the existence

of fetal gluconeogenesis, or a robust ability to adjust rapidly to maternal hypoglycemia (Menon and Sperling, 1988; Marconi et al., 1993; De Leon et al., 2014). Once the placental link is interrupted and glucose is no longer delivered continuously via the umbilical vein, the neonate must maintain normoglycemia and adequate cerebral glucose delivery despite minimal and sporadic enteral carbohydrate intake during the first 24–72 hours of life. Glucose homeostasis is accomplished in a manner generally similar to that in older children who fast: via secretion of the counterregulatory hormones—namely, cortisol, glucagon, growth hormone (GH), and catecholamines—and their actions at target tissues, in combination with the suppression of insulin secretion. In concert, these hormonal changes regulate four different metabolic systems: glycogenolysis, gluconeogenesis, lipolysis, and ketogenesis (Table 99.1). The end result is to facilitate normoglycemia until carbohydrate intake and absorption occur on a more regular basis.

In the first few hours of life, in response to decreased delivery of glucose, glucagon and catecholamine levels rapidly increase, and insulin level falls. This combination shifts metabolic activity from anabolism to catabolism and induces enzymes necessary for glycogenolysis (glycogen phosphorylase) and gluconeogenesis (pyruvate carboxylase and phosphoenolpyruvate carboxykinase) (Granner et al., 1983; Menon and Sperling, 1988; Girard, 1990). Glycogen breakdown plays the largest role in meeting glucose needs during the first 24 hours (approximately 50%) and causes a depletion of glycogen stores from 50 mg/g of liver at birth to less than 10 mg/g of liver at 24 hours of life (De Leon et al., 2014). Gluconeogenesis develops somewhat more slowly and is not fully active until 8–12 hours of life, providing 20%–30% of glucose needs in the first 24 hours (Girard, 1990; Kalhan et al., 2001).

Gluconeogenesis and lipolysis contribute to plasma glucose levels after 8–12 hours of life, with their role increasing as glycogen stores are depleted. Lipolysis produces glycerol, which can enter into the gluconeogenic pathways, and free fatty acids (FFAs) can be oxidized directly by some organs, including the heart, kidney, and skeletal muscle, but long-chain fatty acids cannot cross the blood–brain barrier (Patel and Kalhan, 1992). Importantly, partial oxidation of fatty acids in the liver via ketogenesis produces ketones such as β -hydroxybutyrate (BOHB) and acetoacetate, which the brain can metabolize. However, ketogenesis is impaired in the first 8–12 hours of life, coincident with the known transitional hypoglycemia of infancy discussed later (Stanley et al., 1979, 2015). The importance of gluconeogenesis, lipolysis, and fatty acid metabolism is highlighted in breastfed infants as the macronutrient profile of colostrum favors protein and fatty acids, compared with

TABLE 99.1 Genetic Causes of Hyperinsulinemic Hypoglycemia

| Inheritance | Molecular Defect | Chromosome | Histologic Appearance | Clinical Features | Treatment |
|-------------|---|-----------------|-----------------------|---|--|
| Sporadic | Paternally inherited <i>ABCC8/KCNJ11</i> mutation with somatic loss of heterozygosity | 11p15 | Focal | Macrosomia, moderate to severe hypoglycemia in the first few days to weeks of life | Poor response to diazoxide, local resection for focal and near-total pancreatectomy for the diffuse form |
| AR | <i>ABCC8/KCNJ11</i> (inactivating) | 11p15 | Diffuse | Macrosomia, onset in the first few days to weeks of life, family history or consanguinity may be present | Near-total pancreatectomy |
| AD | <i>ABCC8/KCNJ11</i> (dominant negative, inactivating) | 11p15 | Diffuse | Milder symptoms, may manifest themselves in late infancy | Usually responsive to diazoxide (Pinney et al., 2008) |
| AD | <i>GLUD1</i> (activating) | 10q23 | Diffuse | Modest hyperinsulinemia and hyperammonemia, onset usually >6 months | Diazoxide, restriction of leucine in the diet |
| AD | <i>GCK</i> (activating) | 7p13 | Diffuse | Modest hyperinsulinemia, onset usually >6 months | May respond to diazoxide |
| AR | <i>HADH</i> | 4q25 | Diffuse | Variable clinical presentation, abnormal acylcarnitine profile | Diazoxide |
| AD | <i>HNF4A</i> or <i>HNF1A</i> | 20q13 and 12q24 | Diffuse | Possible Fanconi tubulopathy. May have family history of MODY | Diazoxide |
| AD | <i>UCP2</i> | 11q13 | Diffuse | | Diazoxide |
| AD | <i>HK1</i> | 10q22 | Diffuse | Variable severity and penetrance | Diazoxide |
| AR | <i>PGM1</i> | 1q31 | | Congenital disorder of glycosylation. Bifid uvula, hepatopathy, hypogonadotropic hypogonadism, poor growth, myopathy, dilated cardiomyopathy (Tegtmeyer et al., 2014) | Galactose supplementation may be beneficial |

AD, Autosomal dominant; AR, autosomal recessive; MODY, maturity-onset diabetes of youth.

the relative carbohydrate predominance of mature human milk (Jenness, 1979).

Glucagon level continues to rise gradually over the first few days of life, coincident with the known gradual increase and stabilization of glucose levels that occur in normal infants by 48–72 hours of life (Lubchenco and Bard, 1971; Sperling et al., 1974).

The brain is the most metabolically active neonatal organ, and its demand for glucose is proportional to brain weight (Bier et al., 1977; Zeller and Bougneres, 1992). Glucose uptake and oxidation in the brain occur via insulin-independent facilitated diffusion through glucose transporter (GLUT) channels and is dependent on arterial blood glucose concentration. An in vivo study using rats (which are believed to have GLUT channels with similar kinetics to those of humans) showed that consumption of glucose in the brain outpaces its replacement via diffusion at an arterial concentration of 36 mg/dL (Choi et al., 2001). At this point cerebral blood flow increases markedly, presumably to prevent severe central nervous system glucose depletion and neurologic sequelae. The relatively large size of the neonatal brain, and its high metabolic demand, is associated with a twofold to threefold higher (per weight) hepatic glucose production than in adults. Conditions that interfere with hepatic glucose production therefore place the infant at risk of hypoglycemia, some of which are discussed briefly in this chapter, and others are discussed in Chapter 22.

In total, the combined counterregulatory response and insulin suppression are similar to the starvation response that occurs in older children, with two exceptions: (1) the additional complication that maternal factors and immaturity of the counterregulatory response can interfere with glucose homeostasis in the neonatal period and (2) the decrease in insulin production and release of glycogen stores are not as pronounced as in older children. These latter factors may explain the “transitional hypoglycemia” seen in normal newborns during the first 24 hours of life.

Transitional Neonatal Hypoglycemia

Plasma glucose values in the first few hours of life are frequently lower than accepted thresholds for normoglycemia in older children, a phenomenon known as *transitional neonatal hypoglycemia*. Serial measurements of glucose in the first few days of life in healthy, term appropriate for gestational age (AGA) infants demonstrate average values in the 50s to low 60s (mg/dL) (Lubchenco and Bard, 1971; Srinivasan et al., 1986). If normal is defined as within two standard deviations from the mean, the lower limit of normal may be as low as the high 30s to low 40s in the first few hours of life (Srinivasan et al., 1986; Hoseth et al., 2000). In support of this, Lubchenco and Bard (1971) showed that if feeding is delayed by 3–6 hours from birth, approximately 10% of healthy, term AGA infants will have glucose levels below 30 mg/dL.

• BOX 99.1 Signs of Neonatal Hypoglycemia**Autonomic**

Sweating
 Pallor
 Tachycardia
 Tachypnea
 Tremor
 "Jittery"

Neuroglycopenic

Hypotonia
 Lethargy
 Coma
 Seizure
 Weak suck
 Abnormal cry (weak, high pitched)

A recent review (Stanley et al., 2015) of the data available on transitional hypoglycemia shows that it is characterized by relative hyperinsulinism (HI) as indicated by hypoketosis and preserved glycogen release in response to glucagon (Stanley et al., 1979). An additional factor may be the time required for the enzymatic machinery of gluconeogenesis and glycogenolysis to become active in response to the rise in glucagon and catecholamine secretion after birth.

It is unknown whether the decline in glucose values or the relative HI seen with transitional hypoglycemia serves an adaptive function. The important diagnostic distinction is that the decline in glucose level is transient. Just 2 of the 374 newborns (0.5%) in the Lubchenco and Bard cohort had a glucose level below 50 mg/dL before feeding on day 3 or 4 of life.

Signs and Symptoms of Hypoglycemia

Neonates with hypoglycemia may have no detectable symptoms and may be identified incidentally only on measurement of blood glucose levels or in the monitoring of a high-risk infant. When symptoms occur, they may be seen in a progression due to initial counterregulatory hormone responses (such as adrenergic hormones as well as cortisol and GH) that result in symptoms referable primarily to the autonomic system (autonomic, or "neurogenic," symptoms and signs). When deficient glucose supply to the brain occurs, neurologic dysfunction is detectable and may be considered the symptom of "neuroglycopenia" (Box 99.1). However, the symptoms may be subtle and difficult to recognize clinically and may be accompanied by other, nonspecific symptoms such as apnea, cyanosis, temperature instability (especially hypothermia), and bradycardia.

It is difficult to define a consistent threshold in the neonate below which hypoglycemia produces the aforementioned symptoms and especially neuroglycopenia. One often-quoted study of 17 children showed changes in auditory evoked potentials below a whole blood glucose concentration of 47 mg/dL (2.6 mmol/L) but only included four neonates with hypoglycemia (aged 1–3 days) (Koh et al., 1988). In those four neonates, three had no clinical signs at the time of the abnormal recorded evoked potentials, and one was drowsy. The challenge of defining a true threshold is underscored in these four neonates given that one of them was asymptomatic with normal evoked potentials at a whole blood glucose level of 1.9 mmol/L (34 mg/dL) on day 1 of life, while another neonate was symptomatic at a whole blood glucose level of 2.5 mmol/L (45 mg/dL).

Monitoring of Blood Glucose

While symptomatic, prolonged hypoglycemia in neonates is a risk factor for cerebral injury and poorer neurodevelopmental outcomes,

• BOX 99.2 Maternal and Neonatal Conditions That Increase the Risk of Neonatal Hypoglycemia

Maternal Conditions

Diabetes (gestational or pregestational)
 Administration of drugs (β sympathomimetics, e.g., terbutaline, oral hypoglycemic agents)
 Intrapartum dextrose infusion
 Hypertension/preeclampsia

Neonatal Conditions

Prematurity
 Intrauterine growth restriction
 Hypoxia–ischemia
 Large/small for gestational age
 Sepsis
 Hypothermia
 Polycythemia
 Presence of syndromic features (microphallus, midline defects, Beckwith–Wiedemann syndrome)

the lower limit of normoglycemia in asymptomatic infants has been difficult to elucidate (Burns et al., 2008; Adamkin, 2016). This is complicated by the pattern of transitional hypoglycemia mentioned earlier, during which plasma glucose values may drop to levels considered very low for older children. Maternal and neonatal conditions with a high risk of hypoglycemia are well known, and it is common for newborn nurseries to have screening protocols for these infants (Box 99.2). Such protocols commonly result in the treatment of asymptomatic infants on the basis of point-of-care glucose values, with limited evidence available to define the optimal threshold that minimizes overtreatment while still preventing neuroglycopenia and neurologic damage.

Multiple definitions of the ideal asymptomatic treatment threshold with regard to long-term neurologic outcomes have been proposed: 47 mg/dL (Lucas et al., 1988), 45 mg/dL (Tin et al., 2012), 40 mg/dL (Kaiser et al., 2015), and 30 mg/dL (Kerstjens et al., 2012). It is unlikely that a single threshold exists, as the point at which neurologic injury occurs is likely patient and situation dependent and related to the availability of ketones and other substrates to the brain.

Recently, a prospective investigation into an appropriate glucose treatment threshold for infants was performed using a prospectively evaluated cohort of 404 infants of gestational age at least 35 weeks who were at risk of hypoglycemia (infant of diabetes mother, birth at <37 weeks' gestational age, birthweight <10th percentile or >90th percentile) (McKinlay et al., 2015). Infants also wore blinded continuous glucose monitoring systems to allow the investigators to evaluate them for outcomes related to subclinical hypoglycemia missed on the intermittent point-of-care checks. Infants were treated to maintain a blood glucose concentration of at least 47 mg/dL for at least the first 48 hours of life. Neurodevelopmental outcomes were then assessed at 2 years of age with the Bayley Scales of Infant and Toddler Development, third edition, and tests of executive and visual function. The study authors found no association between hypoglycemic episodes and neurosensory impairment and concluded that neonatal hypoglycemia was not associated with adverse neurologic outcome when treatment was aimed at maintaining a blood concentration of 47 mg/dL in these high-risk infants.

Recent Pediatric Endocrine Society guidelines make glucose threshold recommendations for infants who are at risk of

hypoglycemia but without a known risk of permanent hypoglycemic disorders, such as HI, hypopituitarism, or an inborn error of metabolism. Such neonates should have a treatment threshold of 50 mg/dL in the first 48 hours. On the basis of evidence that suggests average glucose values in normal neonates older than 48 hours of age are no different from those of older children, [Thornton et al. \(2015\)](#) suggest that these infants should demonstrate an ability to maintain glucose levels above 60 mg/dL during a 6–8-hour fast before discharge ([Thornton et al., 2015](#)).

For diagnostic work-up of hypoglycemia after 48 hours of age, we recommend a glucose threshold of 50 mg/dL for collection of a “critical sample” to assess the neonate for the counterregulatory hormone response, insulin level, acidosis, and the presence of important metabolic substrates such as BOHB, lactate, serum amino acids, and FFAs. Perhaps most importantly, a plasma glucose level should be obtained simultaneously to allow accurate interpretation of the critical sample, as the point-of-care test result may be artificially low.

Normoinsulinemic Hypoglycemia

Insulin's role in maintaining normoglycemia is paramount as its activity lowers serum glucose concentration via glucose uptake through insulin-sensitive GLUTs and represses the effects of counterregulatory hormones whose primary function during hypoglycemia is to increase serum glucose values. It is therefore relevant to divide a discussion of the causes of hypoglycemia by those that are associated with appropriate suppression of insulin during hypoglycemia (normoinsulinemic hypoglycemia) and those that are associated with inappropriate, elevated insulin levels at the time of hypoglycemia (hyperinsulinemic hypoglycemia) ([Box 99.3](#)).

Hypoglycemia in Premature and Small for Gestational Age Infants

Premature or small for gestational age (SGA) infants are at high risk of transient hypoglycemia because of immaturity of the metabolic pathways described earlier, exacerbated by inadequate stores of glycogen and triglycerides. The doubling of average fetal weight from 1700 g at 32 weeks to 3400 g at birth is largely due to the accrual of hepatic glycogen and adipose tissue fat stores, which then serve as important reserves of substrates for energy metabolism in the first few days of life. Hypoglycemia can also be caused by delayed maturation of enzymes necessary for gluconeogenesis. Premature infants can have markedly reduced glucose 6-phosphatase activity relative to term infants that may persist for months after birth ([Hume and Burchell, 1993](#)). There is also a lack of glucose level rise after administration of gluconeogenic precursors, which suggests impaired activity of the enzymes of gluconeogenesis ([van Kempen et al., 2003](#)). For these reasons, SGA and premature infants should be screened for asymptomatic hypoglycemia and supported with IV dextrose or nasogastric feeding until their hypoglycemia resolves. It is important to note that hypoglycemia in these infants may be multifactorial, as HI may be a contributing factor as well (discussed later).

Counterregulatory Hormone Deficiency

Hypopituitarism

Deficiencies of cortisol or GH, or their combined deficiency, in the neonatal period can cause hypoglycemia. Often these two deficiencies occur together in the context of hypopituitarism with

• BOX 99.3 Causes of Neonatal Hypoglycemia

Hyperinsulinemic

Transient:

- Infants of diabetic mothers
- Intrapartum dextrose infusion into mother
- Stress in peripartum/postnatal period: trauma, asphyxia, hypothermia
- Small for gestational age infants

ATP-sensitive potassium channel defects

GLUD1 activating mutation

HADH mutation

GCK activating mutation

HNF1A and *HNF4A* mutations

UCP2 mutations

HK1 mutations

Beckwith–Wiedemann syndrome

Fundoplication (dumping syndrome)

Hyperinsulinism in congenital disorders of glycosylation

β-cell adenoma—multiple endocrine neoplasia type 1

Normoinsulinemic

Transient:

Developmental immaturity in adaptation to fasting: prematurity, small for gestational age

Increased metabolic expenditure: sepsis, erythroblastosis fetalis, polycythemia

Maternal conditions: toxemia, administration of tocolytics (β sympathomimetics)

Hypopituitarism

Primary adrenal insufficiency

Inborn errors of metabolism

Glycogen storage disease

Disorders of gluconeogenesis

Defects in fatty acid catabolism and ketogenesis

Organic acidurias

Galactosemia

Hereditary fructose intolerance

adrenocorticotrophic hormone (ACTH) and GH deficiency. Infants with congenital hypopituitarism often have other signs of midline malformations, such as a midline cleft palate, nystagmus (optic nerve hypoplasia), seizures (holoprosencephaly), direct hyperbilirubinemia (thyroid hormone deficiency), or micropenis and undescended testes in a male (gonadotropin deficiency). Brain magnetic resonance imaging (MRI) may reveal the underlying cause of hypopituitarism, which may range from severe midline malformations such as alobar holoprosencephaly to more subtle abnormalities such as an isolated ectopic posterior pituitary bright spot or a hypoplastic pituitary gland.

Biochemical evaluation of hypopituitarism requires careful consideration in the first few months to year of life. Conventional stimulation tests used to diagnose GH deficiency in older children have been used in infants, but normal responses are not well established. The stimulation test believed to be safest for use in infants is the glucagon stimulation test, which causes a rapid rise then rapid fall in glucose level, placing the infant at risk of hypoglycemia. Therefore this testing should only be done under close monitoring, preferably in an intensive care unit setting ([Carillo and Bao, 2009](#)). As an alternative, taking into account normal physiologic processes in the perinatal period, there is evidence that a single random GH measurement in the first week of life can adequately diagnose GH deficiency ([Binder et al., 2010](#)). Using a post hoc defined threshold of 7 μg/L, Binder et al. demonstrated

a sensitivity of 100% and a specificity of 98% for the diagnosis of GH deficiency.

Similarly, standardized testing to diagnose adrenal insufficiency in the neonatal period is currently a matter of debate. Glucagon stimulation testing can be used to simultaneously assess a neonate for GH and ACTH deficiency but carries the risk of hypoglycemia mentioned earlier. The conventional ACTH stimulation test using Cortrosyn has been used in infants; however, the appropriate dose of 125 µg, 1 µg, or 15 µg/kg has not been well established, and physician preference differs. Importantly, infants with ACTH deficiency will not develop classic salt wasting with hyponatremia and hypokalemia because of intact aldosterone production and secretion, which is regulated not by the pituitary but by the renin–angiotensin system. Hyponatremia may occur but is less severe relative to primary adrenal insufficiency and is likely because of reduced free water clearance, for which both cortisol and thyroid hormone play a role.

An important consideration in patients with hypopituitarism is that thyroid hormone should not be replaced until adrenal insufficiency has been either treated or ruled out, as thyroid hormone replacement will increase the metabolism of cortisol and can precipitate an adrenal crisis if done in the context of undiagnosed adrenal insufficiency.

Isolated Adrenocorticotrophic Hormone Deficiency

Isolated ACTH deficiency is very rare and is associated with mutations in the genes responsible for the production and/or modification of the proopiomelanocortin (POMC) precursor polypeptide. The *TBX19* gene codes for T-box 19, which regulates transcription of the *POMC* gene in corticotrophs, and deficiency has been associated with adrenal insufficiency in infants (Vallette-Kasic et al., 2005). A low estriol level on the prenatal triple-marker screen may be a predictor of *TBX19* mutation and ACTH deficiency in general (Weintrob et al., 2006). Deletions of the *POMC* gene itself affect all POMC peptides, including α -melanocyte-stimulating hormone, resulting in red hair and fair skin, in addition to ACTH deficiency, with severe obesity developing within the first few months of life (Krude et al., 1998). Mutations in *PCSK1* result in impaired cleavage of ACTH from POMC, as well as several gut hormones. Deficiency results in severe neonatal diarrhea and failure to thrive and a high risk of ACTH deficiency as well as panhypopituitarism, including central diabetes insipidus (Martin et al., 2013).

Primary Adrenal Insufficiency

Congenital Adrenal Hyperplasia

Cortisol deficiency caused by disease of the adrenal gland (primary adrenal insufficiency) in infants is most commonly due to congenital adrenal hyperplasia (CAH). The most common form of CAH, 21-hydroxylase deficiency (incidence 1 in 10,000 to 1 in 15,000 annually), results in ambiguous, virilized genitalia in an XX infant because of overproduction of adrenal androgens synthesized from precursors upstream of the enzyme block. However, in a XY infant, CAH due to 21-hydroxylase deficiency is more subtle and may present only with hyperpigmentation of the skin, particularly of the scrotum. The classic presentation of hyponatremia, hyperkalemia, and severe dehydration because of the cortisol and aldosterone deficiency of primary adrenal insufficiency often does not develop until 7–10 days of life, so their absence in a newborn with hypoglycemia should not rule out this condition. Newborn screening for the 21-hydroxylase deficiency form of CAH is now widespread, and thus a diagnosis is often suspected early because of elevated

17-hydroxyprogesterone levels in newborn blood spot samples. Where hypoglycemia occurs in an infant, especially with any of the aforementioned features of salt wasting or ambiguous genitalia, confirmation of a normal 17-hydroxyprogesterone level should be sought. Treatment is with hydrocortisone and fludrocortisone for glucocorticoid and mineralocorticoid replacement, respectively, along with sodium chloride supplementation because of the low salt content of breast milk and most formulas.

More rare forms of CAH include 11 β -hydroxylase deficiency (1 in 100,000 or 1 in 5000 in Jews of Moroccan ancestry; Rosler et al., 1992) and the very rare 3 β -hydroxysteroid dehydrogenase deficiency (HSD). As with 21-hydroxylase mutations, 46,XX infants with 11 β -hydroxylase deficiency resulting in hypoglycemia will have ambiguous genitalia. They may also have hypertension due to excessive production of deoxycorticosterone, a potent mineralocorticoid located upstream of the 11 β -hydroxylase enzyme block. 3 β -HSD deficiency results in undervirilized male genitalia, due to deficient testosterone synthesis, but may be associated with virilized female genitalia due to elevated dehydroepiandrosterone levels. Mutations in steroidogenic acute regulatory protein impair the transfer of cholesterol into the mitochondria, disrupting the necessary first step of steroid synthesis, the conversion of cholesterol to pregnenolone. These patients have cortisol deficiency, almost always have aldosterone deficiency, and XY infants can have phenotypically female external genitalia because of impaired androgen synthesis (Meimaridou et al., 2013).

X-Linked Adrenal Hypoplasia Congenita

Dosage-sensitive sex reversal adrenal hypoplasia congenita region of the X chromosome (DAX-1; which is encoded by *NROB1*) is a transcription factor located on the short arm of the X chromosome (Xp21). It is expressed in adrenal, hypothalamic, pituitary, and hypothalamic tissues, but its gene targets are largely unknown (Suntharalingham et al., 2015). Mutations in *NROB1* result in underdevelopment or agenesis of the adrenal gland with the potential to cause severe adrenal insufficiency in infancy. In the first few weeks of life, males classically exhibit vomiting, severe dehydration leading to vascular collapse, and often hyperpigmentation. Some patients are more mildly affected and present later in childhood with more mild adrenal insufficiency. Patients later develop hypogonadotropic hypogonadism, manifesting itself as delayed pubertal development without elevation of gonadotropin levels. However, DAX-1 appears to have a direct role in gonadal development, as there is also a component of primary hypogonadism (Yu et al., 1998; Mantovani et al., 2006). Further implicating its role in gonadal development is the finding that duplication of *NROB1* causes sex reversal with female external genitalia and variable presence of müllerian structures in a 46,XY infant (Sukumaran et al., 2013). Adrenal hypoplasia congenita (AHC) is also seen as a part of Xp21 contiguous gene deletion syndrome, which occurs due to deletion of *NROB1* and nearby genes encoding glycerol kinase, and dystrophin. Affected patients have severe developmental delay and develop Duchenne muscular dystrophy in addition to the primary adrenal insufficiency secondary to AHC.

IMAGe Syndrome

IMAGe syndrome (intrauterine growth restriction, metaphyseal dysplasia, congenital adrenal hypoplasia, and genital anomalies) was first described in 1999 in three boys who exhibited severe adrenal insufficiency shortly after birth (Vilain et al., 1999). The affected infants also had skeletal abnormalities, micropenis, and hypogonadotropic hypogonadism. Linkage analysis and sequencing

in a five-generation family identified a likely causative gene mutation in the gene encoding cyclin-dependent kinase inhibitor 1C (*CDKN1C*) located on chromosome band 11p15 within an imprinted cluster of genes (Arboleda et al., 2012). Affected patients in the pedigree had maternally transmitted mutations, suggesting that imprinting silences the paternal allele of *CDKN1C*. *CDKN1C* is believed to have a role in inhibiting cell cycle progression, and causative mutations are suspected to be gain-of-function mutations. Mutations in *CDKN1C* have also been associated with Beckwith–Wiedemann syndrome (BWS) as well as Russel–Silver syndrome, demonstrating the importance of this gene in regulating growth (Eggermann et al., 2014).

Adrenocorticotrophic Hormone Resistance

Resistance to ACTH at the level of the adrenal gland causes a rare form of primary adrenal insufficiency with retained aldosterone secretion. Cortisol level is low, and ACTH level is elevated. Hyperpigmentation caused by excessive production of alpha-melanocyte-stimulating hormone (α -MSH) and stimulation of melanocortin 1 receptor can be present at birth or develop over time. Almost 50% of cases are due to autosomal recessively inherited mutations in the melanocortin 2 receptor (or ACTH receptor) gene (*MC2R*) and the melanocortin 2 receptor accessory protein gene (*MRAP*) (Meimaridou et al., 2013). Autosomal recessive mutations in the nicotinamide nucleotide transhydrogenase gene (*NNT*) caused ACTH resistance in three consanguineous families, presumably because of increased oxidative stress in the zona fasciculata (Meimaridou et al., 2013).

Adrenal Hemorrhage

Adrenal hemorrhage is a rare occurrence in neonates and rarely results in adrenal insufficiency even when it is confirmed radiographically, likely because adrenal hemorrhage occurs bilaterally in only 5%–10% of cases (Gyurkovits et al., 2015). Maternal diabetes, high birthweight, fetal acidemia, and birth asphyxia are associated with higher rates of adrenal hemorrhage.

Inborn Errors of Metabolism

The neonatal inborn errors of metabolism are covered extensively in Chapter 22, and the key pathways of metabolism are outlined in Fig. 99.1. Hypoglycemia resulting from these conditions often requires fasting for at least 4–6 hours, and therefore they are less likely to present with hypoglycemia in infancy. However, a few conditions can impact early fasting adaptation and therefore can present with hypoglycemia in the neonatal period.

Glycogen Storage Diseases

Many of the glycogen storage diseases (GSDs) become clinically apparent later in childhood; however GSD type 1 can present in neonates as it is associated with hypoglycemia after fasting for just 2–2.5 hours. GSD type 1 results from impaired function of glucose 6-phosphatase, the enzyme responsible for the final step of glycogenolysis and gluconeogenesis in which glucose is produced from glucose 6-phosphate (see Fig. 99.1). Patients have hepatomegaly due to accumulation of fat, as well as elevated serum lactate, lipids, lactate, and uric acid levels. Administration of glucagon 2–4 hours after a carbohydrate meal is helpful diagnostically as it causes a rise in lactate level but no rise in glucose level. Treatment is with frequent feeding as well as more slowly absorbed carbohydrates such as uncooked cornstarch, to help prolong fasting tolerance.

Defects of Fatty Acid Catabolism and Ketogenesis

Medium-chain acyl coenzyme A dehydrogenase (MCAD) deficiency is the most common disorder of fatty acid oxidation (1 in 17,000). Newborn screening for MCAD deficiency is widespread, and infants with MCAD deficiency are often detected on the basis of screening. Defects arise from autosomal recessively inherited mutations in the *ACADM* gene, and MCAD is responsible for the initial dehydrogenation of acyl coenzyme A molecules with a chain length between 4 and 12 carbon atoms. Patients exhibit metabolic decompensation after periods of fasting or exhibit illness, and the levels of C₆–C₁₀ fatty acids are increased on an acylcarnitine profile, particularly octanoylcarnitine. Hypoglycemia is often a late finding, but lethargy, coma, and severe neurologic injury can occur because of the additional lack of ketone bodies available to the brain. Presentation is typically between the age of 3 months and the age of 3 years (average 18 months), but presentation can occur in the first few weeks of life (Derks et al., 2006). Urea, ammonia, uric acid, and liver function test levels are often abnormally elevated as well in the context of a hepatic steatosis.

Galactosemia

Galactosemia is described in more detail in Chapter 22. It occurs because of mutations in one of three genes necessary to metabolize galactose to glucose: the genes encoding galactose 1-phosphate uridylyltransferase (*GALT*), galactokinase (*GALK1*), and uridine diphosphogalactose 4-epimerase (*GALE*). Hypoglycemia can be a clinical presentation of galactosemia, often in the context of the other classic features such as hepatomegaly, jaundice, failure to thrive, cataracts, and *Escherichia coli* sepsis (Hennermann et al., 2011; Karadag et al., 2013).

Hereditary Fructose Intolerance

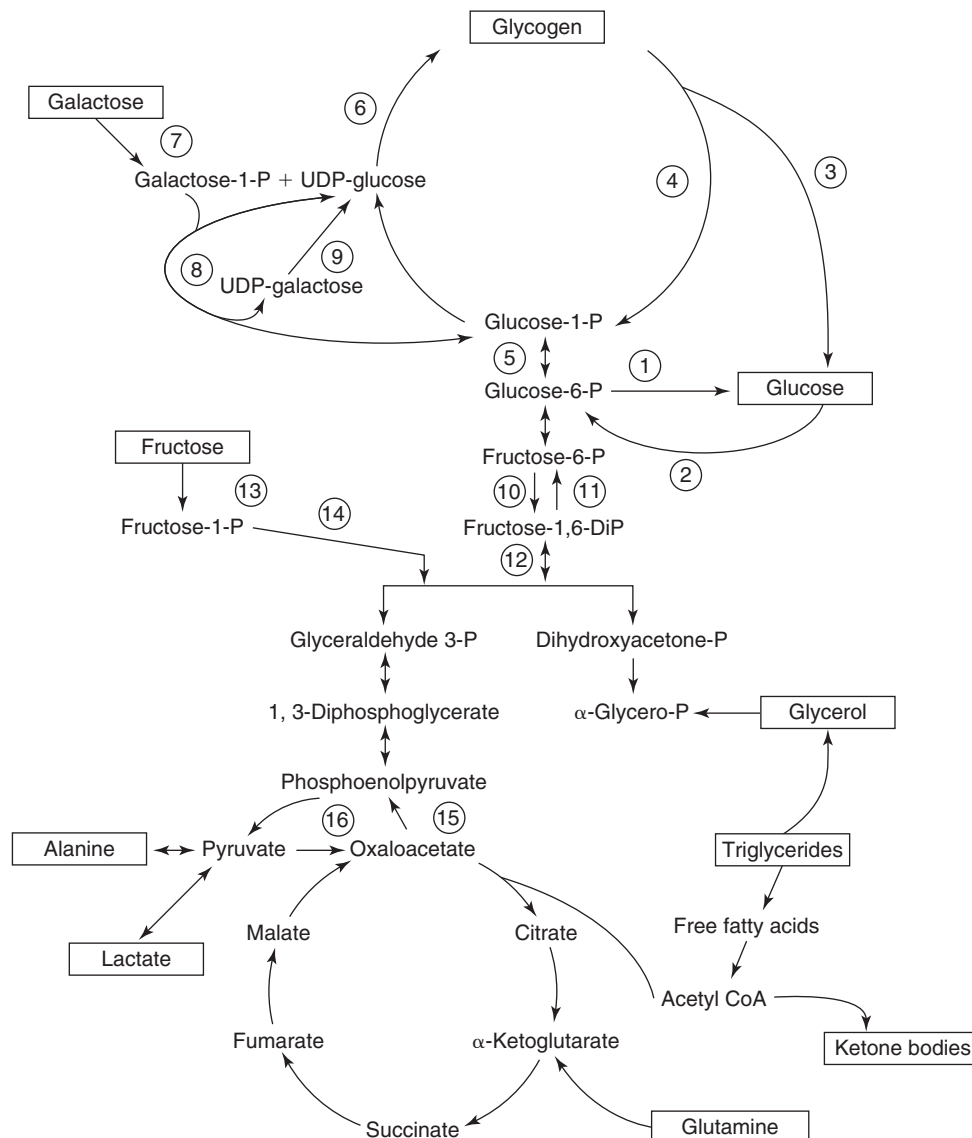
Hereditary fructose intolerance is due to mutations in the gene for aldolase B (*ALDOB*), which is responsible for metabolizing fructose 1-phosphate into substrates for gluconeogenesis. Vomiting, abdominal pain, acidosis, and hypoglycemia result after fructose or sucrose ingestion begins in infancy (Baker et al., 2015). The accumulation of fructose 1-phosphate leads to liver and kidney dysfunction, the extent of which is proportional to the degree of fructose consumption. Hypoglycemia at least partially results from impaired glycogenolysis in the liver, resulting in impaired response to glucagon (Van Den Berghe et al., 1973). Short-term treatment is with IV dextrose administration, and further organ injury can be prevented by avoidance of dietary fructose and high-fructose corn syrup.

Hyperinsulinemic Hypoglycemia

This section focuses on infants with hypoglycemia due to excessive insulin production. The causes include transient as well as permanent disorders of insulin secretion, and single-gene defects predominate in the permanent forms of HI (Table 99.1). A key management distinction among infants with HI is whether they can be treated with diazoxide and, if not, whether they have a focal or diffuse abnormality of pancreatic insulin regulation. Before the causes and management of HI are discussed, it is important to consider the physiology of insulin secretion from the pancreatic β cell.

Mechanism of Insulin Secretion

Depolarization of the pancreatic β cell leads to insulin secretion, and the most important regulator of membrane polarization in the pancreatic β cell is the adenosine triphosphate (ATP)–sensitive

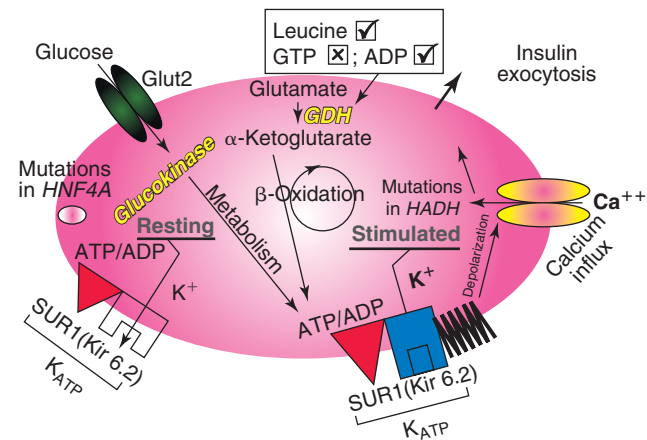


• **Fig. 99.1** Key metabolic pathways of intermediary metabolism: (1) glucose 6-phosphatase; (2) glucokinase; (3) amylase-1,6-glucosidase; (4) phosphorylase; (5) phosphoglucomutase; (6) glycogen synthetase; (7) galactokinase; (8) galactose 1-phosphate uridylyltransferase; (9) uridine diphosphogalactose 4-epimerase; (10) phosphofructokinase; (11) fructose 1,6-diphosphatase; (12) fructose 1,6-diphosphate aldolase; (13) fructokinase; (14) fructose 1-phosphate aldolase; (15) phosphoenolpyruvate carboxykinase; (16) pyruvate carboxylase. CoA, Coenzyme A; dihydroxyacetone-P, Dihydroxyacetone phosphate; FFAs, free fatty acids; fructose-1,6-DiP, fructose 1,6-bisphosphate; fructose-1-P, fructose 1-phosphate; fructose-6-P, fructose 6-phosphate; galactose-1-P, galactose 1-phosphate; glucose-1-P, glucose 1-phosphate; glucose-6-P, glucose 6-phosphate; glyceraldehyde 3-P, glyceraldehyde 3-phosphate; α -glycero-P, α -glycerophosphate; UDP, uridine diphosphate. (Modified from Sperling MA, Menon RK. Differential diagnosis and management of neonatal hypoglycemia. *Pediatr Clin North Am.* 2004;51:703–723.)

potassium channel (K_{ATP} channel) (Fig. 99.2). The K_{ATP} channel is an octameric complex of two different subunits: (1) four inward-rectifying potassium channel subunits (Kir6.2 encoded by the *KCNJ11* gene) and (2) four regulatory sulfonylurea receptor subunits that surround the channel and help regulate the open or closed conformation of the channel (SUR1 encoded by the *ABCC8* gene). The channel is sensitive to the metabolic and energy state of the cell as manifested by the ATP/adenosine diphosphate (ADP) ratio in the cellular cytoplasm. Glucose enters the β cell via an insulin-independent glucose channel (GLUT2 encoded by the *SLC2A2* gene). If the glucose concentration is sufficiently

high, glucose is phosphorylated by galactokinase to glucose 6-phosphate, the first step of glycolysis and therefore the entry point for glucose in the metabolic pathways that produce ATP (see Fig. 99.1). The increased ATP/ADP ratio causes closure of the K_{ATP} channel, resulting in membrane depolarization. In response to depolarization, voltage-gated calcium channels open, and the increase in intracellular calcium concentration causes fusion of the insulin-containing vesicles with the cellular membrane and subsequent release of insulin extracellularly.

The essential role that the K_{ATP} channel plays in regulating insulin secretion is evidenced by the large number of mutations

Pancreatic β cell

• **Fig. 99.2** Mechanisms of insulin secretion by the β cell of the pancreas. Glucose transported into the β cell by insulin-dependent glucose transporter 2 (*Glut2*) undergoes phosphorylation by glucokinase and subsequent metabolism, resulting in an increase in the intracellular adenosine triphosphate (ATP)/adenosine diphosphate (ADP) ratio. The increase in the ATP/ADP ratio closes the ATP-sensitive potassium channel (K_{ATP} channel) and initiates the cascade of events characterized by an increase in intracellular potassium concentration, membrane depolarization, calcium influx, and release of insulin from storage granules. Leucine stimulates insulin secretion by allosterically activating glutamate dehydrogenase (*GDH*) and by increasing the oxidation of glutamate, thereby increasing the ATP/ADP ratio and closure of the K_{ATP} channel. Mutations in the *HADH* gene, which codes for the mitochondrial enzyme L-3-hydroxyacyl coenzyme A dehydrogenase, which catalyzes the penultimate step in the fatty acid β -oxidation pathway, are also associated with hyperinsulinism (HI). Mutations in *HNF4A* cause multiple defects in glucose-stimulated insulin secretion. Ca^{2+} , Calcium ion; Cross (x), inhibition; check mark (✓), stimulation; GTP, guanosine triphosphate; K^{+} , potassium ion; *Kir6.2*, potassium channel inwardly rectifying subunit; *SUR1*, sulfonylurea receptor 1. (Modified from Sperling MA, Menon RK. Differential diagnosis and management of neonatal hypoglycemia. *Pediatr Clin North Am.* 2004;51:703–723.)

in both *KCNJ11* and *ABCC8* that cause either congenital HI (decreasing channel presence in the membrane or favoring the closed conformation) or neonatal diabetes mellitus (NDM; favoring the open channel conformation), both of which are discussed later in this chapter.

Diagnosis of Hyperinsulinism

Estimates of congenital HI differ widely depending on the study population but may be as high as 1 in 2500 in regions with high rates of consanguinity (Stanley, 2016). It is a common form of neonatal hypoglycemia, but the clinical features may be difficult to recognize and even missed until detection in later life or adulthood. However, infants with HI may have recognizable features of fetal hyperinsulinemia such as overgrowth and hypertrophic cardiomyopathy. The hypoglycemia is usually present early but can be initially difficult to distinguish from the transient neonatal hypoglycemia seen in the first few hours of life. However, the hypoglycemia may be immediately apparent as more severe and recalcitrant to early feeding. Confirming the diagnosis requires an index of suspicion on the part of the clinician and a search for historical and physical examination findings such as macrosomia and, potentially, signs suggestive of an underlying syndrome known to be associated with HI such as BWS, Kabuki syndrome, or

• BOX 99.4 Diagnostic Criteria for Hyperinsulinism Based on Critical Sample at Time of Hypoglycemia

- Insulin level $>2 \mu\text{IU/mL}$
- β -Hydroxybutyrate level $<1.8 \text{ mmol/L}$
- Free fatty acid level $<1.7 \text{ mmol/L}$
- Glucose rise $\geq 30 \text{ mg/dL}$ after glucagon administration
- Insulin-like growth factor-binding protein 1 level $\leq 110 \text{ ng/mL}$

Turner syndrome. As a state of excess glucose consumption, the normal physiologic rates of glucose supply are insufficient to maintain euglycemia, and thus a supraphysiologic glucose infusion rate (especially if $>10 \text{ mg/kg}$ per minute) in the hypoglycemic infant strongly suggests HI. Where hypoglycemia is recognized and confirmed, the confirmatory diagnosis of HI hinges on the critical detection of inappropriate hyperinsulinemia, including measurable insulin, and the metabolic markers of a persistent insulin effect, including suppressed FFA mobilization, hypoketosis, and a glycemic response to glucagon (Stanley and Baker, 1976; Stanley, 2016; Box 99.4).

However, important recent data from a retrospective review of infants with hypoglycemia have shown that some children with HI do not have detectable insulin at the time of hypoglycemia; of 28 individuals with congenital HI, only 23 had detectable insulin (median value $6.7 \mu\text{IU/mL}$), but in those where ketosis was measured, none had BOHB levels greater than 1.8 mmol/L (Ferrara et al., 2016). Importantly, bedside BOHB levels show good correlation with laboratory values, suggesting clinical utility in making a rapid diagnosis of HI. Similarly, infants without HI have FFA levels on average three times higher than in HI causes of hypoglycemia. While previous data suggested an FFA concentration cutoff of 0.5 mmol/L , these recent data suggest this misses many individuals with true HI and that an FFA concentration cutoff of less than 1.7 mmol/L provides sensitivity of more than 85%. Other biochemical data that are consistent with HI but may not yield results in as timely a manner as is ideal for rapid clinical confirmation include a C-peptide level of 0.5 ng/mL or higher and an insulin-like growth factor-binding protein 1 (which is suppressed by insulin) level of 110 ng/mL or lower.

These biochemical findings may be challenging to coordinate and collect in the setting of acute hypoglycemia, and so a diagnostic challenge using a provocative fast performed under controlled conditions may be required (Finegold et al., 1980). This should include frequent sampling of plasma glucose, insulin, BOHB, and FFAs. At a point where the glucose level falls below 2.8 mmol/L (50 mg/dL), the test can conclude with a glucagon challenge to test the glycemic response to an IV or intramuscular dose of glucagon (1 mg) followed by collection of glucose levels every 10 minutes for 40 minutes. A rise of 30 mg/dL or more in plasma glucose concentration is considered consistent with HI. This test has high specificity, and while some patients with subsequently confirmed HI in the aforementioned study did not achieve a rise in glucose concentration of 30 mg/dL or more, its sensitivity is greater than relying solely on the detection of serum insulin levels at the time of hypoglycemia. With a controlled provocative test as described herein, however, multiple markers of inappropriate hyperinsulinemia can be collected, and the clinician can thus arrive at a timely and correct diagnosis at the bedside. While the subsequent detection of urine ketones may provide adjunctive data, ketonuria does not exclude HI, and accurate diagnostic results are more likely to be

achieved in a timely fashion at the bedside by blood sampling (Wolfsdorf et al., 1984). These authors thus do not recommend routine reliance on urinary ketosis as a replacement for the protocol described.

Hypoglycemia in Infants of Diabetic Mothers

Transient hyperinsulinemic hypoglycemia is often associated with pregnancies affected by diabetes, including both gestational diabetes and permanent maternal diabetes. Infants are typically macrosomic and, despite high energy stores in the form of glycogen and fat reserves secondary to fetal hyperinsulinemia, hypoglycemia may persist for several days after disconnection from the high maternal glucose supply. This suggests a more persistent hyperinsulinemia, but it may resolve within the first few days to week of life. The severity of neonatal hypoglycemia in this setting is impacted by late pregnancy maternal glycemia, with avoidance of hyperglycemia reducing the severity of fetal hyperinsulinemic hypoglycemia (Andersen et al., 1985; Taylor et al., 2002). Neonates may require high rates of dextrose administration and/or frequent feedings, similar to patients with permanent congenital HI, but may successfully be weaned off high delivered substrate support judiciously during the first week or first 2 weeks of life.

Perinatal Stress and Transient Hyperinsulinism

While the well-described transitional hypoglycemia in normal newborns typically resolves within the first few hours to the first day of life, in stressed infants a persistent form of hypoglycemia may be seen (Hoe et al., 2006). The mechanism, similar to transitional hypoglycemia in healthy newborns, appears to be related to persistent HI, as ketogenesis and lipolysis remain suppressed. While somewhat unclear, it has been postulated that a lower glucose threshold for insulin secretion persists, given that insulin is a key fetal growth factor and thus its persistent secretion may confer a protective effect in the small or stressed neonate (Stanley, 2016). This may be seen in the context of perinatal asphyxia, preeclampsia, or SGA and may require interventions with early frequent feeding, medical therapy including diazoxide directed at the insulinemia, or continuous feeds to avoid the complications of hypoglycemia (Collins and Leonard, 1984; Collins et al., 1990; Thornton et al., 2015). For SGA infants with hyperinsulinemic hypoglycemia, diazoxide treatment is often required for as long as 6 months and has been reported to last as long as 22 months (Arya et al., 2013).

Mutations in the ATP-Sensitive Potassium Channel Genes *KCNJ11* and *ABCC8*

Mutations in the *KCNJ11* and *ABCC8* genes located on chromosome band 11p15.1 are the most common cause of permanent HI, accounting for about 40%–50% of cases in neonates, most of which concern *ABCC8* (de Lonlay et al., 2002; Dunne et al., 2004). Most commonly this is due to mutations that prevent channel formation, trafficking to the cell membrane, or insensitivity to the cellular ATP/ADP ratio. In the absence of a functional K_{ATP} channel, the β cell is permanently depolarized, leading to insulin secretion. Because diazoxide activity requires a functional channel present in the cell membrane, patients with *KCNJ11* or *ABCC8* mutations typically do not respond to diazoxide therapy.

The mechanism of inheritance of the K_{ATP} channel gene mutation strongly correlates with the extent of pancreatic involvement. Patients with two autosomal recessively inherited mutations will have diffuse

involvement of the pancreas, whereas patients with one paternally inherited mutation will have focal disease. The focal lesion arises from somatic loss of the maternal allele in a subpopulation of pancreatic cells, leading to focal unopposed expression of the mutated paternal allele, as well as unbalanced expression of imprinted genes that contribute to cellular hyperplasia (Dunne et al., 2004; Bellanne-Chantelot et al., 2010). Rarely, dominant-negative single allele mutations can result in diffuse disease. As described later in this section, the extent of pancreatic involvement directly impacts treatment. Patients with the focal histologic subtype can be cured by targeted surgical resection, whereas those with diffuse disease may require near-total pancreatectomy for definitive therapy.

Activating Mutation of the *GLUD1* Gene: Hyperinsulinemia–Hyperammonemia Syndrome

After mutations in *KCNJ11* and *ABCC8*, glutamate dehydrogenase (GDH) gene (*GLUD1*) mutations are the second most common cause of genetic HI. GDH is a mitochondrial matrix protein that converts guanosine diphosphate (GDP) to guanosine triphosphate (GTP), which in turn regulates amino acid and ammonia metabolism. Autosomal dominant, gain-of-function mutations increase the GTP/GDP ratio in the β cell, leading to closure of the K_{ATP} channel and subsequent depolarization and insulin secretion. Presentation is typically at between 4 and 12 months of age, but hypoglycemia can present in the first few days of life (De Lonlay et al., 2001). Leucine is an allosteric activator of GDH, causing hypoglycemia to worsen 30–90 minutes after a protein-rich meal. In addition to hyperinsulinemic hypoglycemia, patients also have elevated serum ammonia levels, although this is believed to have no clinical consequence and does not require therapy (De Leon et al., 2014). The hypoglycemia is diazoxide responsive, and carbohydrate loading is encouraged before protein-rich meals to prevent hypoglycemia. Patients may also develop generalized seizures, including absence epilepsy (Raizen et al., 2005).

HADH Mutation

Short-chain L-3-hydroxyacyl-CoA dehydrogenase (SCHAD; encoded by the *HADH* gene) is a mitochondrial enzyme responsible for β -oxidation of fatty acids, but patients with autosomal recessive mutations can have a presentation similar to those with HI–hyperammonemia (HA) syndrome (Clayton et al., 2001). This is due to SCHAD's additional role as a direct inhibitor of GDH activity via protein–protein interaction (Li et al., 2010). These patients share the same hypoglycemic sensitivity to protein loading as patients with HI–HA syndrome; however, they do not have HA and do not have an increased risk of seizure disorder. Affected patients can be identified by the presence of elevated serum 3-hydroxybutyrylcarnitine and elevated urine 3-hydroxyglutaric acid levels (Molven et al., 2004).

GCK Mutations

Glucokinase catalyzes the reaction of glucose to glucose 6-phosphate and serves as the “glucose sensor” of the pancreatic β cell. Dominant, activating mutations lower the glucose threshold for insulin secretion, causing hypoglycemia. Activating *GCK* mutations are usually de novo and not inherited from either parent, so the family history is typically negative. Affected infants are often large for their gestational age and can have severe hypoglycemia in the neonatal

period, but there is a wide spectrum of severity of presentation and hypoglycemia. Diazoxide therapy is effective in some cases, but pancreatectomy is required for some patients (Sayed et al., 2009).

HNF4A and HNF1A Mutations

Hepatocyte nuclear factor 4 α (HNF4 α) and hepatocyte nuclear factor 1 α (HNF1 α) are transcription factors expressed in hepatocytes, β cells, intestinal epithelial cells, and renal tubular cells, and are traditionally associated with maturity-onset diabetes of youth (MODY) types 1 and 3, respectively. Transcriptional regulation targets of HNF4 α and HNF1 α include each other, GLUT2, and possibly Kir6.2, but the exact cause of HI in infants is unclear (Stanescu et al., 2012). Some patients with neonatal HI due to *HNF4A* and *HNF1A* mutations develop diabetes years later in adolescence or young adulthood, consistent with MODY types 1 and 3. Diazoxide is effective as therapy for patients with hypoglycemia secondary to *HNF4A* and *HNF1A* mutations. *HNF4A* HI has also been described in combination with a renal Fanconi tubulopathy and hepatomegaly due to increased glycogen stores (Stanescu et al., 2012).

Other Genetic Causes of Hyperinsulinism

Autosomal dominant mutations in the uncoupling protein 2 gene (*UCP2*) have been shown in a few individuals with diazoxide-sensitive hyperinsulinemic hypoglycemia in infancy (Gonzalez-Barroso et al., 2008). *UCP2* was subsequently shown to regulate mitochondrial and cellular oxidation, and decreased *UCP2* activity is thought to increase the oxidation of glucose, thereby increasing the intracellular ATP/ADP ratio and increasing insulin secretion (Vozza et al., 2014).

With the cooperation of a four-generation pedigree of autosomal dominantly inherited congenital HI, Pinney et al. (2013) identified a new single gene cause of HI: *HK1*, which encodes hexokinase 1. Family members were variously affected, but all who were treated with diazoxide responded well.

Autosomal recessive mutations in the phosphoglucosyltransferase 1 gene (*PGM1*) cause a syndrome of abnormal protein glycosylation associated with a multifactorial hypoglycemia (Tegtmeyer et al., 2014). Patients demonstrate defects in glycogen breakdown as well as a postprandial hyperinsulinemia, both of which have been associated with hypoglycemia in infancy (Stanley, 2016). Other congenital disorders of glycosylation have been associated with HI, including phosphomannomutase 2 deficiency and mannose phosphate isomerase deficiency (Kapoor et al., 2009).

Beckwith–Wiedemann Syndrome

Patients with BWS have characteristic features including macrosomia, macroglossia, hemihypertrophy, ear pits, umbilical hernia, and a high risk of embryonal tumors. It results from epigenetic abnormalities in the imprinted 11p15.5 region. Most patients (85%) with BWS do not have a family history of the disorder. Children conceived by assisted reproductive technologies may be at increased risk of BWS and other imprinted disorders. Up to 50% of patients have hyperinsulinemic hypoglycemia with a wide spectrum of severity, most of which resolves in the first few days of life (DeBaun et al., 2000). The cause of HI in BWS is variable and may depend on the patient's specific genetic abnormality, sometimes including mutations in the *KCNJ11* gene or the *ABCC8*

gene (Kalish et al., 2016). Patients with BWS may respond to diazoxide; however, some patients require partial pancreatectomy.

After Fundoplication

Neonates undergoing fundoplication for gastric reflux or another reason are at risk of postprandial hypoglycemia related to dumping syndrome. Approximately 25% of neonates develop dumping syndrome after fundoplasty, most of which is identified in the first postoperative week (Calabria et al., 2011; Samuk et al., 1996). Hypoglycemia occurs 1–3 hours after a meal because of an exaggerated insulin response to early postmeal hyperglycemia, which is often detectable as well. Treatment is with slower gastric feeds, and sometimes continuous feeds are required. Treatment with uncooked cornstarch, pectin, octreotide, and acarbose has been attempted with various degrees of success as well (Calabria et al., 2011).

Differentiation Between Focal Adenomatous and Diffuse Pancreatic Hyperplasia

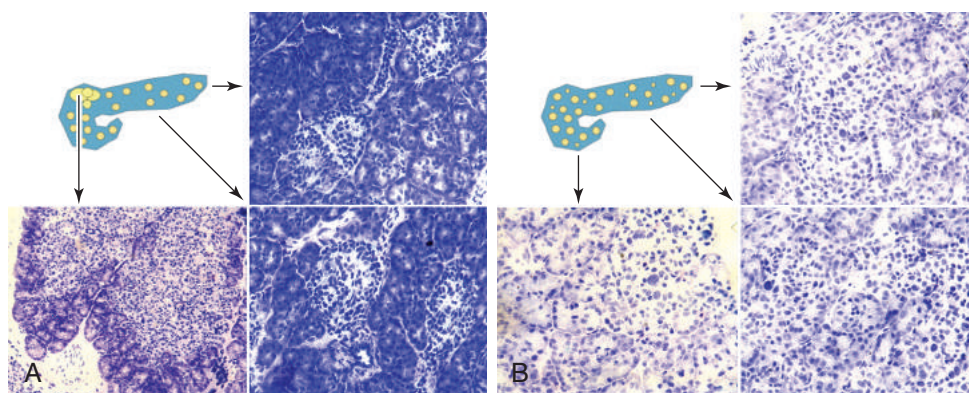
Patients with hyperinsulinemic hypoglycemia who are not responsive to diazoxide therapy should undergo specialized imaging to evaluate them for a focal pancreatic lesion that can be cured surgically. Positron emission tomography (PET) with [^{18}F]fluoro-L-dopa uses the fact that pancreatic β cells take up the radiolabeled L-dopa (Fig. 99.3). Simultaneous PET and MRI therefore provide visualization of the hyperactive β cells within the pancreas and allows the differentiation of focal and generalized hyperactivity (see Fig. 99.3). This distinction is important as it is far preferable to perform a curative focal resection rather than a “near-total” pancreatectomy, which has a more variable outcome ranging from continued HI to diabetes (Lord et al., 2015).

Approximately half of patients requiring surgery will have a focal lesion (De Lonlay et al., 1999, 2002). Such imaging is often successful in identifying the appropriate surgical management in those patients with HI refractory to medical management (Yang et al., 2012).

Management of Hyperinsulinemic Hypoglycemia

The goal of treatment is to minimize hypoglycemia frequency and duration to prevent impaired neurologic development. First-line treatment is with diazoxide, which opens the β -cell K_{ATP} channel, thereby decreasing insulin secretion. Common side effects include hypertrichosis and fluid retention, sometimes requiring diuretic use. Pulmonary hypertension has also been described in a small number of patients receiving diazoxide (Nebesio et al., 2007).

As mentioned earlier, not all hyperinsulinemic patients will respond to diazoxide (particularly those with K_{ATP} channel mutations), and second-line therapies include subcutaneous octreotide administration and glucagon infusion (Table 99.2). Octreotide is known to result in tachyphylaxis, causing diminishing efficacy of the medication. There is also a possible association with necrotizing enterocolitis, which requires special consideration in the neonatal population (Hawkes et al., 2016). Glucagon infusion is effective but not sustainable as a long-term treatment strategy because of the need either for IV infusion or for continuous delivery via a subcutaneous catheter. Continuous subcutaneous delivery systems



• **Fig. 99.3** (A) Frozen sections obtained from three pancreatic sites during surgery for a focal form of hyperinsulinism in the head. Specimens taken from tail and body show islets of Langerhans at rest with little cytoplasm, leading to crowded nuclei, whereas the focal form aspect from the head is that of multilobular involvement with local signs of β -cell hyperactivity: abundant cytoplasm and large, abnormal nuclei (toluidine blue stain; original magnifications $\times 200$ and $\times 100$). (B) Frozen sections obtained from three pancreatic sites during surgery for the diffuse form of hyperinsulinism. On each biopsy specimen, there is one islet showing hyperfunctional signs with abundant cytoplasm and irregular nuclei more than four times the size of the acinar nuclei nearby used as an internal control. The disease involves the whole pancreas and, in consequence, can be called *diffuse*, which does not mean that all islets are involved in the same manner (toluidine blue stain; original magnification $\times 200$). (Reproduced from Delonlay P, Simon A, Galmiche-Rolland L, et al. Neonatal hyperinsulinism: clinicopathologic correlation. *Hum Pathol*. 2007;38:387–399.)

TABLE 99.2

Drugs Used in the Management of Neonatal Hyperinsulinism

| Drug | Dose/Route | Mechanism of Action | Adverse Effects |
|--|---|--|---|
| Diazoxide | 5–20 mg/kg per d in three divided doses orally | Binds to SUR1 subunit, opens K_{ATP} channel | Fluid retention, hypertrichosis, rarely eosinophilia, leukopenia, hypotension |
| Chlorothiazide (in conjunction with diazoxide to decrease fluid retention) | 7–10 mg/kg per d in two divided doses orally | Synergistic response to diazoxide | Hyponatremia, hypokalemia |
| Octreotide | 5–25 μ g/kg per d in 6–8-hourly SC injections or IV infusions | Inhibits insulin secretion by binding to somatostatin receptors and inducing hyperpolarization of β cells, direct inhibition of voltage-dependent calcium channels | Anorexia, nausea, abdominal pain, diarrhea, tachyphylaxis |
| Glucagon | 1–20 μ g/kg per h, SC injection or IV infusion | Increases glycogenolysis and gluconeogenesis | Nausea, vomiting, paradoxical insulin secretion at high dose |

K_{ATP} , ATP-sensitive potassium; IV, intravenous; SC, subcutaneous.

have been described, but they are limited by crystallization of the glucagon and obstruction of the tubing (Mohnike et al., 2008). Sirolimus, a known cause of transplant-related diabetes, has also been used experimentally with some success (Senniappan et al., 2014).

Nutritional factors should also be considered, by increasing the carbohydrate content of feeds or by altering the length and frequency of feeding breaks. Families should be taught how to use a glucometer and to be vigilant for signs and symptoms of hypoglycemia. Parental education on the administration of glucagon is necessary before discharge.

Patients who require pancreatectomy have a high risk of future development of insulin-dependent diabetes and neurobehavioral

problems (Lord et al., 2015). Lord et al. (2015) recently reported the outcomes for 121 children with congenital HI treated with pancreatectomy, finding that 36% developed diabetes during long-term follow-up, and 20% of these patients developed diabetes in the immediate postoperative period. The risk of diabetes was associated with the percentage of pancreatectomy: 93% of those with diabetes had a 95% pancreatectomy or greater, while those without diabetes had a median 65% pancreatectomy. From parent-reported questionnaires, 48% had neurobehavioral concerns, including 21% of patients with psychiatric or behavioral concerns, 18% with speech delay, and 16% with learning disability. Quantitative measures of adaptive behavior were abnormal in 27% of patients.

The finding that these neurodevelopmental outcomes were similar for the focal and diffuse groups suggested to Lord et al. that a shared exposure to recurrent hypoglycemia in the neonatal period may be causative.

Hyperglycemia in the Neonate

Insulin is a critical mediator of fetal growth, and therefore infants with hyperglycemia due to persistent insulin deficiency are almost universally born SGA and have a history of intrauterine growth restriction. It is thought that the autoimmune process leading to β -cell destruction takes at least 6 months to manifest itself, and therefore the classic, autoimmune-mediated type 1 diabetes earlier than 6 months of age does not occur, with the notable exception of immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome-related diabetes (Iafusco et al., 2002). Persistent hyperglycemia that meets the criteria for diabetes (glucose level >125 mg/dL fasting or >200 mg/dL) in an infant younger than 6 months strongly suggests a monogenic cause, and genetic testing is positive in at least 80% of cases (De Franco et al., 2015). About 50% of infants with diabetes in the first few months of life will have resolution by 1–2 years of age (TNDM), but a significant portion of these patients will have relapse of diabetes later in childhood or adolescence (von Muhlendahl and Herkenhoff, 1995; Rubio-Cabezas et al., 2014). A family history of early adulthood-onset diabetes mellitus, perhaps reported as or assumed initially to be type 1 diabetes mellitus, type 2 diabetes mellitus, or MODY, can suggest an inherited abnormality of insulin secretion, but a family history is not present in 70% of molecularly confirmed cases (De Franco et al., 2015). Elucidation of the specific genetic mutation has immediate implications for treatment, as those with mutations in the *KCNJ11* or *ABCC8* genes, which encode the components of the K_{ATP} channel, are best treated with orally administered sulfonylurea rather than insulin.

Transient Stress-Related Hyperglycemia

More commonly, neonatal hyperglycemia is transient and caused by physiologic stress such as sepsis or other acute illness, particularly in infants receiving continuous IV dextrose infusions (Mittal et al., 2013; Box 99.5). Cortisol and catecholamines secreted during acute illness increase gluconeogenesis, glycogen breakdown, and insulin resistance, all of which increase the plasma glucose concentration. Hyperglycemia will resolve as the neonate's overall clinical status improves and as glucose infusion rates are minimized to the

lowest necessary amount. Insulin infusions may be necessary to control hyperglycemia in the short term.

Genetic Causes of Neonatal Diabetes

NDM is a genetically heterogeneous disease, with 22 known genetic subtypes. The different types of NDM differ widely in their prognosis, from mild self-resolving hyperglycemia to permanent forms associated with severe neurodevelopmental features. Some types of NDM respond to sulfonylureas, while others do not. Thus early referral for genetic testing should be made as soon as a clinical diagnosis of NDM is made. It is important to recognize that the absence of a family history does not exclude a genetic cause, as most NDM-causing mutations arise spontaneously (De Franco et al., 2015).

Transient Neonatal Diabetes Mellitus Due to Chromosome Band 6q24 Anomalies

Most patients (70%) with TNDM have an abnormality of the imprinted region of chromosome band 6q24. Within this imprinted region are two genes, *PLAGL1* and *HYMAI*, that share a promoter. This promoter is differentially methylated depending on the parent of allelic origin. In the normal situation, only the paternally inherited allele is expressed, while the maternally inherited allele is methylated and not expressed. Situations that lead to a relative increase in the expression of *PLAGL1* and *HYMAI* cause TNDM. These include paternal uniparental disomy, duplication of 6q24 on the paternal allele, or hypomethylation of the maternal allele, producing inappropriate *PLAGL1* and *HYMAI* expression from the maternal allele. The mechanism by which overexpression of *PLAGL1* and *HYMAI* leads to TNDM is not fully understood. A second, nonimprinted promoter also controls *PLAGL1* expression, which has led to the hypothesis that the postnatal remission of TNDM is due to a switch over to this nonimprinted promoter postnatally. This form of TNDM resolves at a median age of 3 months, but in about half of patients, diabetes will reappear during adolescence. Management is with subcutaneous insulin therapy until remission. Associated clinical features include macroglossia (50%), umbilical hernia (25%), facial dysmorphism (20%), cardiac and renal anomalies (9%), and hand anomalies (8%) (Docherty et al., 2013).

Transient Neonatal Diabetes Mellitus Due to ATP-Sensitive Potassium Channel Gene Mutations

About 25% of patients with TNDM have normal methylation at 6q24. Most of these 6q24-normal TNDM patients have heterozygous activating mutations in either *KCNJ11* or *ABCC8*, which encode the two components of the K_{ATP} channel as described earlier. Activating mutations in these same genes can also cause PNDM (see later). Although some studies have suggested that TNDM-causing mutations are functionally less severe than PNDM-causing mutations, some mutations in *KCNJ11* have been reported in both PNDM patients and TNDM patients, so an absolute genotype–phenotype correlation may not exist (Ashcroft, 2005; Gloyn et al., 2005). Compared with 6q24-associated TNDM, infants with K_{ATP} channel-associated TNDM have higher birthweight, later diagnosis, later remission, and earlier relapse of hyperglycemia (Flanagan et al., 2007). No significant clinical differences have been noted between *KCNJ11* and *ABCC8*-related TNDM, both of which respond favorably to sulfonylureas.

• BOX 99.5 Differential Diagnoses of Neonatal Hyperglycemia

Common Causes

Excessive intravenous glucose infusion
Impaired glucose homeostasis in preterm/sick/small for gestational age infants
Sepsis
Stress
Corticosteroids

Rare Causes

Transient neonatal diabetes
Permanent neonatal diabetes

Additional Genetic Causes of Transient Neonatal Diabetes Mellitus

Homozygous mutations within the promoter of the insulin gene (*INS*) have been reported in four unrelated TNDM patients (Garin et al., 2010). These mutations involve DNA elements within the promoter that serve as binding sites for neurogenic differentiation 1 (NEUROD1) and Gli-similar (GLIS)3, two transcription factors that are rare genetic causes of PNDM (see later). Promoter mutations within the *INS* gene can also cause PNDM. Even within the same family, the same *INS* promoter mutation (c.-331C>G) has been reported to cause TNDM in some individuals and PNDM in others. The reason for this variability is unknown.

Homozygous mutations in the gene *SLC2A2*, encoding GLUT2, which transports glucose into the β cell, have been reported in four unrelated patients with TNDM; the parents were first cousins in three of these patients (Sansbury et al., 2012). Biallelic mutations in *SLC2A2* also cause Fanconi–Bickel syndrome (FBS), whose features include renal Fanconi syndrome, poor growth, hepatomegaly, and impaired utilization of glucose and galactose (Santer et al., 2002). More than 95% of patients with biallelic *SLC2A2* mutations have symptoms of FBS without evidence of NDM, but the reason for this is unknown.

Nonsyndromic Causes of Permanent Neonatal Diabetes Mellitus

Infants with NDM without evidence of remission in the first year or first 2 years of life are classified as having PNDM. The most common cause of PNDM is heterozygous, activating mutations in the K_{ATP} channel subunit genes *KCNJ11* and *ABCC8* (more commonly *KCNJ11*), accounting for almost half of all patients with PNDM (Gloyn et al., 2004; Babenko et al., 2006). These mutations decrease the K_{ATP} channel's sensitivity to the cellular ATP concentration, keeping the channel inappropriately open and inhibiting insulin secretion. Twenty percent of individuals with PNDM due to *KCNJ11* mutations will also have developmental delay, epilepsy, and neonatal diabetes (DEND) syndrome (Clark et al., 2010). There are clear genotype–phenotype correlations within the *KCNJ11* gene, with some mutations being associated with DEND syndrome and others being associated only with PNDM. Patients with PNDM due to mutations in *KCNJ11* and *ABCC8* typically respond well to sulfonylureas. Some neurologic features of DEND syndrome have also been reported to respond to sulfonylureas, highlighting the importance of the K_{ATP} channel in neuronal cells (Pearson et al., 2006).

Mutations within *INS* are also a common cause of PNDM and are found in approximately 10% of patients with PNDM. *INS* mutations can be homozygous (more common among offspring of consanguineous relationships) or heterozygous, but in both cases the mutations lead to inadequate production of insulin (Edghill et al., 2008; Garin et al., 2010; De Franco et al., 2015).

Rarer genetic causes of nonsyndromic PNDM include biallelic inactivating mutations in *GCK* (which encodes glucokinase) and *PDX1* (Bennett et al., 2011; De Franco et al., 2013). Glucokinase serves as the “glucose sensor” of the β cell, converting glucose into glucose 6-phosphate. *PDX1* encodes a transcription factor necessary for the formation of the pancreas in utero. Heterozygous mutations in *GCK* are a relatively common cause of MODY, accounting for 20%–50% of MODY patients. Therefore *GCK* mutations should be strongly considered in patients with PNDM who have a family history of MODY, mild fasting hyperglycemia, or gestational

diabetes in a nonobese mother. Some patients with homozygous mutations of *PDX1* have pancreatic agenesis, producing exocrine insufficiency in addition to PNDM, but in some *PDX1* mutation patients, pancreatic exocrine function is intact (De Franco et al., 2013).

Syndromic Causes of Neonatal Diabetes Mellitus

In addition to the genes described earlier, there are 17 other known genetic causes of NDM, which are typically considered “syndromic” because they are often associated with other, nonendocrine features. Although some syndromic forms of NDM present with other features (e.g., congenital heart defects), diabetes is often the initial presentation, making early genetic diagnosis helpful as it can guide management and necessary screening. For example, patients with biallelic mutations in *EIF2AK3* have Wolcott–Rallison syndrome, which usually presents with NDM, while other features (skeletal dysplasia, developmental delays, and liver dysfunction) do not develop until later. A quarter of NDM patients whose parents are consanguineous have Wolcott–Rallison syndrome, making it the most common cause of PNDM among this group of patients. The remaining syndromic causes of NDM are listed in Table 99.3. Because of the large number of genetic causes of NDM, sequencing of multiple genes in parallel is typically the most efficient diagnostic approach.

Treatment of Neonatal Hyperglycemia

Treatment of hyperglycemia in the neonatal period is dictated by the clinical scenario and the results of genetic testing when available. Transient hyperglycemia is best managed by treatment of the underlying cause (e.g., sepsis). In addition to close monitoring of glucose levels, exogenous glucose administration can be decreased to approximately 3 mg/kg per minute. If necessary, insulin treatment can commence, starting with a low-dose insulin infusion (e.g., 0.03 units/kg per hour).

Treatment of NDM requires insulin, at least until a genetic diagnosis is made. For those with a mutation in 6p24, insulin requirements usually drop quite quickly, and treatment is often discontinued by 12 weeks of age. Hyperglycemia may recur with intercurrent illness and then recurs in more than half of children, generally at the time of puberty.

Infants with an identified mutation in *KCNJ11* or *ABCC8* can be treated with sulfonylureas. Generally it is best to gradually decrease insulin dosing as sulfonylurea treatment is initiated. Sulfonylurea dosing tends to be higher than typically used in adults. While more than 90% of those with a *KCNJ11* or *ABCC8* mutation can successfully transition from insulin to sulfonylurea and maintain near-normal glycemic control, the factors associated with sulfonylurea failure are the specific genetic mutation and longer duration of diabetes (Babiker et al., 2016). Treatment with sulfonylureas is not only effective in achieving euglycemia but has also led to improvements in neurologic status for patients with DEND syndrome; this appears to be secondary to improved cerebellar perfusion (Fendler et al., 2013).

Acknowledgments

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TABLE 99.3 Syndromic Causes of Neonatal Diabetes

| Gene | Syndrome | References |
|----------------|---|---|
| <i>EIF2AK3</i> | Wolcott–Rallison syndrome | Julier and Nicolino (2010); Rubio-Cabezas et al. (2009) |
| <i>FOXP3</i> | IPEX syndrome: severe diarrhea, type 1 DM, dermatitis, X-linked | Hannibal and Torgerson (2004) |
| <i>GATA4</i> | Neonatal and childhood-onset DM, may have pancreatic hypoplasia, cardiac malformations, and neurocognitive defects | Shaw-Smith et al. (2014) |
| <i>GATA6</i> | Pancreatic agenesis, with or without congenital heart defects | De Franco et al. (2013) |
| <i>GLIS3</i> | NDM with congenital hypothyroidism | Senee et al. (2006); Dimitri et al. (2011) |
| <i>HNF1B</i> | Renal cysts and diabetes, NDM | Yorifuji et al. (2004) |
| <i>IER3IP1</i> | NDM with microcephaly and epileptic encephalopathy | Abdel-Salam et al. (2012); Shalev et al. (2014) |
| <i>MNX1</i> | NDM with neurologic features, Currarino syndrome (sacral agenesis, imperforate anus) | Bonnefond et al. (2013); Flanagan et al. (2014) |
| <i>NEUROD1</i> | NDM with cerebellar hypoplasia, sensorineural hearing loss, visual impairment | Rubio-Cabezas et al. (2010) |
| <i>NEUROG3</i> | NDM with malabsorptive diarrhea | Pinney et al. (2011); Rubio-Cabezas et al. (2011) |
| <i>NKX2-2</i> | NDM with neurologic features | Flanagan et al. (2014) |
| <i>PTF1A</i> | NDM with pancreatic and cerebellar agenesis | Sellick et al. (2004) |
| <i>RFX6</i> | NDM with pancreatic hypoplasia, intestinal atresia, gallbladder hypoplasia (Mitchell–Riley syndrome) | Smith et al. (2010); Sansbury et al. (2015) |
| <i>SLC19A2</i> | NDM with deafness and thiamine-responsive megaloblastic anemia | Shaw-Smith et al. (2012) |
| <i>SLC2A2</i> | NDM with renal dysfunction (Fanconi–Bickel syndrome) | Sansbury et al. (2012) |
| <i>WFS1</i> | Wolfram syndrome (diabetes insipidus, DM, optic atrophy, and deafness), low-frequency sensorineural hearing loss, optic atrophy | Rohayem et al. (2011) |
| <i>ZFP57</i> | NDM with intrauterine growth retardation and neurologic features, complex hypomethylation defects | Mackay et al. (2008) |

DM, Diabetes mellitus; NDM, neonatal diabetes mellitus.

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100

Craniofacial Malformations

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KEY POINTS

- Craniofacial malformations can impact swallowing, breathing, hearing, vision, speech, and development and for some neonates can result in life-threatening airway compromise.
- Early recognition and assessment of craniofacial conditions that include appropriate diagnostic studies, identification of associated health concerns, and family education can have a positive impact on the care of the newborn.
- Timely referral of the newborn with a craniofacial condition for multidisciplinary craniofacial team care is an important step in the provision of coordinated medical and surgical management.

The neonatologist is often the first point of contact for a child born with a craniofacial malformation. Abnormalities of the face and head can be distressing to a new parent, who is immediately wondering, “Is my child going to look, feel, and develop normally?” Having a basic understanding of the relationship between craniofacial abnormalities and feeding, breathing, hearing, vision, speech, and overall development will help the neonatologist begin to counsel a family. Airway compromise is well described in multiple craniofacial syndromes, and early identification can be lifesaving. Prompt recognition of a constellation of anomalies pointing toward a syndrome or diagnosis will result in better targeted evaluations and therapies for that patient. (Tables 100.1–100.2 contain a concise presentation of potential intensive care unit [ICU] issues that may be encountered with certain craniofacial malformations and syndromes.) This chapter highlights the most relevant craniofacial malformations that a neonatologist will encounter. We describe here the epidemiology, genetics, diagnosis, phenotype, and potential ICU issues as well as basic management recommendations to help guide the neonatologist in caring for an infant with craniofacial malformations.

Micrognathia/Robin Sequence

Epidemiology

The triad of micrognathia, glossoptosis, and airway obstruction, originally described in 1923 by Pierre Robin, is known as *Robin*

sequence (RS) or *Pierre Robin sequence*. Whether cleft palate is an obligatory feature of RS is debatable. Approximately one-quarter of infants with cleft palate (CP) were found to have RS in a multisite, population-based, case-control study (Genisca et al., 2009). The tremendous heterogeneity and lack of uniformly accepted diagnostic criteria for, or definitions of, RS make it challenging to know the true prevalence. However, estimates of birth prevalence range from 1 in 8500 to 1 in 14,000 births (Bush and Williams, 1983; Printzlaun and Andersen, 2004).

Phenotype

RS is an etiologically and phenotypically heterogeneous disorder. More than half of children with RS have an associated syndrome, with Stickler syndrome being the most common. While there is great variation in severity, RS is characterized by the following phenotypic features: micrognathia (small and symmetrically receded mandible), glossoptosis (tongue of variable size falls backward into the postpharyngeal space), and resultant upper airway obstruction, often with a cleft palate (Breugem et al., 2016; Fig. 100.1A–B). Caouette-Laberge et al. (1994) described CP (U-shaped CP more common than V-shaped CP) in 90% of 125 individuals with RS. Infants with RS often have airway obstruction, feeding difficulties, and challenges gaining weight, and they may have associated anomalies, including hypotonia and limb reduction defects. Congenital heart defects are present in up to 25% of babies with RS who die in early infancy (Hennekam et al., 2010a). It has been reported that a portion of individuals with RS experience developmental delay, cognitive impairment, and poorer school achievement; overall morbidity and mortality are higher in syndromic RS or RS with associated anomalies compared with isolated RS (Caouette-Laberge et al., 1994; Persson et al., 2013). Clinical judgment can be made about whether the patient represents “isolated RS,” “RS plus,” or a syndromic form of RS, and the diagnostic work-up should include investigation of the common associated anomalies and syndromes (Tan et al., 2013; Gomez-Ospina and Bernstein, 2016).

Intensive Care Unit Concerns

In infants with RS the tongue is displaced toward the posterior pharyngeal wall or up into the cleft, resulting in upper airway

**TABLE
100.1****Craniofacial Syndromes Commonly Associated With Cleft Lip and/or Cleft Palate**

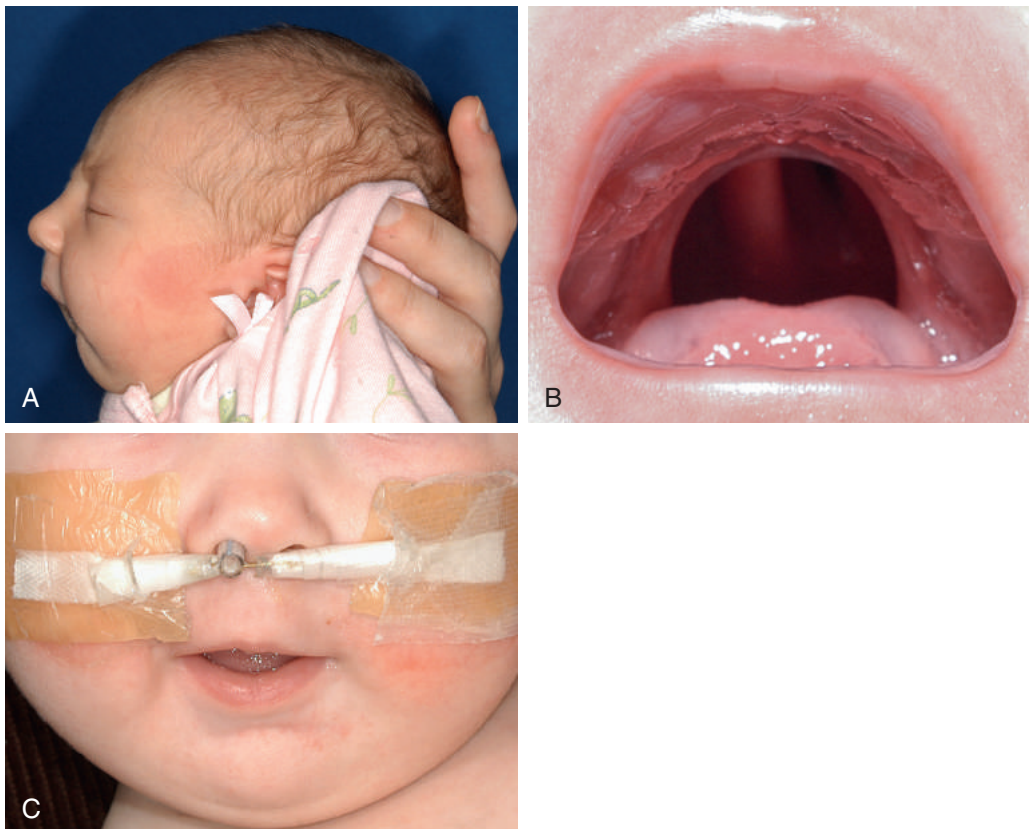
| Syndrome | Phenotype | ICU Issues | OMIM |
|---|--|--|--|
| Robin sequence ^a | Micrognathia, glossoptosis with upper airway obstruction, cleft palate | Airway obstruction, feeding difficulties | na |
| Stickler syndrome ^a | Cleft palate, micrognathia, glossoptosis (Robin sequence), high myopia, risk of retinal detachment and blindness, midface hypoplasia, hearing impairment, arthropathy, pectus, short fourth and fifth metacarpals | Airway obstruction, feeding difficulties | 180300, 604841, 184840, 614134, 614284 |
| 22q11.2 deletion syndrome (velocardiofacial syndrome, DiGeorge syndrome) ^a | Cleft palate and submucous cleft palate, small mouth, myopathic facies, retrognathia, prominent nose with squared-off nasal tip, hypoplastic nasal alae, short stature, slender tapering digits | Cardiac anomalies, airway obstruction, feeding difficulties, aspiration | 192430, 188400, 611867 |
| Opitz oculogenitoharyngeal syndrome (Opitz BBB/G syndrome) ^a | Hypertelorism, telecanthus, cleft lip and/or palate, dysphagia, esophageal dysmotility, laryngotracheoesophageal cleft (aspiration), hypospadias, bifid scrotum, cryptorchidism, agenesis of the corpus callosum, congenital heart disease, mental retardation | Laryngotracheoesophageal clefting (stridor, feeding difficulties, choking, aspiration) | 145410, 300000 |
| Pallister–Hall syndrome ^a | Cleft palate, flat nasal bridge, short nose, multiple buccal frenula, microglossia, micrognathia, malformed ears, hypothalamic hamartoblastoma, hypopituitarism, postaxial polydactyly with short arms, imperforate anus, genitourinary anomalies, intrauterine growth restriction | Laryngotracheoesophageal clefting (stridor, feeding difficulties, choking, aspiration), panhypopituitarism | 146510 |
| <i>IRF6</i> -related disorders (including Van der Woude and popliteal pterygium syndrome) | Cleft lip with or without cleft palate, cleft palate only, lower lip pits or cysts, ankyloglossia; popliteal pterygium syndrome will also have popliteal pterygia, bifid scrotum, cryptorchidism, finger and/or toe syndactyly, abnormalities of the skin around the nails, syngnathia and ankyloblepharon | Not anticipated | 119300, 119500 |
| CHARGE syndrome ^a | Coloboma of the eye, heart malformations, choanal atresia, growth retardation, genital anomalies, ear abnormalities and/or deafness, facial palsy, cleft palate, dysphagia | Airway obstruction in bilateral choanal atresia, cardiac anomalies, feeding difficulties, aspiration | 214800 |
| Smith–Lemli–Opitz syndrome ^a | Cleft palate, micrognathia, short nose, ptosis, high square forehead, microcephaly, hypospadias, cryptorchidism, ventricular septal defect, tetralogy of Fallot, hypotonia, mental retardation, postaxial polydactyly, 2–3 toe syndactyly, defect in cholesterol biosynthesis | Cardiac anomalies, airway hypotonia, and airway obstruction | 270400 |
| Ectrodactyly, ectodermal dysplasia, and clefting syndrome | Cleft lip and/or palate, split-hand/split-foot, ectodermal dysplasia (sparse hair, dysplastic nails, hypohidrosis, hypodontia), genitourinary anomalies | Not anticipated | 129900, 604292, 129400 |
| Ankyloblepharon, ectodermal dysplasia, and clefting syndrome | Cleft lip with or without cleft palate, cleft palate only, intraoral alveolar bands, maxillary hypoplasia, ankyloblepharon (eyelid fusion), ectodermal dysplasia (sparse hair, dysplastic nails, hypohidrosis, anodontia) | Not anticipated | 106260 |
| Orofaciodigital syndrome | Median cleft of upper lip, cleft palate, accessory oral frenula, lobulated tongue with hamartomas, broad nasal root, small nostrils, syndactyly, brachydactyly, postaxial polydactyly, polycystic renal disease, agenesis of the corpus callosum | Not anticipated | 311200 |
| Kabuki syndrome ^a | Cleft palate, arched eyebrow, long palpebral fissures, eversion of lateral third of lower eyelid, brachydactyly, short fifth metacarpal, cardiac anomalies, postnatal growth deficiency/dwarfism, mental retardation | Cardiac anomalies | 147920, 300867 |
| Fryns syndrome ^a | Cleft lip with or without cleft palate, micrognathia, coarse facies, diaphragmatic hernia, distal limb hypoplasia, malformations of the cardiovascular, gastrointestinal, genitourinary, and central nervous systems | Congenital diaphragmatic hernia, pulmonary hypoplasia; cardiac anomalies | 229850 |

**TABLE
100.1****Craniofacial Syndromes Commonly Associated With Cleft Lip and/or Cleft Palate—cont'd**

| Syndrome | Phenotype | ICU Issues | OMIM |
|---|--|--|------------------------|
| Miller syndrome (postaxial acrofacial dysostosis) ^a | Cleft palate (more than cleft lip), malar and mandibular hypoplasia, downslanting palpebral fissures, lower eyelid coloboma, microtia/atresia, conductive hearing loss, postaxial limb deficiency, absent fifth digit | Airway obstruction | 263750 |
| Treacher Collins syndrome (mandibulofacial dysostosis) ^a | Cleft palate, malar and mandibular hypoplasia, downslanting palpebral fissures, lower eyelid coloboma (missing medial lower eyelid lashes), microtia/atresia, conductive hearing loss | Airway obstruction | 154500, 613717, 248390 |
| Aarskog syndrome (faciodigitogenital syndrome) | Hypertelorism, widow's peak, ptosis, downslanting palpebral fissures, strabismus, maxillary hypoplasia, broad nasal bridge with anteverted nostrils, occasional cleft lip and/or palate, floppy ears, brachydactyly, clinodactyly, joint laxity, shawl scrotum | Not anticipated | 100050 |
| Wolf-Hirschhorn syndrome (4p deletion syndrome) ^a | Cleft lip and palate, coloboma, hypertelorism, growth deficiency, microcephaly, mental retardation, cardiac septal defects | Congenital diaphragmatic hernia, cardiac anomalies, seizures, airway hypotonia/obstruction | 194190 |
| Amnion rupture sequence ^a | Cleft lip and palate, oblique facial clefts, focal areas of scalp aplasia, constriction bands with terminal limb amputations and syndactylies, occasional anencephaly, encephalocele, and ectopia cordis | Encephalocele, oropharyngeal/airway deformation | 217100 |

^aPotential ICU issues.

ICU, Intensive care unit; OMIM, online mendelian inheritance in man.



• **Fig. 100.1** (A) Infant with Robin sequence and significant micrognathia. (B) U-shaped cleft palate. (C) Infant with Robin sequence and a nasopharyngeal tube in place.

obstruction. The tongue can act as a ball valve, leading to inspiratory obstruction. In addition to glossoptosis, other mechanisms may contribute to airway obstruction in individuals with RS, such as pharyngeal hypotonia and airway inflammation from associated gastroesophageal reflux. The principal physiologic sequelae of RS are the inability to effectively feed and breathe due to airway obstruction. In the immediate neonatal period, patients with RS may have increased inspiratory work of breathing, cyanosis, and apnea. Obstruction is more common in the supine position and can be exacerbated during feeding and in sleep or in any state where there is loss of pharyngeal tone. Chronic obstruction can lead to failure to thrive, carbon dioxide retention, pulmonary hypertension, and eventually right-sided heart failure (cor pulmonale).

Airway obstruction is the main cause of feeding and growth issues in infants with RS. Feeding problems can also be related to abnormal coordination, primary swallowing dysfunction, and pharyngeal hypotonia, and suction mechanics are complicated by the presence of a cleft palate. Increased energy expenditures because of the increased work of breathing may lead to failure to thrive if the infant is not receiving adequate caloric intake. Gastroesophageal reflux is common in infants with RS, as it is in other infants who have increased work of breathing.

Management

First and foremost, the airway must be addressed. Placement of a nasopharyngeal (NP) airway or endotracheal tube may be required in an emergency, and it is important to realize that severe, life-threatening airway obstruction can present in the delivery room. Although uncommon, a prenatal diagnosis of micrognathia allows the involvement of neonatologists and otolaryngologists in the delivery room (Costello et al., 2010).

A number of therapeutic maneuvers can be used to stabilize the upper airway in RS, ranging from positioning to surgery. Placing the baby in the prone or lateral decubitus position will often open up the airway and decrease the degree of obstruction. This may improve airway patency and air exchange, which decreases the work of breathing and may also improve tolerance of oral feeding. When prone positioning fails to stabilize the airway, alternative approaches include the use of an NP airway, noninvasive positive pressure, treatment with tongue–lip adhesion (TLA), and mandibular advancement through distraction osteogenesis. Children with isolated airway obstruction at the base of tongue without other medical comorbidities may be considered for mandibular distraction osteogenesis (MDO) (Paes et al., 2013). The surgery consists of surgical osteotomy and placement of distraction device that slowly increases mandibular length and ramus height and brings the base of the tongue forward, thereby increasing the airway space. This procedure will not achieve respiratory stabilization in patients with concomitant airway anomalies, lung disease, central apnea, or the need for positive pressure ventilation. Tracheotomy may be necessary to provide a safe and secure airway in some infants. Treatment protocols differ across institutions (Bookman et al., 2012), and an example of the initial evaluation and clinical team discussion for the neonate with tongue-based airway obstruction is provided in Box 100.1. While the threshold for intervention and the management options differ substantially, most providers agree that most neonates with RS can be treated nonsurgically.

An NP airway provides a temporary way to bypass the infant's airway obstruction (see Fig. 100.1C). An endotracheal tube can be

modified so that it can be passed through the nares into the hypopharynx above the epiglottis, allowing oxygenation/ventilation by bypassing the obstruction at the base of the tongue (Parhizkar et al., 2011). The NP airway may prevent the need for more invasive procedures and allows the team to address oral skills and feeding (Wagener et al., 2003). In some institutions the infant is discharged home with an NP airway in place (Abel et al., 2012). Infants are monitored with oximetry, and parents are taught NP airway maintenance (suctioning) and replacement. The NP tube is typically in place for 3 to 6 months or less if symptoms resolve or other interventions become necessary. Airway compromise and stability are assessed by physical examination, CO₂ levels, oxygenation, overnight sleep studies, and growth, monitored over time (Evans et al., 2011).

When airway obstruction is localized to the tongue base and positioning has not improved breathing and feeding, a TLA may be a temporizing measure to minimize obstruction while allowing for mandibular growth (Schaefer and Gosain, 2003). In some institutions, TLA has been shown to have a high initial success rate for correction of airway obstruction in a neonate. However, long-term follow-up indicates that many infants require secondary interventions to manage their feeding and airway (Denny et al., 2004).

The infant's clinical status, perceived need for long-term respiratory support, and failure of less invasive interventions will

• BOX 100.1 Evaluation and Decision Making for Neonates With Tongue-Based Airway Obstruction

Initial Evaluation in the Neonatal ICU:

Physical examination (supine vs prone): attention to craniofacial features, respiratory status, cardiac and limb differences
 Evaluation for presence of glossoptosis, stertor, obstructive apnea, and work of breathing
 Capillary blood gas and total CO₂ level
 Oxygen saturation monitoring
 Growth parameters
 Dysmorphology evaluation
 Craniofacial and otolaryngology consultations
 Consider genetics evaluation if there are multiple anomalies or a concerning family history (micrognathia, cleft palate, childhood hearing loss/myopia/joint problems)
 Consider airway endoscopy (guided by airway severity and response to interventions)
 Consider airway imaging (guided by airway severity and response to interventions)

Multidisciplinary Team Treatment Discussions May Address:

Does the patient need escalation in care to treat airway obstruction?
 Have appropriate subspecialty consults and evaluations been obtained? (Varies by institution, but can include specialists with expertise in neonatal intensive care, craniofacial and pediatric care, airway evaluations, airway surgery, jaw surgery, parent/family support)
 Should the patient undergo CT to assess the possibility of craniofacial skeleton and MDO (if so, when and how to proceed safely)
 Has the distal part of the airway been evaluated to look for other levels of airway obstruction?
 Does the patient need a tracheostomy tube, or is he/she a candidate for mandibular distraction?
 What is the family and social context?
 What will the disposition be once airway has been stabilized?

CT, Computed tomography; ICU, intensive care unit; MDO, mandibular distraction osteogenesis.

determine whether more invasive surgery is indicated (Evans et al., 2011; Cielo et al., 2016). For some neonates, mandibular distraction osteogenesis may be an alternative to tracheostomy. Airway endoscopy helps to delineate the level of obstruction, and computed tomography (CT) of the facial skeleton provides optimal understanding of jaw anatomy and tooth bud position before distraction. Recognition of other airway anomalies or issues, such as laryngotracheomalacia, subglottic stenosis, and poor secretion handling, will affect decision making regarding airway management. Children with RS associated with syndromes, skeletal dysplasia, or neurologic conditions may have more than one factor contributing to their airway obstruction such that a tracheostomy may be the best approach to alleviate respiratory compromise. Thus infants with RS who have airway obstruction unresponsive to positional techniques (side or prone) for whom surgical options are being considered (mandibular distraction versus tracheostomy) should have a comprehensive airway evaluation as well as a diagnostic evaluation for an underlying syndrome or associated malformations that might impact respiratory status and response to therapies.

Nutrition can be maintained with a hypercaloric formula and/or fortified breast milk given by side-lying feeding using a cleft feeder, via a nasogastric feeding tube, or via a gastrostomy tube. Oral feeding can and should be introduced when the airway is stable. Oral stimulation is important to prevent oral aversion. As tone improves, the child gains better control of the tongue, and growth ensues, feeding will become less of a problem. Close observation for symptoms of gastroesophageal reflux with proactive pharmacologic treatment can minimize airway inflammation.

Given the association with cognitive and motor delay, close monitoring of development and referral to early intervention services, such as a Birth to Three program, are recommended.

Stickler Syndrome

The most common syndrome associated with RS is Stickler syndrome. Between 20% and 30% of individuals with RS will have Stickler syndrome (Izumi et al., 2012). Stickler syndrome is most commonly an autosomal dominant (with variable expressivity) connective tissue disorder with ophthalmic, orofacial, auditory, and articular manifestations and has been divided into six types (Stickler syndrome types I and II have ocular findings, type III is nonocular, and types IV to VI are recessive conditions) (Robin et al., 2017).

Stickler syndrome is characterized by cleft palate, hearing loss, arthropathy, joint hypermobility, reduced height, and eye abnormalities, including myopia, cataracts, glaucoma, and retinal detachment. The myopia of Stickler syndrome is usually congenital, nonprogressive, and of high degree. Facial features include flat midface with depressed nasal bridge, short nose, anteverted nares, and micrognathia, telecanthus, and epicanthal folds with a concave facial profile (Fig. 100.2). Sensorineural hearing loss is more common in type II Stickler syndrome.

The diagnosis of Stickler syndrome should be considered in any neonate with RS or a cleft palate, especially when associated with myopia or hearing loss. Spondyloepiphyseal dysplasia is not usually apparent in the newborn period. Mutations affecting one of six genes (*COL2A1*, *COL9A1*, *COL9A2*, *COL9A3*, *COL11A1*, and *COL11A2*) have been associated with Stickler syndrome, and clinical molecular testing by sequence analysis is available for all types. More than 90% of individuals with Stickler



• **Fig. 100.2** Infant with Stickler syndrome, showing a flat face, depressed nasal bridge, and epicanthal folds. This infant also has Robin sequence and required tracheostomy.

syndrome are found to have a mutation in either *COL2A1* (Stickler syndrome type I, Online Mendelian Inheritance in Man [OMIM] 108300) or *COL11A1* (Stickler syndrome type II, OMIM 604841) (Robin et al., 2017). The diagnosis should also be considered in any newborn with a family history of RS or Stickler syndrome features.

In addition to appropriate management of feeding, breathing, and growth (as described earlier in RS), management of Stickler syndrome includes active detection of the ocular features of the syndrome, such as myopia. This is because the associated risk of retinal detachment and blindness are preventable. An initial ophthalmology evaluation is recommended for all children with RS aged between 6 and 12 months or at the time of a definitive molecular diagnosis of Stickler syndrome and then routine surveillance thereafter.

Orofacial Clefting

Epidemiology

Orofacial clefts of the primary and secondary palate are among the most common congenital anomalies. Classified as either cleft lip with or without CP (CL±P) or CP only (CPO), these two phenotypes are thought to be distinct in origin. One case of orofacial cleft occurs in approximately every 500 to 550 births, and on an average day in the United States, 20 infants are born with an orofacial cleft (Tolarova and Cervenka, 1998). Cleft lip and palate is the most common type of orofacial clefting, followed by cleft lip, then CPO, and less prevalent are atypical clefts (macrostomia or lateral cleft, oblique and midline clefts). Unilateral CL±P is more common than bilateral involvement (Genisca et al., 2009). A bifid uvula can be a normal variant, found in 2% to 4% of births, but can also be a sign of an associated submucous cleft palate, which can have the same functional impact as an overt CP (Hennekam et al., 2010a, Ch 21).

The causes of most orofacial clefts are unknown and are nonsyndromic (isolated) in 70%–75% of infants with CL±P and approximately 50% of those with CPO (Tolarova and Cervenka, 1998; Leslie and Marazita, 2013). Neonates with orofacial clefting

who are born prematurely or have low birth weight may have a higher incidence of associated congenital malformations (Milerad et al., 1997). Racial and ethnic variation in the prevalence of clefts has been described, with the highest prevalence of CL±P found in Native Americans, followed by whites and Hispanics, and the lowest overall prevalence of CL±P demonstrated in African Americans (Croen et al., 1998). The cause of nonsyndromic clefts is complex and multifactorial, likely resulting from interaction between environmental and genetic factors. Known environmental risk factors include maternal tobacco smoking, alcohol use, anticonvulsant treatment, and nutritional status. Recognition of contributing genetic factors such as a mutation in *IRF6* resulting in Van der Woude syndrome is increasing, and the impact of folate supplementation as an environmental modulator is under investigation (Mossey et al., 2009; Wehby and Murray, 2010). Although many candidate genes have been described, there is no routinely recommended genetic testing for a child with isolated CL±P in the absence of a family history. Recurrence risk information for the parents of a child with CL±P or for the affected individual is dependent either on the specific syndrome/genetic diagnosis or on empiric risks for those with nonsyndromic clefting. For a family with just one child affected with CL±P, the recurrence risk is 2%–5% for a subsequent child, increasing to 10%–15% if there are other family members with clefts. The recurrence risk is slightly less if the child has CPO (Harper, 2011).

Anatomy

The embryologic development of the primary palate begins very early in gestation, and the upper lip and primary palate have usually fused by the seventh week of gestation. A failure of fusion of the medial and lateral nasal processes with the maxillary process produces CL±P. Clefts can affect the primary palate (lip, alveolus, or anterior portion of the hard palate that extends to the incisive

foramen) and secondary palate (posterior hard palate and soft palate). Clefts of the primary and secondary palate can be unilateral or bilateral and complete or incomplete. A complete cleft of the primary palate leaves no residual tissue between the alar base and the lip, whereas an incomplete cleft does not extend through the floor of the nose (Fig. 100.3A–C, F).

Phenotype

The cleft of the primary and secondary palate will affect facial shape and growth (see Fig. 100.3A–C). Children with CP are at increased risk of eustachian tube dysfunction, recurrent otitis media, and acquired hearing loss, as well as speech issues later in childhood. Associated dental findings include hypodontia and, less commonly, natal teeth. Feeding difficulties, nasal regurgitation of feeds, and difficulty gaining weight may occur in infants with a CP (submucous and overt clefts of the palate).

Lateral facial clefting or macrostomia is pathogenically distinct from isolated cleft lip/palate and is often associated with syndromes, including craniofacial microsomia (CFM) and Treacher Collins syndrome (TCS). Amniotic rupture sequence can be associated with oblique facial clefts and may be associated with underlying central nervous system (CNS) malformations and transverse limb anomalies.

A true median cleft of the upper lip is the rarest type of facial clefting (see Fig. 100.3D). Midline clefting can be associated with other congenital defects as can be seen in orofaciogigital syndrome and frontonasal dysplasia (FND), and CNS malformations are common in children with midline clefts. Some midline clefts are not true clefts but represent hypoplasia or agenesis of the primary palate or premaxillary agenesis, which can be associated with holoprosencephaly (HPE) sequence (see Fig. 100.3E). Infants with HPE often have a depressed nasal tip and a short columella and appear hypoteloric (compared with FND or frontonasal encephalocele, where midline clefting may



• **Fig. 100.3** (A) Infant with a unilateral incomplete cleft lip. (B, C) Infant with bilateral complete cleft lip and palate. (D) Infant with midline cleft and hypertelorism. He also has a frontonasal encephalocele. (E) Infant with premaxillary agenesis and holoprosencephaly. (F) Infant with Van der Woude syndrome with unilateral complete cleft lip and a lip pit (arrow).

be present, but the infant has a broad nasal tip and/or columella and hypertelorism).

Orofacial clefting is rarely associated with clefting of the airway structures, such as cleft larynx or extension of clefting into the trachea. Opitz G/BBB syndrome is a multiple congenital anomaly syndrome characterized by facial anomalies (100% will be hypertelorism and 50% will have CL±P), genitourinary abnormalities (90% will have hypospadias), and laryngotracheoesophageal (LTE) defects (present in 70%) (Meroni, 2011). Autosomal dominant (OMIM 145410) and X-linked recessive (OMIM 300000) forms of Opitz G/BBB syndrome are recognized. Pallister–Hall syndrome (PHS; OMIM 146510) is characterized by a constellation of findings that include hypothalamic hamartoma (resulting in seizures and pituitary dysfunction), polydactyly, airway clefting, and other anomalies (genitourinary, renal, pulmonary, and imperforate anus). Bifid epiglottis is the most common airway manifestation in PHS, although LTE clefts have been reported. LTE defects may range from LTE dysmotility in mild forms to laryngeal or tracheoesophageal clefts in more severe forms.

ICU Concerns

Most infants with CL±P do not require ICU care. Thus an infant with an apparently isolated cleft who develops significant respiratory or electrolyte abnormalities requiring ICU care should be considered syndromic until proven otherwise. In these infants a genetics consultation should be considered.

The newborn with a midline cleft or premaxillary agenesis is at risk of serious underlying CNS anomalies, including HPE. In the presence of HPE, detection of associated medical issues is important. Endocrine abnormalities can arise because the midline malformation affects the development of the hypothalamus and the pituitary gland. Clinical manifestations can include growth hormone deficiency, adrenal hypoplasia, hypogonadism, diabetes insipidus, and thyroid deficiency. Neurologic manifestations that warrant close attention include seizures, hypotonia, spasticity, autonomic dysfunction, and developmental delays.

With an LTE cleft, there is longitudinal communication between the airway and the esophagus, allowing tracheal aspiration of oral contents, including saliva and feeds. Clefting of the larynx may result in stridor, a hoarse cry, respiratory distress, swallowing dysfunction, feeding difficulties, regurgitation, and aspiration, hypoxia, recurrent pneumonias, and eventually severe respiratory compromise if unrecognized. An infant boy with hypertelorism, hypospadias, orofacial clefting, and symptoms of airway obstruction or aspiration should be evaluated for Opitz syndrome. Infants with PHS may also have respiratory distress due to airway clefting, as well as other potentially life-threatening clinical manifestations such as seizures and severe panhypopituitarism. Genetic evaluation and consideration of molecular testing for Opitz syndrome and PHS can be coordinated through a geneticist.

Management

The specifics of management of orofacial clefting are center specific. Because of the potential impact of the orofacial cleft on breathing, eating, hearing, speech, facial growth, and dental health, it is recommended that infants and children with clefts be referred to a multidisciplinary care team for long-term management. In remote areas, the nearest cleft team may be found through the American Cleft Palate–Craniofacial Association (ACPA) team listings (American Cleft Palate–Craniofacial Association, 2017). Overviews of

recommended team care for patients with cleft lip/palate can be accessed electronically (American Cleft Palate–Craniofacial Association, 2009; The Center for Children with Special Needs, 2010).

On the initial assessment, the provider should assess the cleft and examine the infant for dysmorphic features and other anomalies. Hearing should be evaluated by evoked otoacoustic emissions or by brainstem auditory evoked response if the newborn does not pass the initial hearing screen. A neonate with a complete cleft lip should be evaluated by a craniofacial or cleft team in the first 2 weeks of life, and some centers offer taping or presurgical molding (nasal alveolar molding) that can be initiated in this period.

Many mothers will be able to breastfeed an infant born with an isolated cleft lip. Breastfeeding a baby with CP (with or without cleft lip) will prove extremely challenging because the open palate will not generate the negative pressure needed for sucking. Thus infants with CP with or without cleft lip should be offered expressed breast milk or infant formula with use of a specialized cleft feeder. A variety of cleft nipples/bottles have been devised to allow oral feeding, including the CP nurser (squeeze bottle), Haberman feeder, Pigeon bottle, and Dr. Brown bottle with a cleft valve (<http://www.cleftline.org/who-we-are/what-we-do/feeding-your-baby/>). Infants with CP tend to swallow more air during feedings. The child should feed in an upright position, as gravity will help prevent nasal regurgitation. If the child is still having difficulty feeding, a feeding specialist should be consulted. Adequate weight gain is important for overall health and readiness for the surgical procedures that occur in the first year of life. Newborns with clefts are considered nutritionally high risk, and a dietitian should be consulted to help determine caloric needs and to closely monitor growth.

In general, surgical closure of the lip and nasal deformity is done within the first 6 months of life. Palatoplasty typically occurs between 9 and 12 months of age to optimize speech and language development.

If there are concerns about airway clefting or anomalies of the larynx or trachea, a chest X-ray should be obtained and the airway evaluated, in addition to appropriate evaluation of associated anomalies. Microlaryngoscopy with the patient under general anesthesia remains the gold standard in the diagnosis of a laryngeal cleft (Johnston et al., 2014). Given the risk of gastrointestinal manifestations such as gastroesophageal reflux, dysmotility, and aspiration, antireflux precautions should be initiated in infants with suspected or confirmed LTE defects. Early diagnosis and proper repair of the laryngeal cleft are essential to prevent injury to the lungs. Significant LTE defects will need to be managed surgically, and tracheostomy may be necessary initially to ensure airway stability and safety.

In the presence of a midline cleft, it is important to evaluate the patient for underlying CNS malformations such as HPE. In any child with a midline cleft or facial features consistent with premaxillary agenesis/hypoplasia, CNS imaging (CT or MRI) is recommended. Consultation with a geneticist or genetic counselor may provide insight into the genetics, molecular testing options, and recurrence risk of HPE. Treatment of HPE is supportive and based on symptoms. The outcome depends on the severity of HPE and the associated medical and neurologic manifestations.

Syndromes Associated With Cleft Lip and/or Palate

It is estimated that there are more than 400 syndromes associated with orofacial clefts (Hennekam et al., 2010a). The frequency

with which associated malformations are encountered with CL±P is approximately 25% (Genisca et al., 2009). In approaching diagnosis of a syndrome, one should categorize the type of cleft (CL±P, U-shaped or V-shaped cleft palate, or more atypical orofacial cleft) and look for any other malformations. Table 100.1 describes the syndromes most commonly associated with clefting and the key features, potential ICU issues, and OMIM database classification. The OMIM database at the National Library of Medicine is a comprehensive collection of more than 15,000 human genes and genetic phenotypes. A referral to a clinical geneticist is recommended when an underlying diagnosis is suspected but not established.

22q11.2 Deletion Syndrome

Epidemiology and Genetics

22q11.2 deletion syndrome is a genetic condition with an estimated prevalence of 1 in 1000 births in which affected individuals are missing a region (typically 3 Mb, encompassing approximately 40 genes) on one copy of chromosome 22 (Carlson et al., 1997; McDonald-McGinn et al., 2015). Before the availability of genetic testing for this condition, individuals with clinical features of 22q11.2DS were classified under a range of other clinical syndromes, such as DiGeorge syndrome, velocardiofacial syndrome, and Shprintzen syndrome. Subsequently, a subset of children with overlapping features in these conditions (such as congenital heart disease and cleft palate) were also noted to share a deletion on chromosome arm 22q. It was later discovered that the most children in whom either DiGeorge syndrome or velocardiofacial syndrome has been clinically diagnosed share the deletion on one copy of chromosome 22. It has been estimated that more than 90% of individuals with “classic” features of 22q11.2DS have a detectable 22q deletion (McDonald-McGinn et al., 2013). 22q11.2DS is associated with more than 180 clinical features, and phenotypic variation is a hallmark of this genetic condition (McDonald-McGinn et al., 2015).

Phenotype

In neonates, 22q11.2DS presents in various ways. In some infants this condition is diagnosed prenatally. Testing may occur as part of the evaluation for fetuses with congenital heart disease or because of a parental history of 22q11.2DS. The clinical indications for genetic testing for this condition in neonates frequently include congenital heart malformations (particularly conotruncal anomalies), seizures secondary to hypocalcemia, dysphagia, cleft palate, and/or respiratory distress secondary to upper airway obstruction. 22q11.2DS commonly has multiorgan system involvement, including cardiac and palatal abnormalities, immune differences, endocrine and gastrointestinal problems, and later-onset conditions across the life span, including variable cognitive deficits and

psychiatric illness. In this section, we focus on the evaluation of infants with craniofacial characteristics suggestive of 22q11.2DS.

Several craniofacial features have been observed in individuals with 22q11.2DS; however, many of these are subtle and may not be apparent in the newborn period. Common features identified on the newborn physical examination include cleft palate, small, overfolded helices, and tapered fingers. Other clues to the diagnosis include dysphagia and/or nasal regurgitation (even in the absence of an overt cleft palate), congenital heart disease (most commonly conotruncal anomalies), and hypocalcemia.

An estimated 8% of infants with CP have 22q11.2DS (Hennekam et al., 2010b). For this reason, recommendations differ regarding routine testing of infants with isolated cleft palate. Most agree, however, that molecular testing is indicated for children with a CP in combination with any of the other features that can be observed in 22q11.2DS. The accurate identification of 22q11.2-associated disorders impacts medical surveillance, management, and counseling. Clinical testing with chromosomal microarray or multiplex ligation-dependent probe amplification will capture deletions, duplications, and smaller changes including those that would not be detected with fluorescence in situ hybridization for 22q deletion.

Evaluation and Management

Families of infants, for whom there is a high clinical suspicion and those testing positive for this deletion, should receive genetic counseling. Individuals with 22q11.2DS should undergo studies to identify associated health concerns. These screening evaluations include a total lymphocyte count (low absolute lymphocyte count necessitates evaluation of T-cell and B-cell subsets and referral to an immunologist), hematocrit, platelet count, and total and ionized calcium levels to screen the infant for hypocalcemia. Additional studies include echocardiogram to evaluate the infant for congenital heart malformations and renal ultrasonography. Newborns should have a palatal examination to evaluate them for overt or submucous clefting, as well as a diagnostic hearing test. Infants with evidence of dysphagia (even in the absence of a palatal cleft) benefit from an evaluation by a feeding specialist to determine if a swallow study is needed or if a cleft bottle would be helpful. Additional recommendations for screening evaluations and management have been outlined by McDonald-McGinn et al. (2015).

Craniosynostosis

Definitions/Epidemiology

Craniosynostosis refers to the premature fusion of one or more cranial sutures (metopic, sagittal, right or left coronal, or right or left lambdoid) that normally separate the bony plates of the cranium. The birth prevalence of all craniosynostoses is estimated to be 1 in 2500 live births (Boulet et al., 2008).

Typically, patent sutures allow the calvaria to expand as the brain grows, producing the normal head shape and size. If one or more sutures fuse prematurely, there is restricted growth perpendicular to the fused sutures and compensatory growth in the patent sutures, producing an abnormal head shape. Craniosynostosis is a heterogeneous disorder with significant health consequences that range from an abnormal head shape and increased intracranial pressure (ICP) to secondary visual and intellectual impairments. Known causes of primary craniosynostosis include monogenic and chromosomal abnormalities as well as environmental factors. Nonsyndromic single suture craniosynostosis accounts for 85% of patients. Syndromic

• BOX 100.2 Specialties of the Members of a Craniofacial Team

| | |
|------------------|-----------------|
| Pediatrics | Otolaryngology |
| Nursing | Plastic surgery |
| Social work | Neurosurgery |
| Genetics | Ophthalmology |
| Nutrition | Oral surgery |
| Feeding | Dentistry |
| Speech pathology | Orthodontics |
| Audiology | Psychology |

TABLE 100.2 Craniosynostosis Syndromes and Potential Airway Compromise

| Syndrome | Key Features | Tracheal Abnormalities | Midface Hypoplasia | OMIM |
|---|--|--|--|--------|
| Apert syndrome ^a | Craniosynostosis (coronal > lambdoid > sagittal), acrobrachycephaly (steep, wide forehead and flat occiput), proptosis, hypertelorism, exotropia, trapezoid-shaped mouth, prognathism, invariable symmetric syndactyly of hands and feet, variable elbow fusion, cognitive impairment, narrow palate with lateral palatal swellings, widely patent sagittal suture connecting anterior and posterior fontanels | Tracheoesophageal fistula, tracheal cartilaginous sleeve less common | Significant maxillary hypoplasia, obstructive sleep apnea syndrome | 101200 |
| Crouzon syndrome ^a | Craniosynostosis (coronal > lambdoid > sagittal), brachycephaly, prognathism, exophthalmos, papilledema, hypermetropia, divergent strabismus, atresia of auditory canals, Chiari type 1 malformation and hydrocephalus | Solid cartilaginous trachea or tracheal cartilaginous sleeve | Significant maxillary hypoplasia, obstructive sleep apnea syndrome | 123500 |
| Pfeiffer syndrome types I, II, and III ^a | Craniosynostosis (coronal > sagittal > lambdoid), brachycephaly, hypertelorism, proptosis, broad first digits with radial deviation, variable syndactyly and elbow fusion, cloverleaf skull | Solid cartilaginous trachea or tracheal cartilaginous sleeve | Significant maxillary hypoplasia, obstructive sleep apnea syndrome | 101600 |
| Muenke syndrome | Unilateral or bilateral coronal craniosynostosis, brachydactyly, downslanting palpebral fissures, thimble-like middle phalanges, coned epiphysis, carpal and tarsal fusions, sensorineural hearing loss, Klippel–Feil anomaly | | Mild maxillary hypoplasia, no airway compromise anticipated | 602849 |
| Saethre–Chotzen syndrome ^a | Unilateral or bilateral coronal craniosynostosis, acrocephaly, brachycephaly, low frontal hairline, hypertelorism, facial asymmetry, ptosis, characteristic ear (small pinna with a prominent crus), fifth finger clinodactyly, partial 2–3 syndactyly of the fingers, duplicated halluces | | Maxillary hypoplasia | 101400 |
| Carpenter syndrome | Craniosynostosis (coronal > lambdoid > sagittal), hypertelorism, proptosis, brachycephaly, brachydactyly, preaxial polysyndactyly, mental retardation | | Maxillary hypoplasia | 201000 |
| Jackson–Weiss syndrome | Craniosynostosis (coronal), acrocephaly, hypertelorism, proptosis, midface hypoplasia, radiographic abnormalities of the foot including fusion of the tarsal and metatarsal bones, 2–3 syndactyly, broad short first metatarsals and broad proximal phalanges | | Maxillary hypoplasia | 123150 |

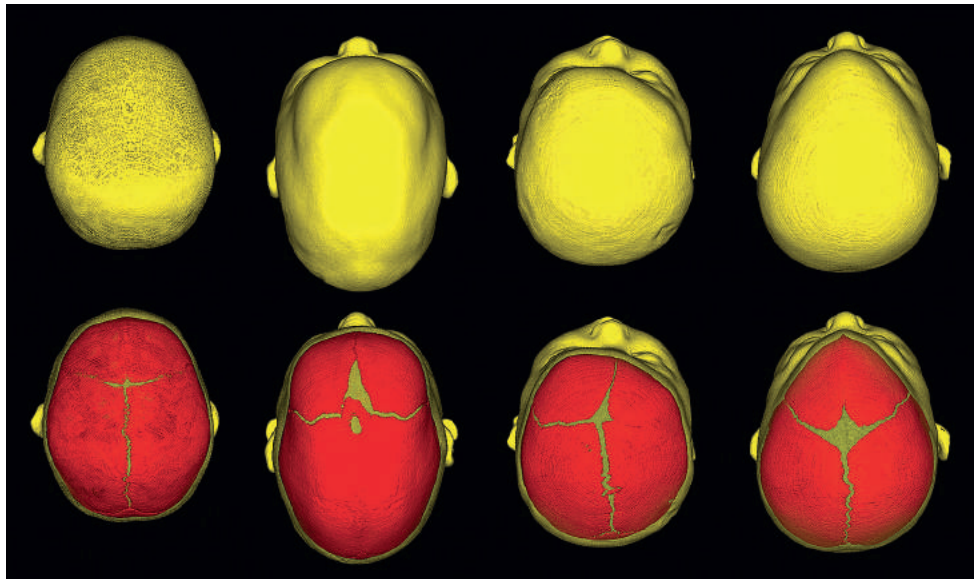
^aSignificant risk of airway morbidity.
OMIM, Online mendelian inheritance in man.

cranosynostosis may involve single or multiple fused sutures, additional anomalies (such as limb, cardiac, CNS, and tracheal malformations), and developmental delay. Multiple suture involvement is usually considered hereditary even when it does not fit a classic pattern of anomalies. Advances in molecular genetics and next-generation sequencing have led to the identification of causative mutations and genetic pathways in these relatively common congenital anomalies (Twigg and Wilkie, 2015a, 2015b). The genetic cause of craniosynostosis in humans is only partially understood. However, for most syndromic forms, identification of the primary genetic cause and contributing factors is possible with use of clinically available genetic tests (Agochukwu et al., 2012).

Single Suture Synostosis

Sagittal synostosis is the most common single suture synostosis (50%–60%), with a prevalence of 1.5 per 10,000 live births (Boulet et al., 2008). Known risk factors include male sex, intrauterine head constraint, twin gestation, thyroid hormone dysregulation,

and maternal smoking. Although uncommon, the most frequently encountered associated anomalies include congenital heart defects and genitourinary tract malformations. Syndromes with synostosis involving only the sagittal suture are rare. Premature union of the sagittal suture hinders normal calvarial expansion, leading to scaphocephaly, an elongated, narrow calvarium, decreased bitemporal diameter, and frontal and occipital bossing (Fig. 100.4). Premature fusion of the suture before birth leads to abnormal head shape in the newborn period. A breech-positioned neonate can have scaphocephaly or dolichocephaly that may mimic sagittal synostosis. However, in sagittal synostosis, frontal bossing and biparietal narrowing progress, whereas the head shape in a breech-positioned infant will normalize in the first month of life. There is a concern that children with single suture synostosis are at risk of elevations in ICP, local brain injury, and later developmental delays. Although school-age children born with single suture craniosynostosis have been found to have evidence of mild developmental delays, the pathogenesis and direct relationship to synostosis have not been determined (Speltz et al., 2015).



• **Fig. 100.4** Head shapes in single suture synostosis. From left to right: normal head shape, sagittal synostosis, coronal synostosis, and metopic synostosis.

Coronal synostosis is the second most common single suture synostosis (20%–30%), with a prevalence of 0.7 per 10,000 live births (Boulet et al., 2008). The skull is notable for a flat supraorbital rim and orbit that appears higher on the affected side, with a frontal bulge on the contralateral side (see Fig. 100.4). The nose often appears to twist away from the coronal fusion. Genetic syndromes are more frequently seen in individuals with coronal synostosis, including Saethre–Chotzen syndrome, Muenke syndrome, and craniofrontonasal dysplasia. All families of children with coronal synostosis should be offered genetic consultation and/or genetic testing to include *FGFR2*, *FGFR3*, *TWIST1*, *TCF12*, and *EFNB1* on the basis of clinical examination.

Metopic synostosis (15%–20% of single suture craniosynostosis) has a prevalence of 0.8 per 10,000 live births (Boulet et al., 2008), although recent reports suggest that metopic synostosis may be as common as coronal synostosis (Lee et al., 2012). Risk factors include male sex, twin gestation, and in utero exposure to valproate. Syndromes, associated anomalies, and chromosomal abnormalities occur in approximately one-quarter of individuals with metopic synostosis (Lajeunie et al., 1998; Azimi et al., 2003). Premature fusion of the metopic suture results in a triangular head shape, or trigonocephaly, which features a midline forehead ridge, fronto-temporal narrowing, pterion constriction, hypotelorism, and an increased biparietal diameter (see Fig. 100.4). Isolated metopic ridging is common in infancy, does not distort forehead shape, and is not associated with metopic synostosis.

Lambdoid synostosis (3% of single suture craniosynostosis) is the least common form of single suture synostosis. It is characterized by flattening of the ipsilateral occiput, posterior–inferior displacement of the ear, bulge of the mastoid process on the fused side, and a skull base tilted downward on the affected side.

Multiple Suture Synostosis

Multiple suture (or *multisuture*) *synostosis* describes patients who have two or more fused sutures. Although children with multisuture synostosis are more likely to have a known syndromic form of

craniosynostosis such as Apert syndrome, Crouzon syndrome, or Muenke syndrome, some have chromosome aberrations or patterns of craniosynostosis with associated anomalies not previously described. With 20 known hereditary forms of craniosynostosis, genetic consultation and counseling are of critical importance in the management of these conditions (Twigg and Wilkie, 2015a, 2015b). Here we briefly discuss select major syndromes with craniosynostosis that may have medical issues in the newborn period. See Table 100.2 for a description of key phenotypic features and potential airway compromise.

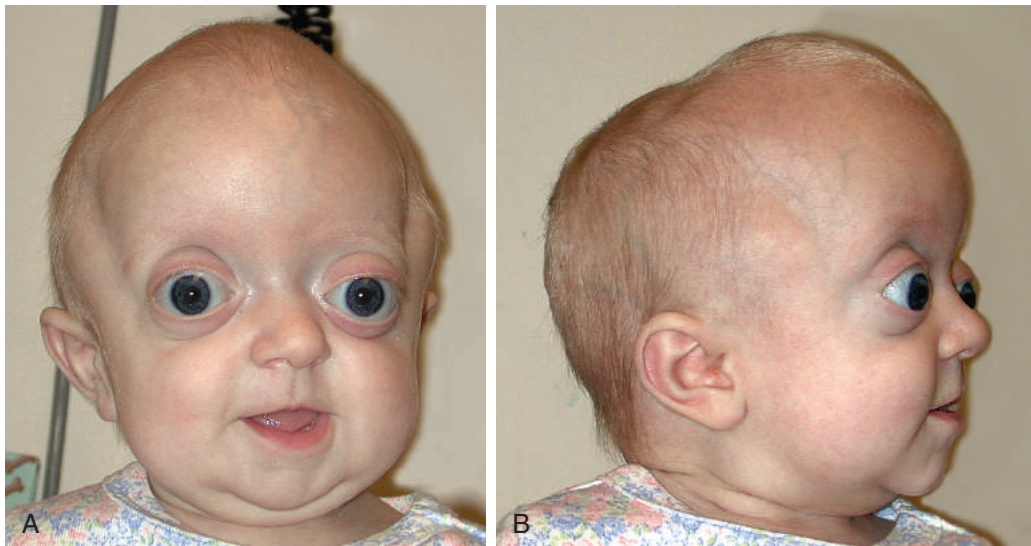
Apert syndrome (OMIM 101200) was initially described as acrocephaly with four-limb syndactyly. It accounts for 4.5% of all craniosynostosis (Hennekam et al., 2010c; Fig. 100.5). It is inherited as an autosomal dominant trait and is associated with advanced paternal age. Neurocognitive outcomes differ, but a moderate to severe degree of cognitive impairment is most common. Four mutations in *FGFR2* causing Apert syndrome have been identified.

Crouzon syndrome (OMIM 123500) is an autosomal dominant condition that demonstrates wide phenotypic variability. Shallow orbits with proptosis are an important diagnostic finding, although this feature may be subtler in the newborn (Fig. 100.6). Significant abnormalities involving the CNS include the frequent presence of a Chiari type 1 malformation, with progressive hydrocephalus resulting in intracranial hypertension. Compared with Apert syndrome, Crouzon syndrome is associated with more extensive suture involvement, smaller cranial volume, and more severe intracranial constraint; however, cognitive development is usually normal. Like Apert syndrome, Crouzon syndrome is caused by mutations in *FGFR2*. A less common form of Crouzon syndrome with acanthosis nigricans skin findings developing in the first 2 years of life is caused by a transmembrane mutation in *FGFR3* (OMIM 612247).

Pfeiffer syndrome (OMIM 101600) is a hereditary craniosynostosis that shares significant overlap, both phenotypically and genetically, with Crouzon syndrome. It is an autosomal dominant inherited disorder with craniosynostosis accompanied by proptosis, broad and deviated thumbs and big toes, and



• **Fig. 100.5** (A) Infant with Apert syndrome, a high and full forehead, proptosis and exotropia, midface hypoplasia, and a trapezoid-shaped mouth. (B, C) Hands and feet in Apert syndrome. Note the syndactyly symmetrically affecting hands and feet. All five digits may be webbed, or a single toe, finger, or thumb may be free.



• **Fig. 100.6** (A) Infant with Crouzon syndrome with brachycephaly. (B) Proptosis is seen in the lateral view.

partial syndactyly of the hands and feet (Fig. 100.7). Mutations in *FGFR1* and *FGFR2* cause Pfeiffer syndrome. Type 1 (i.e., classic) Pfeiffer syndrome involves mild manifestations including brachycephaly, midface hypoplasia, and digital malformations. Type 2 consists of cloverleaf skull, extreme proptosis, digital malformations, elbow ankylosis, developmental delay, and neurologic complications. Type 3 is similar to type 2 but without a cloverleaf skull.

Muenke syndrome (OMIM 602849) is an autosomal dominant syndrome caused by a single P250R mutation in the *FGFR3* gene. Like Apert syndrome, Muenke syndrome is associated with advanced paternal age. Individuals with Muenke syndrome may have coronal craniosynostosis (unilateral or bilateral) or macrocephaly and variable

degrees of proptosis, without significant midface hypoplasia (Fig. 100.8).

Saethre–Chotzen syndrome (OMIM 101400) is caused by a mutation in the *TWIST1* gene on chromosome 7. The inheritance is autosomal dominant, and many children with Saethre–Chotzen syndrome will have an affected parent. In addition to craniosynostosis, affected individuals commonly have a low frontal hairline, ptosis, 2–3 syndactyly of the fingers, cervical spine anomalies, and duplicated halluces. Although learning difficulties may be noted, cognitive impairment is not typical of Saethre–Chotzen syndrome caused by intragenic mutations. Children with deletions rather than point mutations often demonstrate significant developmental delays.



• **Fig. 100.7** (A, B) Infant with Pfeiffer syndrome, brachycephaly, a high forehead, midface hypoplasia, proptosis, and ocular hypertelorism. (C) An older child with Pfeiffer syndrome and the typical broad thumbs with radial deviation.



• **Fig. 100.8** (A, B) Infant with Muenke syndrome, acrobrachycephaly due to bicoronal synostosis, and absence of proptosis. (C) Sibling of the infant in (A, B) also with Muenke syndrome; note the downslanting palpebral fissures.

Cloverleaf skull can result from any form of multisuture craniosynostosis. The skull forms a trilobular appearance, as the cerebrum bulges through the sagittal and squamosal sutures, because of craniosynostosis affecting the coronal, metopic, and lambdoid sutures. Cloverleaf skull can be isolated or more commonly associated with a syndrome, and it is estimated that up to 20% of cases represent Pfeiffer syndrome.

ICU Concerns

The most significant concerns for the newborn with craniosynostosis are airway compromise (specifically, upper airway obstruction) and intracranial hypertension.

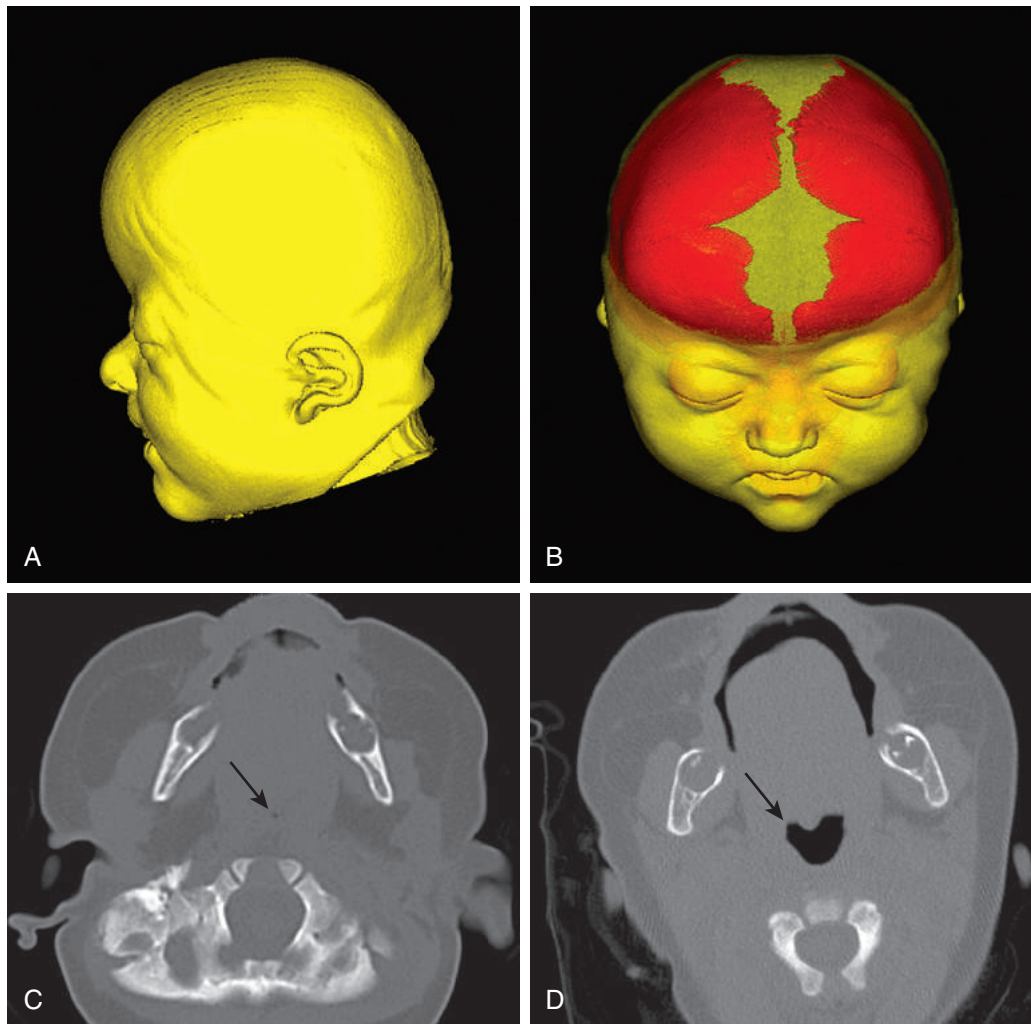
Midface hypoplasia and tracheal anomalies that may be present in syndromic craniosynostosis can lead to significant airway compromise (see [Table 100.2](#)). With midface hypoplasia, there is decreased NP/oropharyngeal space because of a small maxilla, narrowing at the level of the posterior choanae and posterior displacement of bony and soft tissue structures, leading to breathing problems, obstructive sleep apnea, asphyxia, and even death ([Fig. 100.9](#)). Obstructive sleep apnea is common in Apert, Pfeiffer, and Crouzon syndromes.

Cartilaginous tracheal abnormalities can be present in multisuture craniosynostosis syndromes. Vertically fused tracheal cartilage (also referred to as tracheal cartilaginous sleeve, solid cartilaginous trachea, and stovepipe trachea) in Crouzon and Pfeiffer syndromes may produce a rigid trachea resulting in upper airway stenosis, inability to clear secretions, and increased risk of injury because of decreased distensibility. Characteristic tracheal cartilaginous rings are fused to form a continuous sleeve of cartilage, which may extend from

below the subglottis to the carina or bronchus; rarely, the cartilaginous sleeve can begin more proximally, at the level of the cricoid cartilage. Infants with congenital tracheal anomalies may have fixed stridor, apnea, cyanosis, or increased work of breathing because of multilevel airway obstruction.

Neurologic abnormalities such as hydrocephalus and increased ICP may arise, especially in multisuture craniosynostosis. Increased ICP due to constraint of the growing brain within a restricted calvarium is usually of low grade and chronic, causing symptomatic intracranial hypertension when brain growth is rapid during the first 2 years of life. ICP issues in the neonate are not usually life threatening, given the open fontanel and compensatory splaying of normal sutures or erosion of the calvarium, but brain injury and cognitive impairment may result if skull-expanding surgery is not performed.

Hydrocephalus, which is more common in Crouzon and Pfeiffer syndromes compared with other multisuture synostosis syndromes, can occur as a result of obstruction of cerebrospinal fluid at the basal cistern, aqueductal stenosis, or impeded venous flow or when there is an associated Chiari malformation. Hydrocephalus is extremely common in cloverleaf skull. Individuals with multisuture craniosynostosis (particularly Apert syndrome) more commonly have nonprogressive distortion ventriculomegaly or compensated hydrocephalus, which does not require shunting ([Collmann et al., 2005](#)). Abnormalities of the corpus callosum and septum pellucidum have been described in Apert syndrome, and neuroimaging and genetic advances will illustrate links between brain architecture, phenotype, and genotype ([Fernandes et al., 2016](#)). Seizures presenting in multisuture craniosynostosis syndromes are usually due to encephalopathy rather than increased ICP. Epilepsy is more common



• **Fig. 100.9** (A, B) Three-dimensional reconstruction of a child with Apert syndrome with significant midface hypoplasia, leading to upper airway obstruction. Also notable is acrobrachycephaly due to bicoronal synostosis and the typical pattern of sagittal suture patency. (C) Computed tomography (CT) scan axial slice at the level of the skull base in a newborn with Apert syndrome. The arrow pointing to the airway illustrates significant airway obstruction. (D) CT scan of a newborn illustrating a normal airway (arrow).

with increasing number of sutures involved, and seizures occur in approximately 10% of individuals with Crouzon syndrome (Cohen, 2000).

Conductive and mixed hearing loss, most commonly due to middle ear disease, ossicular abnormalities, and external auditory canal stenosis or atresia, can be present in syndromic craniosynostosis. Profound sensorineural hearing loss has been described in Saethre–Chotzen syndrome (Lee et al., 2002).

Evaluation

The evaluation of the patient with craniosynostosis includes recognizing and confirming the type of suture fusion, clinical syndrome identification, evaluation for associated anomalies, and preparedness for surgical repair. A craniofacial team made up of the appropriate specialties allows proper planning and coordination so that the patient may receive the best possible care (McCarthy et al., 2012).

The family and prenatal history, including documentation of affected family members, teratogen exposure, maternal thyroid

disease, and in utero constraint (oligohydramnios, twins, fetal movement), and the birth history should be ascertained, specifically looking for risk factors.

A detailed physical examination should be performed as part of the initial evaluation, looking for any other anomalies, with specific attention to cleft palate, limb defects, heart defects, and ear anomalies. The assessment of cranial and face shape, mobility of the sutures, presence of sutural ridging, skull base symmetry, and ear position is important. Facial appearance, with particular attention to the degree of maxillary hypoplasia, is important in determining the risk of airway compromise due to midface hypoplasia. If concerning airway symptoms are present, such as snoring, stridor, or apnea, consultation with a sleep specialist and polysomnography may help to quantify the presence and severity of obstructive sleep apnea. Consultation with an otolaryngologist and airway endoscopy may help identify the types and degree of airway narrowing (Wenger et al., 2017). Particular attention to the presence of tracheal malformations, such as vertically fused tracheal cartilage, is crucial in some craniosynostosis syndromes.

With the increased awareness of this condition, the diagnosis of these tracheal malformations is increasingly made on direct laryngoscopy/bronchoscopy or with MRI.

Neurologic assessment includes ascertaining the history, brain imaging, an audiologic evaluation (early screening for hearing loss in conjunction with regular otologic examinations), ophthalmologic evaluation, and ongoing developmental assessments. In multisuture craniosynostosis, it is important to monitor the patient for any signs or symptoms of increased ICP. Evaluation of the patient for hydrocephalus should be a part of the initial assessment of all children with multisuture craniosynostosis. CT with three-dimensional reconstruction will ultimately confirm the diagnosis of craniosynostosis, delineate the degree of suture involvement, and help with preoperative planning. MRI may be helpful in defining any associated CNS anomalies. Ophthalmology consultation is valuable in management of proptosis, strabismus, or nystagmus and in determining the presence of papilledema or optic atrophy.

In addition to the foregoing general recommendations, syndrome-specific recommendations are outlined as follows. In Apert syndrome a cardiac and genitourinary evaluation is recommended. If proptosis is present, as can occur in Apert, Crouzon, and Pfeiffer syndromes, ocular lubricants may be helpful in prevention of exposure keratopathy. In Apert, Crouzon, Pfeiffer, and Saethre-Chotzen syndromes, associated vertebral anomalies, especially fusions, may be present, detected on spine radiographs, and more accurately visualized with CT imaging. If any limb abnormalities are seen, as in Apert, Jackson-Weiss, Pfeiffer, and Saethre-Chotzen syndromes, radiographs with orthopedic consultation should be obtained.

All individuals with single suture synostosis and developmental delay or associated birth defects should be evaluated by a geneticist to determine association with a clinical syndrome and the role of genetic testing. The families of children with multisuture synostosis caused by known classic craniosynostosis syndromes should be offered appropriate genetic testing and genetic counseling. The remaining children with multisuture synostosis in the absence of a known syndromic form should be offered genetic consultation and possible molecular genetic testing.

Management

Although the specific timing of the surgical treatment may differ between teams, it is generally accepted that individuals with synostosis should undergo cranial surgery in the first year of life. Cranioplasty involves release of fused sutures and repositioning and reconstruction of the calvaria, so as to prevent increased ICP and progressive abnormal craniofacial development. Several techniques, including endoscopic strip craniectomy, calvaria distraction, and traditional cranioplasty, are currently used.

Early recognition of tracheal malformations can be lifesaving (Letsburapa et al., 2010). Awareness of potential airway compromise and proactive airway management are crucial in many craniosynostosis syndromes. Temporizing measures to bypass airway obstruction include placement of nasal stents, endotracheal intubation, and ultimately tracheostomy. Specific airway management in syndromic craniosynostosis will depend on the level and severity of obstruction. Serious caution must be exercised in the placement and care of tracheostomies in patients with tracheal cartilaginous sleeve malformation because of abnormal tissue healing and granulation tissue formation. Midfacial surgery may be necessary in some children who have problems with airway obstruction, swallowing,

feeding, and dental malocclusion. This is usually performed later in childhood.

For all individuals with craniosynostosis, we recommend involvement of a craniofacial team, including members specializing in pediatrics, neurosurgery, ophthalmology, oral surgery, orthodontics, otolaryngology, nursing, nutrition, plastic surgery, and social work.

Disorders of the First and Second Branchial Arches

Craniofacial Microsomia

Epidemiology and Genetics

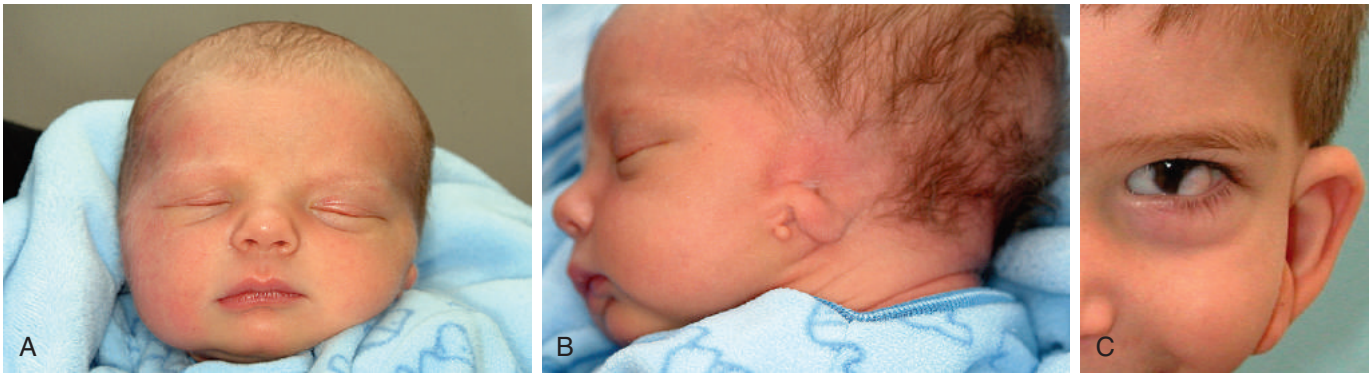
CFM (OMIM 164210), a congenital malformation in which there is asymmetric deficiency in skeletal and soft tissue on one or both sides of the face, is the most frequently encountered form of facial asymmetry. CFM affects approximately 1 in 5600 births (Grabb, 1965). Individuals with features of CFM have been classified under a variety of different diagnoses (hemifacial microsomia, oculoauriculovertebral spectrum, facioauriculovertebral syndrome, first and second branchial arch syndrome, otomandibular dysostosis, Goldenhar syndrome, lateral facial dysplasia) attesting to the phenotypic variability of disorders associated with mandibular hypoplasia. Most often CFM is a sporadic condition with a recurrence risk of approximately 2% for future pregnancies, unless there is a known family history of microtia or CFM (Beleza-Meireles et al., 2014; Heike et al., 2014). Various causes, both environmental and heritable, have been studied, and for most, the cause is thought to be multifactorial.

Phenotype

CFM is primarily a syndrome of the first or second branchial arches, resulting in underdevelopment of the ear, temporomandibular joint, mandibular ramus and body, and mastication muscles. The affected ear may have an external soft-tissue malformation with or without preauricular tags and may be lower in position compared with the ear on the contralateral side. Hearing loss may result from maldevelopment of the ossicular chain and a stenotic or atretic external auditory canal. Second branchial arch defects can involve the facial nerve and muscles of facial expression, which can exacerbate the appearance of facial asymmetry. Even with bilateral facial involvement, there is usually asymmetry (Fig. 100.10A). The presence of microtia can be associated with significant risk of hearing loss on the affected side and increased risk of hearing loss in the contralateral ear (see Fig. 100.10B). Infants with CFM are often born small for their gestational age, and the perinatal history may include polyhydramnios due to fetal swallowing dysfunction.

A common classification system for CFM is the OMENS system, which characterizes the degree of involvement of facial structures: orbital distortion, mandibular hypoplasia, ear anomaly, nerve involvement, soft tissue deficiency (Gougoutas et al., 2007; Birgfeld et al., 2011). Extracraniofacial anomalies associated with CFM, including renal, cardiac, and vertebral anomalies, are common and will affect recommendations for screening and surveillance.

There can be extreme variability of phenotypic expression, ranging from isolated microtia to significant mandibular hypoplasia, bilateral microtia, clefting, and extracranial involvement. Isolated microtia may represent a *forme fruste* of CFM. Other craniofacial features include external auditory canal stenosis or atresia, unilateral



• **Fig. 100.10** (A, B) Infant with craniofacial microsomia, mandibular asymmetry, and left-sided microtia. (C) Child with an epibulbar lipodermoid and craniofacial microsomia.

macrostomia (transverse facial cleft leading to lateral displacement of the oral commissure and the most common form of orofacial clefting in CFM), cleft lip and/or palate, temporomandibular joint ankylosis, ankyloglossia, preauricular or facial pits (most common in the distribution of the facial nerve), midface hypoplasia and malocclusion, epibulbar lipodermoids (see Fig. 100.10C), microphthalmia, eyelid and ocular colobomas, facial palsy, and seventh nerve paresis and other cranial nerve palsies. Goldenhar syndrome has historically been described as a subgroup variant of CFM characterized by vertebral anomalies and epibulbar dermoids in addition to the ear and jaw findings. In CFM, deficient growth of the hypoplastic mandible and the compensatory growth of the contralateral maxilla and zygoma contribute to significant facial asymmetry that progresses with growth. Conversely, facial and skull asymmetry caused by deformation (intrauterine or postnatally with plagiocephaly and torticollis) will often reduce with time, repositioning, and treatment of torticollis.

Other Branchial Arch Malformations

Moebius Syndrome

Moebius syndrome (OMIM 157900) is a rare congenital condition affecting approximately 2000 people worldwide (Broussard and Borazjani, 2008). The sixth and seventh cranial nerves are universally affected. Sixth nerve palsy leads to inability to abduct the eyes beyond the midline. This is usually bilateral but may be unilateral or asymmetric. Paralysis of facial muscles results from the seventh nerve palsy. While newborns may have a “masklike facies,” the presentation may not be recognized in the newborn period (McKay et al., 2016). Feeding difficulties may result from swallowing and sucking problems, aspiration, and palatal weakness related to more widespread cranial nerve involvement. There have been associations with chest wall abnormalities, including absence of the pectoralis muscle, suggesting a pathogenic relationship with the Poland anomaly (OMIM 173800). Exposure conjunctivitis and keratopathy can occur in children with facial paralysis and lagophthalmos and should be prevented with ocular lubricants. Limb defects occur in half of children with Moebius syndrome, most commonly talipes deformity; however, transverse limb anomalies are also seen. Individuals with hypoglossia–hypodactylia or Hanhart syndrome can have severe limb deformities, ankyloglossia, and temporomandibular joint ankylosis, in addition to Moebius syndrome–like features and micrognathia, and are at risk of significant swallowing dysfunction and airway compromise (Yasuda et al., 2003).

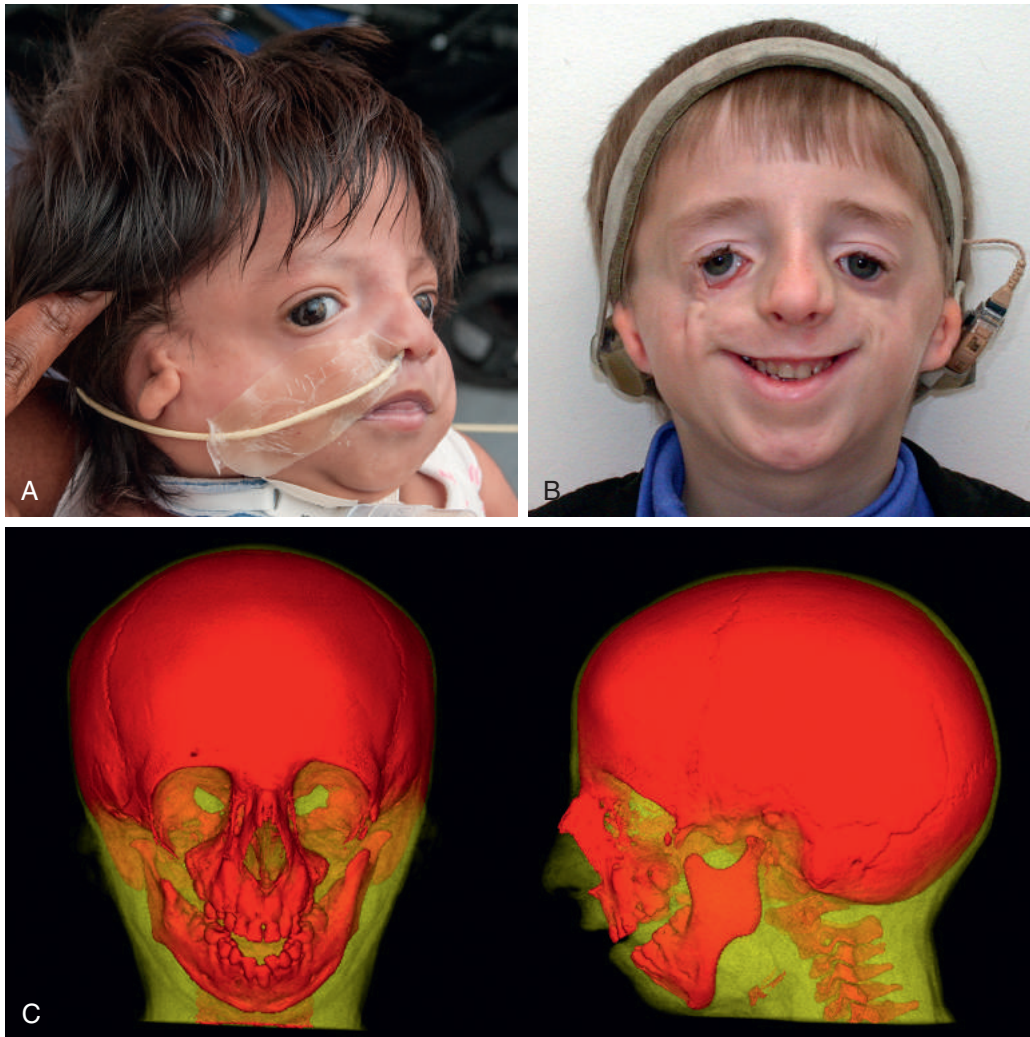
Treacher Collins Syndrome

TCS is most commonly an autosomal dominant disorder of craniofacial development that affects approximately 1 in 50,000 live births (Rovin et al., 1964). As in CFM, the tissues affected in TCS arise from the first and second branchial arches. The major clinical features of TCS include hypoplasia of facial bones (particularly the mandible and zygoma), external ear anomalies or microtia, external auditory canal atresia, bilateral conductive hearing loss, lateral downward sloping palpebral fissures, and lower eyelid colobomas (Fig. 100.11A–B). Hearing loss is present in up to 50% of individuals with TCS (Dixon et al., 2007). In severe cases the zygomatic arch may be absent and CP may occur. Extracraniofacial features are rare in TCS. Mutations in one of three genes *TCOF1*, *POLRIC*, and *POLR1D* are causative of TCS, and mutations in the *TCOF1* gene account for 71%–93% of affected individuals (OMIM 154500). Diagnosis of TCS is usually made clinically and can be confirmed with genetic testing (Katsanis and Jabs, 2012). In newborns with TCS, airway management may be required to address narrowing of the airway or extreme shortening of the mandible (see Fig. 100.11C). When compared with that in CFM, the mandibular hypoplasia in TCS is usually bilateral and symmetric, leading to increased risk of upper airway obstruction, increased need for tracheostomy, and risk of death in the neonatal period. Choanal atresia or stenosis and severe micrognathia with glossoposis can lead to airway obstruction in the infant with TCS (Katsanis and Jabs, 2012).

Intensive Care Unit Concerns

Mandibular hypoplasia in CFM can lead to upper airway obstruction that may be obvious on physical examination, presenting with stertor or stridor and increased work of breathing, or may be more subtle, as with snoring obstructive sleep apnea. Bilateral severe mandibular and malar involvement in TCS leads to airway obstruction at the level of nasopharynx and base of the tongue and substantial respiratory compromise.

Infants with CFM may have feeding difficulties that may be related to macrostomia affecting lip seal, palate dysfunction, or more commonly swallow coordination issues and dysphagia related to hypoglossal dysfunction and muscular and bony underdevelopment. Infants with Moebius syndrome may have cranial nerve palsies that affect swallow and oral coordination. These infants are at higher risk of aspiration and should be monitored clinically,



• **Fig. 100.11** (A) Infant with Treacher Collins syndrome (TCS), microtia, severe mandibular and zygomatic hypoplasia, and airway obstruction requiring tracheostomy. (B) An older child with TCS, downslanting palpebral fissures, eyelid colobomas, and bilateral microtia wearing a hearing augmentation device. (C) Three-dimensional reconstruction of TCS. Note the severe mandibular and zygomatic hypoplasia, which may lead to significant airway compromise. Also notable are the orbital defects seen in TCS.

especially if they are failing to thrive or developing any concerns for aspiration or lower respiratory tract disease.

Management

In newborns with suspected CFM, an evaluation for any associated anomalies should be undertaken. All children with external ear anomalies or any evidence of first or second branchial arch abnormalities should undergo a diagnostic hearing evaluation in the newborn period, with follow-up audiometry in the first year of life. If there is any hearing loss, ongoing monitoring of hearing is routine. It is also important to monitor ear health and eustachian tube function in the patent/hearing ear. CT to assess middle and inner ear anatomy is not recommended in the neonatal period. Consultation for ear reconstruction and atresia repair should occur by 4 years of age, although hearing amplification and aural habilitation in hearing loss can be initiated earlier.

Renal ultrasonography and cardiac examination (echocardiogram) should be undertaken in infancy to identify any serious structural

malformations. Ophthalmology consultation should be sought for appropriate management of epibulbar lipodermoids, colobomas (if present), and risk of exposure keratopathy. Malocclusion and dental issues will need to be addressed as the child gets older. Children should undergo cervical spine screening radiographs to identify vertebral defects in segmentation. If the newborn has no symptoms of cervical spine abnormality, screening four-view cervical spine radiographs can be deferred until the child is 2 to 3 years old, when cervical vertebrae are more easily imaged. Appropriate cervical spine imaging is recommended in children undergoing surgery before 2 years of age and children with head tilt or signs of vertebral anomalies.

Mild airway obstruction in CFM may be reduced or minimized with prone positioning. However, infants with severe bilateral mandibular hypoplasia may have significant airway compromise and require tracheostomy placement. In cases with significant airway compromise, referral to a craniofacial center to determine optimal and safe airway management should be pursued. For treatment of mandibular underdevelopment, surgery timing is dependent on

the degree of mandibular hypoplasia, mandibular growth, occlusion, and airway involvement. For children with severe hypoplasia of the mandible, bone grafting may be necessary for jaw reconstruction before mandible distraction. Oral feeding should be introduced when the airway is stable. Oral stimulation is important to prevent oral aversion. Given the risk of feeding difficulty and aspiration in infants with malformations of the first and second branchial arches, early consultation with both a dietitian and a feeding therapist is recommended.

CHARGE Syndrome

Epidemiology and Genetics

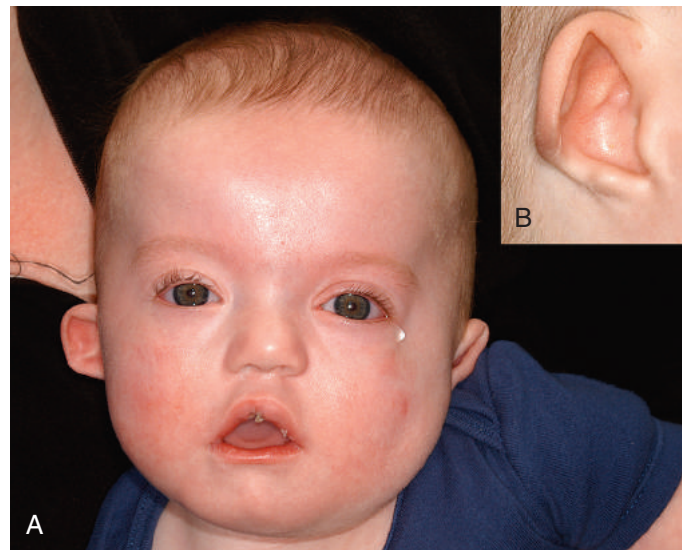
The term *CHARGE* (coloboma, heart defect, atresia choanae, retarded growth and development, genital hypoplasia, ear anomalies/deafness) was first coined by Pagon, given the observation that the associated malformations occurred more frequently together than one would expect on the basis of chance (Pagon et al., 1981). Over time, the facial features and associated malformations were better characterized as a syndrome, with mutations in at least one major gene described (OMIM 214800).

This multiple malformation condition has a prevalence of approximately 1 in 10,000 births (Blake and Prasad, 2006). Although multiple chromosomal aberrations have been reported in children with the phenotype of CHARGE syndrome, mutations in the *CHD7* gene account for 65%–70% of cases. When the diagnosis of CHARGE syndrome is suspected, molecular testing for mutations in the *CHD7* gene can be performed to confirm the diagnosis and provide more information to assist in counseling for the parents and the patient. For children in whom *CHD7* gene testing results are normal, evaluation for chromosomal abnormalities and copy number variants is possible with use of comparative genomic hybridization and single-nucleotide polymorphism array technology (Lalani et al., 2012).

Phenotype

The diagnosis of CHARGE syndrome is based on a combination of major and minor clinical criteria, but the diagnosis should be suspected in any neonate with any of the major characteristics: ocular coloboma (80%–90%), choanal atresia or stenosis (50%–60%), cranial nerve dysfunction or facial palsy (40%–90%, depending on which cranial nerve is involved), or characteristic CHARGE ears (90%–100%) (Lalani et al., 2012). As in other conditions with severe airway obstruction or swallowing dysfunction, polyhydramnios is commonly present prenatally when bilateral choanal atresia is present.

Distinctive ear anomalies (hypoplastic lobes, cupped or lop, position is often low set and posteriorly rotated) or deafness occurs in most individuals with CHARGE syndrome (Fig. 100.12). Hearing loss can be a combination of conductive and sensorineural hearing loss. Other craniofacial features include square face with malar flattening, broad forehead, facial asymmetry, pinched nostrils, full nasal tip, long philtrum, and CP (40%). Ocular colobomas can range from a coloboma of the iris to anophthalmia. Cardiac defects can be a major source of morbidity in infants with CHARGE syndrome and are found approximately 80% of the time. Conotruncal and aortic arch anomalies are the most common congenital heart defects, but atrioseptal defects, ventriculoseptal defects, patent ductus arteriosus, hypoplastic left-sided heart, and vascular rings have also been described.



• **Fig. 100.12** (A) Child with CHARGE syndrome with (B) classic ear malformation—hypoplastic lobes, cupped and low set.

Intensive Care Unit Concerns

The most important postnatal emergency in CHARGE syndrome is bilateral posterior choanal atresia (Blake et al., 2009). Neonate with bilateral choanal atresia will have breathing difficulty and cyanosis within the first hour of life. As with all forms of nasal obstruction, crying relieves the cyanosis because it allows the obligate nose breather to take in air through the mouth; feeding exacerbates respiratory distress. Left untreated, the newborn with bilateral choanal atresia can asphyxiate and die. Symptoms of bilateral choanal stenosis or unilateral atresia may not present until after the newborn period with chronic rhinorrhea or breathing problems associated with respiratory infections. Respiratory distress in a newborn with CHARGE syndrome is usually due to choanal atresia, but other features, including swallowing dysfunction and reflux, can contribute to aspiration and lower respiratory tract disease. These infants may also have micrognathia and glossoptosis, putting them at risk of airway obstruction at the level of the pharynx/hypopharynx. Infants with CHARGE syndrome may require multiple surgical procedures during the first year of life and are at increased risk of postoperative airway events (Blake et al., 2009; Bergman et al., 2010).

Cyanotic heart disease may present in the immediate newborn period because of tetralogy of Fallot, outflow tract anomalies, and interrupted aortic arch. Awareness and recognition of the association of CHARGE syndrome and congenital heart defects are crucial.

A significant cause of morbidity is feeding difficulty. Feeding and secondary growth problems are common in early infancy and may be attributed to swallowing dysfunction, pharyngeal incoordination, gastroesophageal reflux, and aspiration. Cranial nerve palsies (specifically cranial nerves V, IX, and X) may contribute to swallowing dysfunction, and tracheoesophageal fistula (TEF) contributes to aspiration risk. Although it is well described that infants with CHARGE syndrome who survive the newborn period are more likely to survive childhood, the risk of death in infancy remains. Male sex, bilateral choanal atresia, TEF, cyanotic heart disease, atrioventricular septal defects, CNS malformations, and ventriculomegaly have all been associated with reduced life expectancy in individuals with CHARGE syndrome (Tellier et al., 1998; Issekutz et al., 2005; Blake et al., 2009). A study of 77

individuals with CHARGE syndrome found mortality to be 13% (Issekutz et al., 2005).

Management

While the clinical needs will differ, some children with CHARGE syndrome will require intensive medical management and undergo multiple surgical interventions in infancy and early childhood. Early management targets airway stabilization and circulatory support. With this in mind, neonates with CHARGE syndrome require immediate evaluation of their airway and cardiac structure and function. An oral airway should be placed if bilateral choanal atresia is suspected. This can stabilize the airway by bypassing the choanal obstruction. Once the airway has been secured, a confirmatory CT scan of the nasal passages can be obtained; a CT of the temporal bones can be included in conjunction with the facial CT and may reveal the characteristic inner ear findings (Mondini malformation of the cochlea and/or absent or hypoplastic semicircular canals) of CHARGE syndrome. If the oral airway does not allow adequate air entry, endotracheal intubation may be required. In consultation with a pediatric otolaryngologist, transnasal stents may be placed to keep the nasal passages patent in choanal stenosis (and postoperatively after choanal atresia repair). Given the significant risk of cyanotic heart defects, an echocardiogram and cardiology consultation should be obtained to assist in management.

Infants with CHARGE syndrome or suspected CHARGE syndrome should also have audiologic and ophthalmologic evaluations in the neonatal period and should be referred to Birth to Three/early intervention services. Consultation with an immunologist and immune evaluation should occur for the individual with CHARGE syndrome and recurrent infections (Wong et al., 2015). Underdevelopment of the genitals and genitourinary anomalies may be present. If there is a concern for hypogonadism, the pituitary–gonadal axis can be evaluated in infancy and will help determine the option for sex steroid therapy. Screening renal ultrasonography should also be performed (Blake and Prasad, 2006).

Consultations with both a feeding specialist and a dietitian are recommended in the newborn period. If the findings of an oral feeding evaluation or videofluoroscopic swallow study are concerning for swallowing dysfunction or aspiration, supplemental tube feeding should be initiated. With prolonged feeding issues, gastrostomy tube feeding is often necessary. Infants with severe gastroesophageal reflux and/or aspiration risk may be candidates for Nissen fundoplication at the time of gastrostomy tube placement.

Macroglossia/Beckwith–Wiedemann Syndrome

Epidemiology and Genetics

The true prevalence of Beckwith–Wiedemann syndrome (BWS; OMIM 130650) is unknown, but it has been estimated that BWS affects 1 in 13,700 births (Thorburn et al., 1970). This is probably an underestimate, given that there are mild cases of BWS that go undetected. The genetics of BWS is complex and variable. Most cases are sporadic and may result from chromosomal rearrangement, mutations, or epigenetic effects (DNA methylation changes) affecting imprinted genes on chromosome band 11p15.5. Approximately 80% of individuals with features of BWS are found to have an 11p15.5 abnormality by clinically available testing (Shuman et al., 2016). Although there are no consensus criteria for diagnosis of BWS, the presence of macroglossia, overgrowth, and abdominal

wall defects suggests the diagnosis of BWS. As children with BWS are at risk of neoplasms in early childhood, recognition and diagnosis of BWS are consequential. Data suggest a possible link between imprinting disorders and assisted reproduction, and thus infants conceived by in vitro fertilization may be at higher risk of BWS (Maher et al., 2003). If there are features of BWS present or a family history of BWS, geneticists may recommend 11p15 methylation studies and chromosome microarray analysis to identify abnormalities of the 11p15 region. Although genetic testing can provide confirmation of diagnosis in 80% of individuals, clinical suspicion of the diagnosis is sufficient for initiation of medical management and tumor surveillance studies. Currently, the frequency of screening study recommendations is independent of the underlying molecular cause; however, this will likely change in the future as children with BWS due to a gain of methylation at imprinting center 1 and paternal 11p15 uniparental disomy have a higher risk of developing tumors (Mussa et al., 2016). At this time, initiation of screening studies and consultation with genetics are recommended (Brioude et al., 2013).

Phenotype

BWS is a disorder of overgrowth with multiple features, including macrosomia, macroglossia, visceromegaly (involving the kidneys, pancreas, liver, spleen, or adrenal glands), abdominal wall defects (including rectus diastasis, umbilical hernia, and omphalocele), hemihypertrophy (asymmetric overgrowth of one or more regions of the body), renal anomalies (structural anomalies and nephrocalcinosis), and adrenocortical cytomegaly (Fig. 100.13). Macroglossia is the most frequent and most obvious manifestation of BWS (present more than 95% of the time) (Elliott et al., 1994). Other craniofacial features include capillary nevus flammeus, metopic ridge, large fontanel, mandibular prognathism, prominent eyes, anterior earlobe linear creases, and posterior helical pits. Less common findings in BWS include CP, cryptorchidism, and cardiac defects (isolated cardiomegaly is more common than cardiomyopathy). The risk of embryonal tumors (Wilms tumor, hepatoblastoma, neuroblastoma, or rhabdomyosarcoma) in childhood is estimated to be 7.5%, of which 95% present in the first 8 years of life, leading to recommendations for tumor surveillance (Firth and Hurst, 2005).

Some features suggestive of BWS may be present prenatally, including polyhydramnios (caused by swallowing dysfunction), preeclampsia, fetal macrosomia, and a large placenta. Prematurity has been reported in 50% of births (Elliott et al., 1994), and in addition to complications of prematurity, the neonate with BWS may develop hypoglycemia and polycythemia.

Intensive Care Unit Concerns

Hypoglycemia due to hyperinsulinemia and islet cell hyperplasia occurs in up to 50% of neonates with BWS and usually develops in the first few days of life (Munns and Batch, 2001). It is critical to detect and treat hypoglycemia in any neonate with features of BWS to prevent seizures and brain injury. Polycythemia can occur and may need to be treated in the early neonatal period.

Obstructive airway symptoms may present in the newborn period if macroglossia is severe. However, airway obstruction more commonly presents later in infancy, outside the newborn period. The enlarged tongue can occlude the upper airway, leading to respiratory distress, apnea, and hypoxia. A large tongue can also contribute to feeding issues, dysphagia, and aspiration. Upper airway endoscopic evaluation by an otolaryngologist and an



• **Fig. 100.13** (A) Premature newborn with Beckwith–Wiedemann syndrome, macroglossia, and rectus diastasis. (B) Same child at 6 months of age. Macroglissia has increased, and he now has a tracheostomy.

overnight sleep study may help understand the severity of airway compromise and guide airway treatment.

Mortality among infants with BWS has been reported to be as high as 21% and is related to complications of prematurity and macroglossia (Shuman et al., 2010).

Management

Hypoglycemia in newborns should be managed according to standard protocols for treating neonatal hypoglycemia. If hypoglycemia persists or is refractory to therapy, additional biochemical testing and consultation with an endocrinologist should be considered (Roženková et al., 2015). Neonates with an omphalocele may require surgery in the first few days of life.

There is no definitive approach to the management of macroglossia. Airway obstruction may be lessened by the placing of the baby on the side or prone. If the infant requires endotracheal intubation, it is important to exercise caution, because macroglossia can affect visibility of airway structures. If macroglossia results in significant airway obstruction or prolonged intubation, tracheostomy may be needed as a temporizing measure to bypass the obstruction. Tongue growth will slow over time, and as jaw growth accelerates, airway compromise should decrease. Some children may benefit from surgical reduction of the tongue, which is usually performed between 2 and 4 years of age, but may be offered as early as 3 to 6 months at some centers.

Referrals to an infant feeding specialist and dietitian are recommended in the infant with severe macroglossia or if the infant is not gaining weight. Although some infants are able to feed orally, others will benefit from supplemental tube feeding.

Although cardiac defects are rare, it is important to perform a thorough cardiac evaluation, including electrocardiogram and echocardiogram if any cardiac abnormalities are suspected.

Surveillance for tumors begins in the neonate with BWS or at the time of diagnosis. Abdominal ultrasonography to assess the patient for organomegaly and baseline CT or MRI of the abdomen should be performed. Abdominal ultrasonography every 3 months is recommended through 8 years of age. In conjunction, staggered serial serum alpha fetoprotein measurements (every 6 to 12 weeks) are recommended through 4 years of age to assist early identification of hepatoblastomas before detection by screening ultrasonography.

Referral to a craniofacial team may be helpful in the management of the airway obstruction in BWS, including evaluation for tongue reduction and facial hemihypertrophy. A geneticist and genetic counselor may recommend genetic testing for confirmation of the diagnosis and/or recurrence risk counseling.

Frontonasal Dysplasia, Hypertelorism, Encephalocele

Embryology

Frontonasal dysplasia (FND; also known as *frontonasal malformation*, *median cleft face syndrome*, and *frontal nasal syndrome*) is a malformation resulting from abnormal morphogenesis of the frontonasal process. The development of the facial midline is abnormal, leading to ocular hypertelorism and associated craniofacial features. Most cases of FND are sporadic.

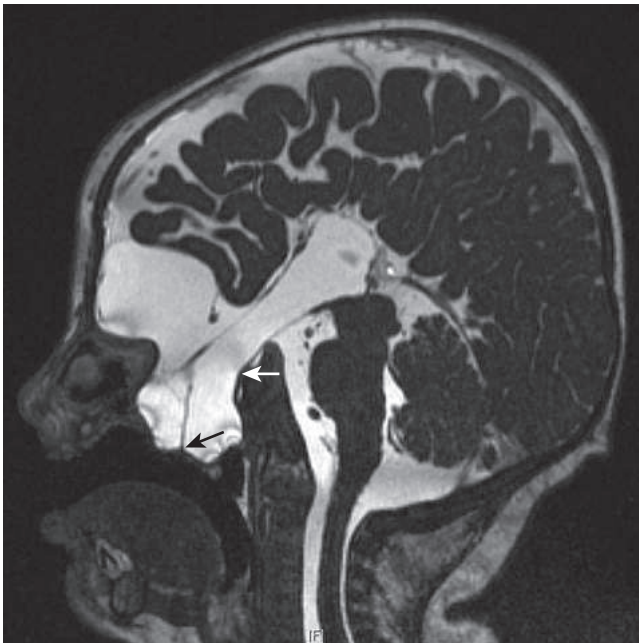
Phenotype and Genetics

FND has been defined phenotypically as containing two or more of the following craniofacial features: ocular hypertelorism; broadening of the nasal root; midline facial cleft affecting the nose, lip, or palate; unilateral or bilateral clefting of the alae nasi; hypoplastic nasal tip; anterior cranium bifidum; and a V-shaped frontal hairline

(Wu et al., 2007). Grading of hypertelorism is best achieved by measurement of the interpupillary distance. In a term newborn an interpupillary distance greater than 4.5 cm is considered hypertelorism (Jones et al., 2013). FND is a heterogeneous condition with genetic forms (OMIM 136760, OMIM 613451, OMIM 613456, associated with mutations in three *ALX* genes), sporadic forms without associated anomalies, and FND phenotype with associated pattern of malformations (subtype of FND) or known genetic syndrome such as craniofrontonasal syndrome (Wu et al., 2007; van den Elzen et al., 2014).

In addition to hypertelorism, eye anomalies, including epibulbar dermoids, colobomas, ptosis, nystagmus, or cataracts, may be present in FND and are associated with a more severe phenotype and an increased incidence of CNS abnormalities (Wu et al., 2007). Associated CNS manifestations include encephalocele, agenesis of the corpus callosum, and abnormal neuronal migration. Developmental delay is a significant risk, especially when there are CNS malformations. When FND is associated with extracephalic anomalies or when ocular hypertelorism is more severe, there is an increased association with cognitive impairment (Hennekam et al., 2010d). Frontonasal encephaloceles (and meningoceles) are the most common encephaloceles in FND (Fig. 100.14).

A subpopulation of patients with frontonasal malformation also have coronal craniosynostosis and variable skeletal and ectodermal defects and have an X-linked condition termed *craniofrontonasal syndrome* (CFNS, OMIM 304110). Similarly to FND, facial features include hypertelorism, frontal bossing, broad nasal bridge, and a bifid nasal tip. Children with CFNS often have significant facial asymmetry due to unicoronal synostosis. In this X-linked condition, females are affected more severely than males (and typically have hypertelorism and grooved nails), and mutations are detected in the *EFNB1* gene. Affected individuals usually have normal intelligence.



• **Fig. 100.14** Magnetic resonance imaging of an infant with frontonasal dysplasia and a midline cleft lip. The scan reveals a moderate-sized meningocele extending into the posterior nasopharynx. The *white arrow* points to midbrain meningocele coming through the cribriform plate; the *black arrow* points to the intraoral meningocele.

Intensive Care Unit Concerns

Intracranial abnormalities associated with FND may put the infant at risk of CNS manifestations such as hydrocephalus or seizures. If the pituitary gland is involved or deficient, as can be seen with HPE sequence, there can be serious endocrine abnormalities (as discussed in Orofacial Clefing). Also, frontonasal encephalocele may contribute to upper airway compromise at the level of the nasopharynx.

Management

In any infant with hypertelorism or features that raise suspicion for FND, awareness of potential underlying malformations is critical, and cranial imaging by CT scan or MRI should be considered. Instrumentation of the nose and mouth, including placement of a nasogastric tube or suction catheter, should be avoided or used with caution until the CNS anatomy has been delineated. Because infants with FND have a high incidence of frontonasal encephalocele or meningocele, placement of these catheters could lead to brain injury. If an infant with FND needs urgent or emergent endotracheal intubation, intraoral structures should be examined carefully to prevent injury to herniating CNS structures if they are present. Management of seizures or any electrolyte derangements should be managed as per the neonatal ICU standard protocol. Consultation with a craniofacial team, including specialists in ophthalmology, can be helpful in understanding the work-up and management (including potential surgical interventions) for individuals with FND.

Prenatal Screening for Fetal Face Anomalies

The exact role of fetal face examination with ultrasonography in a low-risk pregnancy is under evaluation. Routine obstetric surveillance includes a midtrimester anatomic ultrasound examination at 18 to 22 weeks' gestation. Most studies looking at the recognition rate and incidence of ultrasonographic diagnosis of orofacial clefing focus on this anatomic examination. Adequate evaluation of the facial structures with ultrasonography can be achieved by 16 to 17 weeks' gestation. The following facial features can be visualized with two-dimensional routine ultrasonography at 18 weeks' gestation with standard facial views (which include coronal images of the nose, lips, and orbits and sagittal profile views): orbital size and position, eye size, including microphthalmia and anophthalmia, shape of nose, nasal hypoplasia, length of the philtrum, clefts of the upper lip, frontal bossing, retrognathia, micrognathia, macroglossia, and soft tissue abnormalities. Cleft lip with or without CP can be detected by prenatal ultrasonography, whereas isolated CP, which is not typically associated with a cleft of the alveolus, may be obscured by the tongue, which has the same echogenicity as the secondary palate, thus making prenatal diagnosis of CPO more difficult. A retrospective study in a low-risk population demonstrated that routine prenatal ultrasonography with standard facial views performed at 18 weeks' estimated gestational age detected 93% of cases of cleft lip and palate, 67% of cases of isolated cleft lip, and 22% of cases of CPO (Cash et al., 2001). New and increasingly sensitive methods for identifying craniofacial differences prenatally are emerging (Tonni et al., 2015; Rubio et al., 2016). Although the diagnosis is not definitive, prenatal diagnosis is particularly valuable in allowing appropriate prenatal counseling (Maarse et al., 2015). Families who have the opportunity to meet members of a craniofacial team before delivery often appreciate having some understanding of what to

expect in the newborn period and are armed with knowledge to help their new baby receive the best care possible.

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Common Neonatal Orthopedic Conditions

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KEY POINTS

- Development dysplasia of the hip represents a spectrum of disease. Selective screening by physical examination and imaging is recommended.
- Most cases of congenital muscular torticollis resolve spontaneously. Physical therapy and surgery are reserved for recalcitrant cases.
- A variety of foot deformities are common and can be encountered in the neonate. Stretching, casting, or surgery may be required for resolution.
- Torsional and angular deformities of the lower extremities must be differentiated from physiologic variants. Asymmetry and rapid progression are the hallmarks of pathologic variants.
- Congenital vertebral anomalies result from failures of formation or segmentation of spinal elements. Spinal deformities such as scoliosis or kyphosis may ensue.

Although orthopedic afflictions of the newborn are generally not life threatening, they do have the potential to significantly impair functional performance, even when diagnosed and treated early. This chapter discusses the most commonly encountered of these orthopedic problems.

Developmental Dysplasia of the Hip

The term *developmental dysplasia of the hip* (DDH) encompasses a spectrum of disease from acetabular dysplasia, to hips that are located but unstable (femoral head can be moved in and out of the confines of the acetabulum), to frankly dislocated hips in which there is a complete loss of contact between the femoral head and acetabulum. DDH occurs in 11.5 of 1000 infants, with frank dislocations occurring in 1 to 2 per 1000 ([Committee on Quality Improvement, Subcommittee on Developmental Dysplasia of the Hip, 2000](#); [Guille et al., 2000](#)). Studies have suggested that breech positioning at delivery, family history of DDH, limited hip abduction, talipes, female sex, swaddling, large birth size, and first born have all been associated with a higher probability of finding DDH ([American Academy of Orthopedic Surgeons, 2014](#)). The left hip alone is affected in 60% of infants, the right hip alone is affected in 20% of infants, and both hips are affected in 20% of infants ([Guille et al., 2000](#)).

Dislocations can be divided into two groups: syndromic and typical. *Syndromic* dislocations are most frequently associated with neuromuscular conditions such as myelodysplasia and arthrogryposis or with syndromes such as Larsen syndrome. Syndromic dislocations probably occur between week 12 and week 18 of gestation ([Committee on Quality Improvement, Subcommittee on Developmental Dysplasia of the Hip, 2000](#)). *Typical* dislocations occur in otherwise healthy infants in the third-trimester prenatal period or postnatally.

Congruent reduction and stability of the femoral head are necessary for normal growth and development of the hip joint. The natural history of untreated DDH is controversial because newborn instability may resolve or progress to painless dislocation. In cases that progress to subluxation, individuals have significantly increased risk of developing precocious arthritis with moderate to severe hip pain as young adults ([Wedge and Wasylenko, 1979](#); [Cooperman et al., 1983](#)). This pain can be debilitating and the reconstruction difficult. Early detection and treatment of DDH are therefore important in avoiding the devastating sequelae of a late diagnosis.

There are no pathognomonic signs of a dislocated hip. The physical examination requires patience on the part of the examiner and may be facilitated by having the baby feed from a bottle. Evaluation for asymmetry is perhaps the most important key to the evaluation for DDH, although asymmetry is not typically evident in bilateral dislocations. The presence of asymmetric abduction is suggestive of a dislocation, as is a Galeazzi sign. The presence of asymmetric thigh folds may be indicative of DDH but often occurs in unaffected infants. The Galeazzi sign is elicited with the baby placed supine on an examining table so that the pelvis is level, with the hips and knees flexed to 90 degrees. With the baby's hips in neutral abduction, the examiner determines if the knees are at the same height. If one femur appears shorter than the other, the hip may be dislocated posteriorly ([Fig. 101.1](#)). Limitation of hip abduction in babies older than 12 weeks is the most reliable examination finding suggestive of DDH. Adduction of 30 degrees and abduction of 75 degrees should be possible in most newborns. Side-to-side variations should be noted. Each of these signs, individually or in combination, may serve to increase the index of suspicion of the examiner and lower the threshold for further diagnostic studies or referral to a pediatric orthopedist.

There are two common ways of assessing hip stability in the newborn ([Fig. 101.2](#)). The Ortolani test is performed on one leg

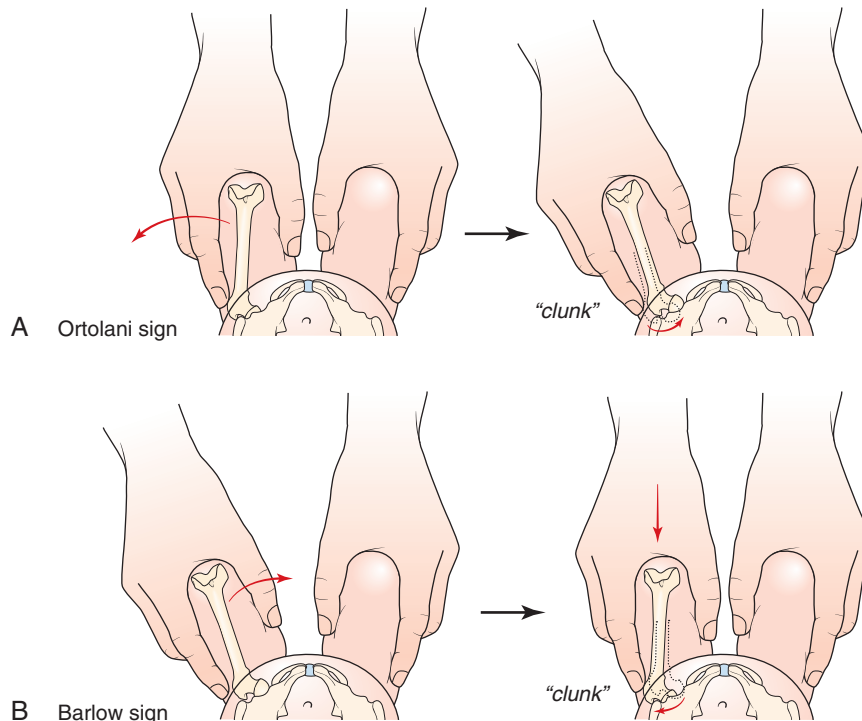
at a time, with the calm newborn supine on the examining table. The index and middle fingers of the examiner are placed along the greater trochanter, while the thumb is placed on the medial aspect of the thigh. The pelvis is stabilized by the placing of the thumb and ring or long finger of the opposite hand on top of both anterior iliac crests simultaneously. Alternatively, the opposite thigh may be held in the same manner as the examined side, thus



• **Fig. 101.1** Presence of Galeazzi sign.

stabilizing the pelvis. The hip is flexed to 90 degrees and gently abducted while the leg is lifted with the hip in neutral external/internal rotation. A palpable clunk is felt as the dislocated femoral head reduces into the acetabulum. This finding is reported as the Ortolani sign (positive result on the Ortolani test). The Barlow test is an attempt to dislocate or subluxate a located but unstable hip. The thigh is held and the pelvis stabilized in the same manner as for the Ortolani test. With the hip in neutral external/internal rotation and at 90 degrees of flexion, the leg is then gently adducted with a mild posteriorly directed pressure applied to the knee. A palpable clunk or sensation of posterior movement constitutes a positive result (i.e., the Barlow sign). Each hip should be examined separately. High-pitched clicks are frequently elicited with hip range of motion. These sounds are most frequently attributed to snapping of the iliotibial band over the greater trochanter and are not associated with dysplasia (Bond et al., 1997). With progressive soft tissue contractures, both the Ortolani test and the Barlow test become unreliable after 3 months of age.

Imaging of the immature hip can be a valuable adjunct to the physical examination. An anteroposterior (AP) radiograph of the pelvis can be difficult to interpret before the age of 4 to 5 months. The femoral head is composed entirely of cartilage until the secondary center of ossification appears. Before the appearance of the secondary center, ultrasound examination is the method of choice for visualizing the cartilaginous femoral head and acetabulum. Static ultrasound images allow visualization of acetabular and femoral head anatomy, while the complementary dynamic images give information on the stability of the hip joint (Graf, 1984; Clarke et al., 1985). The primary limitation of hip ultrasonography is that the results are dependent on the experience and skill of the operator, especially when performed within the first 3 weeks after birth (Marks et al., 1994). For these reasons ultrasonography is



• **Fig. 101.2** Assessing hip stability. (A) Ortolani-positive hips are those where the dislocated hip can be relocated. (B) Barlow-positive hips are reduced but can be dislocated.

recommended as an adjunct to clinical evaluation rather than as an independent screening tool ([Committee on Quality Improvement, Subcommittee on Developmental Dysplasia of the Hip, 2000](#)). Studies conducted before 4 weeks after birth may be useful for confirming equivocal physical examination findings and for monitoring treatment of hips with known dislocations. Clinicians must be aware, however, that ultrasound images in this age group often reveal minor degrees of dysplasia that usually resolve spontaneously and may lead to overtreatment of physiologic hip variations. Ultrasonography is the technique of choice for assessment of infants at high risk of DDH after 4 to 6 weeks of age and again is useful in following up the results of intervention. After 4 months of age, the gold standard remains the AP radiograph of the pelvis.

All newborns should be screened for DDH by a properly trained healthcare provider by physical examination. Risk factors for DDH should be determined by the treating physician. A Cochrane review found that neither universal nor targeted ultrasound screening strategies have been demonstrated to improve clinical outcomes, including the incidence of late-diagnosed DDH and need for surgery ([Shorter et al., 2013](#)). Adding further confusion to the debate over the approaches to optimal DDH screening procedure is a report by the US Preventive Services Task Force, which found “insufficient evidence” to recommend *any* routine DDH screening, including physical examination ([Shipman et al., 2006](#)). This recommendation was based on the lack of clear evidence for the efficacy of infant screening to reduce the incidence of late-presenting DDH. In response to these findings, the American Academy of Orthopedic Surgeons (endorsed by the American Academy of Pediatrics, the Pediatric Society of North America, and the Society for Pediatric Radiology) has published a revised clinical practice guideline to aid in the early diagnosis of and initiation of appropriate intervention for DDH ([Mulpuri et al., 2015](#)). These recommendations are summarized as follows:

1. Universal ultrasound screening. Moderate evidence supports not performing universal ultrasound screening of newborn infants.
2. Evaluation of infants with risk factors for DDH. Moderate evidence supports performing an imaging study before 6 months of age in infants with one or more of the following risk factors: breech presentation, family history, or history of clinical instability.
3. Imaging of the unstable hip. Limited evidence supports that the practitioner might obtain an ultrasound image in infants younger than 6 weeks of age with positive instability examination findings to guide the decision to initiate brace treatment.
4. Imaging of the infant hip. Limited evidence supports the use of an AP radiograph of the pelvis instead of an ultrasound image to assess DDH in infants beginning at 4 months of age.
5. Surveillance after normal findings from an infant hip examination. Limited evidence supports that a practitioner reexamine infants previously screened as having normal hip examination findings on subsequent visits before 6 months of age.
6. Stable hip with ultrasound imaging abnormalities. Limited evidence supports observation without a brace for infants with a clinically stable hip with morphologic ultrasound imaging abnormalities.
7. Treatment of clinical instability. Limited evidence supports either immediate or delayed (2 to 9 weeks) brace treatment for hips with positive instability examination findings.
8. Type of brace for the unstable hip. Limited evidence supports use of the von Rosen splint over Pavlik, Craig, or Frejka splints for initial treatment of an unstable hip.

9. Monitoring of patients during brace treatment. Limited evidence supports that the practitioner perform serial physical examinations and periodic imaging assessments (ultrasound or radiograph depending on age) during management for unstable infant hips.

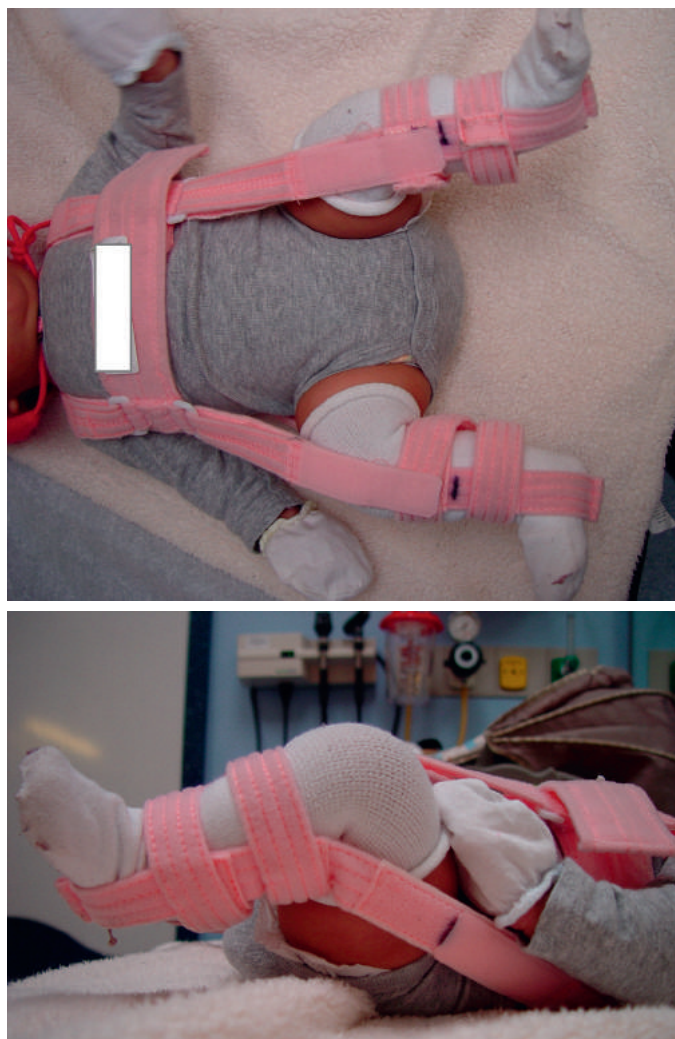
If there are no risk factors, then serial examinations are recommended according to a standard periodicity schedule until the child is 6 months old. If during these periodic visits physical findings raise suspicion of DDH, or if a parental concern suggests hip disease, confirmation is recommended by an expert physical examination, by referral to a pediatric orthopedist (or other practitioner with expertise in medical and surgical management of newborn hip disease), or by age-appropriate imaging. When a positive Ortolani or Barlow test is present at birth and persists beyond the usual age of spontaneous resolution (2 to 9 weeks), the infant should be referred to an orthopedist for management. However, if the positive Ortolani or Barlow test disappears, then age-appropriate imaging (ultrasonography at 6 weeks or radiograph by 6 months) is warranted. If the baby has positive risk factors, such as breech positioning at birth or a family history but stable hip examination findings, then age-appropriate imaging is recommended (ultrasonography at 6 weeks or radiograph at 6 months).

Although previously recommended, triple diapering is not an accepted form of treatment in DDH, and communication between providers is encouraged if the practitioner examining the newborn in the hospital is different from the 2-week follow-up examiner. Despite newborn screening programs, 1 in 5000 will have a dislocated hip detected at 18 months of age or older ([Dezateux and Godward, 1995](#)). It is important to appreciate that not all dislocated hips are present at birth, and not all hips dislocated at birth are detectable in the newborn period.

Treatment of DDH is dependent on the age at presentation. For children aged 0 to 6 months, a reducible hip is treated in a Pavlik harness or other appropriate orthosis. Patients with modest ultrasonographic abnormalities but stable hip examination findings can be monitored, as normalization can be expected without treatment ([Sucato et al., 1999](#)). The Pavlik harness is a dynamic orthosis that allows the infant to actively move the hips through a sphere of motion that encourages deepening and stabilization of the acetabulum ([Fig. 101.3](#)). The harness is applied as soon as possible after the diagnosis of DDH is made. The length of treatment is dependent on the age at presentation. Progress is judged by serial physical examinations and static and dynamic ultrasonography. In the case of a frankly dislocated hip, treatment is abandoned if no improvement is noted within 4 weeks of splint application. Closed reduction under general anesthesia, usually with arthrographic evaluation and subsequent spica casting, is then attempted at 4 to 5 months of age ([Luhmann et al., 2003](#)). For a persistently irreducible dislocation, which is unusual in the 0 to 6-month age group, open operative reduction of the hip with subsequent spica casting is undertaken. The success of Pavlik harness treatment is variable and correlates with the severity of the hip dysplasia. Treatment is successful in nearly 100% of stable hips, greater than 90% of dislocatable (Barlow-positive) hips, 61%–93% of dislocated but reducible (Ortolani-positive) hips, and only 40% of irreducible dislocations ([Viere et al., 1990](#); [Hangen et al., 1995](#); [Sucato et al., 1999](#); [Lerman et al., 2001](#); [Swaroop and Mubarak, 2009](#)).

Torticollis

Congenital muscular torticollis (CMT) manifests itself at birth or soon thereafter and is the most frequent cause of wryneck. However,



• **Fig. 101.3** The Pavlik harness. Lightweight orthotic, useful in treatment of neonatal DDH. The device holds the hip in flexion and abduction, promoting optimal positioning of the femoral head in the acetabulum. Excessive flexion and abduction should be avoided.

other conditions, some more serious, may cause torticollis. These include congenital anomalies of the vertebra and skull (e.g., Klippel–Feil syndrome, hemivertebrae, basilar invagination, craniosynostosis) (Hensinger et al., 1974; Dubousset, 1986; Raco et al., 1999), abnormalities of the central nervous system (e.g., syringomyelia, tumors) (Kiwak et al., 1983), Chiari malformations (Dure et al., 1989), ocular abnormalities (Bixenman, 1981); pharyngeal abscess, and gastroesophageal reflux (e.g., Sandifer syndrome) (Ramenofsky et al., 1978). Patients with CMT can be divided into those who demonstrate a sternocleidomastoid muscle (SCM) “pseudotumor,” those with tightness or fibrosis of the SCM without pseudotumor (termed *muscular torticollis*), and those with all of the characteristic features of congenital torticollis without evidence of contracture or fibrosis of muscle (termed *postural torticollis*) (Cheng et al., 2001).

CMT has been estimated to occur in 0.3%–2.0% of live births (Cheng et al., 2001). It is usually discovered between 6 and 8 weeks after birth. The infant presents with a cock robin appearance, with the head tilted toward and the chin rotated away from the affected SCM. Twenty percent to 30% of patients will have a palpable pseudotumor present in the middle to inferior aspect of

the affected SCM, which spontaneously regresses with time, leaving a fibrous band (Herring, 2002). More than half will have facial asymmetry. The left and right SCMs are affected in equal proportions. CMT probably results from ischemia within the SCM, leading to fibrosis (Davids et al., 1993). The cause of the ischemia is unknown, but intrauterine crowding may play a role, inasmuch as some authors have reported an association of torticollis with other deformations, such as DDH and metatarsus adductus (Morrison and MacEwen, 1982; Tien et al., 2001).

Excellent results with a manual stretching program can be attained in children first seen before 1 year of age (Morrison and MacEwen, 1982; Carenzio et al., 2015). Initially, the parents are instructed in the technique of stretching the contracted SCM by rotating the infant’s chin toward the affected SCM while simultaneously tilting the head away from it. This is completed 10 times each session and held for a count of 10 and done at least 10 times per day. Unfortunately, adherence may be an issue. If the child fails to improve substantially within 3 to 4 weeks, a physical therapist is enlisted to see the child two or three times weekly to supervise the program and reinforce the home therapy. Additionally, the parents are instructed to configure the infant’s crib and toys in such a manner as to encourage active rotation toward the involved side.

There is no justification to undertake surgery in any child younger than 1 year or in any child who has not completed a minimum of 6 months of therapy (Morrison and MacEwen, 1982; Cheng et al., 2001). In a prospective study of 821 children with muscular torticollis, only 8% of patients with a history of a pseudotumor, and 3% of those without, required surgical intervention following a well-structured stretching program (Cheng et al., 2001). Because of the difficulty of monitoring exercise programs, because parental adherence is always in question, and because surgical intervention is infrequent, it is possible that in many patients resolution is spontaneous. No patients with postural torticollis require surgery. Risk factors for surgery include late initial presentation, presence of a pseudotumor, and rotation deficit of greater than 15 degrees.

The timing of surgical intervention remains controversial. In patients with significant plagiocephaly and facial asymmetry, surgery should be considered just before 2 years of age so as to maximize the chance for complete remodeling. For those with either no or mild facial asymmetry, good to excellent results can be expected with surgery up to 6 years of age (Ling, 1976). Acceptable results are reported as late as 12 years of age, but the ability to remodel facial asymmetry appears diminished (Lee et al., 1986). More recent literature suggests good surgical outcomes in neglected CMT even after 15 years of age (Kim et al., 2015). Surgery entails either release or lengthening of the SCM through cosmetically pleasing incisions (Ferkel et al., 1983). The use of a molded helmet to promote facial and skull remodeling is common in some centers. Prospective studies that establish the effectiveness of helmets are lacking.

A less frequent cause of congenital torticollis is osseous fusion between bones in the cervical spine. These fusions may be between the skull and C1 and/or C2 or in the lower cervical spine. They result from failure of the bones to properly segment during embryogenesis. These abnormalities, in combination with a low posterior hairline and a short webbed neck with limited range of motion and head tilt, constitute the triad referred to as *Klippel–Feil syndrome* (Copley and Dormans, 1998). These congenital bone fusions can range from involvement of two segments to involvement of the entire cervical spine. Colloquially, Klippel–Feil syndrome has come to refer to any congenital malformation in the cervical

spine with or without other elements of the triad. In infants and young children the neck may remain quite flexible despite the bone abnormalities. In a newborn with torticollis who does not improve with passive stretching exercises, radiologic evaluation is mandatory. Cervical spine radiographs are not recommended in all patients initially presenting with neonatal torticollis, as these radiographs are quite difficult to interpret in this age group because of the predominance of cartilage in the bones of the neck. Furthermore, many neonates would be subjected to unnecessary ionizing radiation.

The natural history of Klippel–Feil syndrome in most cases is quite favorable, requiring nothing more than periodic observation. In patients with severe involvement, however, the consequences of this disorder can include early spondylosis with the development of pain or stenosis, the development of progressive torticollis and scoliosis, and the occurrence of neurologic compromise and sudden death secondary to even minimal trauma (Herring, 2002). Despite these potentially devastating sequelae, the greatest advantage of early detection of Klippel–Feil syndrome is in being alerted to commonly associated disorders, including congenital heart disease (14%–29%), renal anomalies (25%–35%), scoliosis (60%), audiologic anomalies (80%), including deafness (15%–35%), synkinesis (15%–20%), and, less commonly, posterior fossa desmoid tumors (Muzumdar and Goel, 2001; Herring, 2002). The recognition of a Klippel–Feil anomaly should prompt a thorough evaluation for these associations. Treatment of Klippel–Feil syndrome most often involves periodic observation with activity modification. In the face of progression of deformity or severe deformity, spinal fusion may be warranted.

Sandifer syndrome (gastroesophageal reflux) can also cause a torticollis. With this syndrome the torticollis is intermittent and may change direction, and there is no tightness of the SCM, with normal findings on radiographs (Ramenofsky et al., 1978).

Hemiatlas, or the failure of formation of a portion of the first cervical vertebra, is also a rare cause of torticollis (Dubousset, 1986). In the infant the neck may be quite flexible and the torticollis passively correctable. An open-mouth (odontoid view) cervical spine radiograph reveals this deformity. If the torticollis is progressive or severe, gradual correction of the deformity with a halo vest followed by posterior occiput to cervical spine fusion is necessary. Other potential causes of torticollis in the neonate include central nervous system tumors and syringomyelia. If radiographs appear normal, a thorough neurologic examination and referral to a neurologist are recommended.

Foot Deformities

Congenital deformities of the foot are relatively common but often overlooked in newborns. Consequently, the true incidence of the milder, self-limited deformities is unknown. For identification purposes, congenital foot abnormalities can be divided into those that result in the toes pointing upward (calcaneovalgus, congenital vertical talus), those that result in the toes pointing inward (metatarsus adductus, clubfoot), and those with too many toes or toes stuck together (polydactyly, syndactyly).

Calcaneovalgus is thought to be a postural deformity secondary to intrauterine positioning in which the dorsum of the foot is, or can be, directly apposed to the anterior aspect of the leg (Fig. 101.4). Plantar flexion of the foot is often limited from contracture of the anterior ankle and lateral soft tissues. The estimated incidence of calcaneovalgus is 0.4 per 1000 to 1 per 1000 live births (Wynne-Davies, 1964; Nunes and Dutra, 1986). It appears to be more



• **Fig. 101.4** Calcaneovalgus foot. (From Pediatric Pes Planus JAAOS Oct. 2015, Bouchard M, Mosca V. Flatfoot deformity in children and adolescents: surgical indications and management. *J Am Acad Orthop Surg.* 2014;22:623–632.)



• **Fig. 101.5** Congenital vertical talus. (From Pediatric Pes Planus JAAOS Oct. 2015, Bouchard M, Mosca V. Flatfoot deformity in children and adolescents: surgical indications and management. *J Am Acad Orthop Surg.* 2014;22:623–632.)

common in girls and after breech deliveries (Nunes and Dutra, 1986). There may be an increased association with hip dysplasia, so a thorough hip examination is warranted, as outlined in Developmental Dysplasia of the Hip (Paton and Choudry, 2009). Complete resolution with gentle stretching exercises conducted by the parents can be achieved, although complete resolution generally occurs spontaneously by 3 to 6 months of age. In the more severe calcaneovalgus feet where the ankle cannot be plantar flexed past the neutral position, serial casting to facilitate correction is often required. Calcaneovalgus may be seen in conjunction with external rotation of the tibia and posteromedial bowing of the tibia. A deformity that fails to resolve mandates referral to a pediatric orthopedist.

Calcaneovalgus needs to be differentiated from congenital vertical talus, a rarer condition that is frequently associated with neuromuscular conditions and syndromes such as arthrogryposis and



• **Fig. 101.6** The appearance of the foot with metatarsus adductus. (From the private collection of Dr. Vincent S. Mosca, Department of Orthopedics and Sports Medicine, Seattle Children's Hospital, Seattle, WA.)

spina bifida (Hoffinger, 1996). In congenital vertical talus the hindfoot is fixed in equinus (plantar flexion), giving the sole of the foot a characteristic “rocker bottom” appearance because of dorsal dislocation of the midfoot though the talonavicular joint (Fig. 101.5). Treatment during infancy consists of serial casting to stretch dorsal soft tissues and reduce the midfoot, followed by limited surgical release if needed, pinning of the talonavicular joint, and Achilles tenotomy (Dobbs et al., 2006). Most children have surgery between 6 and 12 months of age, and best outcomes are achieved when surgery is performed before age 2 years (Stricker and Rosen, 1997). When casting fails to reduce the midfoot, more extensive surgical releases are required.

The two common neonatal foot deformities resulting in medial deviation of the toes are metatarsus adductus and talipes equinovarus (clubfoot). Metatarsus adductus is present at birth but frequently diagnosed later during the first year of life. It has been estimated to occur in 1 in 100 births (Widhe et al., 1988). The condition is thought to result from intrauterine crowding. Characteristic features include a concave medial border of the foot with a curved lateral border, a “bean-shaped” sole of the foot, a higher-than-normal-appearing arch, and a neutral heel (Fig. 101.6; Hart et al., 2005). Metatarsus adductus can be classified into cases that undergo passive correction and those that do not. Feet in which passive correction is possible are best left alone and will improve spontaneously. Feet in which passive correction is not possible (the curved lateral border cannot be straightened) should be treated with manipulation and serial casting by age 6 to 9 months. The corrections can then be maintained with reverse or straight-last shoes if necessary. Operative treatment should be considered only in children older than 3 years who have a rigid deformity and have failed to respond to a casting program (Weinstein, 2000).

The term *clubfoot* describes a foot with hindfoot equinus and adduction and supination of the forefoot (Fig. 101.7). Clubfoot deformities range from mild to severe and occur in 1 in 1000 to 2 in 1000 live births (Herring, 2002). A risk factor for clubfoot is early amniocentesis (11 to 13 weeks' gestation), which is hypothesized to cause decreased fetal movement during a critical



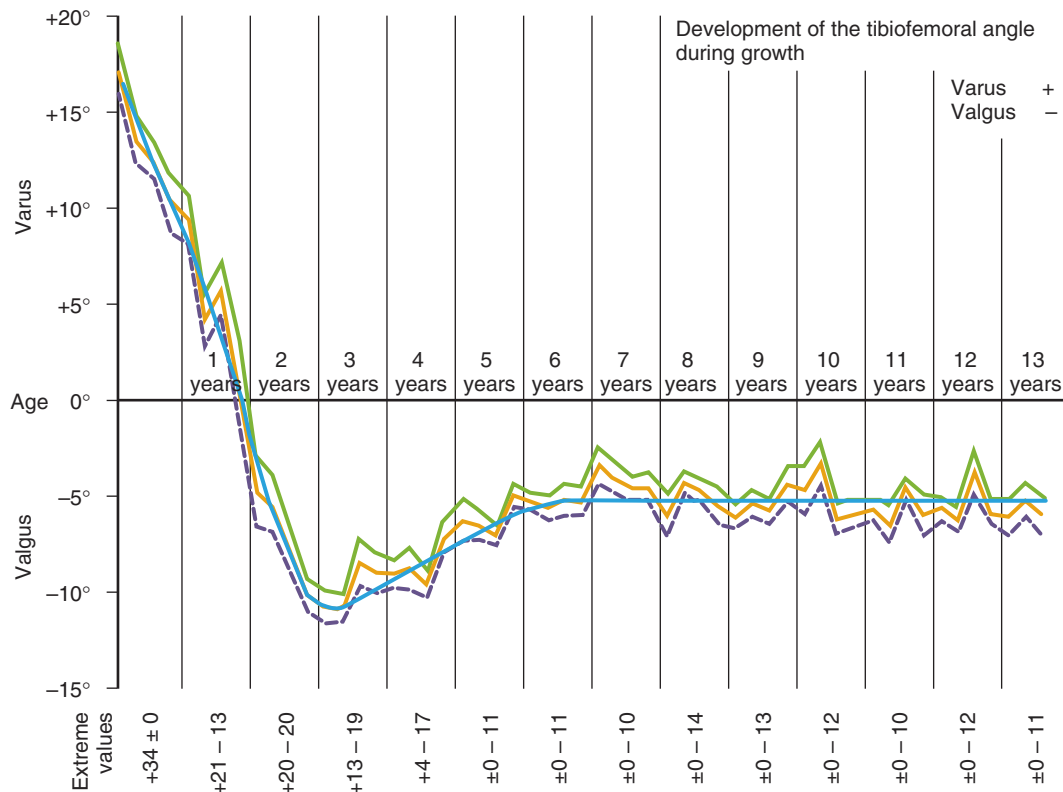
• **Fig. 101.7** The appearance of an untreated newborn clubfoot. (From the private collection of Dr. Vincent S. Mosca, Department of Orthopedics and Sports Medicine, Seattle Children's Hospital, Seattle, WA.)

phase of foot development (Tredwell et al., 2001). Although the cause of clubfoot remains unproven, there appears to be dysplasia of all osseous, muscular, tendinous, cartilaginous, skin, and neurovascular tissues distal to the knee in the affected limb.

The mild, “postural” clubfoot appears to represent a packaging problem due to intrauterine positioning. This deformity is passively correctible, lacks deep medial creasing, demonstrates minimal or no calf atrophy, and resolves spontaneously or responds quickly to a stretching and casting regimen. At the opposite end of the spectrum is the arthrogryptic or neuromuscular clubfoot that demonstrates severe rigidity; absence of skin creases, suggesting early in utero affliction; and failure to respond to nonoperative therapies. Between these two extremes lies the classic, idiopathic clubfoot deformity. The typical clubfoot demonstrates a deep, single medial skin crease, curved lateral border with a high arch, and rigid varus and equinus of the heel with a deep, single, posterior skin crease (Noonan and Richards, 2003). This gives the foot its “down and in” position and toes pointing to the midline. In unilateral cases the affected limb has a smaller foot and calf circumference (see Fig. 101.7).

All clubfoot deformities should be referred to a pediatric orthopedist for treatment. Initial treatment for all cases of congenital clubfoot is nonoperative. Untreated clubfoot has a poor natural history, with development of early degenerative changes in the foot joints. Historically, clubfoot was treated with early and extensive surgical correction. The long-term results, however, are poor, with high recurrence rates (Weinstein, 2000). Consequently, this approach was abandoned, and surgeons began advocating nonoperative methods of clubfoot correction (Kite, 1964; Seringe and Atia, 1990; Ponseti, 1996). These programs differ in the specifics of treatment but share a labor-intensive approach. Although many forms of nonoperative clubfoot treatment exist, the Ponseti method of cast correction has achieved preeminence in this regard. Studies show excellent mid-term to long-term results with decreased stiffness (Ponseti, 1992).

The Ponseti method uses a specific set of manipulations and serial corrective long-leg casts, followed by a prolonged period of bracing. Treatment is ideally commenced within the first few weeks after birth, but successful treatment is commonly achieved when treatment is initiated up to 1 year of age (Dobbs et al., 2004). We prefer to initiate treatment 1 to 2 weeks after discharge from the hospital to allow parental adjustment for the new child in their lives. Every 5 to 7 days, manipulation of the foot is performed with passive stretching, and the correction is maintained with a new long-leg cast, with an average of four to five casts in the idiopathic clubfoot (Dobbs et al., 2006). This is followed by



• **Fig. 101.8** Development of the tibiofemoral angle during growth. (Data from Salenius P, Vankka GK. The development of the tibiofemoral angle in children. *J Bone Joint Surg Am.* 1975;57:259-261.)

percutaneous Achilles tenotomy in most patients and a further 3 weeks of casting. Children are then placed into a foot abduction orthosis full-time for a period of 3 months and then part-time, while sleeping, until approximately age 4 years.

The “French functional method” has also been successfully duplicated in at least one US hospital with good results (Herring, 2002; Faulks and Richards, 2009). This method necessitates daily manipulations by a trained physical therapist for 8 weeks, with the addition of continuous passive motion during the first 4 weeks. This is followed by strapping and continued bracing.

The Ponseti and the French “nonoperative” methods both frequently use Achilles tenotomy and, at times, tendon transfers to attain the ultimate desired result. Recurrences of deformity are common (16%–37%), requiring further casting. A smaller percentage of patients (8%–16%) require surgical release of the hindfoot to various degrees (Faulks and Richards, 2009; Janicki et al., 2009).

Torsional and Angular Deformities of the Lower Extremities

Torsional and angular deformities of the legs constitute the most frequent nontraumatic reason for referral to a children’s orthopedist. Torsional deformities of the lower extremities rarely come to the attention of the physician before the child reaches walking age.

Occasionally a neonate demonstrates bowing of the legs, or genu varum, of a sufficient degree to concern the parents. The true incidence of genu varum is unknown, but in our experience it is extremely common. The overwhelming majority of cases resolve spontaneously, with a small minority of affected children manifesting a pathologic condition. Genu varum and internal tibial torsion

are nearly universal in neonates and spontaneously resolve between 2 and 3 years of age. Internal tibial torsion imparts an appearance of bowing to the tibia (Brooks and Gross, 1995). This is often concerning to both the parent and the physician.

Genu varum is physiologic up to the age of 2 years. Salenius and Vankka (1975) documented the tibiofemoral angles both clinically and radiographically in 979 children on the basis of 1408 examinations between birth and 16 years of age. They noted that newborns demonstrate a mean varus alignment of 15 degrees, which increases and becomes maximal at 6 months of age and then decreases to neutral at approximately 18 months. The maximum valgus (knock knees) of 12 degrees is then achieved by 3 to 4 years. By age 7 years, normal adult valgus alignment is achieved (Fig. 101.8). Natural history studies have demonstrated that physiologic genu varum is a self-limited process. Even genu varum with angulation greater than 30 degrees has been shown to undergo correction spontaneously with growth (Heath and Staheli, 1993).

Management of physiologic genu varum and tibial torsion consists of serial observation, reassurance, and parental education. Treatment with orthotics such as the Denis Browne splint is not indicated and in fact may be harmful to the ligaments of the knee.

Physical examination should include evaluation of the torsional profile (Staheli, 1977), which includes measurements of internal and external rotation of the hips and the thigh-foot angle. Measurement of the thigh-foot angle is performed with the child in the prone position comparing the axes of the sole of the foot with the thigh and is an indicator of tibial torsion. The examiner should also look for evidence of rhizomelic shortening and genu varum, which may herald a diagnosis of achondroplasia or other skeletal dysplasia.

It is important to note whether onset of the varus of the lower extremities is gradual or abrupt and if the deformity can be localized to the distal part of the femur, the proximal part of the tibia, or the midportion of the tibia. Radiographs are indicated only with asymmetric deformities, with short stature, or in infants with progressive deformities. Photographs are the preferred method of follow-up evaluation for progression.

Considerations in the differential diagnosis of genu varum include focal fibrocartilage dysplasia, skeletal dysplasias such as achondroplasia, posttraumatic physal growth arrests, osteogenesis imperfecta (OI), and metabolic bone disease such as vitamin D-resistant rickets, renal osteodystrophy, and tibia vara (infantile Blount disease). Blount disease is bilateral in 80% of affected children and does not occur before walking age, and most clinicians agree that this diagnosis cannot be made before 2 years of age.

Focal fibrocartilaginous dysplasia (FFD) involving the medial aspect of the proximal tibial metaphysis is a relatively rare cause of genu varum in the newborn and infant. It was first reported by Bell et al. (1985) and continues to be recognized in scattered case reports. The pathophysiologic basis for the disease may be abnormal development of fibrocartilage at the insertion of the pes anserinus (medial hamstring tendons). FFD presents before 1 year of age with unilateral bowing of the tibia that has prompted radiologic evaluation. The radiographs demonstrate a lytic defect in the proximal medial metaphysis of the tibia with surrounding sclerosis. The natural history of the deformity is that of progression until 2 years of age, with subsequent resolution by 4 years of age. During the progression the deformity can become quite pronounced and unsettling. Up to 1 cm of tibial length discrepancy is likely. Use of orthotics is not indicated. Surgery is indicated only in patients older than 4 years without evidence of spontaneous resolution (Zayer, 1980).

Tibial bowing can also occur in the sagittal plane. There are two major types of bowing distinguished by the direction of the apex of the bow. Posteromedial bowing has been previously described in conjunction with calcaneovalgus foot position in the neonate. Its cause is unknown, but numerous hypotheses have been proffered, including intrauterine fracture with malunion and in utero malpositioning with subsequent growth retardation and soft tissue contractures (Thompson, 2001). The deformity is unilateral and evident at birth. There is an associated calcaneovalgus foot deformity. Other features include shortening of the tibia and a smaller calf circumference and smaller foot relative to the contralateral side. Frequently there is a dimple at the apex of the deformity. Radiographic examination of the entire extremity from hip to ankle should be performed. Radiographs demonstrate the degree of bowing and in some cases thickening and sclerosis of the diaphyseal cortices on the compression side of the deformity with obliteration of the intramedullary canal. There is no increased fracture risk associated with the deformity.

Posteromedial bowing tends to resolve with growth, such that much of the deformity resolves by 2 years of age, with continued gradual correction beyond that. The shortening of the tibia and fibula persists, however, and progressively worsens during growth. Leg length inequality at skeletal maturity averages 4.1 cm (Hofmann and Wenger, 1981). Early referral to and serial follow-up assessments by a pediatric orthopedist are necessary to appropriately time epiphyseodesis surgery of the normal longer leg to allow equal leg lengths at skeletal maturity.

The second and most serious type of tibial bowing is anterolateral. It is usually identified at the newborn examination. It is unilateral and most frequently associated with congenital pseudoarthrosis of

the tibia. Although its cause is unknown, congenital pseudoarthrosis of the tibia is associated with neurofibromatosis type 1 (NF1) in 40%–80% of cases (Masserman et al., 1974; Paterson, 1989; Thompson, 2001). It is arguably the most challenging congenital malformation to treat in orthopedics. It is estimated to occur in 1 in 140,000 live births (Crawford and Schorry, 1999). Cutaneous signs of NF1 may be evident. If they are not, NF1 should remain a consideration as cutaneous and ocular manifestations may not be present in the newborn, and thus the child should be followed up expectantly.

If fracture has occurred, motion at the pseudoarthrosis site will be apparent. The foot may be normal or slightly small. The ankle may be in slight valgus to compensate for the bowing. The natural history of congenital pseudoarthrosis of the tibia is that of fracture with nonunion and repeated surgical attempts at obtaining union. Most of these attempts fail; if one such procedure succeeds, however, repeated fracture is likely, and the cycle begins again. Frequently, amputation is the end result. Because of this possibility, efforts are best directed at prevention of initial fracture.

Orthopedic consultation for anterolateral tibial bowing is imperative. In the perambulatory child, a total-contact (clamshell) ankle-foot orthosis should be fabricated and worn at all times except for bathing, to diminish the chance of fracture. When the child begins to walk, the orthosis may be extended above the knee with a drop-lock hinge to allow sitting. Bracing is continued until skeletal maturity is attained. Although definite proof that long-term bracing affects the natural history of this condition is lacking, most orthopedists consider that bracing is warranted. Under no circumstances should an osteotomy to correct the bowing of an unfractured tibia be undertaken because development of a pseudoarthrosis is likely to result. Many treatment options exist once a documented pseudoarthrosis occurs. Long-term immobilization, external fixation, internal fixation, bone transport, bone grafting, microvascular bone transfer, and electric stimulation have been attempted (Crawford and Schorry, 1999). High failure rates are commonly reported. Amputation has been advocated as a salvage procedure after failed attempts at union and typically has good outcomes (Jacobsen et al., 1983). Herring et al. (1986) reported that children who underwent Syme amputation had better psychologic and orthopedic functioning than those children who underwent numerous corrective surgical procedures.

Tibial bowing in the anteromedial direction can occur and is typically seen in children with fibular hemimelia, a condition with multiple lower limb anomalies. In addition to a deficient or absent fibula, there is a strong association with absent lateral rays of the foot, a bowed tibia, knee deformities, a short femur, hip dysplasia, and leg length discrepancy. Management might include deformity correction, limb lengthening, and foot, knee, or hip reconstruction.

Tibial bowing may also be confused with a congenital knee dislocation. This is a rare condition, noted at birth, with a reported incidence of 0.017 per 1000 (Jacobsen and Vopalecky, 1985). The cause is unknown. It is most likely related to contracture of the quadriceps muscle. Congenital knee dislocations are associated with clubfoot, arthrogryposis, myelodysplasia, and Larsen syndrome, with ipsilateral hip dislocation occurring in 70%–100% of cases (Curtis and Fisher, 1969). The knee can be hyperextended so severely that the foot might even reach the child's face, and the knee cannot be flexed. Nonsurgical treatment, consisting of manipulation and serial casting, should be started promptly after radiographic diagnosis and before management of the dislocated or dysplastic hip. Surgery is reserved for children who do not respond to nonsurgical treatment

and is best performed at the age of approximately 6 months (Nogi and MacEwen, 1982; Fig. 101.9).

Congenital Vertebral Malformations

Congenital vertebral malformations occur in 0.5 in 1000 to 1 in 1000 live births. Although a minority of cases may be due to genetic inheritance, there are no established gene defects that solely account for these disorders. The syndromes associated with them include Klippel–Feil syndrome, Goldenhar syndrome, and VATER (VACTERL) sequence. Likewise, many congenital vertebral malformations occur in isolation and may be due to intrauterine exposures such as exposure to high levels of blood glucose (hyperglycemia), exposure to carbon monoxide, or exposure to antiepileptic drugs. The ultimate concern with congenital vertebral anomalies is their potential to result in significant spinal deformity; namely, scoliosis or kyphosis or a combination of the two. Many, however, remain asymptomatic throughout life.

Defects can be attributed to a failure of formation, a failure of segmentation, or both. Failures of formation result from asymmetric vertebral body formation and ensuing development of a hemivertebra. Hemivertebrae can be incomplete, with partial retention of the affected side, or complete. When partial retention of the pedicle occurs, a wedge vertebra develops. Complete hemivertebra can be

further categorized. Radiographically, the presence of open disk spaces signifies the presence of growth plates and therefore growth potential. Unsegmented hemivertebrae, in which the segment is fused to one vertebra or both adjacent vertebrae, have less growth potential and therefore less deformity potential. Fully segmented hemivertebrae retain full growth potential from both the cranial end and the caudal end and consequently demonstrate a much greater propensity to result in significant deformity. Failures of segmentation are characterized by bony fusions (bars) between adjacent vertebrae. Bilateral bars result in “block vertebrae” that, because of their symmetry, have minimal potential for deformity.

The propensity to result in a clinically significant deformity depends on the location of the defect, the type of defect, and the age of the patient (Hedequist and Emans, 2007). Curves at the lumbosacral and cervicothoracic junctions may result in more clinically apparent deformities. Prediction of progression is largely driven by the presence of unbalanced defects (McMaster and Ohtsuka, 1982). In order of severity, the risk of progression in congenital spinal deformities is associated with the following defects: unilateral bar with contralateral hemivertebra, unilateral bar, hemivertebra, wedge vertebra, and block vertebra (Fig. 101.10). Additionally, the presence of multiple anomalies at multiple levels (e.g., multiple hemivertebra) can result in additional risk of progression when they are on the ipsilateral side or, conversely, may result in balanced growth when they are on contralateral sides of the spine.

All patients with known congenital spinal deformities should be evaluated for associated cardiac and renal anomalies. Cardiac anomalies are found in approximately 15% of these children and are usually evident on physical examination. Routine screening with an echocardiogram is not recommended unless clinical findings are suggestive (Prybis et al., 2007). Renal anomalies, on the other hand, are often clinically silent and have been reported in up to 37% of children with known congenital spinal anomalies (Riccio et al., 2007). Thus routine sonography of the urinary tract system is recommended for all children with congenital spinal malformations.

Occult intraspinal anomalies are found in up to 30% of children with congenital spinal malformations. These include Chiari malformations, syringomyelia, tethered cord, reduced spinal cord diameter, and diastematomyelia. Associated physical examination findings are those consistent with occult dysraphism, such as dimpling of the skin, pigmentation changes, or the presence of hairy patches or skin tags in the lower back or intergluteal cleft. Changes to the lower extremities such as atrophy, foot deformities,



• Fig. 101.9 Congenital knee dislocation.

| Site of curvature | Type of congenital anomaly | | | | | |
|-------------------|----------------------------|----------------|--------------|---------|----------------------------|--|
| | Block vertebra | Wedge vertebra | Hemivertebra | | Unilateral unsegmented bar | Unilateral unsegmented bar and contralateral hemivertebrae |
| | | | Single | Double | | |
| Upper thoracic | <1°—1° | ★—2° | 1°—2° | 2°—2.5° | 2°—4° | 5°—6° |
| Lower thoracic | <1°—1° | 2°—3° | 2°—2.5° | 2°—3° | 5°—6.5° | 6°—7° |
| Thoracolumbar | <1°—1° | 1.5°—2° | 2°—3.5° | 5°—★ | 6°—9° | >10°—★ |
| Lumbar | <1°—★ | <1°—★ | <1°—1° | ★ | >5°—★ | ★ |
| Lumbosacral | ★ | ★ | <1°—1.5° | ★ | ★ | ★ |

□ No treatment required □ May require spinal surgery □ Require spinal fusion ★ Too few or no curves

Ranges represent the degree of derotation before and after 10 years of age.

• Fig. 101.10 Types of congenital spinal anomaly and risk of progression. (From McMaster MJ, Ohtsuka K. The natural history of congenital scoliosis. A study of two hundred and fifty-one patients. *J Bone Joint Surg Am.* 1982;64:1128–1147.)

and asymmetric or pathologic reflexes are also suggestive of intraspinal defects.

Infants with congenital spine anomalies should initially be evaluated with dedicated plain radiographs of the whole spine. Coned-down views of affected parts of the spine may offer additional information about the anatomy of interest. Rib anomalies should be noted, because they are commonly associated with thoracic spine malformations and may have significant long-term implications with regard to restrictive lung disease (Campbell and Hell-Vocke, 2003). The position of the scapula should also be evaluated, because Sprengel deformity is found in up to 50% of children with congenital cervical spine anomalies (Hensinger et al., 1974). The use of magnetic resonance imaging (MRI) is reserved for those children preparing to undergo surgical intervention or those with clinical evidence of neurologic abnormality (Hedequist and Emans, 2007). Cutaneous anomalies of the lumbar spine in the newborn may be evaluated by ultrasonography. This is a particularly effective method for determining the level of the conus medullaris and thus the presence of tethered cord. Computed tomography is typically not indicated in the newborn owing to concerns of unneeded radiation exposure, but if done for other reasons, it can give additional detail on spinal anatomy.

Obstetric Trauma

Birth trauma can be divided into two categories: fractures and neurologic injuries. Birth fractures most commonly involve the clavicle, with clavicular fractures occurring in 2 per 1000 to 35 per 1000 vaginal births (Sanford, 1931; Farkas and Levine, 1950; Cohen and Otto, 1980; Kaplan et al., 1998). Birth fractures also occur in the proximal part of the humerus (Broker and Burbach, 1990; Fisher et al., 1995), the femur (0.13 per 1000 births) (Morris et al., 2002), and even the thoracic spine. It is important to note that clavicular fracture can be seen in combination with a proximal humeral physal separation or in combination with a brachial plexus injury. Reported risk factors for upper extremity birth fractures include large size of the baby, limited experience of the obstetrician, and a midforceps delivery (Cohen and Otto, 1980). Risk factors for femoral fracture include twin gestation, breech presentation, prematurity, and osteoporosis (Morris et al., 2002). Nadas et al. (1993) have reported an association of long-bone fractures with cesarean delivery, breech delivery with assistance, and low birth weight.

The natural history of isolated birth fractures to the extremities is that of uneventful rapid healing without untoward sequelae. Clavicle fractures may be difficult to diagnose, because the neonate may be asymptomatic. In a study of 300 newborns, radiographs revealed five unsuspected clavicle fractures (Farkas and Levine, 1950). Newborns with either a clavicle fracture or a proximal humeral physal separation often have pseudoparalysis of the upper extremity. Considerations in the differential diagnosis include an obstetric brachial plexus palsy and hematogenous metaphyseal osteomyelitis of the humerus with septic glenohumeral arthritis. Pain with direct palpation of the clavicle may be present with obvious deformity. Pain with motion of the shoulder joint and with palpation of the proximal part of the humerus may be caused by either fracture or infection. Elicitation of neonatal reflexes such as the Moro reflex and asymmetric tonic neck reflex (ATNR) may be helpful in evaluating active upper extremity muscle function (Sanford, 1931). Radiographs should be obtained. Ultrasound evaluation of the proximal part of the humerus may be helpful because the proximal humeral epiphysis is entirely cartilaginous at

birth and therefore radiolucent. Ultrasound examination can detect proximal physal separation, metaphyseal osteomyelitis, and septic shoulder arthritis (Broker and Burbach, 1990; Fisher et al., 1995).

Asymptomatic birth fractures of the clavicle and humerus in neonates can be observed. The fracture will unite in short order, with remodeling of bone occurring with growth. Symptomatic fractures in which the child exhibits pseudoparalysis of the upper extremity should be treated with 7 to 10 days of immobilization in a soft dressing or until symptoms subside. Femoral birth fractures can be treated with a Pavlik harness with good results (Morris et al., 2002). This device provides a simple means of immobilization that is accepted well by new parents. Excellent outcomes with no residual deformities or limb length inequalities can be expected.

The presence of multiple long-bone and rib fractures at birth may herald the presence of OI. Prenatal diagnosis is commonly made by ultrasonographic screening, as early as 13 to 14 weeks' gestation, on the basis of deformity (Cheung and Glorieux, 2008). OI is typically classified by the Sillence classification, with type II and type III being the most common types identified in the perinatal period (Sillence et al., 1979). Type II OI is lethal in the neonatal period, whereas most children with type III OI survive into adulthood with considerable short stature and fracture-related morbidity. Thus prompt genetic consultation is critical to establish a diagnosis and prognosis for an affected child. A diagnosis of Bruck syndrome should be considered when clinical and radiographic findings of OI are coupled with joint contractures (Schwarze et al., 2013). Infants with type III OI often require substantial respiratory support and pain management because of rib fractures, with respiratory failure being identified as the most common cause of death in the neonatal period. Treatment of long fractures is primarily to support pain management and can be achieved by custom splints or merely soft supports, such as pillows or blankets. Patients with multiple fractures at birth who are expected to survive the neonatal period should be considered for bisphosphonate treatment (Shapiro and Sponseller, 2009).

Brachial plexus injuries represent the second category of birth trauma afflicting newborns. The mechanism of injury is a separation of the head from the shoulder by lateral bending of the neck with simultaneous shoulder depression during vaginal delivery resulting in a stretching of the brachial plexus. These injuries occur in 1 per 1000 to 4 per 1000 live births (Hardy, 1981; Greenwald et al., 1984). Risk factors include maternal diabetes, large birthweight, prolonged labor, forceps delivery, and shoulder dystocia during a vertex delivery (Piatt, 2004). They are rarely seen in cesarean deliveries. Brachial plexus palsies are associated with clavicle and humerus fractures, as well as torticollis.

The brachial plexus receives contributions from the anterior spinal nerve roots of C5 through T1, which combine and divide to form the peripheral nerves that supply the motor innervation to the upper extremity. Three major injuries are encountered. The most frequent injury is to the upper trunk that involves the C5 and C6 nerve roots primarily and results in an Erb palsy. Affected infants lack external rotation and abduction of the shoulder. Hand function is preserved. The next most frequently occurring injury is a global plexus palsy involving the C5 through T1 nerve roots. This results in flaccid paralysis of the involved upper extremity, including the hand. An isolated lower plexus injury involving the C8 and T1 nerve roots, termed *Klumpke palsy*, is the least common and may be a manifestation of a recovered global plexus injury (Waters, 1997).

The physical examination has proved to be the most reliable method of assessing the level and severity of the neural injury and

thereby predicting the potential for spontaneous recovery (Waters, 1997; Noetzel et al., 2001). Myelography, computed tomographic myelography, magnetic resonance imaging, and electrodiagnostic studies have not proved useful in predicting recovery (Waters, 1997). Active shoulder, elbow, wrist, and finger motion need to be assessed (Piatt, 2004). Frequently, such assessment can be facilitated by elicitation of the primitive reflexes that are transiently present in normal newborns. The hand grasp reflex is normal in all newborns and disappears between 2 and 4 months. The examiner's little finger is placed on the ulnar aspect of the infant's palm, and the infant's fingers reflexively flex and grasp the examiner's finger. The Moro reflex begins to fade at 3 months of age. It is elicited by the examiner holding the newborn's hands while raising the baby off the table and then suddenly releasing them. In response, the newborn extends the spine, abducts and extends all four limbs and digits, and then subsequently adducts and flexes the limbs and digits. Last, the ATNR, or fencing reflex, can be elicited in a normal newborn until the age of 4 months. With the infant lying supine on an examining table, the head is rotated to one side by the examiner. The infant should respond by extending the elbow on the side toward which the face is looking and by flexing the opposite elbow. In newborns with a brachial plexus injury, some of these reflexes will be abnormal because of lack of motor control. For instance, the newborn with an Erb palsy will, most notably, not be able to actively flex at the elbow during the ATNR or the Moro reflex. The presence or absence of Horner syndrome (contracted pupil, drooping eyelid, and decreased sweating on the affected side) must also be noted.

An affected infant needs repeated serial examinations until 6 months of age. Return of biceps function by 3 months is the most important indicator of brachial plexus recovery (Michelow et al., 1994). When biceps recovery is combined with the return of shoulder abduction, wrist extension, and finger extension, there is a 95% chance of normal function (Michelow et al., 1994). When biceps function recovers later than 3 months, it is rare for the child to have complete recovery of normal function (Waters, 1999). A total plexus palsy or the presence of Horner syndrome also heralds a poor prognosis (Michelow et al., 1994; Waters, 1997).

The initial treatment of obstetric brachial plexus injury is aimed at avoiding contractures of the shoulder, elbow, forearm, and hand with occupational or physical therapy during the observation-for-recovery phase. Fortunately, as few as 1 in 10 infants with brachial plexus palsies at birth will require surgical intervention (Piatt, 2004). With the decision for surgery being based on muscle function recovery, prompt referral to a specialist is recommended to initiate monthly neurologic examinations.

Brachial plexus exploration with subsequent reconstruction is indicated for infants with total plexus involvement, Horner syndrome, and no return of biceps function at 3 months and for infants with a C5 to C6 (Erb) plexopathy and no return of biceps function at 3 to 6 months (Waters, 1997). Surgery is undertaken between 3 and 6 months of age. Using this algorithm prospectively, Waters (1999) operated on six infants at 6 months and found that their results were better than those for the 15 patients with biceps recovery at 5 months but worse than those for the 11 patients with biceps recovery at 4 months. Despite treatment as outlined, some children will have residual deficits. Secondary reconstruction, for chronic brachial plexopathy resulting in a dysfunctional shoulder, can be achieved with a tendon transfer of the latissimus dorsi and teres major to the rotator cuff or by derotational osteotomy of the humerus. These procedures and others designed to correct limitations in hand and forearm function are undertaken after the true scope

of the disability has been assessed. A more recent study suggested that some infants with no biceps recovery by 3 months will eventually achieve adequate biceps and shoulder function without surgery (Smith et al., 2004). The optimal timing of surgical intervention remains controversial. Surgical exploration after 18 months is of little benefit.

Neonatal Osteomyelitis and Septic Arthritis

Osteomyelitis is a bacterial infection of bone, and septic arthritis is a pyogenic infection of a joint. Incidence rates of 0.12 per 1000 live births and 0.67 per 1000 neonatal intensive care unit admissions (Ho et al., 1989) have been reported for septic arthritis. The mortality rate is reported to be 7.3% (Caksen et al., 2000). The hip, knee, and shoulder joints are involved most frequently. Neonates are particularly susceptible to osteomyelitis and septic arthritis because of an immature immune response, resulting in vulnerability to organisms that are not ordinarily virulent in infants and children, and because of delays in expressing the classic physical findings associated with these conditions (Morrissy, 2001).

Two subgroups of neonates are affected: premature neonates requiring prolonged hospitalization and otherwise healthy newborns in whom presentation occurs within 2 to 4 weeks after delivery (Morrissy, 2001). Most cases of neonatal osteomyelitis and septic arthritis result from hematogenous spread, but some occur from direct inoculation during percutaneous arterial blood sampling (especially if obtained from the femoral artery). Acute hematogenous osteomyelitis (AHO) and septic arthritis in hospitalized neonates usually occur in premature infants with multiple peripheral and central vascular catheters (Laborada et al., 2003). These infections are frequently caused by *Staphylococcus aureus* or gram-negative organisms (Deshpande et al., 2004). Up to 40% of these patients may demonstrate multiple areas of involvement, characterized by swelling and tenderness, and are systemically ill (Fox and Sprunt, 1978; Bergdahl et al., 1985).

This presentation contrasts with that of the typical out-of-hospital newborn with AHO and septic arthritis, in whom 2 to 4 weeks after birth swelling, pseudoparalysis (lack of active movement of affected limb) and tenderness of the extremity present and who feeds well and is not systemically ill (Morrissy, 2001). *S. aureus* and group B streptococcus are the most common organisms encountered in this latter population. Because of the immature immune response, neonates frequently do not demonstrate fever, leukocytosis, or elevation in erythrocyte sedimentation rates (Scott et al., 1990). However, C-reactive protein is a reliable indicator of AHO and septic arthritis, with a negative predictive value of 95% (Laborada et al., 2003). Blood culture findings are positive in only 50% of patients; cultures of synovial aspirates identify an organism in only 30% of cases (Lyon and Evanich, 1999).

Imaging studies are critical in diagnosing neonatal osteomyelitis and septic arthritis. Plain radiographs are usually the first study performed although they do not always yield a diagnosis in early infections. X-rays demonstrate soft tissue swelling by 3 days, but bone changes are not present for 1 week after the onset of symptoms (Jackson and Nelson, 1982; Dormans and Drummond, 1994). Ultrasound examination is our preferred radiologic method for evaluating the neonate with suspected AHO and/or septic arthritis, as it detects joint effusions and subperiosteal and soft tissue fluid collections and can guide aspiration. If an infection is still suspected despite negative findings after radiographic and ultrasound examination, nuclear scintigraphy and MRI are considered. Both are highly sensitive but may require that the infant be sedated. Scintigraphy



• **Fig. 101.11** Unrecognized neonatal hip sepsis can result in complete dissolution of the femoral head as demonstrated in this child's left hip radiograph.

is particularly useful in identifying multifocal involvement, which is an important consideration in neonatal osteomyelitis. MRI provides accurate regional information on both the soft tissues and bones and is highly sensitive in identifying early infections (Kothari et al., 2001).

In neonates, and before the appearance of the secondary ossification center, the epiphysis receives blood directly from metaphyseal blood vessels. When the infection occurs in these metaphyseal veins causing osteomyelitis, it can traverse the growth plate into the epiphysis, cause abscess formation, and rupture into the joint (Ogden, 1979). Thus septic arthritis is a sequela of adjacent osteomyelitis in up to 76% of neonates (Fox and Sprunt, 1978; Bergdahl et al., 1985). The hip and shoulder are two of the more common sites of neonatal septic arthritis as they have intra-articular metaphyses, allowing a subperiosteal route of decompression for pus into the joint. Because of this unique ability to spread from the metaphysis through the growth plate into the joint, early detection and treatment are necessary to avoid permanent damage to each of these structures. Infection can result in destruction of the growth plate and articular cartilage in short order, resulting in growth disturbances and precocious arthritis. When an area of involvement is suspected, aspiration should be undertaken (Morrissey,

2001). This may confirm the diagnosis and provide fluid for Gram stain and culture so as to better direct treatment. Osteomyelitis without a pus collection in bone or soft tissue can typically be managed with antibiotic therapy; septic arthritis and subperiosteal and bone abscesses should always be debrided surgically in the operating room (Fig. 101.11).

Suggested Readings

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Skeletal Dysplasias and Heritable Connective Tissue Disorders

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KEY POINTS

- Many skeletal dysplasias as well as many connective tissue disorders look similar in the newborn period, especially in premature infants. Arriving at the most accurate diagnosis possible is essential to proper medical decision making and counseling the family. Utilizing all resources available (medical consultants, texts, online resources, and molecular diagnostics) contributes to this process.
- With respect to molecular diagnostics, (1) it is not essential in all cases, especially where diagnoses can be made clinically by physical examination and/or radiographs, (2) particular mutations within a gene do not always determine prognosis, with notable exceptions (i.e., *FGFR3*, *COL1A1*), (3) diagnostic molecular testing is commercially available in all disorders for which a gene has been identified, and prenatal diagnosis is available if the particular gene mutation has been previously identified in an affected individual, and (4) medical geneticists and genetics laboratory directors can aid in choosing the best approach to a molecular diagnosis (which may take several weeks to complete), so as to accommodate disorders exhibiting genetic heterogeneity.
- As in all cases, the family should be kept updated on current clinical status, what is known, what remains unknown, and the timeline for receiving additional diagnostic and prognostic input.

The skeletal dysplasias, or osteochondrodysplasias, are disorders of the development and growth of cartilage and bone. The connective tissue disorders involve abnormalities of the cells' supporting and connecting structures in the matrix. In one series of 126,316 deliveries monitored for 15 years, the incidence of skeletal dysplasias was 2.14 in 10,000 ([Rasmussen et al., 1996](#)). With the growing use and accuracy of ultrasonography for prenatal care, a greater number of osteochondrodysplasias and connective tissue disorders are diagnosed prenatally.

The most recent classification of skeletal dysplasias into 42 groups is based on radiologic, clinical, and/or molecular criteria ([Bonafé et al., 2015](#)). This chapter focuses on several of the more "common" skeletal dysplasias (see [Tables 102.1](#) and [102.2](#) for an expanded list) and connective tissue disorders ([Table 102.3](#)) that manifest themselves prenatally or perinatally, but the discussion is not exhaustive. The osteochondrodysplasias have been reviewed extensively elsewhere ([Krakow and Rimoin, 2010](#); [Krakow, 2015](#)).

There are many different connective tissue molecules, including collagens (more than 24 types), elastin, fibrillin (two types), and

microfibril-associated glycoproteins. These molecules are components of tissues such as bone, cartilage, skin, vascular media, tendon, ligament, and basement membrane in many organs. The heritable disorders of connective tissue are varied, may be very dissimilar clinically, and may manifest themselves in utero or at any age postnatally. Those that may manifest themselves at birth include the early-onset (neonatal) form of Marfan syndrome, congenital contractural arachnodactyly (CCA; Beals syndrome), cutis laxa, some forms of Ehlers–Danlos syndrome (EDS), and Menkes disease.

Clinical Spectra of Disorders With Common Molecular Bases

The number of clinically distinguishable skeletal dysplasias and connective tissue disorders is extensive. With advances in molecular knowledge, several different dysplasias have been recognized to have mutations in the same genes. In some of these disorders, clinical similarities noted previously suggested a common cause. One such clinical spectrum includes achondroplasia, hypochondroplasia, severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN), and thanatophoric dysplasia, all of which are caused by mutations in the fibroblast growth factor receptor 3 gene (*FGFR3*; [Shiang et al., 1994](#); [Bellus et al., 1995](#); [Wilcox et al., 1998](#); [Vajo et al., 2000](#)). Another spectrum of disorders includes Stickler syndrome, Kniest dysplasia, some forms of spondyloepimetaphyseal dysplasia, spondyloepiphyseal dysplasia congenita (SEDC), hypochondrogenesis, achondrogenesis type II, and recessive multiple epiphyseal dysplasia, all of which are caused by mutations in the gene for collagen type II, *COL2A1* ([Spranger et al., 1994](#)). With other disorders, the common cause is not as obvious clinically: diastrophic dysplasia, atelosteogenesis type II, and achondrogenesis type IB are all caused by mutations in the *SLC26A2* gene ([Hastbacka et al., 1994, 1996](#); [Superti-Furga et al., 1996](#); [Bonafé et al., 2013b](#)). The obverse is also evident, wherein a specific clinical entity (e.g., multiple epiphyseal dysplasia) may be caused by a mutation in one of several genes—a concept known as *genetic heterogeneity*.

Approach to Diagnosis

An early and precise diagnosis is important for prognosis, optimal immediate-term and long-term management, accurate genetic

**TABLE
102.1****Lethal Skeletal Dysplasias Manifesting Themselves Prenatally or Perinatally**

| Dysplasia | Skeletal Features | Nonskeletal Features | Radiographic Features | Inheritance and Genes |
|--|--|---|--|---|
| Achondrogenesis type IB (OMIM 600972) | Soft cranium; short, round chest; very short limbs | Round face; polyhydramnios | Poorly ossified calvarium; short ribs with fractures and beading; nonossified vertebrae; short broad femurs with metaphyseal spikes, short broad tibiae and fibulae | AR; <i>SLC26A2</i> |
| Achondrogenesis type II, hypochondrogenesis (OMIM 200610) | Large head, flat face, cleft palate; short trunk; very short limbs (micromelia) | Fetal hydrops; distended abdomen | Lack of vertebral mineralization; short limbs (all segments); enlarged cranium with normal ossification | AD; <i>COL2A1</i> |
| Asphyxiating thoracic dystrophy (Jeune syndrome) (OMIM 263510) | Narrow, long chest; variable limb shortening | Lethal pulmonary insufficiency | Very short ribs with anterior cupping; short limbs with wide proximal femoral metaphyses; premature ossification of proximal femoral epiphysis | AR; <i>DYNC2H1</i> , <i>IFT80</i> , <i>IFT140</i> , <i>IFT172</i> , <i>WDR19</i> , <i>WDR34</i> , <i>TTC21B</i> |
| Atelosteogenesis type II (OMIM 256050) | Narrow chest, short limbs, joint dislocations, equinovarus deformities, gap between first and second digits | Cleft palate, laryngeal stenosis; patent foramen ovale | Occasional coronal and sagittal vertebral clefts; short ribs; short “dumbbell” humeri and femurs, small fibulae; large second and third metacarpals; small round midphalanges | AR; <i>SLC26A2</i> |
| Campomelic dysplasia (OMIM 114290) | Large cranium; small face, flat nasal bridge, cleft soft palate; small, narrow chest; angulated thighs and legs; dimples on legs | Polyhydramnios, congenital cardiac abnormalities; female external genitalia in XY males | Large dolichocephalic calvarium with shallow orbits; short and wavy ribs, often 11 pairs; hypoplastic scapula; small, flat vertebrae; tall, narrow pelvis; relatively long, thin limbs with bent femurs and short tibiae | AD (most are new mutations); <i>SOX9</i> |
| Chondrodysplasia punctata, rhizomelic type 1 (OMIM 215100) | Flat face; very flat nasal bridge and tip; proximal shortening of limbs | Cataracts; joint contractures; ichthyosiform erythroderma | Wide coronal vertebral clefts; short humeri and femurs; stippled epiphyses of long bones, pelvis and periarticular areas; trapezoid ilia | AR; <i>PEX7</i> |
| Short-rib polydactyly types I and III (OMIM 208500 and 613091) | Hydropic appearance, round flat face, micrognathia, very narrow chest, very short limbs, postaxial polydactyly | Cardiac, renal, and/or anal malformations | Normal calvarium; very short, horizontal ribs; flat, wide intervertebral disk spaces; small pelvis; short limbs with lateral and medial metaphyseal spurs | AR; <i>DYNC2H1</i> (for type III only), <i>IFT80</i> , <i>WDR34</i> |
| Thanatophoric dysplasia (OMIM 187600) | Large cranium, proptosis, flat nasal bridge, narrow chest, very short limbs (all segments) | Polyhydramnios, hydrocephalus, brain anomalies, congenital cardiac abnormalities | Large calvarium, small foramen magnum, cloverleaf skull (type 2); short, splayed, cupped ribs; very flat U-shaped vertebrae; short, flat pelvis; short, bowed limbs; metaphyseal flare with spike | AD (most are new mutations); <i>FGFR3</i> |

AD, Autosomal dominant; AR, autosomal recessive; OMIM, online mendelian inheritance in man.

counseling about recurrence risk, and identification of other possibly affected family members or disease carriers. An example is the group of disorders with punctate calcifications (“stippling”) in epiphyses, called *chondrodysplasia punctata*. There are several types, with three possible modes of inheritance: autosomal recessive, X-linked recessive, and X-linked dominant (see Table 102.1). As in any uncommon genetic condition, multiple components may be required to arrive at the correct diagnosis: a complete physical examination, three-generation family history, radiologic studies, and biochemical and/or molecular tests.

Most skeletal dysplasias cause short stature, which can be proportionate or disproportionate. The disproportion may be evident as a short-limbed or short-trunk form of dwarfism. If the limbs

are affected, there may be segmental shortening of the upper arms and thighs (rhizomelia), forearms and legs (mesomelia), or hands and feet (acromelia). Most skeletal dysplasias that manifest themselves at birth involve short limbs. Accurate measurements of length (on a firm surface) and head and chest circumferences must be plotted on standard growth curves, with measurement of arm span and calculation of upper body/lower body segment ratios to objectively assess disproportion.

Other skeletal characteristics can give important clues for specific disorders:

- Children with achondroplasia and thanatophoric dysplasia have large heads (macrocephaly). Cloverleaf skull deformity is present in some forms of thanatophoric dysplasias.

TABLE 102.2**Nonlethal Skeletal Dysplasias Manifesting Themselves Prenatally or Perinatally**

| Dysplasia | Skeletal Features | Nonskeletal Features | Radiographic Features | Inheritance and Gene |
|--|---|--|---|---|
| Achondroplasia (OMIM 100800) | Large cranium; frontal bossing, flat nasal bridge, short neck; slightly narrow chest; proximal limb shortening (rhizomelia), short trident hands; brachydactyly; joint laxity | Hypotonia: delayed motor milestones; spinal stenosis causing spinal compression; small foramen magnum may cause hydrocephalus and/or apnea | Large calvarium, small foramen magnum; diminished lumbosacral interpedicular space, short pedicles; short ribs with anterior cupping; short humeri and femurs; relatively long fibulas; metaphyseal flare; small iliac wings | AD (most are new mutations); <i>FGFR3</i> |
| Chondrodysplasia punctata, X-linked recessive (OMIM 302950) | Distal phalangeal hypoplasia; severe hypoplasia of nose; short stature | Cataracts; hearing loss; congenital ichthyosis; anosmia and hypogonadism (in contiguous gene deletion patients) | Distal phalangeal hypoplasia; stippled epiphyses of long bones; paravertebral stippling | XLR; <i>ARSE</i> |
| Chondrodysplasia punctata, X-linked dominant (Conradi–Hünemann syndrome) (OMIM 302960) | Asymmetric rhizomesomelia | Congenital cataracts; ichthyosis; patchy alopecia | Stippled epiphyses of long bones; paravertebral stippling; tracheal calcifications | XLD; <i>EBP</i> |
| Diastrophic dysplasia (OMIM 222600) | Cleft palate; micrognathia; normal chest at birth; very short limbs; thumbs proximally placed and adducted (hitchhiker thumb); equinovarus; limited joint movement | Cystic masses in auricles (cauliflower ears) during infancy; deafness caused by lack or fusion of ossicles; narrow external auditory canal | Premature ossification of rib cartilage; narrow L1–L5 interpedicular spaces; scoliosis; short limbs; short ulnae and fibulae (mesomelia); broad flared metaphyses; ovoid first metacarpals; variable symphalangism of proximal interphalangeal joints | AR; <i>SLC26A2</i> |
| Kniest syndrome (OMIM 156550) | Large cranium; flat face with large eyes, flat nasal bridge, cleft palate; proximal limb shortening, enlarged joints, flexion contractures | Infancy: tracheomalacia Childhood: myopia and retinal detachment, hearing loss, delayed motor development | Frontal and maxillary hypoplasia, shallow orbits; slightly short ribs; flat vertebrae with coronal clefts; irregular acetabular roof; short limbs with dumbbell metaphyses lateral bowing of femurs and tibiae | AD; <i>COL2A1</i> |
| Spondyloepiphyseal dysplasia congenita (OMIM 183900) | Flat face, cleft palate, short limbs | Infancy: tracheomalacia Childhood: myopia and retinal detachment, hearing loss | Frontal and maxillary hypoplasia, flat vertebrae, small pelvis with irregular acetabular roof, short limbs; normal hands and feet | AD; <i>COL2A1</i> |

AD, Autosomal dominant; AR, autosomal recessive; OMIM, online mendelian inheritance in man; XLD, X-linked dominant; XLR, X-linked recessive.

- A relatively long, narrow chest is seen in asphyxiating thoracic dystrophy.
- In achondroplasia, the hand is short and the fingers form a trident configuration. In diastrophic dysplasia, there are distinctive “hitchhiker” thumbs.
- Clubfeet may occur in diastrophic dysplasia, Kniest dysplasia, spondyloepiphyseal dysplasias, and osteogenesis imperfecta (OI) type II.
- Postaxial polydactyly occurs in short-rib polydactyly and asphyxiating thoracic and chondroectodermal dysplasias. Occasionally, preaxial polydactyly can also occur in the short-rib dysplasias.
- Multiple joint dislocations can manifest themselves at birth in Larsen syndrome, EDS type VII, atelosteogenesis, and Desbuquois syndrome.

The presence of extraskkeletal abnormalities may provide additional clues to diagnosis, as follows:

- Cleft palate may occur in campomelic, Kniest, spondyloepiphyseal, short-rib polydactyly (Majewski), atelosteogenesis type I and type II, hypochondrogenesis, and diastrophic dysplasias.
- Congenital cataracts are frequent in some forms of chondrodysplasia punctata.
- Congenital cardiac defects occur in short-rib polydactyly dysplasias and Ellis van Creveld syndrome.

Clinical and Molecular Evaluation

Radiographs of the entire skeleton, including the skull, thorax (with rib technique), long bones, hands, feet, pelvis, and lateral

**TABLE
102.3****Heritable Connective Tissue Disorders Manifesting Themselves Perinatally or in Childhood**

| Connective Tissue Disorders | Inheritance | Genes | Key Clinical Features |
|---|---|--|---|
| Marfan syndrome (OMIM 154700) | AD; congenital Marfan syndrome usually sporadic | <i>FBN1</i> | Aortic dilation, joint laxity, arachnodactyly, ectopia lentis, dural ectasia |
| Loeys–Dietz syndrome (OMIM 609192, 610168, 613795, 614816, and 615582) | AD | <i>TGFBR1</i> , <i>TGFBR2</i> , <i>TGFB2</i> , <i>TGFB3</i> , <i>SMAD3</i> | Arterial tortuosity, cardiac anomalies, joint laxity, aneurysms, arachnodactyly |
| Congenital contractural arachnodactyly/distal arthrogryposis type 9 (OMIM 121050) | AD | <i>FBN2</i> | Kyphoscoliosis, joint contractures, crumpled ears, cardiac anomalies |
| Ehlers–Danlos Syndromes | | | |
| Classic type (type I) (OMIM 130000) | AD | <i>COL5A1</i> , <i>COL5A2</i> , <i>COL1A1</i> | Joint laxity, atrophic scarring, easy bruising, premature birth, skin hyperelasticity |
| Vascular type (type IV) (OMIM 130050) | AD | <i>COL3A1</i> | Aortic and medium-sized arterial aneurysm, intestinal rupture |
| Kyphoscoliotic type (type VI) (OMIM 225400) | AR | <i>PLOD1</i> | Scoliosis, joint laxity, congenital hip dislocation, ocular globe rupture |
| Arthrochalasia type (types VIIA and VIIB) (OMIM 130060) | AD | <i>COL1A1</i> , <i>COL1A2</i> | Congenital hip dislocation, joint laxity |
| Dermatosparaxis type (type VIIC) (OMIM 225410) | AR | <i>ADAMTS2</i> | Fragile skin, joint laxity |
| Cutis Laxa | | | |
| Autosomal dominant (OMIM 123700 and 130160) | AD | <i>ELN</i> , <i>FBLN5</i> | Loose redundant skin |
| Autosomal recessive (OMIM 219100, 219150, 614437, 219200, 278250, 612940, 613177, and 613075) | AR | <i>FBLN5</i> , <i>EFEMP2</i> , <i>ATP6V0A2</i> , <i>LTBP4</i> , <i>PYCR1</i> | Cutis laxa, musculoskeletal, genitourinary, vascular, and other systemic features |
| Geroderma osteodysplasticum (OMIM 231070) | AR | <i>GORAB</i> | Premature aged appearance, camptodactyly, bowed legs |
| De Barsy syndrome (OMIM 179035) | AR | <i>PYCR1</i> | Aged appearance, intrauterine growth restriction, cutis laxa |
| Menkes syndrome (OMIM 309400) | XLR | <i>ATP7A</i> | Skin laxity, joint laxity, kinky sparse hair, neurologic degeneration |

AD, Autosomal dominant; AR, autosomal recessive; OMIM, online mendelian inheritance in man. XLR, X-linked recessive.

spine, are essential for accurate diagnosis. Atlases dedicated to skeletal dysplasias are essential for this purpose (Lachman, 2006; Spranger et al., 2012), even for the experienced radiologist or neonatologist. Ultrasound images of the brain, heart, and kidneys may be helpful if anomalies in those organs are suspected. Detailed family history and measurements of family members may be helpful; disorders in more mildly affected members might have gone undiagnosed. Molecular investigations may be necessary to arrive at the proper diagnosis; given their complexity, such analyses should be considered after consultation with a clinical geneticist. The advent of “next-generation” DNA sequencing has led to more widespread availability of multi-gene diagnostic panels, which can be used when the diagnosis cannot be arrived at solely by clinical and radiographic means (see <https://www.genetests.org> or the Genetic Testing Registry at <https://www.ncbi.nlm.nih.gov/gtr/>). Molecular definition is

also helpful in the cases of autosomal recessive and X-linked disorders, as this information may be useful for counseling with respect to recurrence risk and prenatal diagnosis in subsequent pregnancies.

If the infant or fetus dies with the disorder undiagnosed, specimens of cartilage and skin fibroblasts should be obtained for histochemical tests, biochemical assays, and/or molecular analyses; these can be used to make or confirm diagnoses and permit accurate future prenatal diagnosis. Even if the molecular or enzymatic basis of the condition is not understood at the time, the tissue may be useful in the future. If photographs and skeletal radiographs were not obtained before death, they should be obtained after death. Additional information on clinical diagnosis may be obtained from the International Skeletal Dysplasia Registry at the University of California, Los Angeles (<http://ortho.ucla.edu/abouttheisdr>).

Disorders of Bone Fragility

Osteogenesis Imperfecta Types II and III

OI is characterized by increased bone fragility. There are classically four major clinical types: types II and III are the severest, manifesting themselves prenatally and perinatally (Steiner et al., 2013). However, fractures at birth can also occur in OI type I. Further heterogeneity in OI has recently been described.

Presentation

OI type II (perinatal lethal type) is estimated to affect 1 in 20,000 to 1 in 60,000 infants. Affected infants may be born prematurely, with low birthweight and disproportionately short stature. The limbs are short and bowed with extra circular skin creases; the hips are abducted and flexed. The head is soft and boggy, and minimal calvarial bone can be felt. The sclerae are dark blue, and the chest is narrow. The infant cries with handling because there may be many fractures at different stages of healing. Sixty percent of affected babies are stillborn or die during the first day of life, and 80% die by 1 month. With the growing use of ultrasonography, affected fetuses may be detected in the second trimester because of short and bowed or angulated limbs and narrow thoraces (Fig. 102.1).

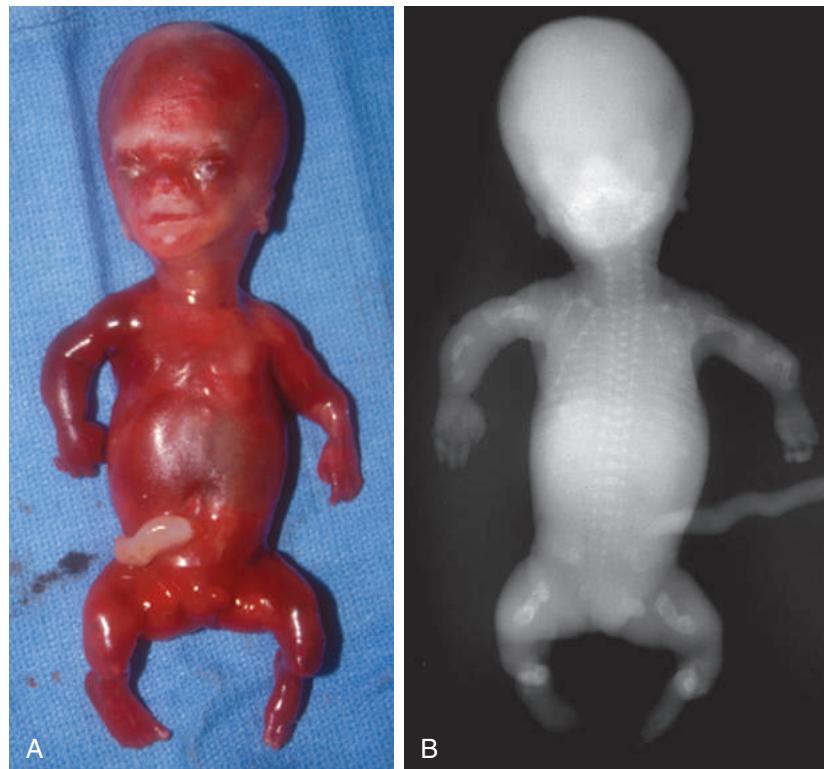
OI type III (progressive deforming type) can manifest itself prenatally, perinatally, and in the first 2 years after birth. Prenatal and perinatal clinical features resemble those in OI type II but are less severe (Fig. 102.2), and perinatal death is not uncommon.

If not present at birth, fractures and deformations of the limbs develop in the first 2 years. The highest prevalence of fractures in OI, up to 200 in a lifetime, occur in type III. Extremely short stature, with an adult height of 92 to 108 cm, can result from microfractures in growth plates. The head may be large because the calvarium is soft with a large anterior fontanel. The sclerae may be blue initially but are white by puberty. The head assumes a triangular shape, with a bossed, broad forehead and a tapered, pointed chin. Later in childhood, dentinogenesis imperfecta and hearing loss may develop. Severe kyphoscoliosis may occur, leading to cardiopulmonary compromise, which is the major cause of early death.

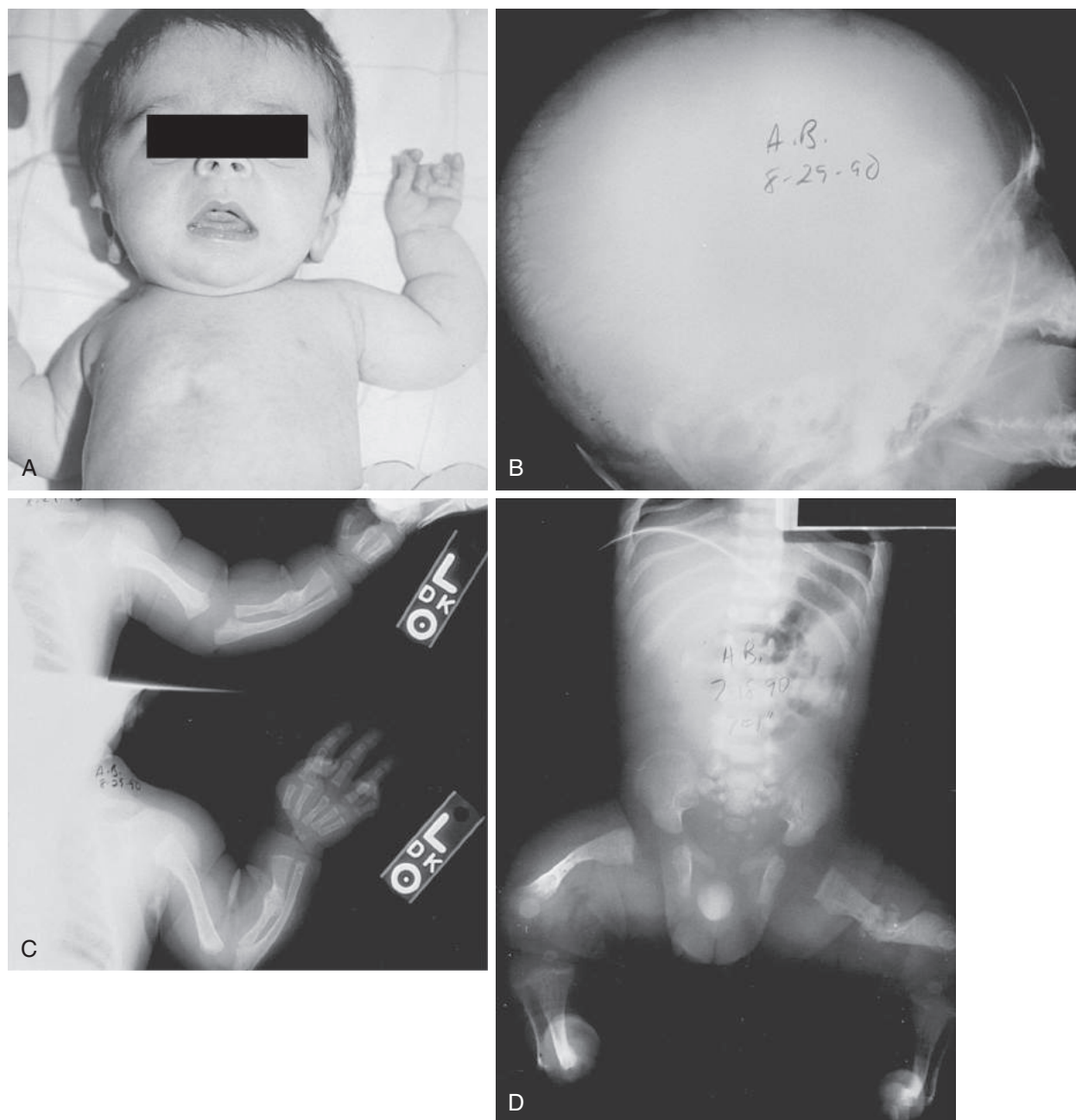
Radiographic Features

Radiographs show the femurs in OI type II to be short, broad, and “telescoped” or “crumpled.” The tibiae are short and bowed or angulated, and the fibulae may be thin (see Fig. 102.1B). There is minimal to no calvarial mineralization. The acetabulae and iliac wings may be somewhat flattened. The ribs are short, wavy, and thin or broad, with “beading” from callus formation at fetal fracture sites.

In OI type III the femurs are short and deformed but not crumpled as in OI type II (see Fig. 102.2B–D). The other long bones are thinner than usual, with healing fractures incurred in utero, bowing, and deformations. The calvarium is undermineralized with a large anterior fontanel, and there are many wormian bones (small islands of bone in the suture spaces; see Fig. 102.2D). The ribs are thin and gracile.



• **Fig. 102.1** Osteogenesis Imperfecta Type II. (A) A 20-week fetus. The limbs are angulated and deformed from multiple fractures. (B) Radiograph of fetus (20 weeks' gestation) showing an absence of ossification in the calvarium, short telescoped or crumpled humeri and femurs, and short and wavy ribs with fractures.



• **Fig. 102.2** Osteogenesis Imperfecta Type III. (A) Neonate with normal face, short neck, and slightly short limbs. (B) Radiograph showing that the calvarium is undermineralized with wormian bones. (C) Radiograph showing the upper limbs, which have bowed humeri and callus in the ulnae. (D) Radiograph showing lower limbs with moderately short, thick femurs, and angulated tibiae and fibulae. (Courtesy of Paige Kaplan, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, United States.)

Etiology

OI is most commonly caused by mutations in one of the two genes encoding type I collagen (*COL1A1* and *COL1A2*), the predominant protein building block of bone. Clinically severe forms of OI are the result of qualitatively abnormal collagen synthesis rather than decreased production (Steiner et al., 2013), as well as the result of recessive disorders affecting noncollagen proteins (Forlino and Marini, 2016).

Inheritance

A fetus or infant with OI type II or III is usually the result of a spontaneous dominant-acting gene mutation, but there is a small risk of recurrence (approximately 6%) in subsequent siblings because of parental somatic or gonadal mosaicism. The parent is usually asymptomatic but may have minimal manifestations, such as short stature. Prenatal diagnosis is available if the particular gene mutation has been identified in the affected individual. Most cases of OI

are inherited as autosomal dominant traits, although rare recessive forms have been shown to be caused by mutations in genes encoding, for example, cartilage-associated protein (*CRTAP*; Barnes et al., 2006), prolyl 3-hydroxylase (*P3H1*), and cyclophylin B (*PPIB*; van Dijk et al., 2009).

Differential Diagnosis

Other lethal skeletal dysplasias may have abnormalities similar to those in OI type II and may be difficult to distinguish by prenatal ultrasonography; however, in experienced hands they can be differentiated on the basis of several ultrasound findings (Krakow et al., 2009). Krakow et al. (2008) published a retrospective analysis of 1500 prenatally diagnosed cases of skeletal dysplasias. The three most common prenatal-onset skeletal dysplasias were OI type II, thanatophoric dysplasia, and achondrogenesis type II, accounting for almost 40% of cases. Postnatal radiographs clearly reveal distinctive differences among thanatophoric dysplasia, campomelic dysplasia, achondrogenesis, and perinatal hypophosphatasia, among other disorders.

Management

If the diagnosis of OI is made prenatally, caesarean delivery has *not* been shown to decrease the fracture rate or increase the survival rate of severely affected fetuses (Cubert et al., 2001). Those severely affected with OI type II are not expected to survive the neonatal period. In OI type III the neonate needs careful handling to minimize pain and prevent further fractures. Analgesia alleviates pain. Consideration can be given to treatment with bisphosphonates (with use of intravenously administered pamidronate), which increase bone density, reduce the frequency of fractures and pain, possibly prevent short stature and deformations, and permit ambulation (Cheung and Glorieux, 2008). It is prudent to treat only severely affected children in whom the clinical benefits outweigh potential long-term effects.

Handling an Infant With Osteogenesis Imperfecta

When the diapers of an infant with OI are being changed, a hand should be placed behind the infant's buttocks with the forearm supporting the legs. Similarly, when the infant is lifted, the buttocks, head, and neck must be supported. The infant can be laid on a pillow to be carried. To transport the infant, an infant seat that reclines as much as possible and allows easy placement or removal should be used. The seat can be padded with egg crating or 1-inch foam. A layer of foam can be placed between the seat's harnesses and the child for extra protection. The car seat must always be placed in the back seat. Sling carriers and "umbrella" strollers should not be used for infants with OI because they do not give sufficient leg, head, and neck support.

Perinatal Hypophosphatasia

Presentation

Perinatal hypophosphatasia is a lethal condition characterized by short deformed limbs, a soft skull, blue sclerae, and under-mineralization of the entire skeleton, so many bones cannot be visualized and may seem absent in radiographs. In the skull, only the base can be visualized radiologically. There may be rachitic changes and fractures. Seizures that are responsive to pyridoxine may occur. There is polyhydramnios during pregnancy, and death can occur in utero. The disorder affects approximately 1 in 100,000 live births, and neonatal death is common (Mornet and Nunes, 2016).

Radiographic Features

The radiographic features of perinatal hypophosphatasia include polyhydramnios (prenatal); underossification, especially of the calvarium and long bones (with marked variability); small thoracic cavity; short, bowed limbs; spurs in the middle portion of the forearms and lower legs; and dense vertebral bodies.

Etiology

Mutations in the *ALPL* gene are responsible for deficiency of the tissue-nonspecific isoenzyme of alkaline phosphatase (TNSALP), thus causing perinatal hypophosphatasia. The serum alkaline phosphatase value is low. Serum values of inorganic pyrophosphate and pyridoxal 5'-phosphate (putative natural substrates for TNSALP) may be elevated, and urinary phosphoethanolamine level is elevated (Mornet and Nunes, 2016).

TNSALP acts on multiple substrates: the essential function of TNSALP is in osteoblastic bone matrix mineralization. TNSALP hydrolyzes inorganic pyrophosphate to phosphate, thought to be critical in promoting osteoblastic mineralization. If TNSALP is deficient, there is extracellular accumulation of inorganic pyrophosphate, which inhibits hydroxyapatite crystal formation and mineralization of the skeleton. TNSALP is also needed for delivery of pyridoxal 5'-phosphate into cells, where it is a cofactor (vitamin B6).

Inheritance

Perinatal hypophosphatasia is inherited as an autosomal recessive trait, with a 25% recurrence risk in future pregnancies. Prenatal diagnosis is optimized with the use of ultrasonography, assay of TNSALP activity in amniocytes, DNA mutation analysis if the previously affected infant's mutations were identified, or a combination of these methods.

Differential Diagnosis

Differential diagnoses include OI type II and achondrogenesis.

Management

Until very recently, treatment was primarily supportive and directed toward minimizing pain and discomfort. Clinical trials with a bone-targeted human recombinant enzyme replacement therapy have yielded promising results, and the drug has recently gained Food and Drug Administration approval (Whyte et al., 2016).

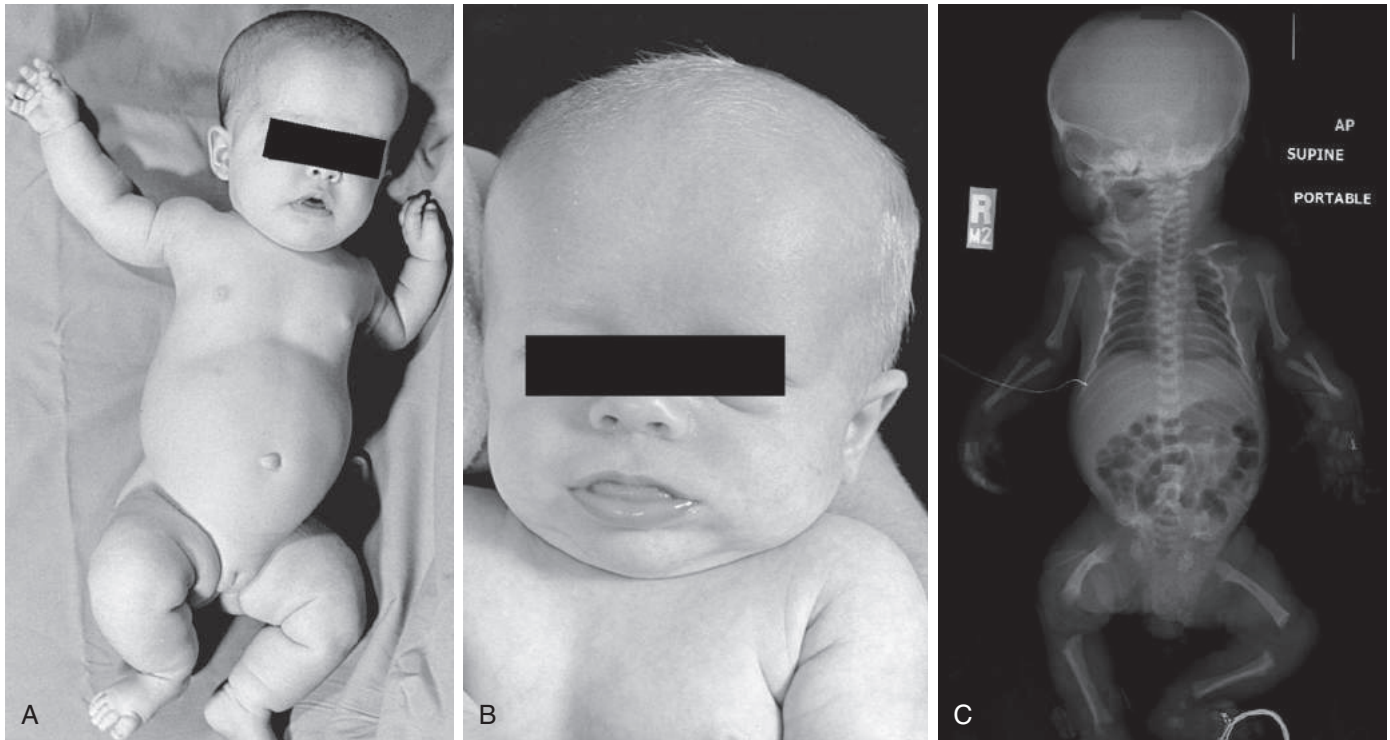
FGFR3 Spectrum

Achondroplasia

Presentation

Achondroplasia is the most common of the nonlethal chondrodysplasias; it affects 1 in 25,000 live births. It is characterized by short stature with short limbs, particularly rhizomelic (proximal) and acromelic (hands) shortening with trident hand configuration, large head with frontal prominence ("bossing"), flat nasal bridge and midface, long narrow trunk, joint laxity, and development of thoracolumbar kyphosis ("gibbus") in infancy (Fig. 102.3).

The foramen magnum and cervical spinal canal may be narrow and can cause compression of the spinal cord. Standards have been published for foramen magnum size in achondroplasia (Hecht et al., 1985). Compression of the lower brainstem and cervical spinal cord can lead to hypotonia, central apnea, retardation, quadriplegia, and (rarely) sudden death (Pauli et al., 1984, 1995). Perinatal or



• **Fig. 102.3** Achondroplasia. (A) Infant with achondroplasia who has macrocephaly and proximal limb shortening (rhizomelia). (B) Infant with achondroplasia who exhibits frontal bossing and flat nasal bridge. (C) Neonatal radiograph of achondroplasia illustrating a large skull, a somewhat narrow chest, short vertebral bodies with a lack of lumbar interpediculate flare, and rhizomelia. ([B] Courtesy Charles I. Scott, Nemours/Alfred I. du Pont Hospital for Children, Wilmington, Delaware, United States.)

infantile death can occur but is unusual (Simmons et al., 2014). Infants often sleep with their neck hyperextended, and symptoms can be exaggerated by neck flexion (Danielpour et al., 2007).

Radiographic Features

The calvarium is large with a relatively small foramen magnum and a short base. The lateral cerebral ventricles may be large, but hydrocephalus is not a common complication. The proximal long bones (humeri and femurs) are short, including the femoral neck. Fibulae are longer than tibiae. There is metaphyseal flaring. The hand is short with a trident configuration of the fingers, with short proximal and middle phalanges. Vertebrae are small and cuboid with short pedicles, and there may be anterior beaking of the first or second lumbar vertebrae; there is lack of widening of the interpedicular distance in the lumbar vertebrae. The pelvis has squared iliac wings (“elephant ear” appearance), a narrow greater sciatic notch, and flat acetabular roofs (see Fig. 102.3C). Compression of the cervical cord, if present, can be ascertained with magnetic resonance imaging cerebrospinal fluid flow studies in flexion and extension.

Etiology

The cause of achondroplasia is a mutation of the *FGFR3* gene, which encodes fibroblast growth factor receptor 3, a membrane-spanning tyrosine kinase receptor, which may form dimers with gene products of other gene family members: *FGFR1*, *FGFR2*, and *FGFR4*. The heterodimers serve as receptors for several fibroblast growth factors (Teven et al., 2014). More than 99% of individuals with achondroplasia have a mutation in the transmembrane domain of the *FGFR3* gene, in which arginine is substituted for glycine

(Gly380Arg; Shiang et al., 1994). The same gene is mutated at different sites in hypochondroplasia, thanatophoric dysplasia, SADDAN, Muenke craniosynostosis, and Crouzon craniosynostosis syndrome with acanthosis nigricans (Vajo et al., 2000). Histopathologic examination demonstrates a defect in the organization and maturation of the cartilage growth plates of long bones because of differing degrees of constitutive activation of the receptor.

Inheritance

The inheritance pattern in achondroplasia is autosomal dominant. Approximately 80% of cases are sporadic occurrences in a family, representing new mutations. Cases may be associated with advanced paternal age, and molecular studies have confirmed that new mutations are of paternal origin (Goriely and Wilkie, 2012). Rare recurrences due to gonadal mosaicism in a parent have been reported. Affected individuals are fertile, and achondroplasia is transmitted as a fully penetrant autosomal dominant trait, meaning that each person who inherits the mutant gene will manifest the condition.

Differential Diagnosis

Differential diagnoses include SADDAN (Vajo et al., 2000) and hypochondroplasia (Bellus et al., 1995).

Management

The infant with achondroplasia is often hypotonic; together with the large head, the hypotonia leads to delayed motor milestones. Development of thoracolumbar kyphosis may be exacerbated by unsupported sitting before truncal muscle strength is adequate; therefore infants should not be carried in flexed positions (including soft sling-carriers and umbrella strollers). Rear-facing car safety

seats should always be used. Most infants lose their kyphosis and develop lumbar lordosis when they begin walking.

Hydrocephalus may occasionally develop during the first 2 years, so the head circumference and body length should be carefully measured and plotted on standard achondroplasia growth charts (Trotter et al., 2005; Ireland et al., 2014). Routine imaging of the skull and brain is not recommended; however, development of hyperreflexia, hypotonia, or apnea may herald the development of clinically significant cord compression. Surgical decompression at the foramen magnum or the upper cervical spine may prevent neurologic damage, although most patients usually gain motor milestones late but spontaneously, because the foraminal diameter expands faster than the cord.

The upper airway in individuals with achondroplasia is small, often leading to obstructive apnea, snoring, and chronic serous otitis media beyond infancy. Treatment may consist of tonsillectomy, adenoidectomy, and placement of myringotomy tubes. Parents should be counseled about the clinical and hereditary aspects of the disorder and given a copy of the guidelines for health supervision of children with achondroplasia issued by the American Academy of Pediatrics (Trotter et al., 2005; Ireland et al., 2014).

Thanatophoric Dysplasia

Presentation

Thanatophoric dysplasia is one of the most common lethal dysplasias (Karczeski and Cutting, 2013), occurring in 1 in 45,000 births. It is characterized by extremely short limbs, long narrow trunk,

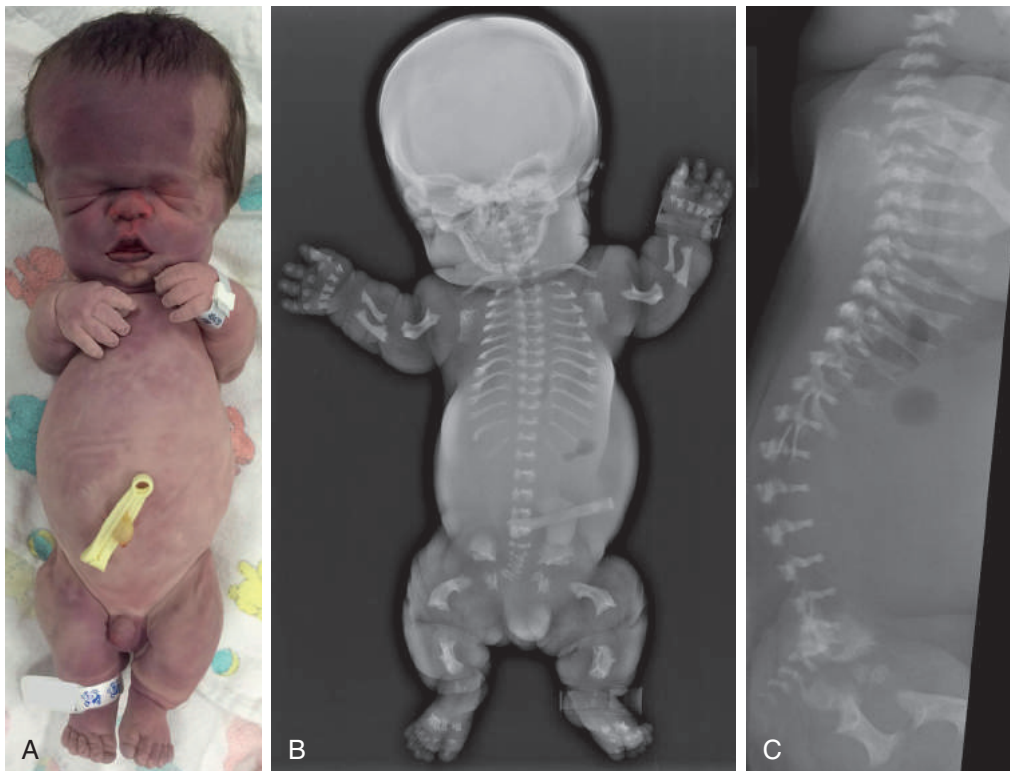
large head with bulging forehead, prominent eyes, flat nasal bridge, wide fontanel, and occasionally cloverleaf skull deformity (Fig. 102.4). It is differentiated into types I and II based on radiologic features and mutation specificity. Death typically occurs in the neonatal period from respiratory insufficiency, although rare survivors have been reported with multiple chronic problems. Polyhydramnios is common during pregnancy.

Radiographic Features

Femurs are short, flared at the metaphyses with a medial spike, and are bowed (type I) or straight (type II); other long bones are also short and bowed (see Fig. 102.4). The calvarium is large with a short base and small foramen magnum; cloverleaf skull is sometimes present in type I thanatophoric dysplasia and is severe in type II thanatophoric dysplasia. Vertebrae are strikingly flat (platyspondyly) with a U shape or an H shape in anteroposterior projection and uniform interpediculate narrowing. Ribs are short, cupped, and splayed anteriorly (Lachman, 2006).

Etiology

Thanatophoric dysplasia represents the severe end of the *FGFR3* spectrum. In thanatophoric dysplasia type I, the most common mutation in the extracellular domain is a substitution of cysteine for arginine at position 248 (Arg248Cys), but other mutations have been described throughout the gene. In all studied cases of thanatophoric dysplasia type II, there is a substitution of glutamate for lysine at position 650 (Lys650Glu; Karczeski and Cutting, 2013).



• **Fig. 102.4** Thanatophoric Dysplasia. (A) Stillborn with thanatophoric dysplasia with a large head with frontal bossing, narrow chest, short limbs, and short fingers (brachydactyly). (B) Radiograph of stillborn with thanatophoric dysplasia demonstrating large cranium, narrow chest, with short ribs, shortening of all long bones (micromelia) with bowing, flattened vertebral bodies (platyspondyly), and normal bone density. (C) Lateral spine radiograph of stillborn with thanatophoric dysplasia demonstrating remarkable platyspondyly.

Inheritance

All cases of thanatophoric dysplasia, as with most cases of achondroplasia and hypochondroplasia, occur sporadically and result from new autosomal dominant mutations. Nevertheless, there may be a small risk of recurrence to siblings of an infant with sporadic thanatophoric dysplasia, possibly caused by gonadal mosaicism. Prenatal diagnosis is available if the particular gene mutation has been previously identified in an affected individual.

Differential Diagnosis

Differential diagnoses include OI types II and III, achondroplasia (severe), achondrogenesis, and hypochondrogenesis.

Management

If the condition is suspected prenatally and diagnosed by molecular means (mutation analysis following amniocentesis), the parents should receive genetic counseling and anticipate neonatal death. If the diagnosis is suggested after delivery and radiographically confirmed, management is solely supportive, with death from pulmonary insufficiency usually occurring within hours to days.

COL2A1 Spectrum

Spondyloepiphyseal Dysplasia Congenita

Presentation

SEDC manifests itself with shortened neck, trunk, and limbs, normal-sized hands and feet, flat facial profile, and occasional cleft palate and clubfoot (Terhal et al., 2015; Fig. 102.5). The name

spondyloepiphyseal dysplasia congenita (SEDC) is derived from the spinal (*spondylo*) and growth plate (*epiphyseal*) involvement. *Congenita* indicates that the condition is present from birth.

Radiographic Features

Radiographic features include ovoid or pear-shaped vertebral bodies in infancy, with platyspondyly more evident at a later age; odontoid hypoplasia evident in early childhood; midface hypoplasia; retrognathia; mild rhizomelia and mesomelia (see Fig. 102.5B–D); absent ossification of the os pubis; apparent decreased bone age caused by epiphyseal involvement; and development of coxa vara, variable kyphosis, and scoliosis in childhood (Spranger et al., 2012).

Etiology

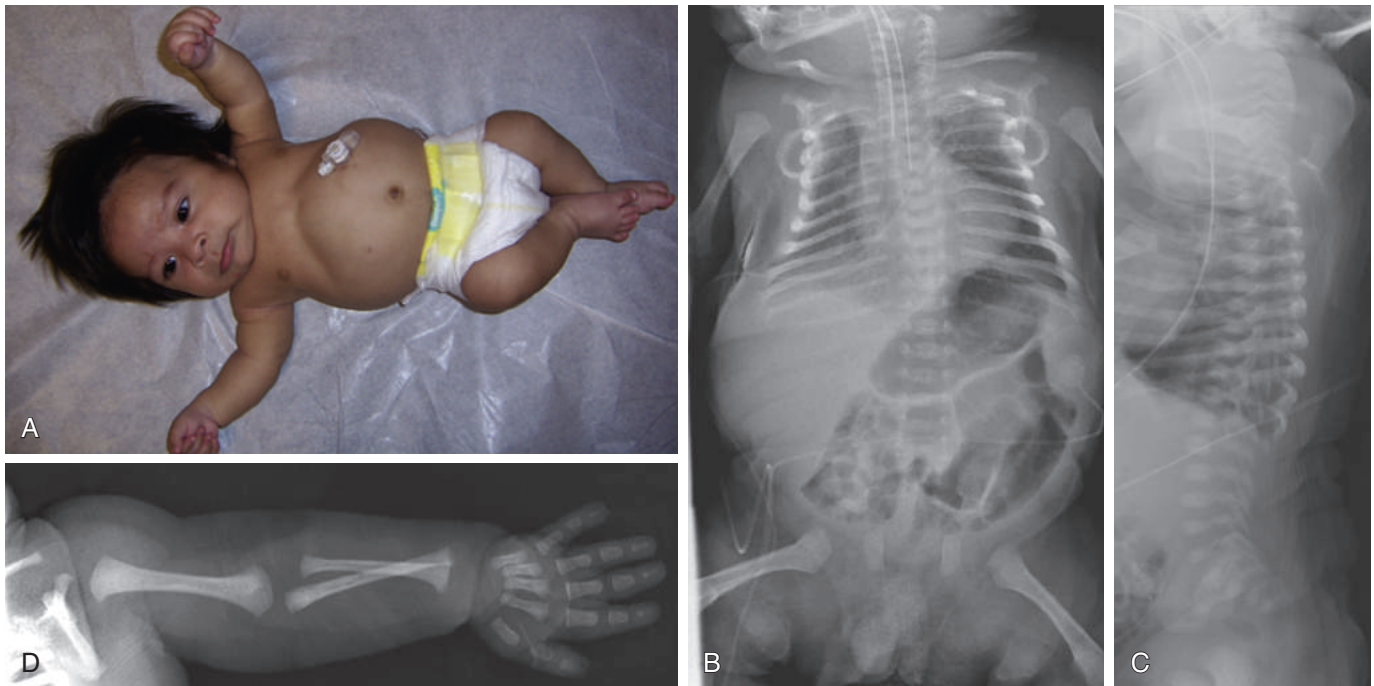
SEDC is caused by mutations in the gene for type II collagen (*COL2A1*), the predominant protein building block of cartilage. Mutations in *COL2A1* are also responsible for Kniest dysplasia, some forms of spondyloepimetaphyseal dysplasia and Stickler syndrome, and the perinatal lethal disorders achondrogenesis type II and hypochondrogenesis (Spranger et al., 1994; Tiller et al., 1995; Mortier et al., 2000).

Inheritance

SEDC is inherited in an autosomal dominant pattern. Offspring of affected individuals are at 50% risk of inheriting the disorder. The recurrence risk for unaffected parents is approximately 6%, considering the possibility of parental gonadal mosaicism.

Differential Diagnosis

Differential diagnoses include the milder form of hypochondrogenesis and Morquio syndrome.



• **Fig. 102.5** Spondyloepiphyseal Dysplasia Congenita. (A) A 2-month-old infant with short neck, trunk, and limbs. Note the flat facial profile and normal size of the hands and feet. (B) Anteroposterior radiograph revealing platyspondyly and short chest. (C) Lateral radiograph revealing platyspondyly. (D) Upper limb radiograph revealing rhizomelia, mesomelia, and a normal-sized hand.

Management

Neonates may require intubation because of upper airway compromise. Care must be given when the cervical spine is manipulated (as in endotracheal intubation), because of odontoid hypoplasia. C1–C2 fusion may be required in early childhood to stabilize the cervical spine. Annual hearing screens are recommended during childhood. Regular ophthalmologic evaluation (semiannually before school age) is essential to detect early development of retinal detachment and to manage myopia. Osteoarthritis is a common feature in early adulthood, often requiring hip arthroplasty.

Achondrogenesis Type II–Hypochondrogenesis

Presentation

The severe end of the *COL2A1* spectrum manifests itself with fetal hydrops and maternal polyhydramnios, severe short trunk and limbs, and fetal or neonatal death caused by pulmonary hypoplasia.

Radiographic Features

Radiographic features include prenatal polyhydramnios, a large calvarium with normal ossification, midface hypoplasia, retrognathia, platyspondyly with underossification of the vertebral bodies (achondrogenesis type II), short chest with a protuberant abdomen, marked shortening of all tubular bones (Fig. 102.6), and small iliac wings.

Etiology

Achondrogenesis type II–hypochondrogenesis is caused by mutations in the gene for type II collagen (*COL2A1*), the predominant protein building block of cartilage (Mortier et al., 2000).

Inheritance

All cases of achondrogenesis type II–hypochondrogenesis are caused by spontaneous dominant-acting mutations in *COL2A1*. The recurrence risk has been reported to be as high as 6% because of parental gonadal mosaicism (Forzano et al., 2007). Prenatal diagnosis is available if the particular gene mutation has been previously identified in an affected individual.

Differential Diagnosis

Differential diagnoses include achondrogenesis type I and OI types II and III.

Management

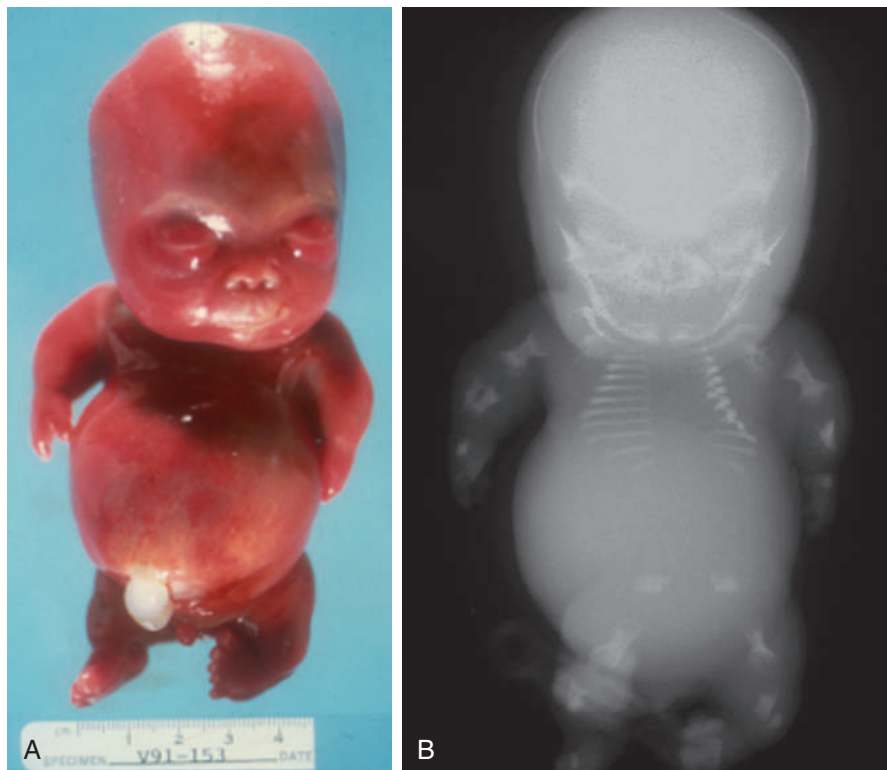
If the condition is diagnosed prenatally, the couple should receive genetic counseling and anticipate neonatal death. If the diagnosis is suggested after delivery and radiographic confirmation is obtained, management is solely supportive, with death from pulmonary insufficiency usually occurring within hours to days.

SLC26A2 Spectrum

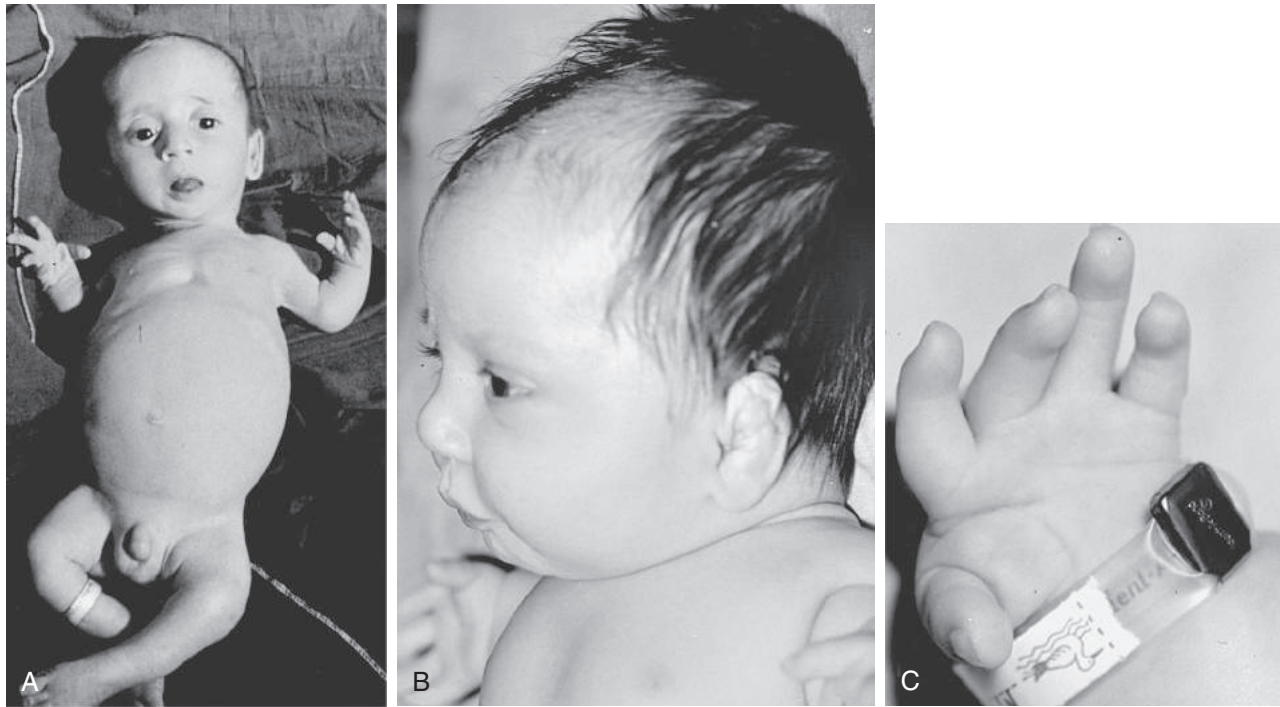
Diastrophic Dysplasia

Presentation

Newborns with diastrophic dysplasia exhibit limb shortening, hitchhiker thumbs, spinal deformities (especially cervical kyphosis), and contractures of the large joints (Fig. 102.7). Clubfoot and ulnar deviation of the fingers may also be present. Cystic ear



• **Fig. 102.6** Achondrogenesis Type II. (A) A 20-week fetus with small chest and short limbs. (B) Radiograph demonstrating poor ossification of vertebral bodies and short limbs.



• **Fig. 102.7** Diastrophic dysplasia. (A) Infant with prominent eyes, small chin, slightly narrow chest, proximally placed angulated thumbs, and short limbs. (B) Neonate profile showing small chin, swollen ears, and short neck. Note the proximally placed angulated thumb. (C) View of the neonate's hand showing the proximally placed angulated thumb and mild syndactyly. (Courtesy Paige Kaplan, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, United States.)

swelling, with subsequent inflammation and calcification, may develop in infancy. On occasion the disease can be lethal at birth, but most individuals survive the neonatal period (Bonafé et al., 2013b).

Radiographic Features

The most characteristic clinical and radiographic feature is the proximally placed hitchhiker thumb, with ulnar deviation of the fingers. Cervical kyphosis is a frequent finding. Long bones are moderately shortened and thick, with mild metaphyseal flaring, rounding of the distal part of the femur, and bowing of the radius and tibia. Severe talipes equinovarus may be present. Iliac wings are hypoplastic, with flat acetabular roofs. The chest can be narrow, bell shaped, or both. Narrowing (lack of flare) of the interpedicular distance in the lumbar spine is reminiscent of achondroplasia.

Etiology

Diastrophic dysplasia is caused by mutations in the *SLC26A2* gene (formerly referred to as the diastrophic dysplasia sulfate transporter gene, *DTDST*), which also cause the lethal disorders achondrogenesis type IB and atelosteogenesis type II, as well as a rare recessive form of multiple epiphyseal dysplasia. The gene product is a sulfate–chloride exchanger of the cell membrane (Superti-Furga et al., 1996); this affects incorporation of sulfate into proteoglycans (mucopolysaccharides), especially chondroitin sulfate B-containing proteoglycans, which are prevalent in cartilage.

Inheritance

Diastrophic dysplasia is inherited in an autosomal recessive pattern. Siblings of affected individuals are at 25% risk of inheriting an abnormal allele from both carrier parents.

Differential Diagnosis

Differential diagnoses include atelosteogenesis type II (part of the *SLC26A2* spectrum), spondyloepiphyseal dysplasia, and arthrogryposis.

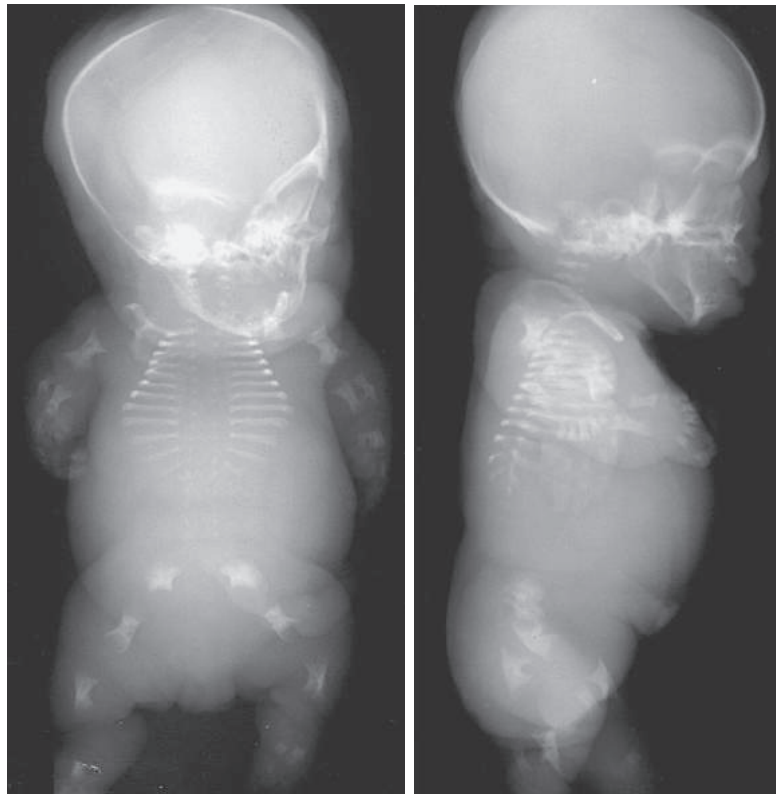
Management

Mechanical ventilation may be required because of small chest circumference and a floppy airway. Maintenance of joint mobility and proper positioning through physical therapy is essential. Serial casting and/or surgical correction of clubfeet may be required. Cervical kyphosis can impede endotracheal intubation and can result in cord compression but may resolve spontaneously during infancy. Preferred treatment of auricular cysts is application of a compression mold rather than incision and drainage (Cushing et al., 2011).

Achondrogenesis Type IB

Presentation

Achondrogenesis type IB is characterized by short stature, extremely short limbs, a relatively large head with a round face, short nose, small mouth, soft skull, and a very short neck (Bonafé et al., 2013a). Polyhydramnios during pregnancy, premature delivery,



• **Fig. 102.8** Achondrogenesis Type IB. Cervical, thoracic, and lumbar vertebral bodies are not ossified, the sacrum is not ossified, the ribs are short, and the limbs are extremely short with medial femoral metaphyseal spikes. (Courtesy Elaine Zackai, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, United States.)

and hydrops are common. The affected infant is stillborn or dies within hours of birth.

Radiographic Features

The long bones are extremely short, with square, globular, or triangular shapes and medial spikes in the metaphyses of the femurs (Fig. 102.8). The calvarium and vertebrae are poorly ossified (type IB), and the ribs are short.

Etiology

Achondrogenesis type IB is caused by mutations in the *SLC26A2* gene (see [Diastrophic Dysplasia](#) earlier).

Inheritance

Achondrogenesis type IB is inherited in an autosomal recessive pattern. Siblings of affected individuals are at 25% risk of inheriting an abnormal allele from both carrier parents.

Differential Diagnosis

Differential diagnoses include atelosteogenesis type II (part of the *SLC26A2* spectrum), achondrogenesis type II, and hypochondrogenesis.

Management

If the condition is diagnosed prenatally, the couple should receive genetic counseling and anticipate neonatal death. If the diagnosis is suggested after delivery and radiographic confirmation is obtained,

management is solely supportive, with death from pulmonary insufficiency usually occurring within hours to days.

Other Skeletal Dysplasias

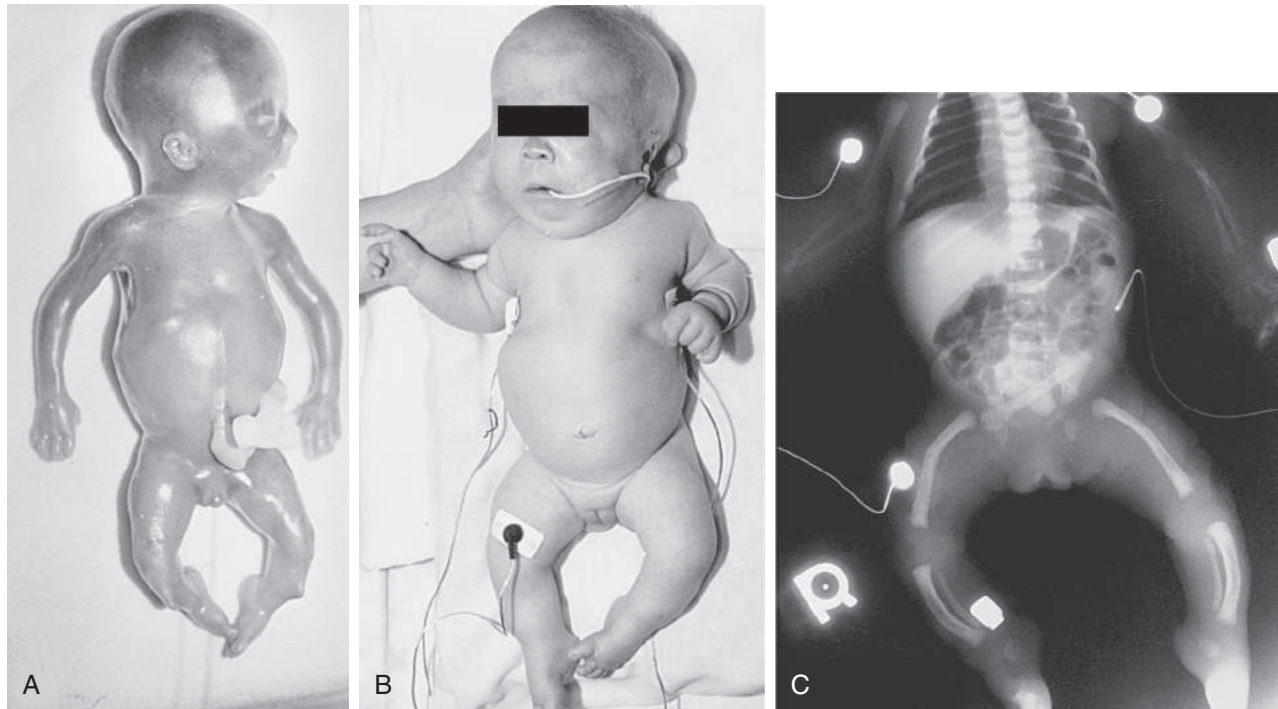
Campomelic Dysplasia

Presentation

Campomelic dysplasia is characterized by short stature (birth length of 35–49 cm), large dolichocephalic skull, large anterior fontanel, high forehead, flat face, widely spaced eyes with short palpebral fissures, low-set ears, cleft soft palate, micrognathia, relatively long and slender thighs and upper arms, short bowed legs with dimples in the midshaft (in most cases), narrow chest, and kyphoscoliosis (Fig. 102.9). Sex reversal or ambiguous genitalia affects 75% of the chromosomal males; there may be internal and external genital abnormalities (from mild anomalies to complete sex reversal) in XY males (Meyer et al., 1997). Absence of the olfactory bulbs and tracts as well as heart and renal malformations may occur. Death, usually in infancy, results from pulmonary hypoplasia, tracheomalacia, or cervical spinal instability. Rare survivors are usually globally developmentally delayed. A few more mildly affected people without bowed limbs have been reported (Unger et al., 2013).

Radiographic Features

The most characteristic finding is midshaft angulation (campomelia) of the femurs, although it is not a constant finding. Other features



• **Fig. 102.9** Campomelic Dysplasia. (A) A 22-week-old 46,XY female fetus with normal head, long philtrum, micrognathia, low-set ears, mild narrowing of the chest, proximally placed thumbs, and bowed or angulated lower limbs resembling those of osteogenesis imperfecta type II but less shortened. The external genitalia are female. (B) Neonate with the long-limb form of the disorder who has a relatively large head, micrognathia, a narrow chest, and bowing of the lower limbs with characteristic dimpling of the lower leg. (C) Radiograph showing the narrow chest, the relatively long, thin limb bones with bowing of the femurs and tibiae, and a long, narrow pelvis. (Courtesy Paige Kaplan, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, United States.)

include hypoplastic, undermineralized cervical vertebrae and thoracic pedicles; narrow iliac wings with dislocated hips; brachydactyly; clubfeet; anterior bowing of tibiae; bell-shaped chest with thin, wavy ribs (with only 11 pairs); and scapular hypoplasia (see Fig. 102.9C).

Etiology

Campomelic dysplasia is caused by mutations in or near the sex-determining region Y-box 9 gene (*SOX9*; Foster et al., 1994). *SOX9* is homologous to the *SRY* gene and encodes a transcription factor involved in both bone formation and testis development.

Inheritance

Campomelic dysplasia is an autosomal dominant trait. Most cases are new sporadic occurrences in a family; recurrence caused by gonadal mosaicism has been reported (Smyk et al., 2007).

Differential Diagnosis

Differential diagnoses include OI types II and III, diastrophic dysplasia, kyphomelic dysplasia, thanatophoric dysplasia, and SEDC (severe).

Management

Survival beyond the newborn period is rare; therefore support is primarily directed toward comfort measures. In survivors, care must be given to the cervical spine, which may be unstable. Chromosomal studies to determine sex and pelvic ultrasonography

to examine internal genitalia may be performed. For survivors, cleft palate may be repaired in those able to feed orally, and clubfeet may require casting or surgical correction.

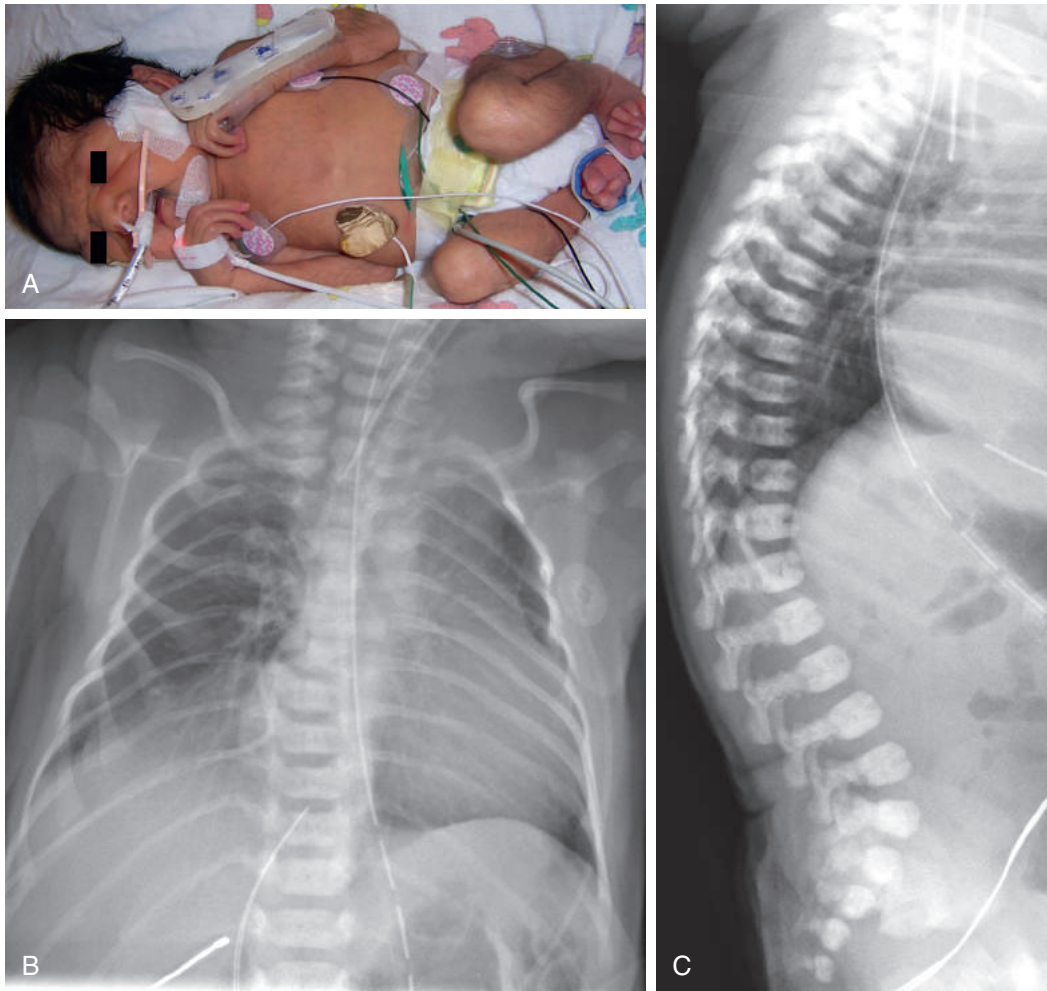
Heritable Connective Tissue Disorders

Genetic disorders of connective tissue represent a large and expanding group of pleiotropic diseases that may involve the musculoskeletal, cardiovascular, and pulmonary systems as well as the eyes and skin. These diseases are caused by mutations in genes that function in extracellular matrix assembly and/or homeostasis and often result in serious clinical consequences (Donkervoort et al., 2015; Vanakker et al., 2015). Several of these disorders present in the newborn period with dramatic joint hypermobility and/or abnormal skin findings (see Table 102.3).

Early-Onset /Rapidly Progressive (Congenital; Neonatal, Infantile) Marfan Syndrome

Presentation

Infants with early-onset/rapidly progressive Marfan syndrome have a long, thin body and can have an aged appearance because of a lack of subcutaneous tissue and wrinkled, sagging skin (Morse et al., 1990; Fig. 102.10). The craniofacial features include dolichocephaly, deep-set eyes with large or small corneas (and occasionally cataracts), high nasal bridge, high palate, small pointed chin



• **Fig. 102.10** Congenital Marfan Syndrome. (A) Neonate with furrowed brow and lax facial skin, thin simple ears, small chin, short neck with redundant skin (not shown), multiple joint flexion contractures, and striking arachnodactyly of fingers and toes. The neonate also had megalocornea, pectus carinatum, mitral valve prolapse, aortic root dilation, and an *FBNI* gene missense mutation. (B) Chest radiograph revealing thin ribs and serpentine clavicles. (C) Lateral spine radiograph demonstrating exaggerated thoracic kyphosis.

with a horizontal skin crease, and large simple or crumpled ears. The fingers and toes are long and thin (arachnodactyly). Some joints are hyperextensible, and others have flexion contractures causing clubfoot, dislocated hips, or adducted thumbs. Infants tend to exhibit hypotonia with low muscle mass. Lenses are usually not subluxated at birth. The most important cause of morbidity and mortality is severe cardiovascular disease, which affects almost every neonate with congenital Marfan syndrome (cMS)—namely, mitral and tricuspid valve prolapse and insufficiency and aortic root dilation. The ascending aorta may be dilated and tortuous. Many infants die of congestive heart failure in the first year of life. Survivors have chronic hypotonia and contractures, are unable to walk, and require many surgical procedures.

Radiographic Features

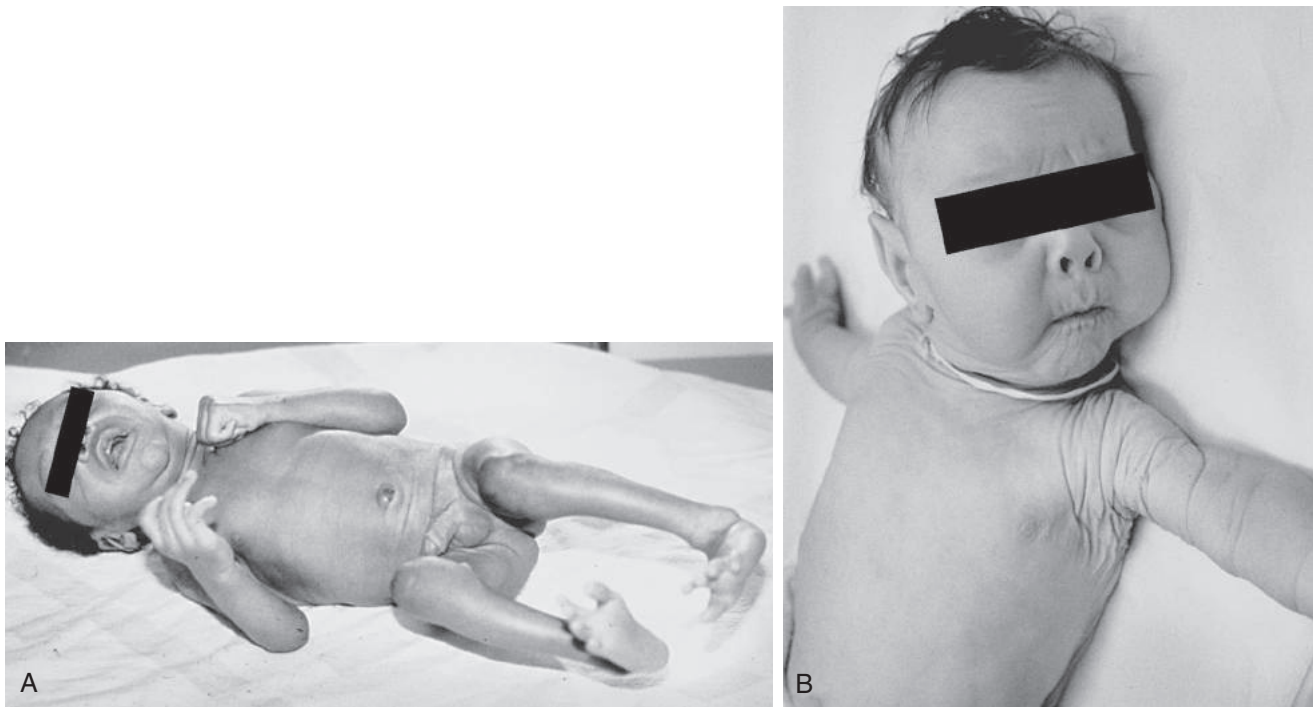
Radiographic features include pectus deformity, spontaneous pneumothorax, dural ectasia, aortic root dilation, and mitral valve prolapse. Many of these features may not be present in the newborn period.

Etiology

Congenital Marfan syndrome is caused by mutations in the gene encoding fibrillin 1 (*FBNI*; Dietz et al., 1991). Fibrillin is a glycoprotein associated with microfibrils, which form linear bundles in the matrices of many tissues, such as aorta, periosteum, perichondrium, cartilage, tendons, muscle, pleura, and meninges. There are two regions in *FBNI* in which many mutations causing cMS occur; these lie among exons 24 to 27 and exons 31 and 32 (Dietz, 2017). Molecular analysis does not yield mutations in all cases.

Inheritance

Marfan syndrome is an autosomal dominant disorder. In most neonates with cMS, occurrence is sporadic within a family (Morse et al., 1990). However, there is one well-documented neonate with cMS whose father had classic Marfan syndrome except for average height (Lopes et al., 1995). There are also reports of familial cMS due to parental gonadal mosaicism (Tekin et al., 2007).



• **Fig. 102.11** (A) Congenital contractural arachnodactyly (Beals syndrome). This infant has a long, thin trunk and limbs, contractures of the joints, and crumpled ears. (B) Infant with cutis laxa. (Courtesy Montreal Children's Hospital, Montreal, Quebec, Canada.)

Differential Diagnosis

Differential diagnoses include CCA, autosomal recessive cutis laxa, and Loeys–Dietz syndrome.

Management

Patients require annual ophthalmologic and cardiac evaluation throughout childhood. Use of cardioselective beta-blockers, such as atenolol, is often implemented at the first signs of aortic root dilation. The angiotensin II antagonist losartan has also shown promise in this regard and may be used in combination with beta-blockers (Brooke et al., 2008; Dietz, 2014). Children should be screened for the development of scoliosis.

Congenital Contractural Arachnodactyly (Beals Syndrome; Distal Arthrogryposis Type 9)

Presentation

CCA (Beals syndrome; distal arthrogryposis type 9) is characterized by a thin, wasted appearance with minimal muscle and fat mass (similar to neonatal Marfan syndrome). Distinctive features include arachnodactyly with contractures of the large and small joints (Fig. 102.11A), as well as crumpled, overfolded helices of the external ear. Cardiovascular involvement is usually limited to mitral valve prolapse, but aortic root dilation may occasionally develop (Godfrey, 2012).

Radiographic Features

Features are nonspecific and include elongated proximal phalanges; contractures of digits, ankles, knees, and hips; thin, gracile tubular bones; and gradual development of kyphoscoliosis.

Etiology

CCA is caused by dominant-acting mutations in the gene encoding fibrillin 2 (*FBN2*).

Inheritance

CCA is inherited in an autosomal dominant manner, with most patients representing the result of spontaneous mutations. Offspring of affected individuals are at 50% risk of inheriting the condition. Gonadal mosaicism has been described in CCA (Putnam et al., 1997).

Differential Diagnosis

Differential diagnoses include cMS, Stickler syndrome, homocystinuria, and distal arthrogryposis.

Management

Proper nutrition is essential to ensure adequate weight gain. Joint contractures respond to physical therapy, but occasionally surgical release may be required. Surveillance for development of spinal curvature and aortic root dilation, although rare, is essential throughout childhood (Godfrey, 2012).

Ehlers–Danlos Syndromes

Presentation

The EDSs are a clinically and genetically heterogeneous group of connective tissue disorders that are characterized by various degrees of joint and skin hypermobility, excessive bruising, abnormal wound healing, and fragility of tissues (Malfait et al., 2011; Donkervoort et al., 2015). This group of disorders was formally reclassified in 1997 (Beighton et al., 1998), but several new rare variant forms

have been reported since then, and an updated reclassification is forthcoming (Donkervoort et al., 2015). The types that are more likely to present in the newborn period include the classic type (formerly type I), the kyphoscoliotic type (formerly type VI), the dermatosparaxis type (formerly type VIIc), and the arthrochalasis type (formerly types VIIa and VIIb). The classic type often presents with premature delivery of an affected fetus as a result of a rupture of the fragile amniotic membranes. The infant may be floppy and in the breech position, and there may be joint laxity and joint instability (Malfait et al., 2011). The kyphoscoliotic type often presents with severe hypotonia and scoliosis with risk of subsequent ocular involvement. In the arthrochalasis type the major involvement is of the ligaments and joint capsules. Large and small joints are hypermobile and dislocatable; severe congenital dislocation of hips occurs. The findings in the dermatosparaxis type include soft, fragile, and redundant skin with some joint hypermobility.

In vascular (formerly type IV) EDS the greatest danger is to the pregnant affected woman, for whom there is a high risk of uterine and arterial rupture. Although there is a 50% risk that the fetus will be affected, the problems of blood loss and prematurity are more important in the newborn period than the disorder itself.

Radiographic Features

Radiographic features are dependent on the particular type of EDS. Congenital hip dislocation may be evident on plain films. Hydronephrosis, bladder diverticula, and spontaneous pneumothorax may occur occasionally. Aortic dilation and arterial aneurysms may be evident by echocardiography and other imaging modalities but occur only in patients of school age or older.

Etiology

Mutations in two of the genes for type V collagen (*COL5A1* and *COL5A2*) are demonstrable in some cases of classic EDS (Malfait and de Paepe, 2005). The vascular type is caused by mutations in the gene for type III collagen (*COL3A1*). The arthrochalasis type is caused by mutations in either gene for type I collagen (*COL1A1* or *COL1A2*), which result in loss of the N-proteinase cleavage site of the protein. The kyphoscoliotic type is caused by mutations in the gene for a procollagen cross-linking enzyme (*PLOD1*). The dermatosparaxis type is caused by mutations in a gene for a procollagen proteinase (*ADAMTS2*).

Inheritance

Most types of EDSs are inherited as autosomal dominant traits. Each child of an affected person has a 50% chance of inheriting and manifesting the disorder, although there can be marked intrafamilial variability (Malfait and de Paepe, 2005). The kyphoscoliotic and dermatosparaxis types of EDSs are inherited in an autosomal recessive manner.

Differential Diagnosis

Differential diagnoses include cMS, CCA (distal arthrogryposis type 9), Larsen syndrome, and other rare variant forms of EDS (Donkervoort et al., 2015; Vanakker et al., 2015).

Management

Trauma should be avoided because of skin fragility. Effective closure of surgical wounds is challenging because of a tendency for dehiscence.

Cutis Laxa

Presentation

Cutis laxa is a group of genetically heterogeneous disorders that primarily affect the assembly and homeostasis of elastic fibers. As such, the presentation can be highly varied. Infantile forms may exhibit loose, furrowed skin, a large anterior fontanelle, hypotonia, hernias, and congenital hip dislocation (see Fig. 102.11B; Vanakker et al., 2015; Van Maldergem et al., 2015).

Radiographic Features

Radiographic features are in part dependent on the genetic form of the disorder. Nonspecific features include a large anterior fontanel, congenital hip dislocation, and hernias. The X-linked form may exhibit occipital horns of the skull. Arterial tortuosity, aortic root dilation, and cortical and cerebellar anomalies may be seen in some forms, as well as gastrointestinal tract and urinary tract diverticula.

Etiology

The relatively mild, autosomal dominant form of cutis laxa is caused by mutations in the elastin gene (*ELN*). The X-linked recessive form (occipital horn syndrome) is caused by mutations in the *ATP7A* gene (allelic with Menkes syndrome). Autosomal recessive forms may be caused by mutations in the fibulin 4 gene (*FBLN4*), the fibulin 5 gene (*FBLN5*), or the gene that encodes the A2 subunit of vacuolar H⁺-ATPase (*ATP6V0A2*). Biochemical clues as to the cause in a particular patient may include decreased serum copper and ceruloplasmin levels (X-linked form), and abnormal serum sialotransferrin isoelectric focusing results in cases caused by *ATP6V0A2* mutations.

Inheritance

Because cutis laxa is genetically heterogeneous, the modes of inheritance include autosomal dominant, autosomal recessive, and X-linked recessive. The latter two modes are usually responsible for forms with neonatal and infantile presentation.

Differential Diagnosis

Differential diagnoses include EDS, Menkes syndrome, geroderma osteodysplastica, and de Barsy syndrome.

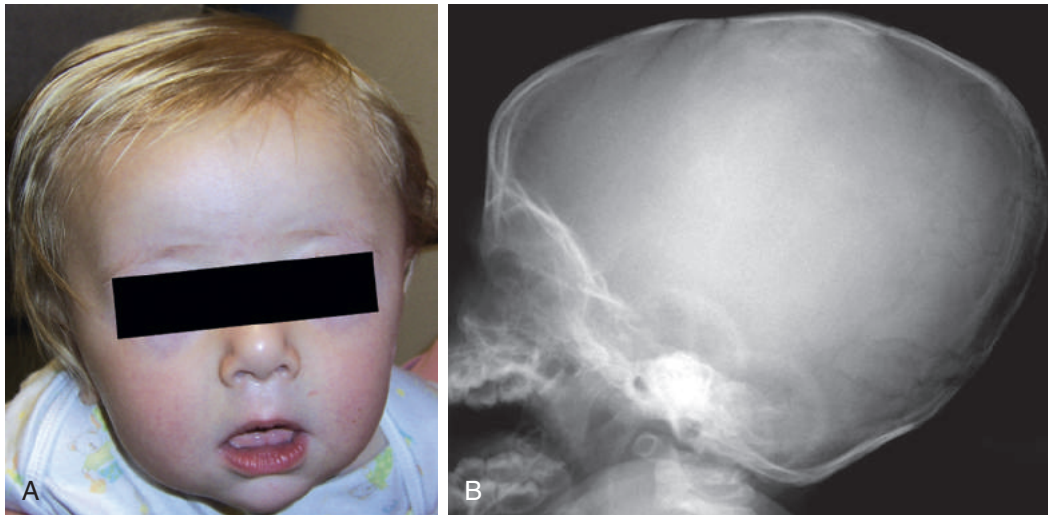
Management

Serious childhood complications include developmental delay, pulmonary emphysema, aortic root dilation, and arterial tortuosity. Annual ophthalmologic and cardiac examinations are essential, and referral to special education programs may be indicated.

Menkes Syndrome

Presentation

Menkes syndrome often appears in the newborn period with nonspecific neurologic manifestations. Typically, developmental delay is evident in the first 2 to 3 months of life, with failure to thrive, seizures, and severe ocular manifestations. Changes in the appearance of the hair include hypopigmentation, brittleness, patchy alopecia, and twisted shafts seen by light microscopy (i.e., pili torti; Fig. 102.12A). Early death is common and may occur in infancy (Kaler, 2010). Serum copper and ceruloplasmin concentrations are low, and the plasma dopamine-to-norepinephrine ratio may be elevated (Goldstein et al., 2009).



• **Fig. 102.12** Menkes Syndrome. (A) Note the blonde hair, fair complexion, and epicanthal folds in this 11-month-old Hispanic boy. (B) Note multiple wormian bones near the occiput.

Radiographic Features

Features may evolve during infancy and may include bladder diverticula (seen on bladder ultrasonography and voiding cystourethrogram), tortuous vessels (on echocardiogram, magnetic resonance imaging with contrast agent), gastric polyps (on upper gastrointestinal [GI] contrast study), metaphyseal spurring, osteopenia, and wormian bones on plain radiographs (Lachman, 2006; see Fig. 102.12B).

Etiology

Menkes syndrome is caused by mutations in the gene encoding a copper-transporting adenosine triphosphatase (*ATP7A*; Kaler et al., 1994). This enzyme participates in the final processing of several copper-dependent enzymes, including dopamine β -hydroxylase, tyrosinase, lysyl oxidase, superoxide dismutase, and cytochrome *c* oxidase. As a result, several physiologic processes and cellular functions are impaired, including collagen cross-linking, pigment production, and neurotransmission (Goldstein et al., 2009).

Inheritance

Menkes syndrome is an X-linked recessive disorder; therefore only males are affected. Female carriers may exhibit pili torti in some hair shafts because of lyonization (Moore and Howell, 1985). Sons born to carrier females have a 50% risk of manifesting the disease.

Differential Diagnosis

Differential diagnoses include cutis laxa (the occipital horn form is allelic), EDS, neonatal cMS, biotinidase deficiency, mitochondrial myopathies, nutritional copper deficiency, and some organic acidurias.

Management

Early diagnosis allows copper supplementation therapy (Kaler, 2013), but this is not effective in all patients. Patients should be monitored for the development of seizures, as well as a propensity for bone fragility, poor wound healing, and vascular fragility leading to excessive bleeding, hemorrhagic strokes, and subdural hematomas. Bladder diverticula may result in urinary retention

and urinary tract infections and should be surgically corrected. Patients are at risk of moderate to severe developmental delay, and they should be referred to infant stimulation and early intervention programs.

Family Support and Education

Many of the conditions described in this chapter are rare, and parents (and sometimes medical staff) are unfamiliar with or bewildered by such conditions. Providing appropriate educational materials is of utmost importance for making important medical decisions and preparing for the future. Support groups such as the Little People of America (<http://www.lpaonline.org>) and the Osteogenesis Imperfecta Foundation (<http://www.oif.org>) are helpful in this regard, as the medical content is reviewed and updated by experts in the field. In addition, individual entries in GeneReviews (<https://www.ncbi.nlm.nih.gov/books/NBK1116/>) frequently include links to support groups as well as to educational pieces written in lay language, such as the NIH Genetics Home Reference (<https://ghr.nlm.nih.gov/condition>).

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103

Newborn Skin Development:
Structure and Function

ROBERT SIDBURY

KEY POINTS

- Healthy full-term infant skin is well developed and serves as an effective barrier. Premature infants, especially those of very low birth weight, have an ineffective barrier that increases risk of invasive infection, dehydration, cutaneous injury, and toxic absorption.
- Birthmarks may have neurologic implications that require magnetic resonance imaging. In some cases newborns and very young infants can be imaged using a “feed and wrap” technique without sedation. Awareness of this window of opportunity may help avoid unnecessary general anesthesia.
- Early application of emollients in certain infants can improve barrier function, decrease risk of infection, and potentially prevent atopic outcomes.
- Blaschko's lines are patterns formed by migration of skin cells during embryogenesis. They are distinct from dermatomes. Cutaneous findings in this distribution are a clue to an underlying mutational event.

The skin serves several critical roles including primary barrier against infection, thermoregulation, and electrolyte homeostasis. Disruption as a result of genetic mutation, injury, or prematurity can be life-threatening. This chapter details the stages of human skin development, the potential challenges of prematurity, and an approach to important clinical presentations.

Cutaneous morphogenesis is complex and incompletely understood. Advances have come from traditional murine model work but also in “reverse” by identifying mutations causing disorders that manifest with developmental defects (e.g., aplasia cutis congenita) (Shimomura, 2016). Human skin develops from apposed tissue of both mesodermal and ectodermal origin. From mesoderm arise fibroblasts, vascular cells, adipocytes, and immune-presenting Langerhans cells, which ultimately reside in the epidermis. Ectoderm-derived tissue includes keratinocytes and neural crest-derived melanocytes. The assembly of the epidermis, dermoepidermal junction (DEJ), dermis, and fat, along with epidermal appendages and immigrant cells, is a complex dynamic with potential for clinically significant disruption at every step. Table 103.1 enumerates

these critical stages. The clinician can use this ontogeny to guide assessment: any newborn with evident cutaneous pathology should prompt additional scrutiny of hair, nails, teeth (natal teeth can suggest specific disorders), and the central nervous system.

Epidermis

The epidermis derives entirely from ectoderm. A single-layered epithelium spans the embryo from gastrulation. Subsequent development proceeds in discrete stages: embryonic (5–8 weeks), embryonic/fetal transition (9–10 weeks), then to early (11–14 weeks), mid (15–20 weeks), and late (20 weeks–birth) fetal period (Holbrook and Odland, 1975). Differentiation and formation of appendages occurs predominantly during the second trimester. It is during the late fetal stage that the skin first becomes functional. The stratum corneum, or outer epidermis, has been likened to “bricks and mortar” (Elias, 1983). Epidermal keratinization begins first on the head, face, and palms. Anucleate corneocytes flatten and form “bricks,” while a mixture of cells, adhesion proteins, and lipids form the semipermeable mortar. One such protein, filament aggregating protein, or filaggrin, has recently been linked to atopy. Patients with filaggrin mutations are at greater risk for eczema, asthma, and allergies (Palmer et al., 2006). Decreased ability to retain moisture with resulting fissures in the stratum corneum may promote epicutaneous allergen exposure and sensitization. Emerging evidence suggests that early aggressive application of thick emollients to at-risk infants decreases their chance of developing atopic dermatitis (Simpson et al., 2014).

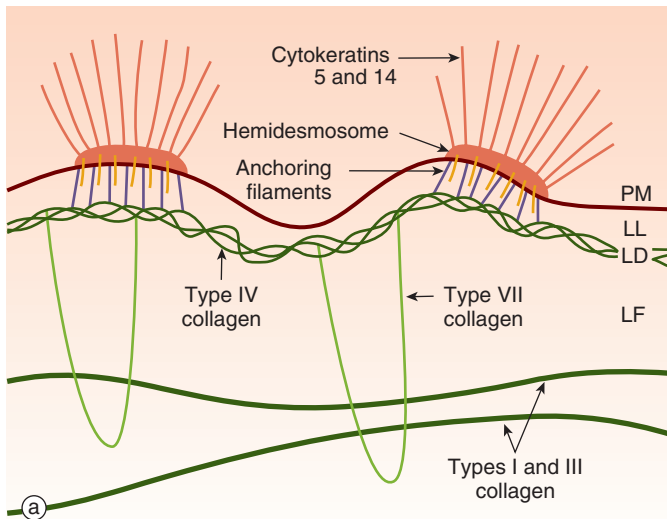
Dermoepidermal Junction

The DEJ is the interface between basal layer keratinocytes of the epidermis and the dermis and helps the skin resist shearing forces. The critical elements of the DEJ form by 8–10 weeks of gestation. The DEJ is comprised of hemidesmosomes, anchoring filaments, anchoring fibrils, and type VII collagen, which combine to tether the dermis to the epidermis (Fig. 103.1). Epidermolysis bullosa (EB) is an inherited disorder of cutaneous fragility that results from defective proteins in this complex. The type of EB depends on the affected protein, and outcomes can vary widely.

TABLE 103.1 Milestones in Fetal Skin Embryogenesis

| Milestone | Gestational Week |
|--|------------------|
| Expression of epidermal keratins | 6 |
| Development of specialized cells including melanocytes, Langerhans | 8 |
| Formation of dermoepidermal junction | 8–10 |
| Beginning of nail development | 10 |
| Beginning of hair development | 12 |
| Palmoplantar sweat gland formation | 10–12 |
| Formation of fat in subcutis | 15 |
| Sweat gland formation in rest of body | 24–26 |

Adapted from: Holbrook KA, Odland GF. The fine structure of developing human epidermis: light, scanning, and transmission electron microscopy of the periderm. *J Invest Dermatol.* 1975;65:16–38



• **Fig. 103.1** Dermoepidermal Junction. (Courtesy of Basicmedical [key.com](http://www.basicmedical.com).) a, Collagen; LD, lamina densa; LF, lamina fibroreticularis; LL, lamina lucida; PM, plasma membrane.

Dermis and Subcutis

The dermis is derived from diverse tissue types. Embryonic mesenchymal cells capable of becoming multiple cell types are enmeshed in a hyaluronic matrix called a *cellular dermis*. At 8 weeks' estimated gestational age (EGA) dermis is distinguishable from underlying tissue. By 15 weeks collagen fibers accumulate, and by 22–24 weeks elastin fibers are first detectable by electron microscopy (Holbrook and Odland, 1975). Immature dermis will not scar when traumatized (such as with a fetal skin biopsy), whereas the increasing tensile strength that accompanies maturation also connotes potential to scar; this occurs at roughly the end of the second trimester. Blood vessel plexi are discernible by 12 weeks' EGA but do not fully mature until after birth. Vasculogenesis, the formation of endothelial cells and then vessels from angioblasts, has been completed by 20 weeks' gestation; angiogenesis follows as existing vessels give rise to new ones. Sprouting angiogenesis may add new

vessels to tissue previously lacking vascularity; this involves spouts of endothelial cells growing toward an angiogenic stimulus such as vascular endothelial growth factor or basic fibroblast growth factor. Intussusceptive angiogenesis is characterized by a splitting process where the vessel wall invades the lumen, causing the vessel to divide in two. This type of angiogenesis occurs only in areas of preexisting vascularity. Both types of angiogenesis can occur in virtually all tissues and organs (Adair and Montani, 2010). Neural networks develop toward the end of the first trimester and follow a vascular pattern. Adipose tissue develops beneath the dermis, starting in the second trimester (Visscher et al., 2015).

Goltz syndrome (focal dermal hypoplasia) is an X-linked dominant disorder caused by mutations in *PORCN*. Affected individuals are 90% female and manifest with striking linear atrophic plaques with distinctive fat herniation due to dermal atrophy.

Appendages

Hair, nail, sweat gland, and apocrine gland development relies on coordinated dermal–epidermal interaction that commences around 10 weeks' gestation. Hair follicle formation is initiated in the dermis, which directs overlying epidermal basal cells to aggregate in the form of a placode. Hair follicles differentiate during the second trimester, and hair canals are formed by 20 weeks when scalp hairs are just visible. Hairs cycle between growth (anagen), resting (catagen), and shedding (telogen). The first shedding cycle occurs around 28 weeks' gestation, after which they reenter anagen phase. The final shedding phase generally occurs postnatally, at the occiput, leading to occipital alopecia often misattributed to pressure or friction.

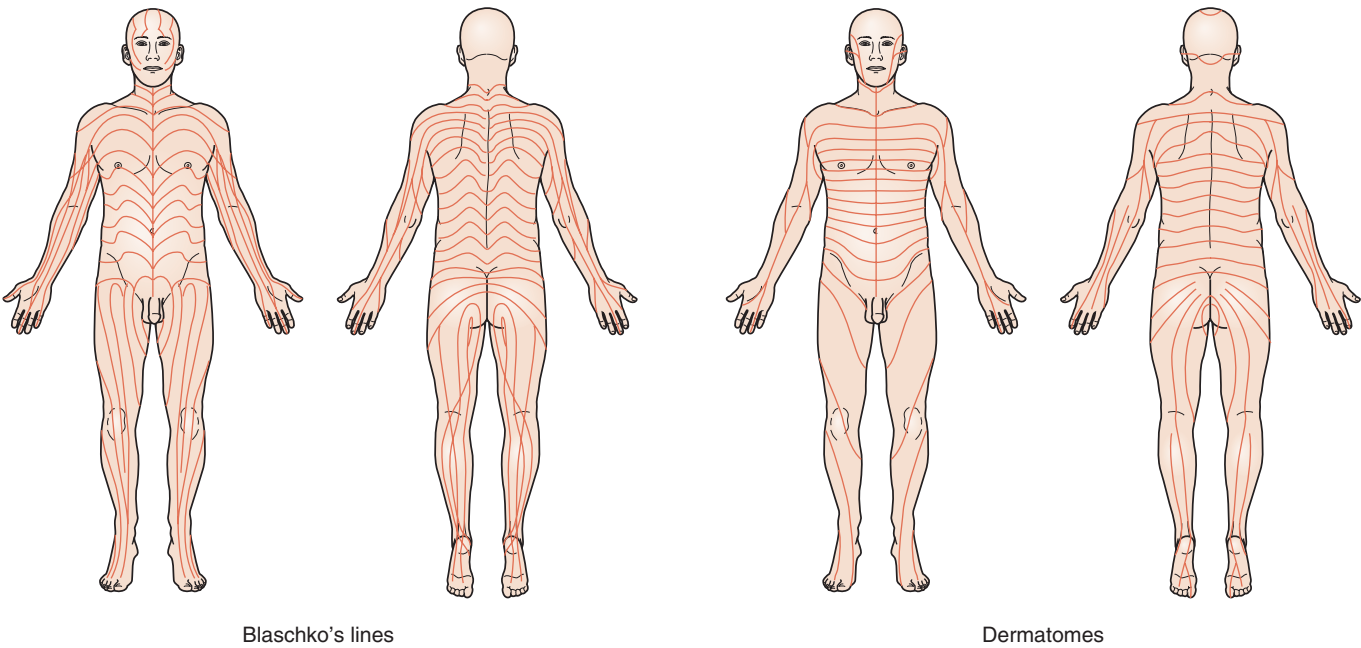
Nails begin development at 8 weeks' EGA, slightly earlier than the hair follicles. A keratinized nail has completely covered the nail plate by 5 months' EGA. Hair and nail keratins have greater structural integrity than those in the epidermis.

Sebaceous glands develop contemporaneously with hair follicles. At 13–16 weeks' EGA characteristic sebaceous gland bulges can be seen with hair follicles, just above the insertion of the arrector pili muscle. Maternal hormones induce sebaceous gland production of sebum during the late second and third trimester. Sebum and shed corneocytes form vernix, which progressively coats the fetus in a cephalocaudal orientation. Fetal lung maturity parallels sebaceous gland activity, and increased physiologic concentrations of surfactant emulsifies surface vernix.

Palmoplantar eccrine sweat glands begin to develop at 55–65 days' gestation and are fully developed by the second trimester. Interfollicular sweat glands and apocrine glands do not form until midsecond trimester. Ectodermal dysplasias are a heterogeneous group of disorders with striking abnormalities in skin appendages. Hydrotic ectodermal dysplasia, or Clouston syndrome, is due to mutations in *GJB6* (connexin 30), which directs formation of gap junctions found notably in adnexal structures.

Specialized Skin Cells

Melanocytes, Langerhans cells, and Merkel cells comprise the primary epidermal immigrant cells. Melanocytes derive from the neural crest and migrate along Blaschko's lines to populate the entire fetal epidermis. Melanocytes are first identifiable at 50 days' EGA and are fully present at birth, but melanogenesis continues such that skin is not fully pigmented at birth and will darken in the first months. Blaschko's lines are distinct from dermatomes, and clinical abnormalities in this distribution can be helpful diagnostically (Fig.



• Fig. 103.2 Blaschko's Lines or Developmental Lines of Epidermal Migration.

103.2). Incontinentia pigmenti, an X-linked dominantly inherited gene dermatosis due to mutations in *NEMO*, presents with a series of cutaneous findings over time, first vesicles and then a warty hyperkeratotic phase, followed by dyspigmentation all along Blaschko's lines. Knowledge of this pattern can help discriminate from fundamentally different processes such as herpes zoster, which would present with vesicles in a dermatomal distribution.

Langerhans cells first appear at 40 days' gestation and serve as antigen-presenting cells. Merkel cells appear at 8 weeks' gestation and serve a mechanoreceptor function.

Epidermal Stem Cells

The skin must constantly repair and renew itself. There are several different types of stem cells within the skin including basal keratinocytes, hair follicles, and melanocytes. Basal keratinocytes can be grown in sheets and used for therapeutic barrier purposes in burn and chronic wound patients (e.g., in EB). Such grafts are useful but do not contain appendages such as hair follicles or sweat glands. Improved understanding of the interaction among various cutaneous stem cells, their microenvironments, and their pluripotent is driving advances in stem cell technology (Hsu and Fuchs, 2014).

Impact of Prematurity

The stratum corneum reaches functional maturity around 33 weeks' EGA. The combination of a developmentally and functionally incompetent barrier, absence or diminished vernix caseosa, large body surface area to mass ratio, and an immature immune system places premature infants, particularly those born at less than 33 weeks' gestation, at significant risk for invasive infection and skin injury. A unique consideration in premature infants is the risk of percutaneous toxicity. Topical medications, cleansers, and even emollients (e.g., lactic acid) can lead to dangerous systemic levels

| TABLE 103.2 The Impact of Prematurity on Skin Physiology | | |
|--|--|--------------------------|
| | Premature | Term |
| Skin thickness | 0.9 mm | 1.2 mm |
| Epidermal thickness | 20 μ | 40 μ |
| Epidermal covering | No vernix before 28 weeks | Vernix |
| TEWL | High (at 26 weeks TEWL = 45 g/m per h) | Low (TEWL = 5 g/m per h) |
| Acid mantle | Incomplete | Complete |
| Percutaneous Absorption | Very high | Normal |

TEWL, Transepidermal water loss.

in premature infants. A particularly seductive risk is that of cutaneous anesthesia when used for small invasive procedures. Minimizing pain is important, but careful attention to safe limits of topical anesthetic is vital (Tran and Koo, 2014). Table 103.2 compares premature and term infant skin. Table 103.3 summarizes important potential cutaneous absorption risks.

Care of Newborn Skin

Emollients can serve several purposes beyond epidermal hydration. Application of cream-based emollients in infants older than 29 weeks' EGA has been shown to enhance skin barrier development (Keichl-Kohlendorfer et al., 2008). In infants at high risk for atopic dermatitis, early application of an emollient decreased the incidence

**TABLE
103.3****Reported Hazards of Percutaneous Absorption in the Newborn**

| Compound | Reference | Product | Toxicity |
|------------------------------------|---|--|--|
| Aniline | Rutter, 1987 | Dye used as a laundry marker | Methemoglobinemia, ^a death |
| Mercury | Dinehart et al., 1988 | Diaper rinses, teething powders | Rash, hypotonia |
| Phenolic Compounds | | | |
| Pentachlorophenol | West et al., 1981 | Laundry disinfectant | Tachycardia, sweating, hepatomegaly, metabolic acidosis, death |
| Hexachlorophene | — | Topical antiseptic (pHisoHex) | Vacuolar encephalopathy, death |
| Resorcinol | West et al., 1981 | Topical antiseptic | Methemoglobinemia ^a |
| Boric acid | Goldbloom and Goldbloom, 1953 | Baby powder | Vomiting, diarrhea, erythroderma, seizures, death |
| Lindane | Rutter, 1987; West et al., 1981 | Scabicide | Neurotoxicity |
| Salicylic acid | Abidel-Magid and El Awad Ahmed, 1994; West et al., 1981 | Keratolytic emollient | Metabolic acidosis, salicylism |
| Isopropyl alcohol (underocclusion) | Rutter, 1987 | Topical antiseptic | Cutaneous hemorrhagic necrosis |
| Silver sulfadiazine | Payne et al., 1992 | Topical antibiotic (Silvadene) | Kernicterus (sulfa component), argyria (silver component) |
| Povidone–iodine | Rutter, 1987; West et al., 1981 | Topical antiseptic (Betadine) | Hypothyroidism, goiter |
| Neomycin | Rutter, 1987 | Topical antibiotic | Neural deafness |
| Corticosteroids | Rutter, 1987; West et al., 1981 | Topical antiinflammatory (Lotrisone) | Skin atrophy, adrenal suppression |
| Benzocaine | Gelman et al., 1996 | Mucosal anesthetic (teething products) | Methemoglobinemia ^a |
| Prilocaine | Frayling et al., 1990; Reynolds, 1996 | Epidermal anesthetic (EMLA) | Methemoglobinemia ^a |

^aHeritable glucose-6-phosphate deficiencies are associated with an increased susceptibility to methemoglobinemia, as is coadministration of several drugs such as sulfonamides, acetaminophen, nitroprusside, phenobarbital, and phenytoin.
EMLA, Eutectic mixture lidocaine anesthetic.

of that disorder at 6 months of age by 50% (Simpson et al., 2014). Sunflower seed oil application reduced the incidence of nosocomial infections by 41% when applied to infants less than 33 weeks' EGA in a study from Bangladesh (Darmstadt et al., 2005). Oils such as safflower, sesame, coconut, and apricot contain fatty acids that may have antiinflammatory and antibacterial properties. Olive oil can promote pityrosporum growth so it may not be the optimal choice (Siegfried and Glenn, 2012). Box 103.1 details general principles of neonatal skin care.

Bathing newborns has myriad potential hygienic, social, and cultural benefits but can potentially be detrimental to newborn skin. The timing of vernix removal varies considerably. Vernix is a complex mixture of water, protein, and lipids. Vernix is hydrophobic and hydrating and contains lysozyme and lactoferrin, which are antimicrobial. Delayed bathing of newborns beyond 6 hours after birth is recommended by the World Health Organization in high-risk settings. The pH of the skin surface of term and preterm infants is more alkaline (6.5–7.5) but declines in the 1st week to values comparable to adult skin (4.0–5.5). The establishment of this “acid mantle” is important for many reasons and can be interrupted or delayed by use of alkaline or irritating soaps. Gentle cleansers, neutral in pH, and lacking fragrances and excessive preservatives or abrasives, can be used safely. Caregivers should practice good hand hygiene, preferably with chlorhexidine, for bacterial decontamination, though 10% povidone–iodine is

advantageous for *Candida* species. Chlorhexidine may provide the most effective agent for umbilical stump care to prevent omphalitis and sepsis. Chlorhexidine 0.5% is superior to povidone–iodine in reducing peripheral catheter colonization in neonates (Visscher et al., 2015).

Morphologic Approach to Skin Pathology

Any skin examination should begin by attempting to identify a primary morphology. This helps to limit differential diagnosis and avoid the diagnostic confusion that can arise from secondary findings such as erosions and ulcerations (Table 103.4). Newborns present most commonly with benign, transient skin findings. These and other common neonatal presentations are detailed in subsequent chapters. There are several distinctive presentations that are important for pediatricians and neonatologists to recognize.

Collodion Membrane

Collodion membrane (CM) is a congenital cutaneous phenotype that has both immediate and longer term implications (Prado et al., 2012). Clinically, a “collodion baby” presents with a tight “plastic wrap–”like encasement on the skin (Fig. 103.3). This taut skin can lead to eversion of the lips (eclabium) and eyelids (ectropion) as well as dysregulation of cutaneous homeostasis, mandating

• BOX 103.1 Proposed Guidelines for Basic Skin Care in the Newborn

Use adhesives sparingly.

- Place protective dressing (e.g., DuoDerm or Tegaderm) at sites of frequent taping (endotracheal tube and nasogastric tube placement).
- Use nonadhesive electrodes, and change them only when they become nonfunctional.

Limit bathing

- Defer initial cleansing until body temperature has stabilized.
- Avoid cleansing agents for the first 2 weeks.
- Use warm water and moistened cotton pledgets in a humid environment.
- Surface cleansing is required no more than twice per week.
- If antimicrobial skin preparation is required, use short-contact chlorhexidine (except on the face).

Be aware of the composition and quantity of all topically applied agents.

- These agents include antimicrobial cleansers, diaper wipes, adhesive removers, and perineal products.
- Dispense from single-use containers, if possible.

Ensure adequate intake of protein, essential fatty acids, zinc, biotin, and vitamins A, D, and B.

- Erosive periorificial dermatitis is a sign of nutritional deficiency.

Apply a simple cream or ointment emollient every 8 hours.

Guard against excessive thermal and ultraviolet exposure.

- Use thermally controlled water for bathing.
- Avoid surface monitors with metal contacts.
- Use Plexiglas shielding over daylight fluorescent phototherapy.

Protect sites of cutaneous injury with the appropriate occlusive dressing.

- Use a film dressing on nonexudative sites.
- Use a hydrogel dressing on exudative wounds.
- Maintain appropriate hydration at the skin–dressing interface.
- Remove necrotic debris with each dressing change.



• **Fig. 103.3** A Newborn With Taut Skin Representing a Collodion Membrane.

exfoliation of the newborn). Diagnosis may be facilitated by skin biopsy and/or genetic testing, but evolution of the skin examination over time may also point toward the ultimate diagnosis noninvasively.

Vesicopustular and Bullous Eruptions

An infant born with widespread vesicles, pustules, bulla, or erosions presents a daunting differential diagnosis (Box 103.2) including infectious, inflammatory, metabolic, and genetic disorders (Avram et al., 2007). Treatable infectious etiologies such as herpes simplex virus (HSV), varicella, *Staphylococcus aureus*, and candida infections should be first ruled out by careful history and cultures. Subsequent consideration may be given to other dermatoses including mastocytosis and histiocytosis. Transient benign dermatoses that present with a vesicopustular morphology such as erythema toxicum (Fig. 103.4) or transient neonatal pustular melanosis (TNPM) should be considered and may be identified by simple laboratory studies (e.g., eosinophils on smear of erythema toxicum vs neutrophils on smear from TNPM). Widespread blistering and erosion, particularly at friction-prone areas such as the hands and feet, raise the uniquely important specter of EB (Fig. 103.5). EB is an inherited disorder of cutaneous fragility, and early awareness can avoid unnecessary iatrogenic exacerbation because of inappropriate handling, adhesives, leads, or monitors (Gonzalez, 2013). It is impossible to determine EB subtype from clinical examination alone. Relatively mild blistering at birth can occur in infants with severe subtypes so providers should be cautioned against trying to predict eventual phenotype based upon initial presentation.

Blueberry Muffin Baby

A so-called blueberry muffin baby presents with distinctive red-purple macules, papules, and plaques that have historically prompted consideration of congenital infections recalled by the TORCH acronym: *Toxoplasmosis*, *Other*, *Rubella*, *Cytomegalovirus* and *HSV*. These entities should be ruled out by history, evaluation, appropriate cultures, and supportive findings such as dysmorphism. Several noninfectious conditions should be added to the clinical differential. Histiocytosis, neuroblastoma, leukemia cutis, transient myeloproliferative disorder, mastocytosis, and multifocal vascular

TABLE 103.4 Morphologic Approach to Diagnosis

| Morphology | Description | Example |
|---------------|---|--|
| Macule/patch | Flat, no epidermal change | Capillary malformation |
| Papule/plaque | Raised, discrete edge, epidermal change | Superficial hemangioma |
| Nodule/tumor | Raised, sloping border, no epidermal change | Dermoid cyst |
| Vesicle/bulla | Clear, fluid filled | Epidermolysis bullosa |
| Pustule | Turbid, fluid filled | Candidiasis |
| Wheal | Edematous, red plaque | Neonatal onset multiinflammatory disease |

careful monitoring of fluids, electrolytes, and thermoregulation. Affected infants should be kept in the neonatal intensive care unit in a controlled environment as the membrane spontaneously sheds over the first weeks after birth. The majority of CM infants will ultimately have some form of chronic skin disorder, most commonly autosomal recessive congenital ichthyosis; however, other disorders including Gaucher disease and ectodermal dysplasia can present as CM. Rarely, CM infants may go on to have normal skin (lamellar

• BOX 103.2 Differential Diagnosis of Cutaneous Vesicles, Bullae, and Erosions in the Neonate

Infectious Vesiculopustular Dermatoses

Herpes simplex virus infection
 Varicella infection
 Cytomegalovirus
Candida infection
 Scabies
Aspergillus infection
 Bacterial infection
 Group B streptococcus
 Group A streptococcus
 Haemophilus influenzae type B
 Staphylococcus aureus
 Listeria
 Treponema pallidum
 Pseudomonas

Noninfectious Transient Conditions With Vesicles and Erosions

Erythema toxicum neonatorum
 Transient neonatal pustular melanosis
 Miliaria
 Neonatal acne
 Eosinophilic pustular folliculitis
 Acropustulosis of infancy
 Sucking blister
 Trauma

Nontransient Bullous Dermatoses

Epidermolysis bullosa
 Incontinentia pigmenti
 Epidermolytic hyperkeratosis
 Hyper-IgE syndrome
 Herpes gestationis
 Pemphigus vulgaris
 Langerhans cell histiocytosis
 Mastocytosis

(From: Avram MM, Gobel V, Sepehr A. Case records of the Massachusetts General Hospital. Case 30-2007: A newborn girl with skin lesions. N Engl J Med. 2007;357:1327–1325.)

lesions can all mimic blueberry muffin lesions (Wolfe et al., 2003). A skin biopsy may be necessary to discriminate and appropriately triage such infants.

Erythroderma

Erythroderma is defined as diffuse erythema with or without associated findings like scaling. Newborns have extremely reactive skin so it may take time to discern normal physiologic change, including cutis marmorata, from actual pathology. Diffuse erythema can herald many underlying conditions, and appropriate assessment must be comprehensive, not unlike the approach to a newborn with vesicles and pustules. While infectious causes of erythroderma are less common, all treatable triggers should be eliminated. More commonly, erythroderma in a newborn may herald genetic or inflammatory conditions such as ichthyosis, psoriasis, or seborrheic dermatitis. Metabolic disorders, primary immunodeficiency, and autoinflammatory syndromes must also be considered. Timely



• Fig. 103.4 Small Eosinophil-Rich Pustules on an Inflamed Base Consistent With Erythema Toxicum.



• Fig. 103.5 Blistering on the Foot of a Newborn With Epidermolysis Bullosa.

assessment is essential even when infectious etiologies have been ruled out, as timely intervention can mitigate morbidity in some disorders (such as Menkes disease) (Galve et al., 2012).

Birthmarks With Neurologic Implications

Large congenital melanocytic nevi may be associated with neurocutaneous melanosis (NCM) (Fig. 103.6). The risk of NCM is increased if the nevus is located on the scalp, or anywhere in the midline, or if infants have multiple (>20) satellite nevi. Magnetic resonance imaging (MRI) with and without contrast is necessary to rule out NCM. Early infancy may offer a unique opportunity to obtain unsedated (feed and wrap) MRI, potentially avoiding general anesthesia later (Antonov et al., 2016).



• **Fig. 103.6** Giant Melanocytic Nevus on the Back.

Cutaneous stigmata, including pigmented or vascular lesions, excessive hair, or abnormal dimpling/clefting in the midline sacrum, can all herald underlying occult spinal dysraphism (OSD). Two or more such lesions, dimples more than 5 mm at the base, or farther than 2.5 cm from the anus all increase OSD risk (Kriss and Desai, 1998). Ultrasound, before complete vertebral ossification, can permit assessment of the brain and spine; however, the false negative rate is high.

Facial vascular anomalies such as capillary malformations may herald underlying ipsilateral angiomatosis and glaucoma (Sturge–Weber syndrome). An MRI with contrast may show leptomeningeal malformation that can predispose toward seizures and developmental delay. Computed tomography may show “tram-track calcifications” in the temporal and occipital cortex though these may not be present initially.

Segmental infantile hemangiomas, also typically on the face, can be associated with posterior fossa abnormalities such as Dandy–Walker malformation and cerebral artery dysmorphology, which predispose toward stroke, aortic coarctation, and ocular abnormalities (PHACE syndrome) (Garzon et al., 2016) (Fig. 103.7). An MRI with angiography looking for distinctively



• **Fig. 103.7** Large Segmental Infantile Hemangioma in the Setting of PHACE Syndrome.

tortuous, dysmorphic cerebral vessels can be diagnostic. As noted earlier, the potential for unsedated feed and wrap imaging may exist.

Suggested Readings

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Complete references used in this text can be found online at www.expertconsult.com

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Congenital and Hereditary Disorders of the Skin

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KEY POINTS

- Genodermatoses are a broad spectrum of heritable disorders that affect the skin and may or may not affect other organ systems.
- Classification and nomenclature of these disorders are evolving as we learn more about the genetic basis and pathogenesis of these conditions.
- Genodermatoses that present in the neonatal period are discussed in this chapter.

Heritable disorders of the skin, also known as *genodermatoses*, encompass a diverse array of conditions that can affect the color, texture, and structural integrity of the epidermis, epidermal appendages, and connective tissue. Some of these diseases affect only the skin, but many are associated with anomalies of other organ systems. Knowledge of the molecular genetic basis of the genodermatoses is rapidly evolving, with the hope that this will lead to novel and more efficacious therapies. Table 104.1 provides a list of resources and support groups for patients with genodermatoses and their families.

Genetic mosaicism refers to an organism composed of two or more genetically distinct populations of cells. Mosaicism affecting the skin can lead to unique cutaneous patterns. One example of this is Blaschko lines, which refers to a linear and whorled pattern with midline demarcation, thought to represent migration of embryonic cells (Fig. 104.1; Lombillo and Sybert, 2005). Blaschko lines are different from dermatomes, skin tension lines, or lines of lymphatic drainage. There are various heritable skin disorders that present with skin lesions that follow Blaschko lines, including pigmentary disorders, incontinentia pigmenti (IP), and Goltz syndrome, which will be discussed in this chapter.

Ichthyoses

The ichthyoses, also referred to as *disorders of keratinization or cornification*, are a group of skin disorders that are characterized by scaly skin and/or hyperkeratosis (thickened stratum corneum). Historically they have been classified by their clinical and sometimes histologic features, but nosology is evolving as the molecular basis of these disorders is being discovered. In 2009 an international consensus conference was held to revise the nomenclature and

classification of inherited ichthyoses, which were subdivided into two principal groups: nonsyndromic and syndromic forms, summarized in Tables 104.2 and 104.3, respectively, with exceedingly rare or minor variants that are beyond the scope of this chapter (Oji et al., 2010). The most striking neonatal presentations are discussed next.

Collodion Baby

The term *collodion baby* refers to a neonate born encased in shiny, thickened skin that resembles parchment or plastic wrap (Fig. 104.2). The taut skin leads to various distorting features such as ectropion (eversion of eyelids), eclabium (eversion of lips), flattening or hypoplasia of the nose and ears, and pseudocontractures of the digits. These infants have an ineffective cutaneous barrier against transepidermal water loss and invasion of pathogenic organisms and are at risk of multiple complications, including temperature instability, dehydration, electrolyte instability, infections, poor sucking, failure to thrive, keratitis, ear canal obstruction, percutaneous toxicity from topical medications, pneumonia from aspiration of squamous material in amniotic fluid, and distal limb or digital ischemia (Prado et al., 2012).

These neonates need diligent supportive care in a humidified incubator with close monitoring of temperature, fluid and electrolyte balance, and caloric intake. Surveillance for signs of cutaneous or systemic infection is important, but prophylactic antibiotics or antifungals are not recommended. Skin care is somewhat controversial, but many advocate application of a bland petroleum-based or water-in-oil emollient at least every 6 to 8 hours. Caution is advised with use of topical medications or keratolytics because of increased percutaneous absorption and risk of toxicity. If there are erosions or fissures, bathing with normal saline may be more comfortable than with plain water. Ophthalmology and otolaryngology consultations should be considered for eye and ear involvement (Prado et al., 2012).

Collodion baby refers not to a specific disease but rather to an initial phenotype seen in several forms of ichthyosis (Table 104.4). Ichthyosis prematurity syndrome, Netherton syndrome (NS), and Sjögren–Larsson syndrome must also be considered in the differential diagnosis. The collodion membrane is typically shed in 3 to 4 weeks, and the eventual phenotype depends on the underlying genetic mutation. More than half of collodion babies have autosomal

**TABLE
104.1****Resources for Patients With Genodermatoses and Their Families**

| Disorder | Resource(s) | Website |
|--|--|--|
| Ectodermal dysplasia | National Foundation for Ectodermal Dysplasias | http://www.nfed.org |
| Ehlers–Danlos syndrome | Ehlers-Danlos National Foundation | http://www.ednf.org |
| Epidermolysis bullosa | Dystrophic Epidermolysis Bullosa Research Association EB Research Partnership | http://www.debra.org https://ebresearch.org |
| Ichthyoses/disorders of keratinization | Foundation for Ichthyosis & Related Skin Types | http://www.firstskinfoundation.org |
| Incontinentia pigmenti | Incontinentia Pigmenti International Foundation | http://www.ipif.org |
| Neurofibromatosis | Neurofibromatosis Network | http://www.nfnetwork.org |
| Oculocutaneous albinism | National Organization for Albinism and Hypopigmentation | http://www.albinism.org |
| Porphyria | American Porphyria Foundation | http://www.porphyrifoundation.com |
| Tuberous sclerosis | Tuberous Sclerosis Alliance | http://www.tsalliance.org |
| Xeroderma pigmentosum | Xeroderma Pigmentosum Family Support Group | http://www.xpfamilysupport.org |

EB, Epidermolysis bullosa.



• **Fig. 104.1** Blaschko lines demonstrated in a girl with extensive epidermal nevi.

recessive congenital ichthyosis (ARCI), discussed in more detail later. About 10% have almost normal skin after the collodion membrane sheds, which has been referred to as *self-healing collodion baby*. It is difficult to predict the long-term prognosis during the neonatal period. The diagnostic work-up should include a detailed family history and consideration of microscopic examination of scalp and eyebrow hair to look for tiger-tail banding seen on polarized microscopy in trichothiodystrophy (TTD). The role of skin biopsy before the collodion membrane has shed is controversial, and the underlying diagnosis may become evident with time, which will help determine appropriate genetic testing or the need for biochemical or metabolic assays.



• **Fig. 104.2** Collodion baby born encased in shiny, thickened skin that resembles plastic wrap.

Autosomal Recessive Congenital Ichthyosis: Harlequin Ichthyosis, Lamellar Ichthyosis, and Nonbullous Congenital Ichthyosiform Erythroderma

ARCI encompasses a spectrum of nonsyndromic autosomal recessive ichthyoses that includes phenotypes ranging from lamellar ichthyosis (LI) to nonbullous congenital ichthyosiform erythroderma (CIE) to harlequin ichthyosis (HI).

The classic LI phenotype is characterized by coarse, yellow to brown-black, platelike scales (Fig. 104.3) with varying degrees of underlying erythema, ectropion, and eclabium. Many patients with LI are born with a full or partial collodion membrane, discussed earlier. The classic CIE phenotype is characterized by more prominent erythroderma and finer, white scales that may not be apparent until the collodion membrane sheds. Neonates with CIE

TABLE 104.2 Nonsyndromic Inherited Ichthyoses

| Disorder | Inheritance | Gene(s) | Newborn Cutaneous Features | Childhood/Adult Cutaneous Features | Extracutaneous Features |
|---|-----------------|---|---|--|---|
| Common Ichthyoses | | | | | |
| Ichthyosis vulgaris | AD ^a | <i>FLG</i> | Onset usually after 2 to 6 months of age | Generalized scaling; hyperlinear palms and soles | Association with atopy |
| Recessive X-linked ichthyosis | XLR | <i>STS</i> | May have mild scaling and/or erythroderma or mild collodion membrane | Generalized brown or gray rhomboid or fine scaling sparing folds; prominent neck involvement; abates in summer | Cryptorchidism (5%–20%); corneal opacities (~50%); prolonged labor; contiguous gene syndromes |
| Autosomal Recessive Congenital Ichthyosis, Major Types^b | | | | | |
| Lamellar Ichthyosis | AR | <i>TGM1, ALOXE3, ALOX12B, ABCA12, CERS3, CYP4F22, NIPAL4 (ICHTHYIN), PNPLA1</i> | Collodion membrane; ectropion; eclabium | Generalized large platelike scaling; palmoplantar keratoderma, with or without scarring alopecia; hypohidrosis | With or without short stature |
| Congenital ichthyosiform erythroderma | AR | <i>TGM1, ALOXE3, ALOX12B, ABCA12, CERS3, CYP4F22, NIPAL4 (ICHTHYIN), PNPLA1, LIPN</i> | Mild scaling and erythroderma or mild collodion membrane | Erythroderma with fine scaling; with or without scarring alopecia; hypohidrosis | With or without short stature; failure to thrive |
| Harlequin ichthyosis | AR | <i>ABCA12</i> | Severe collodion membrane with armor-like scales; ectropion; eclabium; contractures | Erythroderma with fine scaling similar to that in congenital ichthyosiform erythroderma; scarring alopecia; skin infections; severe hypohidrosis | Contractures; failure to thrive; short stature |
| Keratinopathic Ichthyosis, Major Types^b | | | | | |
| Epidermolytic ichthyosis | AD | <i>KRT1, KRT10</i> | Erythroderma, bullae, or erosions, mild scaling | Thick, verruciform scale accentuated in areas of friction; PPK; bacterial colonization/infections | With or without growth failure |
| Superficial epidermolytic ichthyosis ^c | AD | <i>KRT2</i> | Erythroderma, widespread superficial bullae or erosions | Fine molting scale | None |
| Ichthyosis with confetti-like spots ^d | AD | <i>KRT1, KRT10^e</i> | Erythroderma with fine scaling; PPK | Similar to epidermolytic ichthyosis with pale confetti-like spots | With or without growth failure |
| Other Types of Ichthyosis | | | | | |
| Loricrin keratoderma | AD | <i>LOR</i> | Congenital ichthyosiform erythroderma-like or collodion membrane | Honeycomb PPK with digital constriction (pseudoainhum); generalized mild scaling accentuated over joints | None |
| Erythrokeratoderma variabilis ^f | AD | <i>GJB3, GJB4</i> | Persistent cutaneous findings may be present at birth or within the first year. | Transient, migratory erythematous patches with fine scale and fixed, well-demarcated, hyperkeratotic plaques; with or without PPK | None |

Continued

TABLE 104.2 Nonsyndromic Inherited Ichthyoses—cont'd

| Disorder | Inheritance | Gene(s) | Newborn Cutaneous Features | Childhood/Adult Cutaneous Features | Extracutaneous Features |
|---|-------------|--------------------------|---|--|---|
| Peeling skin disease | AR | <i>CDSN</i> (and others) | Congenital ichthyosiform erythroderma-like features | Generalized erythroderma with migratory exfoliative or peeling scale; skin infections; resembles atopic dermatitis | Atopic diathesis with elevated IgE concentration and eosinophilia |
| Keratosis linearis–ichthyosis congenita–keratoderma | AR | <i>POMP</i> | Scaling or mild collodion membrane | Linear, hyperkeratotic papules and plaques in skin folds; PPK with digital constriction bands | None |

^aAutosomal semidominant.^bMinor variants are not included.^cPreviously known as *ichthyosis bullosa of Siemens*.^dAlso known as *congenital reticular ichthyosiform erythroderma*.^eConfetti-like spots that appear during childhood are due to mitotic recombination leading to somatic reversion of *KRT1* or *KRT10* mutations.^fOverlap with progressive symmetric erythrodermatitis.

AD, Autosomal dominant; AR, autosomal recessive; IgE, immunoglobulin E; PPK, palmoplantar keratoderma; XLR, X-linked recessive.

Modified from Oji V, Tadini G, Akiyama M, et al. Revised nomenclature and classification of inherited ichthyoses: results of the first Ichthyosis Consensus Conference in Sorèze 2009. *J Am Acad Dermatol*. 2010;63:607–641.**TABLE 104.3** Syndromic Inherited Ichthyoses

| Disorder | Inheritance | Gene(s) | Newborn Cutaneous Features | Childhood/Adult Cutaneous Features | Extracutaneous Features |
|---|-------------|---|---|--|--|
| Prominent Hair Abnormalities | | | | | |
| Netherton syndrome | AR | <i>SPINK5</i> (encodes the serine protease inhibitor LETK1) | Erythroderma, scaling | Ichthyosis linearis circumflexa characterized by migratory, polycyclic erythematous plaques with double-edged scale; hair shaft abnormalities (trichorrhexis invaginata, aka <i>bamboo hair</i> or <i>ball-and-socket</i> deformity); severe pruritus; bacterial or viral (HPV) infections | Atopic diathesis with elevated IgE concentration and eosinophilia; failure to thrive |
| Ichthyosis–hypotrichosis–sclerosing cholangitis syndrome ^a | AR | <i>CLDN1</i> | Mild scaling; jaundice | Mild scaling; frontotemporal scarring alopecia | Sclerosing cholangitis or congenital paucity of bile ducts |
| Trichothiodystrophy ^b | AR | <i>ERCC2</i> (<i>XPD</i>), <i>ERCC3</i> (<i>XPB</i>), <i>GTF2H5</i> (<i>TTDA</i>) | Collodion membrane or erythroderma and scaling | Fine to platelike scaling; brittle hair (trichoschisis or trichorrhexis nodosa on light microscopy; “tiger-tail” bands on polarized microscopy); photosensitivity; with or without atopic dermatitis; with or without PPK or nail dystrophy | Developmental delay; short stature; cataracts; facial dysmorphism; bone abnormalities; gonadal abnormalities; recurrent infections |
| Ichthyosis follicularis–atrichia–photophobia | XLR | <i>MBTPS2</i> | Mild collodion membrane; congenital atrichia (alopecia) | Generalized follicular hyperkeratosis; atrichia (alopecia); mild hypohidrosis | Severe photophobia (vascularizing keratitis); developmental delay; short stature; atopic diathesis; other |

TABLE
104.3

Syndromic Inherited Ichthyoses—cont'd

| Disorder | Inheritance | Gene(s) | Newborn Cutaneous Features | Childhood/Adult Cutaneous Features | Extracutaneous Features |
|--|-------------|--|--|---|--|
| Prominent Neurologic Abnormalities | | | | | |
| Sjögren–Larsson syndrome | AR | <i>ALDH3A2</i> (encodes fatty aldehyde dehydrogenase) | Scaling accentuated on scalp and neck, with or without erythema | Velvety (acanthosis-like) or lamellar hyperkeratosis accentuated in folds and areas of friction | Spastic paraplegia; mental retardation; ocular findings (retinal glistening white dots) |
| Mental retardation–enteropathy–deafness–neuropathy–ichthyosis–keratoderma syndrome | AR | <i>AP1S1</i> | EKV-like features, may present at birth or within the first few weeks of life | EKV-like (transient, migratory erythematous patches with fine scale and fixed, well-demarcated, hyperkeratotic plaques; with or without PPK) | Congenital sensorineural deafness; peripheral neuropathy; developmental and growth delay; chronic diarrhea |
| Refsum disease | AR | <i>PHYH (PAHX)</i> | Usually normal | General fine scaling usually starts in childhood or adolescence; accentuated palmoplantar markings | Tetrad of retinitis pigmentosa, peripheral neuropathy, cerebellar ataxia, and elevated protein levels in the cerebrospinal fluid; anosmia; progressive deafness; other |
| Prominent Skeletal Abnormalities | | | | | |
| Conradi–Hünemann–Happle syndrome (CDPX2) | XLD | <i>EBP</i> | Severe scaly erythroderma or collodion membrane | Streaky erythema then hyperpigmentation and follicular atrophoderma following Blaschko lines; erythematous scaly plaques in folds; patchy scarring alopecia | Chondrodysplasia punctata with asymmetric skeletal hypoplasia; cataracts; short stature |
| Congenital hemidysplasia–ichthyosiform erythroderma or nevus-limb defects ^c | XLD | <i>NSDHL</i> | Persistent cutaneous findings may present at birth or within the first few months | Unilateral sharply demarcated erythematous scaly plaques that may follow Blaschko lines; with or without unilateral alopecia or nail dystrophy | Ipsilateral skeletal hypoplasia (X-ray may show punctate epiphyseal dysplasia); with or without renal, cardiac, or CNS anomalies |
| Fatal Disease Course | | | | | |
| Gaucher syndrome type 2 | AR | <i>GBA</i> | Mild collodion membrane or CIE-like features | Generalized scaling or normal | Hydrops fetalis; progressive neurologic deterioration; hepatosplenomegaly |
| Multiple sulfatase deficiency | AR | <i>SUMF1</i> | RXLI-like (may have mild scaling and/or erythroderma or mild collodion membrane) or presents later | RXLI-like (generalized brown or gray rhomboid or fine scaling sparing folds; prominent neck involvement; abates in summer) | Metachromatic leukodystrophy and mucopolysaccharidosis with progressive neurologic deterioration |
| Cerebral dysgenesis–neuropathy–ichthyosis–keratoderma | AR | <i>SNAP29</i> | Normal at birth, presents between 5 and 11 months of age | Generalized lamellar hyperkeratosis sparing folds; PPK; fine, sparse hair | Sensorineural deafness; cerebral dysgenesis; neuropathy; microcephaly; neurogenic muscle atrophy; optic nerve atrophy; cachexia |
| Arthrogryposis–renal dysfunction–cholestasis syndrome | AR | <i>VPS33B</i> | Scaling within a few days of birth or mild collodion membrane | Generalized fine or platelike scaling | Arthrogryposis; intrahepatic bile duct hypoplasia with cholestasis; renal tubular degeneration with metabolic acidosis; cerebral malformation; abnormal platelets |

Continued

TABLE 104.3 Syndromic Inherited Ichthyoses—cont'd

| Disorder | Inheritance | Gene(s) | Newborn Cutaneous Features | Childhood/Adult Cutaneous Features | Extracutaneous Features |
|--|-------------|----------------------|---|---|--|
| Other Abnormalities | | | | | |
| Keratitis–ichthyosis–deafness syndrome | AD | <i>GJB2, GJB6</i> | Erythematous, thickened skin; with or without congenital alopecia | Generalized stippled or spiky hyperkeratosis; well-demarcated verrucous plaques; PPK; nail dystrophy; sparse hair or scarring alopecia; hidradenitis suppurativa; recurrent infections; increased risk of squamous cell carcinoma | Congenital sensorineural deafness; progressive keratitis with photophobia |
| Neutral lipid storage disease with ichthyosis ^a | AR | <i>ABHD5 (CGI58)</i> | Mild collodion membrane or CIE-like or EKV-like features | CIE-like features (erythroderma with fine scaling) | Vacuolated granulocytes (Jordan anomaly); hepatosplenomegaly; myopathy; cataracts; hearing loss; developmental delay |
| Ichthyosis prematurity syndrome | AR | <i>SLC27A4</i> | Thick, desquamating scale (vernix caseosa-like) with accentuation on scalp and eyebrows | Scaling with follicular keratosis; atopic dermatitis | Polyhydramnios with opaque amniotic fluid (from shedding epidermis); respiratory distress at birth; asthma; eosinophilia |

^aAlso known as *neonatal ichthyosis sclerosing cholangitis syndrome*.
^bAlso known as *ichthyosis–brittle hair–impaired intelligence–decrease fertility–short stature syndrome*; there are forms of trichothiodystrophy not associated with congenital ichthyosis.
^cThis syndrome was not included as an ichthyosis in the 2009 classification of inherited ichthyoses.
^dAlso known as *Chanarin–Dorfman syndrome*.
AD, Autosomal dominant; AR, autosomal recessive; *CDPX2*, chondrodysplasia punctata (X-linked dominant) type 2; *CIE*, congenital ichthyosiform erythroderma; *CNS*, central nervous system; *EKV*, erythrokeratoderma variabilis; *HPV*, human papillomavirus; *LETKL*, lymphoepithelial Kazal-type-related inhibitor; *PPK*, palmoplantar keratoderma; *RXLI*, recessive X-linked ichthyosis; *XLD*, X-linked dominant; *XLR*, X-linked recessive.
Modified from Oji V, Tadini G, Akiyama M, et al. Revised nomenclature and classification of inherited ichthyoses: results of the first Ichthyosis Consensus Conference in Sorèze 2009. *J Am Acad Dermatol*. 2010;63:607–641.



• **Fig. 104.3** Three-week-old male with generalized large scales characteristic of lamellar ichthyosis after shedding of collodion membrane.

often have a less severe collodion membrane or may be born without a membrane (Oji et al., 2010). The principles of neonatal management for these phenotypes are the same as those discussed earlier for a collodion baby.

HI is the most severe clinical phenotype of ichthyosis in a newborn. These neonates are born with an “armor” of thick, fissured, platelike scales (Fig. 104.4). They have severe ectropion and eclabium and flattened, hypoplastic nose and ears. Constricting bands lead to contractures and digital ischemia. They are at high risk of the complications discussed earlier for collodion babies but have a higher rate of infant mortality. Historically, almost 50% of babies with HI have died in the neonatal period, more than half in the first 3 days of life. The cause of death is most commonly attributed to sepsis and/or respiratory failure (Rajpopat et al., 2011). Intensive, multidisciplinary, supportive care as detailed earlier for collodion babies is critical for neonates with HI. Early initiation of therapy with a systemically acting retinoid (such as isotretinoin, 0.5 to 1 mg/kg per day, or acitretin, 0.5 mg/kg per day) before day of life 7 may improve the survival rate. Topical retinoids and surgical release of constricting bands may also be considered (Milestone and Choate, 2013). Patients who survive infancy have a lifelong ichthyosis that resembles CIE.



• **Fig. 104.4** Newborn with harlequin ichthyosis born with thick, fissured, platelike scales, severe ectropion and eclabium, and hypoplastic nose and ears.



• **Fig. 104.5** Newborn with epidermolytic ichthyosis who exhibited widespread erythema and superficial blisters.

TABLE 104.4 Disorders That May Present as a Collodion Baby

| | |
|-------------------------|--|
| Nonsyndromic ichthyoses | <p>Autosomal recessive congenital ichthyosis: lamellar ichthyosis and congenital ichthyosiform erythroderma phenotypes</p> <p>Ichthyosis vulgaris</p> <p>Recessive X-linked ichthyosis</p> <p>Epidermolytic ichthyosis</p> <p>Bathing suit ichthyosis</p> <p>Self-healing collodion baby</p> <p>Keratosis linearis with ichthyosis congenital and sclerosing</p> <p>Keratoderma syndrome</p> |
| Syndromic ichthyoses | <p>Ichthyosis follicularis–atrachia–photophobia syndrome</p> <p>Neutral lipid storage disease with ichthyosis</p> <p>Trichothiodystrophy with ichthyosis</p> <p>Conradi–Hünemann–Happle syndrome</p> <p>Keratitis–ichthyosis–deafness syndrome</p> <p>Loricrin keratoderma</p> <p>Arthrogryposis–renal dysfunction–cholestasis syndrome</p> |
| Metabolic diseases | <p>Holocarboxylase synthetase deficiency</p> <p>Gaucher disease type 2</p> |
| Other diseases | <p>Hypohidrotic ectodermal dysplasia</p> <p>Congenital hypothyroidism</p> <p>Koraxitrachitic syndrome</p> <p>Palmoplantar keratoderma with anogenital leukokeratosis</p> |

Modified from Prado R, Ellis LZ, Gamble R, Funk T, Arbuckle HA, Bruckner AL. Collodion baby: an update with a focus on practical management. *J Am Acad Dermatol.* 2012;67: 1362–1374.

hyperkeratosis or *bullous CIE*, presents with generalized erythroderma and bullae. The term *epidermolytic hyperkeratosis* describes the histopathologic or ultrastructural features seen on skin biopsy characterized by marked hyperkeratosis (thickened stratum corneum) with clumping and lysis (disintegration) of the epidermal cells above the basal layer (Ross et al., 2008). Newborns with EI typically present with widespread erythema and superficial blistering, as a result of the fragility of the epidermis from abnormal keratin production (Fig. 104.5). These neonates may receive a misdiagnosis of epidermolysis bullosa (EB) or staphylococcal scalded skin syndrome. Subtle skin thickening over the elbows, knees, palms, or soles may be diagnostic clues. During the first few months of life, the phenotype gradually evolves into more pronounced skin thickening with verruciform, ridged scales, accentuated in areas of friction, including flexural and intertriginous areas. Malodorous bacterial colonization is common.

Treatment of neonates with EI requires supportive care with attention to gentle handling to minimize blister formation, including use of nonadherent dressings. They are at risk of temperature instability, dehydration, and electrolyte instability and should be closely monitored for bacterial infections.

EI is caused by autosomal dominant mutations in *KRT1* or *KRT10*, either inherited or caused by a spontaneous mutation. It is also possible that the parent of a child with EI may have a congenital epidermolytic epidermal nevus caused by a somatic mosaic keratin mutation in *KRT1* or *KRT10*. Gonadal involvement of the keratin mutation, more common in those with widespread epidermal nevi, results in an offspring affected by generalized EI. Clinically, an epidermolytic epidermal nevus is indistinguishable from other types of epidermal nevi, but a biopsy would show features of EI. Prenatal diagnosis is possible if there is a known mutation (Chassaing et al., 2006).

Epidermolytic Ichthyosis

The 2009 classification scheme for ichthyosis proposed the term *keratinopathic ichthyosis* to encompass ichthyoses that are a result of keratin mutations, most of which are autosomal dominant. Epidermolytic ichthyosis (EI), previously referred to as *epidermolytic*

Diagnosis of Ichthyoses

Cutaneous and extracutaneous clinical features are critical in narrowing down the differential diagnosis for a neonate born with scaly or hyperkeratotic skin and can sometimes be sufficient for diagnosis. Family history may be helpful, particularly for ichthyoses

with a dominant inheritance pattern. The gold standard for diagnosis is genetic mutation analysis.

Histopathologic findings from a skin biopsy in many patients with ichthyosis are nonspecific, with a few exceptions. EI has characteristic histologic findings of “epidermolytic hyperkeratosis” discussed earlier. Biopsy specimens from an individual with ichthyosis vulgaris, NS, TTD, Refsum syndrome, or Conradi–Hünemann–Happle syndrome (chondrodysplasia punctata X-linked dominant type 2) may show reduced or absent stratum granulosum. Histopathology of loricrin keratoderma is notable for parakeratosis and hypergranulosis (Oji, 2010). Special immunohistochemical stains or ultrastructural analysis by electron microscopy may be considered in certain cases.

Examination of hair shafts can be helpful for diagnosing NS and TTD. Trichorrhexis invaginata (“bamboo hair” or “ball-and-socket” deformity) is characteristic of NS but is not usually present until after 1 year of age and even then is invariably present. The hair of patients with TTD may show trichoschisis (clean transverse fracture) or trichorrhexis nodosa (nodes from longitudinal splitting of fibers) on light microscopy and characteristic tiger-tail banding on polarized microscopy from low sulfur content.

Further work-up for syndromic ichthyoses is typically based on extracutaneous findings.

Prognosis and Treatment of Ichthyoses

Distinguishing the type of ichthyosis is crucial for offering prognostic information to the family, as prognosis is highly variable. Neonatal care for severe phenotypes, including collodion baby, LI, HI, and EI, was discussed earlier. For all types of ichthyosis, skin care typically involves application of bland emollients to hydrate the stratum corneum. Emollients with keratolytics such as urea, salicylic acid, α -hydroxy acids, and propylene glycol are usually avoided in infancy because of risk of toxicity from absorption. Oral retinoids are primarily considered for patients with HI or rarely in those with severe collodion membrane with delayed shedding.

Epidermolysis Bullosa

EB encompasses a group of mechanobullous disorders characterized by skin fragility and blister formation. The clinical spectrum is broad, but in the neonatal period most patients have vesicles, bullae, or erosions. Most forms of EB are caused by genetic mutations that result in absent or reduced levels of adhesion proteins normally present at the interface of the epidermis and dermis, which is called the *basement membrane zone* (BMZ). As a result, the epidermis separates from the dermis with less friction or force than usual, resulting in blister formation. In some EB subtypes, the epithelia of the external eye, ear, nose, upper airway, and gastrointestinal and genitourinary tracts are involved. The nature of the mutated protein and the severity of the mutation typically determine the phenotype: whether blistering is localized or generalized and the extent of extracutaneous involvement. As a general rule, less severe forms of EB tend to be more common and autosomal dominantly inherited, whereas more severe forms are rarer and autosomal recessive (Fine and Mellerio, 2009a; Gonzalez, 2013).

Classification of Epidermolysis Bullosa

EB classification and nomenclature have evolved over many years and were most recently updated at a 2013 international

consensus meeting (Fine et al., 2014). EB is classified into four major types: EB simplex, junctional EB, dystrophic EB, and Kindler syndrome. EB type is determined by the level of the skin at which mechanical fragility and blister formation occur. EB simplex is the most superficial form, with the level of skin cleavage occurring in the epidermis, usually in basal keratinocytes. In junctional EB, blisters form deeper, within the midportion (lamina lucida) of the skin's BMZ. Skin cleavage in dystrophic EB occurs just below the BMZ in the most superficial layer of the dermis (the sublamina densa). In Kindler syndrome, which is very rare, fragility and blister formation are seen at multiple depths within the skin. EB is further subdivided into more than 39 subtypes on the basis of clinical presentation, ultrastructural features, immunohistochemical findings, and the presence of specific genetic mutations (Fine et al., 2014). Only the more common or more notable subtypes are discussed in this chapter (Tables 104.5–104.8).

Epidermolysis Bullosa Simplex

EB simplex most commonly results from genetic mutations affecting keratins 5 and 14 in the basal layer of the epidermis (Table 104.5). Blisters typically heal without scarring. Most patients with EB simplex have autosomal dominantly inherited forms and a normal life span. However, some of the recessive forms, including those associated with muscular dystrophy and pyloric atresia, carry a poor prognosis and are associated with early death (Gonzalez, 2013). Patients with generalized subtypes of EB simplex (intermediate and severe types) present with blistering in the newborn period. Severe generalized EB simplex is characterized by extensive blistering in herpetiform (herpes-like) or arcuate clusters. Mucosal involvement may be seen, nail dystrophy is common, and progressive palmoplantar keratoderma is characteristic. In the intermediate subtype of generalized EB simplex, bullae are most common over pressure points such as the elbows, knees, legs, feet, and hands and may be widespread following the trauma of birth. Mucosal involvement may occur during the newborn period but reduces with age. Nails may be lost but regrow. Localized EB simplex is the most common form of EB. Patients with localized EB simplex are usually asymptomatic during the newborn period, with onset of acral blisters in early childhood.

Junctional Epidermolysis Bullosa

Junctional EB is caused by gene mutations that affect expression of proteins integral to the lamina lucida of the BMZ. All known subtypes of junctional EB are autosomal recessive, and clinical severity is highly variable (Table 104.6). Tooth enamel hypoplasia is characteristic. Generalized severe junctional EB, previously known as *Herlitz subtype*, is the most severe form and carries the highest risk of early death of all forms of EB (Fine et al., 2008). Patients present at birth with widespread mucosal and skin blistering, most pronounced over pressure points (Fig. 104.6). Nails are dystrophic or absent. Exuberant granulation tissue often develops periorally and on the upper back. Airway involvement is typical, and the eyes and gastrointestinal and genitourinary systems are also commonly affected. Prognosis is extremely poor. Most patients die during the first 2 years of life secondary to failure to thrive, sepsis, or respiratory failure (Gonzalez, 2013). The presentation of the generalized intermediate subtype of junctional EB is variable and less severe than that of the generalized form. Localized subtypes are also seen.

TABLE 104.5 Subtypes of Epidermolysis Bullosa Simplex

| Subtype and Previous Eponym | Inheritance | Gene(s)/Protein(s) | Clinical Features |
|--|-------------|--|--|
| EBS, generalized severe (EBS, Dowling–Meara) | AD | <i>KRT5</i> /keratin 5 <i>KRT14</i> /keratin 14 | Presentation: birth Bullae: herpetiform, generalized Mucosal involvement: present or absent Nails: dystrophic Prognosis: progressive PPK, blistering abates with age |
| EBS, generalized intermediate (EBS, Koebner) | AD | <i>KRT5</i> /keratin 5 <i>KRT14</i> /keratin 14 | Presentation: birth to early infancy Bullae: pressure points Mucosal involvement: during infancy Nails: may be lost, but regrow Prognosis: abates with age |
| EBS, localized (EBS, Weber–Cockayne) | AD | <i>KRT5</i> /keratin 5 <i>KRT14</i> /keratin 14 | Presentation: early childhood Bullae: acral Mucosa involvement: mild to none Prognosis: abates with age |
| EBS with muscular dystrophy | AR | <i>PLEC</i> /plectin | Presentation: newborn–neonatal period Mucosal involvement: present Extracutaneous: congenital or delayed-onset muscular dystrophy; tooth enamel hypoplasia Prognosis: early death |
| EBS with pyloric atresia | AR | <i>PLEC</i> /plectin <i>ITGA6</i> or <i>ITGB4</i> /integrin $\alpha_6\beta_4$ | Presentation: birth Bullae: generalized Extracutaneous: pyloric atresia, GU anomalies Prognosis: neonatal death |

AD, Autosomal dominant; AR, autosomal recessive; EBS, epidermolysis bullosa simplex; GU, genitourinary; PPK, palmoplantar keratoderma.

TABLE 104.6 Subtypes of Junctional Epidermolysis Bullosa

| Subtype and Previous Eponym | Inheritance | Gene(s)/Protein(s) | Clinical Features |
|--|-------------|---|---|
| JEB, generalized severe (JEB, Herlitz) | AR | <i>LAMA3</i> , <i>LAMB3</i> , or <i>LAMC2</i> / laminin 332 | Presentation: birth Bullae: widespread with nonhealing granulation tissue Nails: dystrophic or absent Extracutaneous: airway (25%), GI tract, GU systems, eyes, teeth Prognosis: death by 2 years of age because of sepsis, failure to thrive, or airway compromise |
| JEB, generalized intermediate (JEB, non-Herlitz) | AR | <i>LAMA3</i> , <i>LAMB3</i> , or <i>LAMC2</i> / laminin 332 <i>COL17A1</i> /collagen XVII | Presentation: birth Nails, hair, teeth: commonly involved Prognosis: neonatal death to normal life span |
| JEB with pyloric atresia | AR | <i>ITGA6</i> or <i>ITGB4</i> /integrin $\alpha_6\beta_4$ | Presentation: birth Bullae: severe, generalized Extracutaneous: pyloric atresia, GU anomalies Prognosis: neonatal death |

AR, Autosomal recessive; GI, gastrointestinal; GU, genitourinary; JEB, junctional epidermolysis bullosa.

Dystrophic Epidermolysis Bullosa

The dystrophic subtypes of EB are all caused by mutations affecting the expression of collagen VII, which forms the anchoring fibrils, critical for adhesion of the basement membrane to the upper dermis. Blistering occurs just below the BMZ, resulting in significant scarring and milia formation (Fig. 104.7). Dystrophic EB can be

categorized into autosomal dominant and recessive variants. In general, recessively inherited forms are associated with absence of collagen VII and are more severe than dominant forms, in which collagen VII expression is decreased or abnormal (Table 104.7).

The generalized severe recessive variant is the most severe subtype of dystrophic EB. Patients present at birth with generalized blistering of the skin and mucosa. Involvement of multiple organ systems,

TABLE 104.7 Dystrophic Epidermolysis Bullosa

| Subtype and Previous Eponym | Inheritance | Gene/Protein | Clinical Features |
|--|-------------|-----------------------------|--|
| RDEB, generalized severe (RDEB, Hallopeau–Siemens) | AR | <i>COL7A1</i> /collagen VII | Presentation: birth Blistering: widespread, involving skin and mucosa Nails: dystrophic or absent Extracutaneous involvement: ocular, GI tract, GU system, kidneys, heart Sequelae: mutilating scarring, pseudosyndactyly of digits, aggressive SCC Prognosis: poor, death most commonly caused by metastatic SCC |
| RDEB, generalized intermediate (RDEB, non-Hallopeau–Siemens) | AR | <i>COL7A1</i> /collagen VII | Presentation: birth Blistering: widespread but less severe involvement of skin and mucosa Nails: dystrophic or absent Extracutaneous involvement: variable to absent Sequelae: variable pseudosyndactyly, SCC Prognosis: lifelong blistering, risk of early death |
| DDEB, generalized (DDEB, Cockayne–Touraine, and Pasini) | AD | <i>COL7A1</i> /collagen VII | Presentation: birth Blistering: most pronounced on hands, feet, knees, and elbows Nails: dystrophic or absent Extracutaneous involvement: variable GI tract involvement Prognosis: lifelong blistering but normal life span |
| Bullous dermolysis of the newborn | AR, AD | <i>COL7A1</i> /collagen VII | Presentation: birth Blistering: generalized or localized; spontaneous or induced by trauma Prognosis: spontaneous resolution in first year of life |

AD, Autosomal dominant; *AR*, autosomal recessive; *DDEB*, dominant dystrophic epidermolysis bullosa; *GI* gastrointestinal; *GU*, genitourinary; *RDEB*, recessive dystrophic epidermolysis bullosa; *SCC*, squamous cell carcinoma.

TABLE 104.8 Kindler Syndrome

| Inheritance | Gene/Protein | Clinical Features |
|-------------|-------------------------|--|
| AR | <i>KIND1</i> /kindlin 1 | Presentation: birth Blistering: split at multiple levels of the skin; induced by trauma Marked photosensitivity and poikiloderma of sun-exposed skin Extracutaneous: gingivitis, colitis, mucosal stenosis, pseudosyndactyly Prognosis: blistering reduces and poikiloderma worsens with age; normal life span |

AR, Autosomal recessive

including the eyes, gastrointestinal tract, kidneys, genitourinary tract, and the heart, can be seen. Scarring of the hands and feet can lead to functionally limiting pseudosyndactyly and bone deformation. Anemia and failure to thrive are characteristic (Fine and Mellerio, 2009b). Aggressive squamous cell carcinomas arising in chronic wounds are the most common cause of death in patients with recessive dystrophic EB and develop as early as the teenage years (Fine et al., 2008).



• **Fig. 104.6** Newborn with junctional epidermolysis bullosa who exhibited bullae in the diaper area and other areas of friction.

In patients with dominantly inherited forms of dystrophic EB, blistering is typically induced by trauma and limited to the hands, feet, elbows, and knees. Scarring and milia formation occur but do not typically cause functional impairment. Nail dystrophy or loss is common and may be the only manifestation of disease. Life expectancy is usually normal (Gonzalez, 2013). There is a subtype of dystrophic EB called *bullous dermolysis of the newborn* where



• **Fig. 104.7** Four-month-old with dystrophic epidermolysis bullosa characterized by bullae, erosions, and milia.



• **Fig. 104.8** Bullous dermolysis of the newborn with absent skin on legs and feet.

skin fragility is usually transient and spontaneously resolves within the first year of life (Radkevich-Brown and Shwayder, 2013). These patients are often born with absent skin (aplasia cutis), especially on the lower extremities (Fig. 104.8).

Diagnosis of Epidermolysis Bullosa

The differential diagnosis for skin blisters or erosions in the neonate is broad but should include EB. Traumatic and infectious causes of blistering should be ruled out. A thorough history, including family history of blistering disorders, should be obtained. Physical examination findings are an unreliable indicator of EB type during the neonatal period, and discussion of long-term prognosis should be deferred until the subtype of EB is known.

Differentiation between the types of EB is usually done by specialized examination of a skin biopsy specimen and cannot be done by routine light microscopy of hematoxylin- and eosin-stained sections, which would show blister formation at the dermal-epidermal junction in most types of EB. Skin biopsy should be performed at the junction of a fresh blister and normal skin. Ideally, a blister is induced by the rubbing of a pencil eraser against the skin until erythema develops. The level of blister formation (i.e.,

intraepidermal, lamina lucida, or superficial dermis) can be determined by transmission electron microscopy (TEM) or immunofluorescence antigen mapping (IFM) of newly induced blisters. TEM allows detailed visualization of the epidermal-dermal junction to determine whether blister formation occurs superficial to, within, or deep to the BMZ. IFM uses monoclonal antibodies directed against proteins in the BMZ to help determine the level of split and in some cases will show decreased or absent expression of certain proteins mutated in EB. IFM is being increasingly used rather than TEM, but both should be performed by a laboratory experienced in performing these studies on patients with EB. Following TEM or IFM, genetic testing for specific mutations can provide molecular diagnosis of the EB subtype. Sometimes genotype-phenotype correlations are available for known mutations. A list of laboratories that provide testing for EB can be found at <http://www.ncbi.nlm.nih.gov/gtr/tests/>. Unfortunately, cost is still a barrier to routine genetic testing. In families with a known history of EB, methods of prenatal or preimplantation diagnosis can be considered.

Management of Epidermolysis Bullosa

While molecular treatments for EB are on the horizon, supportive measures remain the mainstay of therapy. A multidisciplinary approach is important, involving specialists in fields such as dermatology, surgery, gastroenterology, otolaryngology, dentistry, hematology/oncology, wound care, pain management, occupational and physical therapy, nutrition, and psychology (Fine and Mellerio, 2009b; El Hachem et al., 2014). EB patients who die in infancy and early childhood most often succumb to sepsis, followed by failure to thrive and respiratory failure. Those with severe subtypes of junctional EB are at markedly higher risk of early death (Fine et al., 2008), and in some patients it may be appropriate to emphasize comfort measures over aggressive treatments that prolong life (Yan et al., 2007).

In the neonatal period, prevention of new blisters and prevention of infection are of the utmost importance. Gentle handling and avoiding of frictional trauma should be emphasized. Infants should be rolled or moved with a sheet when possible to avoid friction. When lifting is necessary, one hand should gently support the head and neck, with the other under the buttocks. Ambient temperature should be cool, and routine use of incubators should be avoided, as heat may increase skin fragility. Bedding should be soft, and silicone dressings or padding should be applied to bony prominences and inside diapers. Intact bullae should be gently ruptured and drained with a sterile needle, leaving the roof intact as a biologic dressing. Wounds should be covered with nonadhesive dressings, and these should be changed daily or every other day. Tape and adhesives should never be applied to the skin. Nonadhesive wound dressings can be placed under pulse oximeter probes and electrocardiogram leads. Prevention of infection often involves use of chlorhexidine, dilute bleach, and/or dilute vinegar baths. Topical antibiotics should be used sparingly to avoid resistance. Pain control is essential (El Hachem et al., 2014).

Aggressive nutritional support is essential, especially in patients with feeding difficulties caused by significant mucosal involvement, or pyloric atresia, or those with increased fluid and energy requirements because of widespread blistering (El Hachem et al., 2014). Soft nipples used for feeding infants with cleft palates should be used in those with oral involvement. EB patients are prone to cutaneous infection, most commonly with *Staphylococcus*, which may disseminate. Indwelling catheters and overuse of topical

antibiotics such as mupirocin should be avoided when possible (Fine and Mellerio, 2009a). Tracheostomy may be indicated in patients with airway involvement (Yan et al., 2007; Fine and Mellerio, 2009b). Surgical repair of pyloric atresia enhances survival in affected patients, particularly those with relatively mild skin involvement (Fine and Mellerio, 2009b). Patients with severe disease should also be monitored for iron-deficiency anemia.

Incontinentia Pigmenti

Incontinentia pigmenti (IP), also referred to as *Bloch–Sulzberger syndrome*, is an X-linked neuroectodermal disorder that variably affects the skin, hair, nails, teeth, eyes, and central nervous system (CNS). The disorder is caused by mutations in the *IKBKG* gene, also called *NEMO* (which encodes nuclear factor κ B essential modulator), localized to chromosome band Xq28. The vast majority of patients with IP are female, suggesting causative mutations in *IKBKG* are lethal in males, but there are rare reports of affected males with Klinefelter syndrome (XXY genotype), somatic mosaicism, or hypomorphic alleles (Ardelean and Pope, 2006; Buinauskaitė et al., 2010). Females with a missense mutation in *IKBKG* may have a son with X-linked ectodermal dysplasia with immunodeficiency, discussed later in this chapter. Females with IP are functionally mosaic because of random X chromosome inactivation early in embryologic development (lyonization), which explains the variability in clinical phenotype. Cutaneous lesions of IP demonstrate mosaicism and typically follow Blaschko lines.

Cutaneous Findings

Almost all patients with IP will exhibit cutaneous features at birth or within the first few weeks of life. Four classic stages of skin lesions have been described, but not all patients will exhibit each stage, and there can be overlapping of stages. Stage 1 is characterized by erythema and vesiculobullous or pustular lesions in a linear and whorled pattern following Blaschko lines (Fig. 104.9). This stage can last from weeks to months and then resolves spontaneously but can recur even years later, often precipitated by infection. The distribution along Blaschko lines can help distinguish cutaneous lesions of IP from herpes or bacterial infection.

In stage 2 there are linear, hyperkeratotic, verrucous papules and plaques that may or may not have evolved from previous vesiculobullous lesions. The verrucous stage is usually distributed on the extremities and is most pronounced on the hands or feet. These lesions often resolve by about 6 months of age but rarely persist or recur.

Stage 3 is characterized by linear and whorled brown to slate-gray hyperpigmentation along Blaschko lines and typically involves both the trunk and extremities or just the trunk. The distribution does not usually correspond to prior inflammatory or verrucous lesions. This stage begins within the first few weeks to months of life and may last for years, often resolving spontaneously by adolescence or adulthood.

Less than half of patients exhibit stage 4, with atrophic, hypopigmented, or hypovascular streaks lacking adnexa (hair follicles or sweat glands). These are often located on the posterior lower extremities and may be subtle and thus underreported. The atrophic lesions are persistent and may be the only cutaneous finding in adulthood (Berlin et al., 2002).

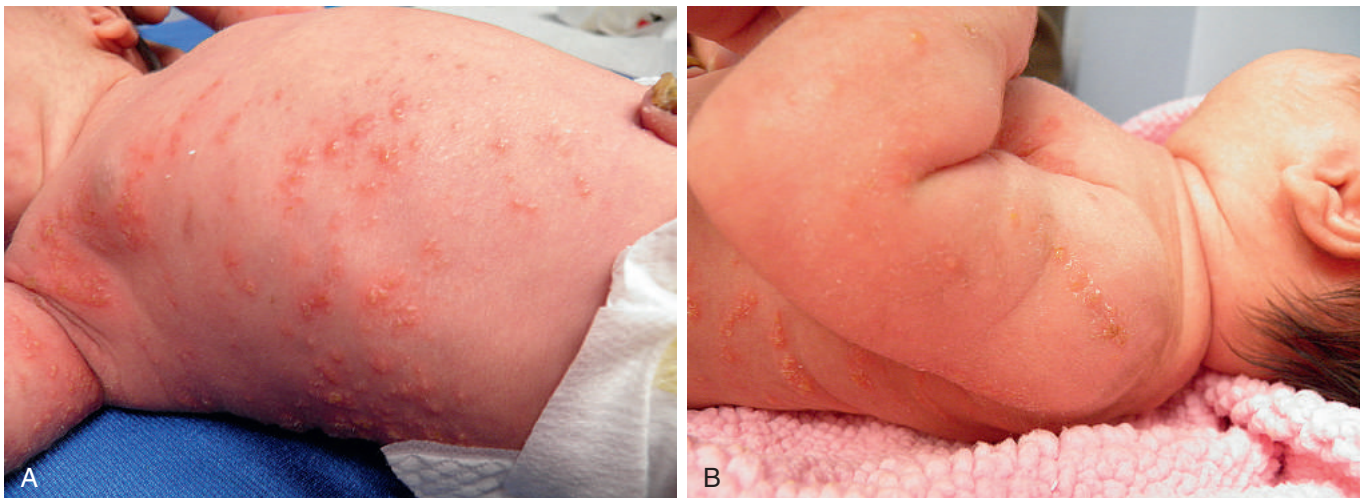
Patients with IP may have variable alopecia (especially of vertex of scalp) and nail dystrophy.

Extracutaneous Findings

Dental anomalies are the most common extracutaneous manifestation of IP and may affect both deciduous and permanent teeth. Ocular abnormalities (both retinal and nonretinal) are highly variable but can be vision threatening and usually present by 1 to 2 years of age. CNS involvement is estimated to occur in about 30% of patients with IP and may include seizures, spastic paralysis, or developmental delay. Rare breast, cardiac, or skeletal abnormalities have been reported.

Diagnosis

The diagnosis of IP can often be made on the basis of clinical findings (Landy and Donnai, 1993) and confirmed by genetic testing for mutations in the *IKBKG* gene. A biopsy of cutaneous lesions in stage 1 will reveal characteristic infiltration of the epidermis by eosinophils with intraepidermal vesicles and dyskeratosis. Stage



• **Fig. 104.9** Newborn with incontinentia pigmenti who exhibited extensive vesiculobullous lesions on the trunk (A) and extremities (B), some following Blaschko lines.

1 may also be associated with a striking peripheral leukocytosis and eosinophilia. These findings can help differentiate vesiculobullous or pustular lesions of IP from infectious causes (such as herpes, varicella, or bullous impetigo), Langerhans cell histiocytosis, EB, autoimmune bullous diseases, bullous mastocytosis, or child abuse. Histopathology of stage 2 lesions shows papillomatosis, hyperkeratosis, and dyskeratosis; however, these same findings can be seen in epidermal nevi. Skin biopsy findings from stage 3 or stage 4 lesions are nonspecific.

Prognosis and Treatment

The cutaneous findings in IP, other than stage 4 atrophic or hypopigmented lesions, resolve spontaneously. Vesiculobullous lesions may require dressings or wound care, with monitoring for superinfection. All patients with suspected IP should have a thorough ophthalmology evaluation soon after birth. Careful neurologic examination is essential, and neuroimaging and electroencephalographic studies should be performed in patients with signs of CNS involvement. Dental evaluations should begin by age 2 years (Berlin et al., 2002).

Focal Dermal Hypoplasia (Goltz Syndrome)

Focal dermal hypoplasia, also known as *Goltz syndrome*, is a rare condition with skin, skeletal, ocular, and other manifestations. It is an X-linked dominant disorder caused by mutations in the *PORCN* gene and predominantly affects females (90%) as mutations are often lethal in males. Cutaneous lesions are usually present at birth, including linear streaks of dermal hypoplasia associated with telangiectasias or pigmentary changes that follow Blaschko lines, yellow to red-brown nodules or outpouching caused by herniation of fat through hypoplastic dermis, and ulcers caused by congenital absence of skin (aplasia cutis). Erythematous papillomas affecting the skin or mucosa may appear later. Nail hypoplasia or ridging, alopecia, and dental abnormalities are common. Skeletal manifestations include craniofacial findings and limb malformations such

as ectrodactyly or syndactyly. X-ray of long bones reveals longitudinal striations of the metaphyses (osteopathia striata). A number of developmental abnormalities of the eye have been associated with focal dermal hypoplasia. Other developmental anomalies, including congenital diaphragmatic hernia, have been described. Most individuals have normal development, but developmental delay has been reported. Treatment of affected individuals requires a multidisciplinary approach (Sutton and Van den Veyver, 2016; Bostwick et al., 2016).

Ehlers–Danlos Syndrome

Ehlers–Danlos syndrome (EDS) is a heterogeneous group of disorders with variable manifestations of cutaneous elasticity, joint hypermobility, and tissue fragility. The revised Villefranche classification from 1997 is still widely used (Table 104.9), but new variants and advances in molecular classification have since been described (Beighton et al., 1998; De Paepe and Malfait, 2012). Most forms of EDS are caused by mutations in genes encoding fibrillar collagens or enzymes that modify these collagens. Premature rupture of membranes is more common in infants with EDS or in pregnancies of affected mothers.

The most common type is classic EDS. These individuals have soft, velvety skin with a doughy texture that is hyperextensible but returns to the normal position after being stretched (in contrast to cutis laxa, discussed later) (Fig. 104.10A). The skin is also fragile with a tendency to form widened, atrophic scars often referred to as *fish-mouth* or *cigarette-paper scars*. Vessel fragility leads to easy bruising (see Fig. 104.10B). Hematomas can become fibrotic and may result in soft subcutaneous nodules referred to as *pseudotumors* or hard calcified nodules referred to as *spheroids*. Classic EDS is also characterized by joint hypermobility and resulting complications (Sobey, 2015).

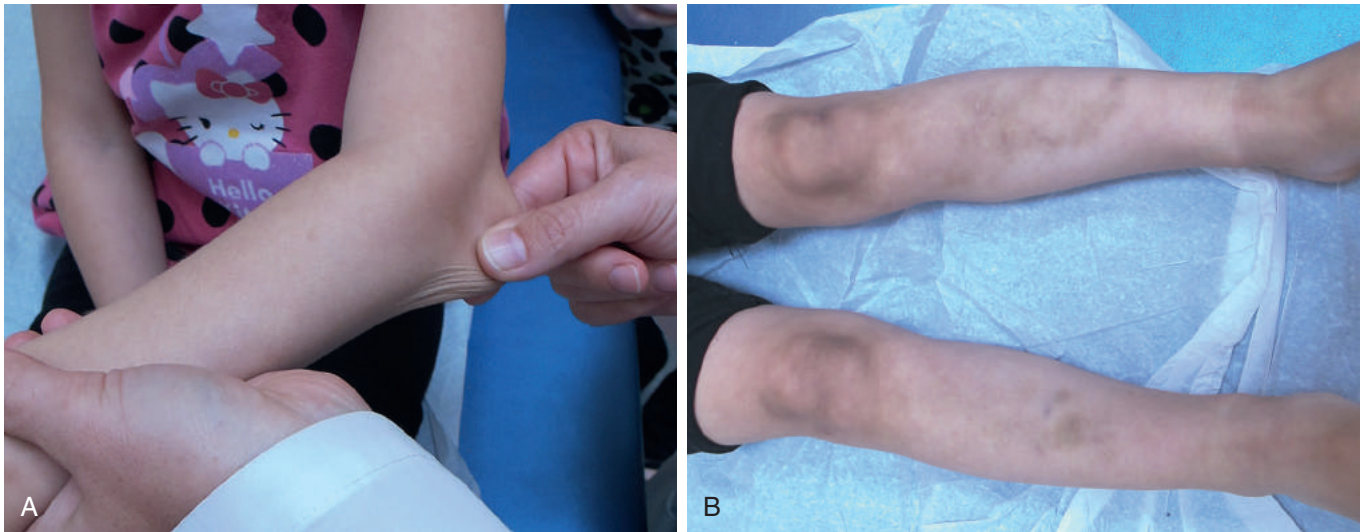
The vascular type has the most severe prognosis as there is risk of life-threatening arterial or organ rupture. The cutaneous findings include thin, translucent skin and extensive bruising but not skin hyperextensibility. Joint hypermobility usually affects just the digits.

TABLE
104.9

Types of Ehlers–Danlos Syndrome in the Revised Villefranche Classification (Beighton et al., 1998)

| Type | Inheritance | Gene(s) | Cutaneous Features | Extracutaneous Features |
|-----------------|-------------|---|--|--|
| Classic | AD | <i>COL5A1</i> , <i>COL5A2</i> , <i>COL1A1</i> | Hyperextensibility, fragility, widened atrophic scars, easy bruising, pseudotumors, calcified subcutaneous nodules (spheroids) | Joint hypermobility |
| Hypermobile | AD | Unknown | Velvety skin, mild hyperextensibility | Joint hypermobility |
| Vascular | AD | <i>COL3A1</i> | Thin translucent skin, easy bruising, early varicosities, acrogeria (premature aged appearance) | Arterial dissection, aneurysm, and rupture; organ rupture; typical facies; pneumothoraces; joint hypermobility limited to digits |
| Kyphoscoliosis | AR | <i>PLOD1</i> | Hyperextensibility, fragility, widened atrophic scars, easy bruising | Congenital progressive kyphoscoliosis; hypotonia; gross motor delay; joint hypermobility; scleral fragility; vascular rupture |
| Arthrochalasia | AD | <i>COL1A1</i> , <i>COL1A2</i> | Hyperextensibility, with or without atrophic scars, easy bruising | Severe joint hypermobility; congenital bilateral hip dislocation; hypotonia; gross motor delay; dysmorphic features |
| Dermatosparaxis | AR | <i>ADAMTS2</i> | Severe fragility, redundant sagging skin | Congenital skull fractures; blue sclera; umbilical hernia; delayed fontanelle closure; short stature |

AD, Autosomal dominant; AR, autosomal recessive.



• **Fig. 104.10** Three-year-old girl with classic Ehlers-Danlos syndrome characterized by hyperextensible skin (A) and easy bruising (B).

Vascular EDS must be differentiated from other subtypes of EDS and Loeys-Dietz syndrome, an autosomal dominant condition caused by mutations in genes affecting transforming growth factor β signaling that is characterized by joint hypermobility, translucent velvety skin, and widespread vascular tortuosity with a high risk of aneurysms and dissections (MacCarrick et al., 2014).

The dermatosparaxis type is a very rare but severe phenotype that often presents at birth and has been associated with congenital skull fractures and skin lacerations (Solomons et al., 2013).

Cutis Laxa

Cutis laxa can be congenital or acquired. Inherited forms may be autosomal recessive, autosomal dominant, or X-linked, and mutations in at least nine different genes have been reported to cause variants of cutis laxa. The autosomal dominant form is caused by mutations in the *ELN* gene, which encodes elastin, and other genes that affect proteins involved in production of elastic fibers. The skin of patients with cutis laxa is characterized by loose, redundant, hypoelastic skin that does not recoil after stretching. The cutaneous findings are often evident at birth in autosomal recessive cutis laxa. Elastic fibers in extracutaneous sites can also be affected, resulting in varying clinical phenotypes depending on the genetic mutation (Berk et al., 2012).

The X-linked form of cutis laxa, once classified as a type of EDS, is also referred to as *occipital horn syndrome*. This disorder is caused by mutations in the *ATP7A* gene leading to impaired copper metabolism and is allelic with Menkes disease. Cutaneous manifestations of these allelic conditions may include soft, doughy skin and hypopigmented, fragile, kinky hair (pili torti).

Ectodermal Dysplasias

The ectodermal dysplasias comprise a heterogeneous group of more than 200 inherited conditions characterized by congenital defects in one or more ectodermal structures and appendages: hair, teeth, nails, and eccrine (sweat) glands. Many of these syndromes are associated with nonectodermal abnormalities (Itin, 2014; Pagnan and Visinoni, 2014). The classification of ectodermal dysplasias is evolving with elucidation of the genetics and molecular pathways

involved (Salinas et al., 2014). In 2009 a new classification scheme for ectodermal dysplasias broadly classified them into two groups on the basis of molecular data and clinical phenotype. Group 1 ectodermal dysplasias are caused by aberrant signaling between the ectoderm and mesenchyme, leading to abnormal tissue differentiation and development of ectodermal structures. The most common pathways affected are the tumor necrosis factor/tumor necrosis factor receptor and p63 pathways, and the most common phenotype is hypohidrotic ectodermal dysplasia (HED). In group 2 ectodermal dysplasias, differentiation of embryonic tissues is normal, and downstream defects in gene expression or transcription are the cause of ectodermal anomalies. The most common group 2 ectodermal dysplasia is Clouston syndrome (Paller, 2011).

Characteristic dysmorphic features and hypotrichosis are often the earliest recognized signs of ectodermal dysplasia. Temperature dysregulation is a common complication of hypohidrosis (reduced sweating) and may lead to recurrent fevers of unknown origin. The clinical features of the ectodermal dysplasias are diverse. The most common or notable forms are summarized in Table 104.10.

Hypohidrotic Ectodermal Dysplasia

HED is the most common type of ectodermal dysplasia and most often results from an X-linked recessive mutation of the ectodysplasin A gene (*EDA*). The classic triad of associated clinical features is hypohidrosis, alopecia, and hypodontia (reduced number of teeth). In X-linked HED, males are hemizygous for the *EDA* mutation and generally display the complete triad, as well as a propensity for respiratory disorders. Most are identified within the first 2 years of life. Females are typically heterozygous for the *EDA* mutation. Their presentation may be subtle and diagnosis delayed.

Infants with X-linked HED may exhibit failure to thrive, nasal congestion, decreased saliva production, and gastroesophageal reflux. Eczema usually presents during the first year of life. Males are more likely to suffer from respiratory problems, including chronic, malodorous nasal congestion, wheezing, and recurrent sinusitis (Fete et al., 2014).

HED with immunodeficiency (HED-ID) is most commonly caused by mutations in the *IKBKKG* gene on chromosome band Xq28. Female carriers of more severe mutations in this same gene

have IP, as discussed earlier. Patients with HED-ID usually present with recurrent infections.

Management of Ectodermal Dysplasias

Management of ectodermal dysplasia hinges on establishing the diagnosis, referral to appropriate specialists on the basis of symptoms

and known associations, and supportive measures. Early involvement of a geneticist is important, and ideally molecular confirmation of the diagnosis is obtained. For children with hypohidrotic forms of ectodermal dysplasia, avoidance of overheating is paramount. Eye drops and nasal irrigation should be used to lubricate the ocular and respiratory mucosa. Dental assessment should be performed early in life as intervention may be initiated before age

TABLE 104.10 Ectodermal Dysplasias (Paller, 2011)

| Syndrome | Inheritance | Gene/Protein | Clinical Features |
|--|-------------|--|--|
| Group 1 | | | |
| Tnf/Tnfr Pathway | | | |
| Hypohidrotic ectodermal dysplasia | XLR | <i>EDA</i> /ectodysplasin A (most common) | <ul style="list-style-type: none"> • Mostly males • Hypohidrosis • Hypotrichosis • Dental anomalies |
| | AD, AR | <i>EDAR</i> /ectodysplasin A receptor | <ul style="list-style-type: none"> • Periorbital hyperpigmentation and dermatitis • Atopic dermatitis |
| | AD, AR | <i>EDARADD</i> /ectodysplasin A receptor–associated death domain | <ul style="list-style-type: none"> • Facies: square forehead, frontal bossing, large nostrils, wide cheekbones, flat malar ridge, thick, everted lower lip; prominent chin; small, pointed, low-set ears • Decreased salivary, lacrimal gland production • Recurrent fevers |
| Nuclear Factor κB Inhibitors | | | |
| Hypohidrotic ectodermal dysplasia with immunodeficiency | XLR | <i>IKBKG</i> /nuclear factor κ B essential modulator | <ul style="list-style-type: none"> • Same as other hypohidrotic ectodermal dysplasias (see above) • Immunodeficiency • Recurrent pulmonary infections |
| | AD | <i>NFKBIA</i> | <ul style="list-style-type: none"> • Inflammatory colitis |
| Transcription Factors | | | |
| | | | <p>p63-Related Unifying Features</p> <ul style="list-style-type: none"> • Orofacial clefting • Midface or maxillary hypoplasia • Limb malformation • Dry, itchy, hypopigmented skin • Sparse, wiry hair • Dystrophic nails • With or without hypohidrosis • Failure to thrive • Hypospadias • Short stature <p>Distinguishing Features</p> |
| Rapp–Hodgkin syndrome | AD | <i>TP63</i> | <ul style="list-style-type: none"> • Narrow dysplastic nails • Conical teeth, hypodontia • Hearing loss, recurrent otitis media • Hyperthermia in early childhood |
| Ankyloblepharon–ectodermal dysplasia–clefting (Hay–Wells syndrome) | AD | <i>TP63</i> | <ul style="list-style-type: none"> • Ankyloblepharon (congenital fusion of the eyelids) • Skin erosions • Conical teeth, hypodontia • Cardiac anomalies |
| Ectrodactyly–ectodermal dysplasia–cleft lip/palate syndrome | AD | <i>TP63</i> | <ul style="list-style-type: none"> • Ectrodactyly (split hand/foot deformity), syndactyly • Dental caries • Hearing loss, small or malformed auricles • Renal agenesis • Endocrine disorders |
| Trichodontoosseous syndrome | AD | <i>DLX3</i> | <ul style="list-style-type: none"> • Hair: kinky, curly hair at birth • Teeth: small, widely spaced, pitted, eroded, discolored; early caries • Nails: thick, brittle • Facies: dolichocephaly, frontal bossing, square jaw • Increased bone density |
| Trichodental (Witkop) syndrome | AD | <i>MSX1</i> | <ul style="list-style-type: none"> • Hair: fine, sparse or normal • Teeth: small • Nails: slow-growing, small, spoon-shaped nails |
| Ellis van Creveld syndrome | AR | <i>LBN, EVC</i> | <ul style="list-style-type: none"> • Nail dysplasia • Polydactyly • Partial cleft lip • Neonatal teeth, hypodontia • Short limbs, skeletal anomalies |

Continued

TABLE 104.10 Ectodermal Dysplasias (Paller, 2011)—cont'd

| Syndrome | Inheritance | Gene/Protein | Clinical Features |
|---|-------------|--------------------------|---|
| Group 2 | | | |
| Clouston syndrome (hidrotic ectodermal dysplasia) | AD | <i>GJB6</i> /connexin 30 | <ul style="list-style-type: none"> • Most common hidrotic ectodermal dysplasia • French-Canadian families • Nails: dystrophic • Hair: normal at birth; becomes sparse, brittle • Teeth prone to caries • Ocular abnormalities • Hyperkeratosis of palms, soles, knees, elbows, knuckles |
| Oculodentodigital dysplasia | AD | <i>GJA1</i> /connexin 43 | <ul style="list-style-type: none"> • Curly hair, sometimes with trichorrhexis nodosa • Teeth: small, many caries • Cleft lip and palate • Ocular anomalies • Fourth and fifth finger syndactyly • Focal keratoderma • Hypoplastic nasal ala • Neurologic, hearing deficits • Cardiac abnormalities |
| Clefting–ectodermal dysplasia | AD | <i>NECTIN1</i> | <ul style="list-style-type: none"> • Nails: spoon shaped, slow growing • Hair: pili torti • Mental retardation • Malformed ears • Partial syndactyly |

AD, Autosomal dominant; AR, Autosomal recessive; TNF, tumor necrosis factor; TNFR, tumor necrosis factor receptor; XLR, X-linked recessive.

2 years. Ectodermal dysplasia associated with immunodeficiency may be treated with hematopoietic stem cell transplant, and more targeted molecular therapies may be available in the future (Paller, 2011).

Disorders With Generalized Hypopigmentation

Oculocutaneous albinism (OCA) is characterized by generalized hypopigmentation of the skin, hair, and eyes, with additional ocular abnormalities. OCAs are classified into “classic” nonsyndromic types and syndromic forms with additional systemic manifestations. There are seven identified forms of nonsyndromic OCA, each associated with a specific gene mutation. The implicated gene mutations affect proteins integral to melanin production or distribution. Severity of pigment loss and ocular manifestations differ depending on the OCA type and mutation severity. For example, the most severe type of OCA, OCA type 1A, is caused by a nonsense mutation in the tyrosinase gene, resulting in complete absence of melanin production. OCA type 1B is caused by a missense mutation in the same gene, allowing various amounts of melanin production. OCA types 1 to 4 account for most cases, and their pathogenesis and clinical features are summarized in Table 104.11 (Kamaraj, 2014).

Hermansky–Pudlak, Chédiak–Higashi, and Griscelli syndromes are syndromic forms of OCA. Hermansky–Pudlak syndrome is most commonly observed in the Puerto Rican population. In addition to OCA, patients have abnormal platelet function and are prone to bleeding. The intestines, lungs, kidneys, and heart may also be affected. Patients with Chédiak–Higashi syndrome

have impaired phagocytosis and are prone to pyogenic infections. Griscelli syndrome is associated with neurologic or immunologic abnormalities. Patients with metabolic disease may also present with generalized hypopigmentation. For example, those with Menkes disease are unable to metabolize copper and therefore lack this essential cofactor for tyrosinase activity and melanin production (Montoliu et al., 2014).

Diagnosis of Oculocutaneous Albinism

In patients with severe forms of OCA the diagnosis is often clinically apparent during the neonatal period. However, presentation may be subtle in patients with some melanin production, such as those with OCA types 2 and 3 born to darker-skinned parents. Comparison of skin, hair, and eye color with that of other family members may be helpful. Genetic testing performed at specialized centers currently identifies causative mutations in approximately 80% of OCA patients (Montoliu et al., 2014).

Treatment of Oculocutaneous Albinism

Treatment of OCA is geared toward addressing visual impairment and preventing sun-induced carcinogenesis. Early referral to and regular monitoring by an ophthalmologist is critical. Vigilant sun avoidance and protection, with use of sunscreen, UV-protective clothing, and eyewear, is essential. Sunscreens with physical blockers—namely, zinc oxide and titanium dioxide—are generally considered to be safest in infants and children. Clinical trials of medications designed to enhance melanin production in patients with albinism and related disorders are currently under way (Kamaraj, 2014).

TABLE 104.11 Congenital Disorders With Generalized Loss of Skin Pigmentation (Kamaraj and Purohit, 2014; Montoliu et al., 2014; Que et al., 2015)

| Syndrome | Pathogenesis | Clinical Features |
|---------------------------|---|--|
| OCA types 1A and 1B | AR mutation in the tyrosinase (<i>TYR</i>) gene, which codes for the key enzyme in melanin production | <ul style="list-style-type: none"> Most common OCA type in white Europeans Complete loss of (OCA type 1A) or significantly reduced (OCA type 1B) pigmentation: white or very light skin and hair Ocular: pink irides at birth become blue gray in adulthood, reduced visual acuity, strabismus, photophobia, nystagmus, misrouting of the optic nerves at the chiasm, foveal hypoplasia |
| OCA type 2 (brown OCA) | AR mutation in the <i>OCA2</i> gene, which codes for melanocyte-specific transporter protein, affecting melanin production and distribution | <ul style="list-style-type: none"> Most common OCA type in equatorial Africans Minimal to moderate skin pigmentation Hair white, golden, or red Irises translucent blue to light brown Ocular: similar to OCA type 1 |
| OCA type 3 (rufous OCA) | AR mutation in the <i>TYRP1</i> gene, leading to destabilization and compromised function of tyrosinase | <ul style="list-style-type: none"> Most common OCA type in southern Africans Skin red, freckled, or hypopigmented Reddish hair Light eyes Ocular: nystagmus, strabismus, positive red reflex Can be subtle: comparison with parents can aid in diagnosis |
| OCA type 4 | AR mutation in the <i>SLC24A5</i> gene, which may impair transport of substances integral to melanin synthesis | <ul style="list-style-type: none"> Most common in Japanese Light skin and hair Translucent irides Ocular: mild nystagmus and photophobia, decreased visual acuity, absent macula |
| Hermansky–Pudlak syndrome | AR mutation in the <i>HPS1</i> , <i>AP3B1</i> , <i>HPS3</i> , <i>HPS4</i> , <i>HPS5</i> , <i>HPS6</i> , <i>DTNBP1</i> , <i>BLOC1S3</i> , or <i>BLOC1S6</i> gene; these genes play a role in lysosome-related organelles | <ul style="list-style-type: none"> OCA, similar to OCA type 1 Abnormal platelets Easy bruising and bleeding Pulmonary fibrosis Cardiomyopathy Intestinal inflammation and hemorrhage Renal failure Most common in Puerto Ricans |
| Chédiak–Higashi syndrome | AR mutation in the <i>LYST</i> (<i>CHS1</i>) gene, which codes for lysosomal trafficking regulator protein, leading to defective lysosomal trafficking and phagocytosis | <ul style="list-style-type: none"> Variable skin hypopigmentation Ocular: similar to OCA type 3 Gingivitis Hepatosplenomegaly Recurrent pyogenic infections Progressive neurologic decline |
| Griselli syndrome | AR mutation in the <i>MYO5A</i> , <i>RAB27A</i> , or <i>MLPH</i> gene, leading to defective melanosome transport within melanocytes | <ul style="list-style-type: none"> Partial albinism, with hypopigmented skin, silver-gray hair Visual problems Neurologic impairment Immunodeficiency, hemophagocytic syndrome |
| Menkes disease | XLR mutation in the <i>ATP7A</i> gene (copper transporter), leading to impaired copper absorption and metabolism | <ul style="list-style-type: none"> “Doughy” inelastic, hypopigmented skin Hairs short, sparse, twisted (pili torti) Microcephaly, brachycephaly, pudgy cheeks Small stature Neurodegeneration: hypotonia, seizures, hypothermia Failure to thrive Impaired vision |

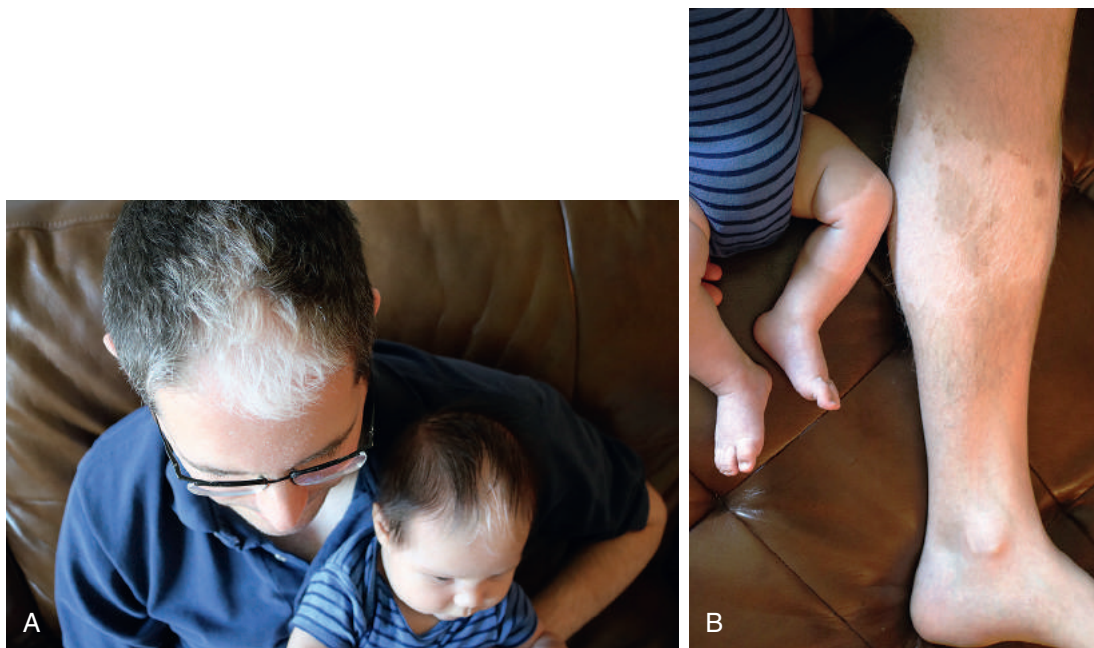
AD, Autosomal dominant; AR, autosomal recessive; OCA, oculocutaneous albinism; XLR, X-linked recessive.

Disorders With Localized Hypopigmentation

Piebaldism

Piebaldism is an autosomal dominant disorder characterized by circumscribed areas of leukoderma (absence of skin pigment). The anterior hairline is affected in 80%–90% of cases, resulting in a

white forelock (poliosis circumscripta) (Fig. 104.11A). This is frequently the sole manifestation (Grob and Grekin, 2016). Well-demarcated depigmented patches with islands of normal pigmentation and hyperpigmentation are typically present at birth and remain stable through adulthood (see Fig. 104.11B), although a few cases of spontaneous repigmentation have been reported (Frances et al., 2015). Characteristically, the ventral body is more affected,



• **Fig. 104.11** Father and son with piebaldism manifesting itself as white forelock (A) and well-demarcated depigmented patches with islands of normal pigmentation and hyperpigmentation (B).

particularly the central forehead, anterior trunk, arms, and legs. The dorsal midline, hands, feet, and periorificial regions are typically spared. Diagnosis is clinical and can be confirmed with molecular analysis of the *KIT* gene. Skin biopsy of depigmented areas is not necessary but when performed reveals absent or markedly reduced numbers of melanocytes. In the neonatal period, management of piebaldism hinges on vigilant photoprotection of amelanotic skin. Camouflaging makeup may be used for cosmetic purposes. Surgical and laser treatments are evolving and may be considered later in life.

The differential diagnosis for an infant with localized loss of skin or hair pigmentation includes disorders with systemic complications such as Waardenburg syndrome and tuberous sclerosis (Table 104.12) as well as nonsyndromic pigmentary anomalies. *Hypomelanosis of Ito* is a historical term used to describe linear and whorled hypopigmentation along Blaschko lines that may be an isolated cutaneous finding or associated with extracutaneous abnormalities, including neurologic or musculoskeletal conditions. Hypomelanosis of Ito is no longer considered a distinct entity but rather encompasses a group of disorders associated with various mosaic defects, and many prefer the term *pigmentary mosaicism* to describe the associated cutaneous findings. Other terms have been used to describe congenital hypopigmentation or depigmentation, including *patterned dyspigmentation*, *segmental pigmentation disorder*, and *nevus depigmentosus*. Congenital vitiligo has also been rarely reported. In any patient born with localized hypopigmentation or depigmentation, a thorough history, family history, and physical examination should be conducted, with further work-up directed by any abnormalities found (Lombillo and Sybert, 2005; Hogeling and Frieden, 2010).

Porphyrias

The porphyrias are a group of disorders caused by mutations in enzymes involved in heme biosynthesis that result in accumulation

of porphyrin or porphyrin precursors. Most porphyrias are autosomal dominant, but some are autosomal recessive, X-linked, or have more complex inheritance patterns. Cutaneous manifestations are due to the photosensitizing effects of porphyrins. Phototoxicity can be immediate with burning pain, erythema, and edema and/or delayed with blisters, dyspigmentation, and scarring. Traditionally, the porphyrias have been classified on the basis of the organ in which porphyrins or porphyrin precursors accumulate as erythropoietic (accumulation in bone marrow erythroid cells), hepatic (accumulation in liver), or mixed (i.e., hepatoerythropoietic porphyria). The porphyrias may be also categorized on the basis of primary symptoms, with “acute porphyrias” predominated by neurovisceral attacks and “cutaneous porphyrias” predominated by skin photosensitivity (Balwani and Desnick, 2012).

The erythropoietic porphyrias (congenital erythropoietic porphyria [CEP], erythropoietic protoporphyria [EPP], X-linked EPP [XLP]) and hepatoerythropoietic porphyria (HEP) can present in early infancy with severe photosensitivity and are reviewed in the following sections. Box 104.1 lists disorders that can present with photosensitivity in infancy. Phototherapy-induced eruptions because of transient porphyrinemia may be seen in neonates with hemolytic disease of the newborn (By et al., 2014; Villanueva et al., 2016).

The hepatic porphyrias are categorized by neurovisceral attacks, with variable cutaneous photosensitivity, and usually present later in childhood or adulthood. Exceptions include homozygous variants of hereditary coproporphyria, variegate porphyria, acute intermittent porphyria, and aminolevulinic acid dehydratase porphyria, which are exceedingly rare but can present in infancy.

Congenital Erythropoietic Porphyria

CEP, also known as *Günther disease*, is an autosomal recessive erythropoietic cutaneous porphyria caused by mutations in the gene encoding uroporphyrinogen III synthase. The disease is

TABLE 104.12**Syndromes With Congenital Localized Loss of Skin Pigmentation (Que et al., 2015; Grob and Grekin, 2016)**

| Syndrome | Pathogenesis | Clinical Features |
|----------------------|---|--|
| Piebaldism | AD mutation in the <i>KIT</i> proto-oncogene, affecting melanoblast migration, proliferation, differentiation, and survival | <ul style="list-style-type: none"> White forelock (poliosis circumscripta) Depigmented macules and patches (leukoderma) Heterochromic irides Rare: Hirschsprung disease, deafness |
| Waardenburg syndrome | AD mutation in one of the following genes: <i>PAX3</i> , <i>MITF</i> , <i>SOX10</i> , <i>EDN3</i> , or <i>EDNRB</i> , which all affect melanocyte development | <ul style="list-style-type: none"> White forelock Early graying (by age 35 years) Heterochromic, bichromic, or bright blue irises Depigmented macules or patches Congenital sensorineural deafness Bony anomalies Facies: dystopia canthorum, synophrys, broad nasal root, nose hypoplasia, smooth or shortened philtrum Hirschsprung disease (type 4) |
| Tuberous sclerosis | AD mutation in tumor suppressor genes <i>TSC1</i> and <i>TSC2</i> , leading to unregulated cell proliferation and formation of hamartomas in multiple organ systems | <ul style="list-style-type: none"> Hypomelanotic ash leaf spots, “confetti-like” hypomelanotic macules Facial angiofibromas Collagenomas/shagreen patches Forehead fibrous plaques Periungual fibromas Neurologic: cortical tubers, subependymal nodules or astrocytomas, developmental delay, seizures, infantile spasms, behavioral disorders Other: retinal achromic patch or hamartomas, renal angiomyolipomas or cysts, cardiac rhabdomyomas, lymphangioleiomyomatosis, etc. |
| Pigmentary mosaicism | Variable genetic mosaicism | <ul style="list-style-type: none"> Hypopigmented patches, whorls, or streaks along Blaschko lines Associated with various neurologic, ocular, dental, or musculoskeletal abnormalities or isolated cutaneous finding |

AD, Autosomal dominant.

• BOX 104.1 Disorders That Present With Photosensitivity in Infancy

Porphyrrias
 Transient porphyrinemia
 Xeroderma pigmentosum
 Cockayne syndrome
 Bloom syndrome
 Rothmund–Thomson syndrome
 Smith–Lemli–Opitz syndrome
 Hartnup disease
 Neonatal lupus erythematosus
 Phototoxic or photoallergic reaction

characterized by severe photosensitivity and hemolytic anemia with splenomegaly. CEP may present in utero as nonimmune hydrops fetalis. Severe photosensitivity usually begins in infancy and can sometimes present in the neonatal period with blistering after phototherapy for hyperbilirubinemia. Scarring, hyperpigmentation, hypertrichosis, and disfigurement develop in photoexposed areas over time (Fig. 104.12). There are variable ocular manifestations. Urine may be faint pink to dark red from accumulation of uroporphyrin. The teeth also develop a reddish-brown color (erythrodonia). Teeth, urine, and stool may fluoresce under Wood lamp examination.

The diagnosis of CEP can be made on the basis of elevated uroporphyrin and coproporphyrin levels in erythrocytes, urine, and stool and can be confirmed by measurement of uroporphyrinogen III synthase activity or gene mutation analysis. Historically, CEP has been managed primarily with photoprotection and long-term transfusions. More recently, CEP has been cured with hematopoietic stem cell transplant, but transplant complications are not insignificant, including death, and thus may not be appropriate for all patients with CEP (Katugampola et al., 2012).

Erythropoietic Protoporphria and X-Linked Erythropoietic Protoporphria

EPP is the most common form of porphyria in childhood and is caused by autosomal recessive mutations in the gene encoding ferrochelatase. XLP, also referred to as *X-linked dominant protoporphyria*, is an X-linked form that results from mutations in the *ALAS2* gene, the product of which catalyzes the first committed step of heme biosynthesis (Whatley et al., 2008). XLP has a phenotype very similar to that of EPP but with higher concentrations of erythrocyte protoporphyrin and a higher incidence of liver disease (Seager et al., 2014).

Clinical manifestations of EPP include immediate painful photosensitivity to sunlight and sometimes fluorescent lighting. In infancy, this may manifest itself as episodes of crying within minutes of UV exposure, and older children may complain of stinging or a burning sensation in exposed areas. Prolonged exposure



• **Fig. 104.12** Two-year-old male with congenital erythropoietic porphyria who has bullae, crusted erosions, hyperpigmentation, and hypertrichosis in sun-exposed areas. He initially exhibited blistering after phototherapy for hyperbilirubinemia as a neonate.

may lead to erythema, edema, and petechiae and vary rarely vesicles or bullae. Long-term UV exposure results in shallow atrophic scars and thickened, leathery skin around the mouth (pseudorhagades) and overlying knuckles.

Hepatic involvement can range from mild liver dysfunction to rare liver failure. Cholelithiasis may cause severe abdominal pain. Anemia is usually mild or absent.

Diagnosis of EPP and XLP can be made on the basis of elevated erythrocyte protoporphyrin concentration, and red blood cells will fluoresce under Wood lamp examination. Increased stool protoporphyrin concentration may also be detected. The diagnosis is confirmed by genetic testing. Management in infancy includes sun avoidance and protection, with symptomatic treatment of photosensitivity reactions. Patients should be monitored for liver disease and microcytic anemia (Lecha et al., 2009; Balwani and Desnick, 2012).

Hepatoerythropoietic Porphyria

HEP is an exceedingly rare disorder caused by homozygous or compound heterozygous mutations in the gene encoding uroporphyrinogen decarboxylase. Heterozygous mutations or sporadic mutations in the same gene result in porphyria cutanea tarda, the most common porphyria in adults. HEP usually presents with severe bullous photosensitivity before age 2 years, with subsequent hypertrichosis and scarring, resembling CEP. The diagnosis can be made on the basis of highly elevated erythrocyte zinc protoporphyrin concentration, along with elevated urine and stool porphyrin concentrations, and can be confirmed by genetic testing (Liu et al., 2013).

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KEY POINTS

- Owing to their cutaneous, immunologic, and renal immaturity, newborns (especially premature neonates) are at increased risk of infection.
- As a group of potentially life-threatening but often treatable diseases, infections must always be considered in a newborn with skin lesions.
- Prompt diagnosis and initiation of therapy are crucial to prevent devastating long-term sequelae, particularly in instances of disseminated disease.
- *Staphylococcus aureus*, *Streptococcus* species, *Candida albicans*, and herpes simplex virus are the most common causes of skin infections in the neonate.

Staphylococcus aureus Infections

Staphylococcus aureus is ubiquitous and harbored as a commensal organism in about one-third of the population, with a predilection for the nares (especially in children), perineum, and other moist cutaneous surfaces (Chen and Tsao, 2013). It is more likely to be present early if the child is born via cesarean delivery rather than vaginally, reflecting colonization via the bacterial microbiota of adult skin (Dominguez-Bello et al., 2010). However, by 1 week of age, most newborns will exhibit nasopharyngeal or umbilical colonization by *S. aureus*.

Invasive infection of infants by *S. aureus* remains a healthcare challenge. A retrospective study of staphylococcal infections in 348 neonatal intensive care units (NICUs) demonstrated that the incidence of invasive *S. aureus* infection (defined as a positive culture result from blood, cerebrospinal fluid [CSF], sterile fluid, or an abscess) was 44.8 per 10,000 infants. Most of these infections were caused by methicillin-sensitive strains (Ericson et al., 2015). Cutaneous signs of invasive *S. aureus* infection are mediated by local or circulating bacterial toxins; *S. aureus* is therefore responsible for skin lesions of protean morphology (Neylon et al., 2010).

Impetigo

Impetigo is a group of superficial skin infections caused by *S. aureus*, group A streptococcus (GAS), or both. Neonatal bullous impetigo is caused by *S. aureus* and can occur in nursery-based, epidemic patterns, often attributed to nasal carriage of *S. aureus*.

Clinical Findings

Impetigo is one of the most common neonatal skin infections. Bullous impetigo manifests itself as flaccid vesicles/bullae or pustules on an erythematous base, most often seen in the diaper area, periumbilically, or in skin folds (Fig. 105.1). Ruptured lesions leave behind a moist red base with a characteristic collarette of scale (Darmstadt and Lane, 1994; Hartman-Adams et al., 2014). Lesions of bullous impetigo are usually not closely grouped, which distinguishes this infection from herpes simplex virus (HSV) infection. Aggressive cases of *S. aureus* bullous impetigo may also present as widespread desquamation in a degloving pattern (Nguyen et al., 2016).

In contrast, nonbullous impetigo is characterized by thin-walled vesicles or pustules, often at sites of broken skin on the extremities or face. These lesions rapidly burst and assume their characteristic appearance of variably pruritic, eroded, red patches with a superimposed honey-colored crust. In both forms of impetigo, affected areas heal without scarring.

Etiology

S. aureus is the exclusive cause of bullous impetigo and the primary cause of nonbullous impetigo. GAS may also be associated with the nonbullous form. Bullous impetigo is mediated by local production of exfoliative toxin A (ETA) or B (ETB); these toxins cleave the desmosomal protein desmoglein 1 in the superficial layers of the epidermis. This loss of adhesion between keratinocytes results in characteristic flaccid blisters.

Diagnosis

Diagnosis is supported by the presence of gram-positive cocci in clusters on Gram stain of a swab from a pustule, vesicle, or crusted plaque of impetigo. Confirmation is made by bacterial culture taken from blood, skin, and soft tissues.

Treatment

Bullous impetigo is benign if treated early, although extensive local proliferation with exotoxin production or dissemination can be life threatening. Treatment should be instituted promptly and isolation maintained until lesions have ceased to spread, no longer have associated crusting, and have begun to reepithelialize. Infants should be closely monitored, and a high index of suspicion should be maintained for evidence of systemic disease. Infants with periumbilical lesions are at risk of bacterial omphalitis. Extremely limited infections may be treated with topical mupirocin, but this form of therapy should be used with caution in neonates. More extensive lesions require a systemically (most recommend



• **Fig. 105.1** Bullous Impetigo. Widespread superficial erosions with characteristic collarettes of scale in a periumbilical distribution.

parenterally) administered penicillinase-resistant antibiotic for 7 to 10 days. Antibiotic choice should ultimately be guided by sensitivities of cultured organisms, especially with the rising incidence of methicillin-resistant strains. In those cases, clindamycin, vancomycin, or linezolid would be considered.

Importantly, *S. aureus* outbreaks in nurseries continue to be documented, and it has been suggested that these epidemics may be underreported (Cimolai, 2003; Neylon et al., 2010; Paranthaman et al., 2014). These outbreaks may present as multiple cases of staphylococcal scalded skin syndrome (SSSS), pustulosis, bullous impetigo, abscesses, or other varied presentations of staphylococcal infection (Cimolai, 2003). Factors that have been proposed to increase the likelihood of staphylococcal outbreaks include crowded nurseries, poor adherence to hand washing by medical staff, inadequate umbilical cord care, nursery staff carriage of the organism, and lack of isolation early in the course of an outbreak. As such, universal hand-washing protocols, a reduction in overcrowding, increased nurse-to-patient ratio, and staff monitoring for carriage have all been proposed to mitigate the risk of nursery outbreaks. Aggressive monitoring of patients via active surveillance cultures and subsequent treatment of *S. aureus*-colonized neonates with topical mupirocin therapy and chlorhexidine baths have also been successful in limiting staphylococcal infections (Popoola et al., 2016).

Staphylococcal Scalded Skin Syndrome

In contrast to the localized effects of the exfoliative toxins that mediate bullous impetigo, SSSS is a generalized manifestation of circulating toxin produced by *S. aureus*. Early diagnosis and treatment of SSSS can be lifesaving.

Clinical Findings

SSSS is most common in full-term infants and young children, although cases of affected premature infants have also been reported



• **Fig. 105.2** Staphylococcal Scalded Skin Syndrome. Widespread erosions centered around the inguinal folds in a newborn. Note the diffuse background erythema.

(Kapoor et al., 2008; Arora et al., 2011). Affected infants demonstrate abrupt onset of temperature instability, lethargy, and irritability, with subsequent generalized skin tenderness and erythema. Initial erythema tends to occur on the face but subsequently becomes more marked in intertriginous areas (Li et al., 2014; Fig. 105.2). Facial swelling, conjunctivitis, and significant periorificial crusting is common; crusting accompanied by radial fissuring about the mouth gives afflicted children a characteristic appearance. Notably, mucosal surfaces are spared in SSSS. Following these initial signs and symptoms, focal or widespread flaccid bullae may develop within hours to days, with subsequent desquamation. This is easily elicited by light stroking of intact skin (Nikolsky sign). Following desquamation, affected areas appear as painful, shiny, denuded patches. At this stage, diffuse skin involvement may cause problems with thermoregulation, fluid and electrolyte balance, and superinfection (Neylon et al., 2010).

SSSS must be distinguished from toxic epidermal necrolysis (TEN), a life-threatening condition involving full-thickness epidermal necrosis, most commonly secondary to medication administration (Ladhani and Joannou, 2000). A distinguishing feature of these two conditions is the presence of mucosal blistering and hemorrhagic crusting in TEN.

Etiology

The signs and symptoms of SSSS are the result of circulating exfoliative toxins, produced from an often subclinical focus of *S. aureus* infection. Infants are thought to be more at risk of developing SSSS because of immunologic and renal immaturity, which allows excess accumulation of exfoliative toxin within the circulation. Specifically, significantly lower anti-exfoliative toxin antibodies have been noted in patients with SSSS compared with healthy controls (Saida et al., 2015). In contrast to bullous impetigo, which is more commonly associated with ETA, SSSS is more frequently

caused by ETB. Additionally, while *S. aureus* is identifiable in the blisters of bullous impetigo, in SSSS *S. aureus* is present at a primary distant site such as the nose, mouth, conjunctiva, umbilicus, or circumcision site. Fresh skin lesions are therefore sterile and blister fluid is culture negative.

Diagnosis

If the diagnosis is in question, a skin biopsy sample prepared for frozen section can be examined emergently. The presence of an intraepidermal rather than a full-thickness blister with separation at the dermal–epidermal junction distinguishes SSSS from TEN, allowing rapid initiation of appropriate therapy. If the clinical impression is strong, swabs from potential niduses of infection (nasopharynx, conjunctiva, umbilicus, etc.) may identify the primary focus. Gram staining may be performed emergently, while bacterial culture will confirm the diagnosis. Bullous lesions do not contain organisms. Blood culture specimens should be obtained because sepsis, although uncommon, may occur.

Treatment

Systemic administration of a penicillinase-resistant penicillin such as oxacillin is the treatment of choice, although other agents, such as clindamycin, may be considered. Clindamycin is a useful first-line agent for treatment of SSSS because of its superior cutaneous penetration, as well as its ability to inhibit bacterial toxin production. Nevertheless, reports assessing antibiotic resistance in SSSS have shown that there are certain geographic areas in which clindamycin-resistant strains predominate. As such, empiric treatment with both oxacillin and clindamycin may be useful until antibiotic sensitivities return (Braunstein et al., 2014). In cases of widespread disease, fluid and nutritional support and maintenance of normal body temperature may be required. Close monitoring for secondary superinfection (particularly with gram-negative organisms) is also warranted. Approximately 2 to 3 days after initiation of therapy, denuded areas become dry and desquamation ensues. Crusted, flaky areas may be treated with normal saline compresses. Application of a bland ointment emollient may promote skin healing and resolution. Additional treatment of family members may be considered in instances of recurrent staphylococcal skin infections. As in bullous impetigo, the intraepidermal cleavage plane is superficial and therefore does not result in scarring in the absence of secondary infection or other complications.

Streptococcus Species Infections

Cutaneous streptococcal infections occur in the newborn but are less common than staphylococcal infections. Nevertheless, outbreaks of GAS in nurseries have been reported (Nelson et al., 1976; Lehtonen et al., 1987), following introduction of the organism via maternal carriers or nursery personnel. Omphalitis is a common manifestation, although cellulitis, pustular eruptions, and paronychia may also be seen. GAS dissemination may occur and often presents as respiratory distress, a toxic shock–like syndrome, lethargy, abdominal distension, and poor oral intake (Miyairi et al., 2004). However, certain classic signs of invasive disease, such as fever, may not be present, particularly in neonates less than 5 days old. Meningitis is more commonly seen in affected individuals 5 days of age or older. Since sepsis and meningitis can result, infected infants should be identified and treated promptly, with strict isolation. Disinfection of the umbilical stump reservoir, and penicillin prophylaxis for carriers and exposed infants, have been the most

effective measures in preventing spread. Active GAS infections respond readily to penicillin.

Group B streptococcus (GBS) is now one of the most frequently encountered pathogens in the newborn nursery and is a primary cause of neonatal sepsis. Early-onset disease (during the first week of life), probably acquired during labor and delivery, most commonly becomes manifest as septicemia with respiratory distress and shock. Late-onset disease (7 days to 3 months) is acquired after birth and is more commonly associated with meningitis and adverse neurodevelopmental outcomes (Oh, 2013).

Skin infections caused by GBS are uncommon but have been documented. The most common skin manifestation of GBS is cellulitis, often of the face and neck (Albanyan and Baker, 1998; Strunk and Burgner, 2015). This so-called GBS cellulitis–adenitis syndrome typically occurs from 1 to 11 weeks of age and is characterized by progressive erythema and swelling. Extracutaneous symptoms include fever, irritability, and poor oral intake. Erosions, abscesses, and necrotizing fasciitis have also been noted with GBS. A diagnosis of GBS can be made via blood culture, which is often positive; CSF cultures are also recommended. Penicillin G or ampicillin is effective as first-line therapy.

Omphalitis

Omphalitis is a bacterial infection of the umbilical stump that presents around day 3 of life. It is commonly caused by *S. aureus*, *Staphylococcus epidermidis*, *Streptococcus* species (spp.), *Escherichia coli*, *Clostridium difficile*, *Klebsiella*, and *Pseudomonas*. Outbreaks in nurseries have been described and are often attributable to staphylococcal or streptococcal infections. Risk factors for the development of omphalitis include prematurity, low birth weight, umbilical cord catheterization, prolonged rupture of membranes, and perinatal maternal infection (Fraser et al., 2006).

Clinical Findings

Omphalitis is characterized by edema, erythema, and tenderness around the umbilicus. Purulent discharge may be present. Infection can evolve to cellulitis or lymphangitis or extend deeper into the subcutis or along the abdominal wall, causing necrotizing fasciitis. Other potential complications include sepsis, peritonitis, abscesses, and hepatic vein thrombosis.

Diagnosis

Gram stain and bacterial culture from moist umbilical stump fluid can be performed, but because this area can be contaminated easily, clinical correlation is needed. Infection must be differentiated from embryonic duct remnants.

Treatment

Systemically acting ampicillin and gentamicin can be used empirically to treat both gram-positive and gram-negative organisms until culture and sensitivities are obtained. Intravenous antibiotics can be switched to enteral antibiotics once the skin improves clinically. A Cochrane review analyzing the use of chlorhexidine antiseptic solution in the prevention of omphalitis indicated that cleansing of the umbilical cord with chlorhexidine, when compared with dry umbilical cord care, likely reduces the incidence of omphalitis and infections in the hospital setting, although it has no impact on infant mortality. In contrast, this practice does cause

reductions in omphalitis, infection, and mortality when used in the community setting (Sinha et al., 2015).

Candida Species Infections

Candida albicans is a yeast species that typically exists with humans as a commensal organism. Colonization of the gastrointestinal, respiratory, and cutaneous surfaces occurs rapidly after birth and is thought to occur primarily via the maternal genitourinary tract, although skin contact may contribute. Nevertheless, decreased host immunity, altered surface microbiota, and epidermal immaturity all contribute to *Candida* pathogenesis and invasive disease (Hube, 2004; Kelly et al., 2015). Development of active infection may occur in utero, during delivery, or postnatally.

Localized Candida Infection (Primary Cutaneous)

Oral Candidiasis (Thrush)

Oropharyngeal candidiasis is characterized by adherent white patches on a normal or erythematous base, most typically on the buccal mucosa or the tongue. These patches are characteristically recalcitrant to physical removal and may be asymptomatic or cause discomfort, resulting in decreased oral intake (Rowen, 2003). Although the peak incidence of *C. albicans* colonization of the oropharynx is estimated to occur at 4 weeks of age (Russell and Lay, 1973), most of the infants affected do not subsequently develop oral thrush. In a study of 650 infants in a NICU in India, however, approximately 3% developed symptomatic oral candidiasis at a mean age of 10.5 days, with birth asphyxia noted to be the only significant risk factor for this occurrence (Gupta et al., 1996). A more recent prospective study evaluating oral lesions of Turkish children in an outpatient setting found a 13.6% incidence of oral candidiasis in neonates less than 1 month of age (Yilmaz et al., 2011). Notably, thrush is more common in neonates born to mothers with symptomatic *Candida* vulvovaginitis rather than those who are simply colonized.

Candida Diaper Dermatitis

Candida diaper dermatitis is exceedingly common. Cutaneous candidiasis is characterized by widespread, confluent erythema with scalloped edges and characteristic involvement of moist, intertriginous areas. Peripheral white scale and satellite papules or pustules may be evident (Rowen, 2003). Alternatively, candidiasis may present as multiple pink papules with overlying scale that merge into broader plaques. In addition to the diaper area, intertriginous sites including the neck folds and axillae may also be infected with *Candida*, as may the nails.

Diagnosis of Localized Cutaneous Candida Infection

Presumptive diagnosis can be made by physical examination and history, but microscopic examination of scrapings suspended in 10% potassium hydroxide for yeast and pseudohyphae is useful. The diagnosis may be confirmed by identification of the organism in culture.

Treatment

Nystatin is a polyene antifungal with activity against *Candida* but not dermatophytes. Oral lesions usually respond promptly to a course of nystatin suspension, 100,000 to 200,000 units, administered by mouth four times daily for 14 to 21 days. In refractory

cases an increased dosage of nystatin or systemic therapy may be instituted (Hebert and Esterly, 1986). Orally or intravenously administered fluconazole has also been used for the treatment of recalcitrant oral thrush or in infants at high risk of dissemination. Nevertheless, its higher relative cost makes it a less suitable first-line treatment.

Localized cutaneous candidiasis in an otherwise healthy infant is most easily treated with topical agents, including nystatin, an imidazole antifungal (e.g., miconazole, ketoconazole), or ciclopirox olamine (Gibney and Siegfried, 1995). A recent study examining the efficacy of a 7-day course of 0.25% miconazole nitrate ointment in the treatment of diaper candidiasis showed a clinical cure rate of approximately 50%; cure rates for recurrent episodes were even higher and did not result in antifungal resistance (Blanco and van Rossem, 2013). Importantly, if a breastfeeding mother is also affected, treatment of the mother with nystatin cream or orally administered fluconazole may be indicated. Gentian (crystal) violet is an antiseptic dye effective against *Candida* species. In a 0.5% or 1% aqueous solution, it has proven to be a safe and effective treatment for thrush but is less commonly used because of side effects, including transient purple staining of the skin and mucosal ulceration. Carcinogenicity in mice has been reported.

Congenital (Intrauterine) Candidiasis

Congenital candidiasis refers to widespread candidiasis (rarely with extracutaneous involvement) that results from ascending intrauterine infection where *Candida* spp. cross the fetal membrane and infect surfaces that contact amniotic fluid. Congenital candidiasis occurs within 6 days after birth, although it is most commonly evident on the first day of life.

Clinical Findings

Lesions of congenital candidiasis may at times be seen on the placenta and fetal membranes, including characteristic miliary granulomas of the umbilical cord (Hebert and Esterly, 1986). These umbilical cord lesions are discrete yellow-white flat-topped papules, measuring 0.5 to 4 mm in diameter. Congenital cutaneous candidiasis (CCC) in affected infants is characterized by erythematous macules, papules, and vesicopustules with intense background erythema, often in various stages of development. The back, extensor extremities, and intertriginous areas are most commonly affected, while the face, oral mucosa, and diaper area are comparatively spared. Palmar and plantar pustules, paronychia, and nail involvement help distinguish this condition from more common, benign neonatal dermatoses (Darmstadt et al., 2000). A scald-like erythema reminiscent of disseminated neonatal candidiasis (see later) has also been reported in full-term infants but is more characteristic of CCC presenting in premature neonates with a birth weight below 1000 g (Darmstadt et al., 2000; Tieu et al., 2012). Skin lesions usually resolve with desquamation within 1 to 2 weeks, and nail changes improve spontaneously with time. The prognosis is good in full-term infants, but that of affected low-birth-weight infants is guarded (Gibney and Siegfried, 1995).

Diagnosis

The differential diagnosis for CCC is broad and includes other vesicopustular conditions (Box 105.1). Rapid bedside diagnosis can be made via a potassium hydroxide preparation that reveals budding yeasts and pseudohyphae. Positive results on cultures from an intact pustule or skin scrapings, as well as evidence of fungal elements present in the stratum corneum on histologic

• BOX 105.1 Causes of Vesicopustular Eruptions in the Newborn

- Transient neonatal pustular melanosis
- Erythema toxicum neonatorum
- Staphylococcal impetigo
- Congenital candidiasis
- Herpesvirus infections
- *Listeria*
- Syphilis
- Langerhans cell histiocytosis

analysis of skin biopsy tissue, are also diagnostic. Cultures of skin, blood, urine, and CSF are usually negative; however, they are indicated when systemic disease is clinically suspected, as well as in all preterm infants.

Treatment

In full-term infants with an uncomplicated course, active nonintervention with close monitoring may be sufficient, as CCC resolves spontaneously within 1 to 2 weeks (Tieu et al., 2012). Alternatively, topical antifungals may be considered. In low-birth-weight infants, systemic treatment with amphotericin B should be considered (Johnson et al., 1981). Other risk factors that may warrant systemic treatment of at-risk neonates include antibiotic or corticosteroid therapy, indwelling catheters, and parenteral nutrition. Clinical findings including respiratory distress, widespread burn-like dermatitis, or other signs or symptoms of systemic infection also warrant more aggressive therapy.

Disseminated/Invasive Candidiasis

In contrast to congenital candidiasis, invasive candidiasis (IC) is a disseminated form of neonatal candidiasis characterized by *Candida* infection in otherwise sterile body fluid such as blood, urine, or CSF. It is more commonly seen in premature, very low-birth-weight (VLBW) infants, and is typically acquired via the vaginal canal or handling in the nursery, although congenital onset may also occur. In contrast to congenital candidiasis, invasive neonatal candidiasis usually presents after the first week of life. While IC is uncommon in infants weighing more than 2500 g, its incidence increases to 5%–10% in neonates weighing less than 1000 g. The most common causes of IC are *C. albicans* and *C. parapsilosis* species (Smith et al., 2005).

The risk factors for systemic infection include VLBW (less than 1500 g), indwelling catheters, endotracheal tubes, broad-spectrum antibiotic therapy (most specifically with third-generation cephalosporins), steroid administration, and parenteral nutrition with intravenous lipid emulsions (Benjamin et al., 2010). Infection rates differ across medical centers, owing to differences in the aforementioned modifiable risk factors. Mortality is estimated at approximately 20%–30% across all affected neonates, with even greater rates of accompanying morbidity in survivors (Benjamin et al., 2010; Greenberg and Benjamin, 2014).

Clinical Findings

Skin manifestations include erosive, burn-like dermatitis followed by desquamation, scaly and erythematous patches, papules, pustules, and abscesses (Fig. 105.3). Intertriginous accentuation and



• **Fig. 105.3** Congenital Candidiasis. “Sunburn-like” erythema with accompanying superficial desquamation on the back of a neonate.

involvement of the oral mucosa is often appreciated (Tieu et al., 2012). Systemic involvement occurs via hematogenous spread from gastrointestinal and cutaneous reservoirs, most frequently involving the kidney, central nervous system (CNS), and eyes. The spleen, liver, heart, bones, and joints may also be affected. Respiratory distress, temperature instability, apnea, bradycardia, abdominal distension, and hypotension are suggestive of IC and should prompt a thorough evaluation. Supportive laboratory features may include an elevated white blood cell count and thrombocytopenia, as well as persistent hyperglycemia and glycosuria (Smith et al., 2005).

Diagnosis

The differential diagnosis of IC with cutaneous involvement is similar to that for CCC and includes neonatal vesiculopustular eruptions that range from benign, self-limited cutaneous processes to rapidly progressive, life-threatening disease (Box 105.1). Early and correct diagnosis is essential. Organisms from skin are usually demonstrable on potassium hydroxide or calcofluor white preparations and cultures of scrapings from involved skin. The diagnosis of disseminated candidiasis can be expedited by a positive touch preparation of a punch biopsy specimen of skin. Using this technique, the practitioner firmly imprints the dermal side of the specimen on a microscope slide and then assesses it for yeast after potassium hydroxide preparation or Gram staining. Histologic examination of specimens from the placenta and umbilical cord prepared with the appropriate stains may also be supportive if fungal elements are demonstrated.

Disseminated disease can be difficult to diagnose, as culture of the organism from blood or CSF is estimated to have a sensitivity of 50% or less, and fungal antigen detection systems appear to have low sensitivity (and have also not been tested in neonates) (Smith et al., 2005; Greenberg and Benjamin, 2014). Although urine culture often leads to false-positive results, such findings are strongly associated with systemic disease in infants at risk and should be interpreted in the clinical context. In addition to blood, urine, and CSF cultures, ophthalmologic examination, chest x-ray for pulmonary involvement, echocardiogram, and head and abdominal ultrasonography should be considered.

Prognosis and Treatment

Systemic infection with *C. albicans* in premature infants is a serious infection with high morbidity and mortality. Sequelae of IC include neurodevelopmental impairment, deafness, retinopathy of prematurity, chronic lung disease, and renal failure (Smith et al., 2005; Barton et al., 2014). Minimizing the aforementioned risk factors for IC and instituting meticulous hand hygiene are considered effective means of prevention. Probiotic supplementation has also been posited to provide protection against IC (Kumar and Singhi, 2016). Empiric first-line treatment should be initiated while diagnostic results are awaited in patients for whom suspicion of IC is high, as this results in improved clinical outcomes. Among the various antifungals used to treat IC, fluconazole and amphotericin B are the agents of choice (including liposomal amphotericin B if urinary tract involvement is excluded) (Pappas et al., 2009; Greenberg and Benjamin, 2014). Echinocandins such as caspofungin are less favored but may also be considered, although they are inappropriate for cases with ophthalmologic involvement as they are unable to penetrate the vitreous. Fluconazole prophylaxis has been recommended for neonates weighing less than 1000 g cared for in NICUs with high rates of IC ($\geq 15\%$). However, a randomized controlled trial, evaluating the effects of fluconazole prophylaxis in patients weighing less than 750 g in NICUs with lower rates of IC (including most of those in the United States and European Union), showed no reduction in the rates of IC or death, suggesting that universal administration of fluconazole prophylaxis in this population may not be appropriate (Benjamin et al., 2014). Therapy is recommended for 21 days after microbiologic clearance as documented by culture and imaging.

Primary Cutaneous Aspergillosis

Primary cutaneous aspergillosis (PCA) is an increasingly common cutaneous infection in neonates, particularly the premature. This increased incidence is due in part to improved survival of extremely premature infants, as well as increased exposure to fungal spores made airborne by construction in and around hospitals (Vonberg and Gastmeier, 2006). PCA is thought to occur primarily via traumatic insult, with subsequent inoculation through intravenous line placement or use of adhesive tape, elastic dressings, armboards, or contaminated gauze. Contributing factors also include an immature skin barrier and immune system, frequent use of systemic corticosteroids in NICU patients, and administration of broad-spectrum antibiotics (Woodruff and Hebert, 2002; Simpson et al., 2015).

Clinical Findings

PCA typically presents as an erythematous papule, patch, or plaque that with time can become pustular and may eventuate in cutaneous ulceration with crusting and, at times, purpura (Fig. 105.4). The differential diagnosis for PCA includes other deep fungal infections, as well as cutaneous *Pseudomonas* infection or inflammatory conditions, including cutaneous polyarteritis nodosa or pyoderma gangrenosum (Woodruff and Hebert, 2002).

Diagnosis and Treatment

A diagnosis of PCA is made via skin biopsy for histologic analysis and tissue culture. Systemic involvement must also be excluded, particularly when lesions are multifocal. Specific evaluation including



• **Fig. 105.4** Primary Cutaneous Aspergillosis. Erythematous plaques and broad pustules, many with dusky centers, on the back of a premature neonate.

blood culture, chest x-ray, echocardiogram, funduscopic examination, lumbar puncture, and abdominal ultrasonography should be considered when one is evaluating a patient with known or suspected PCA.

The treatment of choice for PCA is amphotericin B, with consideration of use of its liposomal formulation. Voriconazole may be used as an alternative. For single lesions, surgical debridement should be considered to prevent systemic dissemination (Woodruff and Hebert, 2002; Simpson et al., 2015).

Tinea Capitis and Tinea Corporis

Although uncommon in neonates, dermatophytoses such as tinea corporis and tinea capitis are important infections to recognize as they can be clinically mistaken for more serious disorders. Most commonly caused by superficial invasion of the epidermis by *Trichophyton* and *Microsporum* species, dermatophytoses are thought to occur in infants in part because of epidermal and immune immaturity. Other predisposing factors in hospitalized infants (particularly in the NICU setting) include moist, humid environments, administration of broad-spectrum antibiotics, and frequent application of tapes and instrumentation (Atanasovski et al., 2011).

Clinical Findings

Clinically, neonatal tinea capitis presents with a variable combination of alopecia, erythema, and scale. An annular morphology may be appreciated, and lymphadenopathy may also be present. It may or may not be accompanied by tinea corporis (dermatophytosis of the body), which classically appears as an annular patch or thin plaque with central clearing and peripheral erythema and scale (Gilaberte et al., 2004; Fig. 105.5). Other more atypical presentations reported include pustules, nodules, and vesicles (Battin and Wilson, 2005). As such, the differential diagnosis for neonatal tinea includes neonatal lupus, Langerhans cell histiocytosis, impetigo, HSV infection, and benign diagnoses including seborrheic dermatitis.

Diagnosis and Treatment

Consideration of dermatophyte infection in the neonate is important as recognition of this infection can prevent costly, unnecessary



• **Fig. 105.5** Tinea Corporis. Annular erythema with central clearing on the forehead of an infant. Note the presence of erythematous papules and superficial scale on the periphery of the lesion.

procedures and evaluations to exclude more serious diagnoses, including neonatal lupus. Potassium hydroxide preparations are a useful bedside diagnostic tool to quickly diagnose tinea corporis or tinea capitis, while dermatophyte culture and skin biopsy may also be considered in more atypical presentations.

Treatment consists of either topical or oral antifungal therapy. Topical therapy two or three times daily with agents including azoles or terbinafine can help clear even scalp infections in neonates and is an appropriate first treatment choice as the risk of systemic absorption and potential side effects is low.

Herpes Simplex Virus Infection

HSV exists as two viral types, HSV-1 and HSV-2. Historically, HSV-2 was associated with genital eruptions, while HSV-1 was associated with orolabial infections, although the incidence of HSV-1-associated genital herpes appears to be increasing (James and Kimberlin, 2015). Neonatal infection can be associated with either serotype. Despite a high prevalence of HSV infections in adults, neonatal herpes is relatively uncommon. Nevertheless, early recognition and prompt initiation of therapy for neonatal herpes are critical, as the consequences of delaying therapy can be devastating. While approximately 5% of cases of HSV infection in the neonatal period are due to in utero exposure, with another 10% occurring after birth, most occur in the perinatal period.

Clinical Findings

Neonatal HSV infection is classified on the basis of the extent of involvement: skin, eye, and/or mouth (SEM) disease; CNS disease; or disseminated disease. Infection typically presents within the first week to 2 weeks after birth, with a slightly later onset of CNS disease at approximately 16 to 17 days. Neonatal HSV infection may present with temperature instability, lethargy, irritability, or poor oral intake or with a sepsis-like syndrome that may include new-onset seizures, pneumonia, and disseminated intravascular coagulation. Approximately 60% to 80% of afflicted neonates exhibit characteristic skin lesions or develop them during the course of the disease. Nevertheless, there remains a significant proportion



• **Fig. 105.6** Congenital Herpes Simplex Virus Infection. Broad ulceration with peripheral vesiculation and desquamation on the left arm of a neonate. (Photo courtesy of Albert Yan, MD; The Children's Hospital of Philadelphia, Philadelphia, PA, USA.)

of patients who never develop skin vesicles throughout their disease course (Kimberlin et al., 2001a; James and Kimberlin, 2015).

Approximately 45% of infected infants exhibit SEM disease. Characteristic cutaneous lesions begin as erythema that quickly evolves into isolated or grouped vesicles on an erythematous base. Continued evolution may result in pustules, crusts, or erosions. Vesiculation typically occurs at sites of trauma, including the presenting part and sites of fetal electrode placement (Arvin and Prober, 1992). Conjunctivitis is relatively common in SEM disease, although oral involvement remains rare. With early institution of therapy, infants with SEM disease have an excellent prognosis, with low rates of morbidity and a mortality rate of zero. Nevertheless, if infection is left untreated, it will progress to disseminated or CNS disease in three-quarters of all cases (Arvin and Prober, 1992). Additionally, infants with SEM disease and three or more recurrences of cutaneous vesicles in the first 6 months of life appear to be at greater risk of neurologic impairment (Kimberlin et al., 2011).

In contrast, approximately 30% of infected infants have CNS disease, with an increased associated mortality and risk of neurodevelopmental abnormalities. Prematurity and the presence of seizures at onset of therapy appear to portend a worse prognosis in infants with CNS disease, even with therapy (acyclovir era). The remaining 25% of infants have evidence of disseminated disease (sepsis, liver failure, encephalitis, disseminated intravascular coagulation, respiratory distress). Forty percent of patients in this group do not manifest any cutaneous findings (Pinninti and Kimberlin, 2014a). The prognosis of disseminated disease is worse than for other forms of neonatal HSV infection; treatment with high-dose acyclovir has improved outcomes compared with historical measures, but mortality associated with disseminated disease is still estimated at approximately 30%. Almost 20% of survivors have neurodevelopmental delays.

Infants infected in utero (congenital HSV infection) have a distinct clinical presentation (Fig. 105.6). Skin lesions are almost always present at birth and include widespread erosions and bullae, scars, hyperpigmentation, hypopigmentation, and aplasia cutis. Other frequent findings include microcephaly, hydranencephaly, intracranial calcification, chorioretinitis, optic atrophy, and microphthalmia (Arvin and Prober, 1992; James and Kimberlin, 2015).

Etiology

Most cases of neonatal herpes simplex are the result of vertical transmission in the perinatal period. Most cases of neonatal HSV infection in developed countries are secondary to HSV-1 infection, reflecting the increased prevalence of HSV-1 associated with genital herpes. The usual route of infection is via intrapartum contact with genital mucosa during a phase of active viral shedding, which may be symptomatic or asymptomatic. Nevertheless, physicians should also be aware of uncommon means of HSV transmission associated with certain ethnic or religious groups. For instance, postnatal transmission of HSV has been reported in ultra-orthodox Jewish populations in which orogenital suctioning of blood is performed following ritual circumcision (Tzvi-Behr et al., 2016).

Epidemiology

In developed countries, the incidence of neonatal HSV infection ranges from 1.6 to 33 per 100,000 live births (Pinninti and Kimberlin, 2014a). When compared with the risk of genital herpes reactivation, the risk of neonatal herpes is greater with maternal first episode primary or first episode nonprimary infection (the latter category reflects acquisition of an HSV serotype in individuals with preexisting protective antibodies versus the other serotype) (Brown et al., 2003). Unfortunately, it has been estimated that up to 80% of mothers who transmit HSV to their infants have no known history of genital herpes (James and Kimberlin, 2015). Vaginal delivery, prolonged rupture of membranes, and maternal infection with HSV-1 are additional risk factors for acquisition of HSV in the neonate.

Diagnosis

A high index of suspicion is required, particularly in cases without cutaneous manifestations. When characteristic herpetic lesions are present, the differential diagnosis includes other causes of vesicopustular eruptions (Box 105.1). A Tzanck smear test or direct fluorescent antibody detection using skin scrapings from the base of a fresh vesicle is a relatively rapid means of detecting cutaneous HSV infection, but the diagnostic yield of both is variable, largely influenced by the age, quality, and handling of the specimen. Viral culture and polymerase chain reaction (PCR) for HSV DNA are the preferred methods for diagnosing neonatal HSV infection; the sensitivity of PCR in identifying HSV DNA in CSF samples is 75% to 100% (Pinninti and Kimberlin, 2014a). Obtaining specimens from the CSF, oropharynx, anus, conjunctivae, and nasopharynx is recommended; evaluation of peripheral blood via PCR is also useful in establishing a diagnosis of neonatal herpes although it does not assist in identifying the extent of disease. Notably, HSV serologic tests are not a useful diagnostic adjunct. If they are elevated, serum alanine aminotransferase levels may indicate disseminated disease.

Treatment

Strategies to decrease the risk of vertical transmission of HSV include cesarean delivery, maternal prophylactic antiviral therapy, and limiting use of invasive monitoring techniques in mothers shedding HSV at the time of delivery (Brown et al., 2003). Specifically, birth via cesarean delivery for women with active lesions or prodromal symptoms has been shown to reduce the risk of neonatal HSV infection (Pinninti and Kimberlin, 2014b). Maternal

suppressive therapy with acyclovir is known to reduce the frequency of genital lesions near term and the frequency of cesarean delivery, but whether this reduces acquisition of HSV by the neonate remains unclear.

Early treatment with antiviral agents is critical to decrease the risk of serious complications and death. All cases of presumptive neonatal HSV infection should be treated with intravenous acyclovir therapy, with a recommended dosage of 60 mg/kg per day divided every 8 hours (Kimberlin et al., 2001b). A notable side effect of this therapy, however, is transient neutropenia that warrants ongoing monitoring. Nephrotoxicity has also been documented in patients treated with acyclovir, although this appears less common in patients with normal renal function whose fluid status is closely monitored. Treatment is recommended for 14 days for SEM disease and 21 days for disseminated and CNS disease. In cases of CNS involvement, repeated evaluation of CSF for HSV infection via PCR should be performed at the conclusion of therapy, and, if the findings are positive, it should be continued for an additional week.

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Common Newborn Dermatoses

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KEY POINTS

- Papulopustular lesions on the palms and/or soles in the first month of life likely represent a self-limited inflammatory process such as eosinophilic pustular folliculitis or infantile acropustulosis, but scabies should be ruled out.
- Neonatal acne (aka *cephalic pustulosis*) is typically multifactorial and self-limited. Treatment is not necessary, but topical agents aimed at reduction of the commensal yeast *Malassezia* can be beneficial.
- Evidence suggests that early use of emollients in infants at risk of developing atopic dermatitis may prevent later disease.
- Subcutaneous fat necrosis of the newborn is generally benign and self-limited but when extensive can cause hypercalcemia.

This chapter describes a group of cutaneous disorders commonly found in neonates. Most of these disorders are benign and are either self-limited or treated with ease. Recognition of these common neonatal dermatoses is important so as to distinguish them from more serious conditions, sparing patients unnecessary work-up and treatment. Each section includes common clinical features and presentation, notes on establishing the diagnosis (with a short differential diagnosis), the cause if known, and a brief discussion of basic treatment and prognosis.

Erythema Toxicum Neonatorum

Clinical Findings

Erythema toxicum neonatorum (ETN) is a benign inflammatory condition affecting newborns. Estimates of incidence range from 7%–41%, and it is seen more commonly in white newborns with a higher birth weight and greater gestational age (Monteagudo et al., 2012). The condition presents in the first 1–3 days after birth as irregularly shaped erythematous macules that can develop overlying vesicles or pustules (Fig. 106.1). ETN most commonly affects the trunk but can involve the face and extremities as well. The palms and soles are typically spared. Affected infants are otherwise healthy. The lesions persist for 1–2 weeks and then spontaneously resolve without sequelae.

Diagnosis

The diagnosis is often clinical but can be further supported by the scraping of a pustule, smearing the contents onto a slide, and

staining with Wright or Giemsa stain (Lucky, 2001). This will reveal a large number of eosinophils. The differential diagnosis of ETN includes other benign entities such as transient neonatal pustular melanosis, miliaria, infantile acropustulosis, and eosinophilic pustular folliculitis. Additionally, infections such as folliculitis, candidiasis, impetigo, and herpes should be considered and ruled out. If a biopsy sample is obtained, histopathologic features include intraepidermal vesicles filled with eosinophils and a mixed intra-dermal inflammatory infiltrate localizing around the superficial pilosebaceous follicle.

Etiology

The cause of ETN remains unclear. Studies have shown the presence of inflammatory mediators, as well as increased eosinophil and macrophage activity (Marchini et al., 2005, 2007) in affected skin compared with unaffected skin. This has led to the theory that ETN could represent a cutaneous immunologic response to microbial colonization of the hair follicles after birth (Marchini et al., 2007).

Treatment and Prognosis

No treatment is required for this benign condition. It is usually asymptomatic. Parents can be reassured and informed that the condition will spontaneously resolve in a matter of weeks. Prolonged courses or recurrence is rare.

Transient Neonatal Pustular Melanosis

Clinical Findings

Transient neonatal pustular melanosis is a benign, self-limited condition that presents at birth in affected infants. It is more common in African-American infants (Badia et al., 2015). There are three clinical phases of transient neonatal pustular melanosis. At birth the neonate has very fragile and superficial 2- to 10-mm pustules located most commonly on the forehead, under the chin, on the low back, and on the shins. These pustules are often wiped off during the initial cleaning after birth. Next, a fine collarette of scale is noted around the resolving pustule. Last, hyperpigmented brown macules develop at the site of the previous pustules (Lucky, 2001; Fig. 106.2). The first two phases usually last 1–2 weeks, but hyperpigmentation can persist for several months (Ghosh, 2015).



Fig. 106.1 Erythema Toxicum Neonatorum. Pinpoint pustules overlying ill-defined erythema on the trunk and extremity of a healthy newborn consistent with erythema toxicum neonatorum.



• **Fig. 106.2** Transient Neonatal Pustular Melanosis. Hyperpigmented macules on the lower back and buttocks, some of which are encircled by scales.

Diagnosis

Diagnosis is clinical, but it can be supported by a scraping from the pustule, with Wright staining of the contents. This will reveal a predominance of neutrophils. The differential diagnosis includes ETN, and these conditions can overlap. Additional considerations include miliaria, acropustulosis of infancy, and infectious diseases such as candidiasis, impetigo, and herpes, which should be ruled out.

Etiology

The underlying cause of transient neonatal pustular melanosis is unknown. Studies linking it with ETN suggest a similar cause.

Treatment and Prognosis

No treatment is required for this benign and self-limited condition. Parents should be informed that while the initial skin findings resolve quickly, the residual hyperpigmentation may take months to completely resolve.

Eosinophilic Pustular Folliculitis

Clinical Findings

Eosinophilic pustular folliculitis (EPF) is a vesiculopustular eruption that can occur in several distinct settings. The infantile form is relatively rare and presents with pruritic follicular lesions typically on the extremities or scalp, although truncal involvement is described. Crops of lesions will occur discretely, not in an annular array as in adults, and persist for days to weeks before resolving without sequelae. EPF tends to present somewhat later than erythema toxicum but has been described on the first day after birth (Buckley et al., 2001).

Diagnosis

Diagnosis is clinical, and persistence past several weeks generally distinguishes EPF from erythema toxicum, although peripheral eosinophilia may be supportive. Histologic examination demonstrates an eosinophilic follicular infiltrate.

Etiology

The cause is unknown.

Treatment and Prognosis

Treatment is symptomatic and can include emollients, topical corticosteroids, and antihistamines. Topical indomethacin therapy has been used in refractory cases (Hashizume et al., 2014).

Acropustulosis of Infancy

Clinical Findings

Acropustulosis of infancy is a relatively uncommon pruritic, vesiculopustular rash that occurs on the hands and feet with spread to the wrists and calves. First described in 1979, this rash is closely related to preceding infections and infestations, with scabies preceding approximately 50% of cases (Paloni et al., 2013; Silverberg, 2015). The eruption is most common in African-American, Hispanic, and Asian children and presents at less than 1 year of age. Crops of intensely pruritic vesicles and pustules appear on the hands and feet, persist for around 2 weeks, and then resolve. Frequent recurrences are the norm, with each eruption decreasing in intensity until eventual complete resolution around 2 to 3 years of age.

Diagnosis

Diagnosis is primarily clinical, and a history of preceding scabies can be quite helpful in making the diagnosis. If a biopsy sample is obtained, it shows necrolysis of the keratinocytes with intraepidermal pustules filled with neutrophils and eosinophils (Vignon-Pennamen and Wallach, 1986). The differential diagnosis includes scabies (concurrent infestation must be ruled out and treated if present), pustular psoriasis, coxsackievirus infection, eosinophilic folliculitis, and less likely, an allergic contact dermatitis.

Etiology

The cause of acropustulosis is unknown, but the condition is known to frequently follow prior scabies infestation or coxsackievirus infection.



• **Fig. 106.3** Numerous grouped milia on the chin of a newborn.

Treatment and Prognosis

The recurrent episodes of acropustulosis of infancy will eventually resolve around 2–3 years of age, but episodes can be managed. Ruling out scabies and treating it if it is present should be a first step (Good et al., 2011). Topical treatment with mid-potency to high-potency corticosteroids is effective for discrete episodes but will not help to prevent relapses (Silverberg, 2015). Historically, acropustulosis of infancy was treated with orally administered dapsone, but this is no longer frequently used because of the risk of hemolysis and methemoglobinemia in patients with glucose 6-phosphate dehydrogenase deficiency.

Milia

Clinical Findings

Milia are an extremely common benign entity, seen at birth in 40%–50% of newborns. They are more common with longer gestations and in infants born to multigravidas (Gupta et al., 2011). Milia (singular, *milium*) are tiny, white monomorphic papules with a smooth surface. They can number from several to dozens. They are an entity distinct from miliaria, which is discussed next. Milia can be present anywhere on the body but occur most commonly on the face, predominantly on the forehead, cheeks, and chin (Fig. 106.3).

Diagnosis

A useful diagnostic tool is expression of the milia contents, which will resemble a tiny white pearl. This is the keratinocyte debris that forms the interior of the cyst. The differential diagnosis includes sebaceous hyperplasia, which would also present on the face but tend to be more yellow, and miliaria, which presents primarily on the body and in greater numbers with distinct clinical variants.

Etiology

Milia are tiny inclusion cysts that form in the epidermis. The epidermal tissue invaginates, often around a vellus hair, and forms a cyst with a wall of keratin-producing cells.

Treatment and Prognosis

Treatment of milia is not required. They tend to resolve spontaneously over several months. Of note, the presence of

multiple large or persistent milia can rarely be associated with syndromes such as epidermolysis bullosa or orofaciocutaneous syndrome.

Miliaria

Clinical Findings

Miliaria is a benign rash due to obstruction of the eccrine duct occurring in the first 1–2 weeks of life, with rapid resolution within days. There are multiple variants depending on the location of the eccrine duct obstruction, with differing clinical presentations. Miliaria crystallina occurs when eccrine ducts within or below the stratum corneum are obstructed and causes small clear vesicles that can be easily wiped away. Miliaria rubra is caused by obstruction of the eccrine duct at the level of the epidermis and is thought to be related to overheating. This causes erythematous papules or papulopustules primarily on the head, neck, face, and trunk. Dermal inflammation contributes to the clinical presentation. Miliaria profunda is caused by occlusion of the eccrine duct at or below the dermal–epidermal junction and causes a mildly inflammatory papular eruption that is rare in newborns.

Diagnosis

The diagnosis of miliaria is made clinically, although scraping of a vesicle or pustule with subsequent Wright staining will reveal few to no cells in miliaria crystallina and lymphocytes in miliaria rubra. The differential diagnosis includes ETN, transient neonatal pustulosis, milia, eosinophilic folliculitis, and infectious entities. Recurrent episodes of pustular miliaria rubra could be a sign of the rare and potentially fatal condition type I pseudohypoaldosteronism, which is an inherited disorder of mineralocorticoid resistance leading to a salt-wasting crisis.

Etiology

Miliaria is caused by obstruction of the eccrine duct, possibly by sweat accumulation. Neonates can have incompletely canalized eccrine ducts, predisposing them to developing obstruction and subsequent miliaria (Dixit et al., 2012).

Treatment and Prognosis

No treatment is required for this benign condition, which will self-resolve. Prevention of overheating can reduce the incidence of miliaria rubra.

Epstein Pearls and Bohn Nodules

Clinical Findings

Epstein pearls and Bohn nodules are very common and benign cysts that form in the mouths of neonates. They present as single or clustered 1- to 2-mm smooth white to yellow papules. Epstein pearls are more often seen in infants born to multigravidas, after longer gestations, and with higher birth weights (Seminario and Ivancakova, 2004). Bohn nodules are often mistaken for natal teeth, prompting referral.

Diagnosis

Epstein pearls are found on the palate, typically at the midline of the junction between the hard and soft palate. Bohn nodules are found on the alveolar ridge, most commonly in the maxillary region. They are distinguishable from neonatal teeth in their location in addition to their appearance; neonatal teeth are usually located in the region of the lower incisors. Radiographic examination would distinguish between these diagnoses (Seminario and Ivanakova, 2004).

Etiology

Both Epstein pearls and Bohn nodules are inclusion cysts. They are thought to originate from remnants of odontogenic epithelium or to potentially be remnants of salivary glands (Dutta et al., 2014).

Treatment and Prognosis

Spontaneous resolution will occur with time, typically within the first several weeks to months after birth. No treatment is required.

Sebaceous Hyperplasia

Clinical Findings

Sebaceous hyperplasia is a common finding, seen in approximately half of newborns (Kanada et al., 2012), and is typically most noticeable surrounding the nose and upper lip. It presents as regular, smooth white to yellow papules that can be grouped around follicles.

Diagnosis

The differential diagnosis includes milia and neonatal acne (neonatal cephalic pustulosis).

Etiology

Sebaceous hyperplasia in newborns is thought to occur secondary to maternal androgens, which stimulate the sebaceous glands in utero (Marchini et al., 2007; Kanada et al., 2012).

Treatment and Prognosis

No treatment is required, and the findings will spontaneously resolve in the first few weeks of life.

Neonatal Cephalic Pustulosis (Neonatal Acne)

Clinical Findings

Neonatal cephalic pustulosis, which is also known by the more common term of *neonatal acne*, consists of erythematous papules and pustules on the cheeks, chin, and forehead of infants (Fig. 106.4). It occurs within the first 30 days after birth and can last up to several months. Comedones are absent. It is an entity distinct from infantile acne, which occurs at 3–6 months of age and can last for years.



• **Fig. 106.4** Neonatal Cephalic Pustulosis (Neonatal Acne). Small monomorphic papules and pustules on the head and neck of a newborn consistent with neonatal cephalic pustulosis or neonatal acne. (From Sidbury R, Paller AS. The diagnosis and management of acne. *Pediatr Ann.* 2000;29:17–24.)

Diagnosis

Diagnosis is made primarily on the clinical appearance, although Wright stain of a smear will reveal neutrophil predominance. The differential diagnosis includes miliaria, milia, ETN, or rarely, severe infectious processes such as candidiasis or folliculitis.

Etiology

The cause of neonatal cephalic pustulosis is debated but is thought to be related to skin colonization with *Malassezia* species. *Malassezia* colonization begins at birth and increases during the first few weeks after birth from approximately 5% of newborns colonized during the first week to 30% colonized by the second to fourth week (Ayhan et al., 2007). This increased *Malassezia* colonization is thought to trigger an inflammatory reaction that some believe becomes neonatal cephalic pustulosis (Bernier et al., 2002; Lucky, 2001). However, others suggest that the degree of skin colonization by *Malassezia* does not differ between affected and unaffected infants and that there is no correlation between the severity of disease and *Malassezia* isolation (Ayhan et al., 2007). If, and why, *Malassezia* colonization causes an inflammatory reaction in some neonates but not in others is undetermined.

Treatment and Prognosis

No treatment is required for this benign self-limited condition. However, the lesions can take months to resolve. Topical treatment with imidazole antifungals, such as clotrimazole, econazole, or ketoconazole, may be effective at shortening the duration of the eruption.

Seborrheic Dermatitis

Clinical Findings

In newborns, seborrheic dermatitis (SD) is an extremely common finding and presents as irregular salmon pink patches with waxy scaling on the scalp, forehead, and nasolabial folds. It can also involve the diaper area. Appearing around 2–4 weeks of age, this condition usually resolves or abates by 1 year of age. Severe cases can become secondarily infected. A strong association between atopic dermatitis (AD) and infantile SD has been postulated, suggesting that infantile SD may precede the development of AD or that these conditions may be on the same spectrum of disease (Alexopoulos et al., 2014).

Diagnosis

Diagnosis is clinical, and the differential diagnosis includes AD, psoriasis, Langerhans cell histiocytosis, or another superficial fungal infection. AD may overlap with SD, as discussed previously, and psoriasis does not typically present this early. Langerhans cell histiocytosis is a more serious, but fortunately rare, condition.

Etiology

In adults, SD is related to *Malassezia* overgrowth, but it is unclear if this is the pathogenesis behind the disorder in infants, although that has been suggested by several historical studies (Broberg and Faergemann, 1989; Ruiz-Maldonado et al., 1989).

Treatment and Prognosis

This condition often resolves spontaneously or abates by 1 year of age. Both topical antifungals and topical steroids have been used to treat this condition. Ketoconazole was shown to be equally as efficacious at treating SD as steroid creams such as hydrocortisone (Cohen, 2004), and therefore this could be a viable treatment option. Treatment of the scalp with olive oil should be avoided, as this medium can encourage yeast growth (Siegfried and Glenn, 2012).

Atopic Dermatitis

Clinical Findings

AD is an extensive topic, spanning all ages of childhood into adulthood. This section will focus on infantile AD, as there are multiple informational sources for this common and important condition as it pertains to later childhood. AD, commonly known as *eczema*, is a chronic, relapsing condition defined by distinctive cutaneous manifestations and associations with additional atopic conditions such as asthma and allergic rhinitis. It is extremely common, affecting 15%–20% of the population, particularly in developed countries. In more than half of cases, the onset of AD is in infancy (Kay et al., 1994). In infants the most common areas of involvement include the cheeks (often seen concurrently with an irritant dermatitis secondary to saliva), neck, flexural folds, wrists, and ankles. In infantile eczema, involvement of the extensor surfaces (skin on the opposite side of a joint) and trunk is seen more commonly than flexural involvement. Erythema, scaling, lichenification, and excoriation are common clinical findings. Itch is the predominant symptom. The diaper area and nasal tip are

notably spared. Superinfection can be common. There is significant overlap with SD, as discussed previously. As infants age, the “typical” presentation of AD often evolves with prominent involvement of the antecubital and popliteal fossae with associated diffusely dry skin.

Diagnosis

Diagnosis is made clinically, and biopsy is rarely performed. The differential diagnosis of AD is broad and includes common entities such as SD, contact dermatitis, and irritant dermatitis. More rarely, severe AD can be associated with recurrent infections of various forms in multiple rare immunologic disorders, including Wiskott–Aldrich syndrome, chronic granulomatous disease, hyper-immunoglobulin E (IgE) syndrome, and severe combined immunodeficiency. Severe recalcitrant eczema with other unusual infections should raise awareness of these serious conditions particularly in the setting of failure to thrive. If lesions appear superinfected with crusting, bacterial culture should be performed.

Etiology

The exact cause of AD remains elusive. At its core, AD appears to be a condition of defective skin barrier function and immune alteration. In AD, there is a prominence of the T-helper2–T-cell subsets, which produce interleukin-4 and promote production of IgE, eosinophilia, mast cell proliferation, and release of histamine. Loss of filaggrin mutations has been linked to individuals with early-onset and persistent AD (Palmer et al., 2006; Brown et al., 2008). Filaggrin acts in the top layer of the skin and maintains skin barrier function. When the skin’s barrier function is impaired, it is increasingly susceptible to exogenous triggers. Nearly all patients with AD carry *Staphylococcus aureus* on their skin, and staphylococcal exotoxins can act as superantigens that stimulate T-cell responses (Leung, 1995). The impact of food and food allergy, a common question from parents, is only relevant in a minority of patients. Food allergies are more common in atopic individuals, and in some cases of AD recalcitrant to treatment, food allergies may play a role in the persistence of the disease. A Cochrane review stated that maternal dietary antigen avoidance during pregnancy had no impact on the development of AD in the first 18 months of life and could increase the risk of preterm birth or decreased birth weight (Kramer and Kakuma, 2014). Breastfeeding during the first 4 months of life has been shown to reduce the incidence and severity of AD in high-risk infants (Blattner and Murase, 2014), although prolonged breastfeeding has not impacted its incidence (Flohr et al., 2011).

Treatment and Prognosis

There is no cure for eczema; therefore the goal of treatment is to manage signs and symptoms and reduce recurrences. Spontaneous resolution often occurs as children age. Maintenance therapies include gentle bathing techniques such as limiting a gentle soap to soiled areas and avoidance of long, hot bathing. Diligent moisturization at least twice daily and always after bathing with a thick, bland emollient is important to restore cutaneous moisturization and maintain barrier function. Treatment of active rash with appropriate strength topical corticosteroids, rarely stronger than class 6 or 7 (e.g., hydrocortisone 2.5% or 1%, respectively) is first-line treatment for symptomatic AD refractory to emollients



• **Fig. 106.5** Diaper dermatitis.

and good skin care. Second-line agents include topical calcineurin inhibitors such as tacrolimus or pimecrolimus, but these agents are not approved for use in children younger than 2 years. Adjunctive topical or systemic antibiotics address concurrent skin infection when present, and dilute bleach baths may be preventive (Huang et al., 2009). Treating inflammation as described earlier is the best way to reduce itch, but when there is sleep disruption, a sedating antihistamine such as diphenhydramine or hydroxyzine may help, if it is age appropriate.

Diaper Dermatitis

Clinical Findings

Diaper dermatitis (DD) is likely the most common skin condition of infants. The vast majority of affected infants are otherwise healthy, but occasionally DD can suggest an underlying systemic disorder. DD is primarily an irritant process, occurring when the skin in contact with the diaper becomes inflamed because of friction and contact with urine, feces, cleansing materials, and other irritants. The clinical presentation includes erythema, with mild scaling of the gluteal crease, buttocks, and convex surfaces of the pubic area and perianal rim (Fig. 106.5). There is often relative sparing of the skinfolds, which are protected from direct contact with the diaper. Erosions or plaques can be present in more severe cases, and discrete ulcerations are seen in a severe form of DD known as *Jacquet dermatitis*. This distinctive noduloerosive presentation is seen more frequently in association with cloth diaper use.

Diagnosis

DD is a clinical diagnosis, and as such, histopathology is usually not helpful. Other infectious and noninfectious causes of rashes in the diaper area should be excluded. Infectious causes of diaper rashes include congenital syphilis and infections caused by *Candida* or other fungi, *Streptococcus*, *Staphylococcus* (including staphylococcal scalded skin syndrome), herpes simplex virus, and human immunodeficiency virus. These infectious diaper area eruptions are usually clinically distinct. *Candida* infections present with bright red, relatively well-demarcated patches and plaques with satellite lesions in the groin region and perianal rim. Staphylococcal infection presents most commonly in the diaper area as bullous

impetigo with scattered bullae and vesicles. Streptococcal infections present as a brightly erythematous perianal patch. Skin findings caused by infections are often found in areas additional to the diaper region. Other considerations include infantile SD, allergic contact dermatitis, nutritional deficiencies (such as acrodermatitis enteropathica in zinc deficiency), Langerhans cell histiocytosis, and rare metabolic disorders. Similar findings will often be identified in other nondiapered skin areas, which can be a distinguishing feature.

Etiology

DD is exceedingly common, with most infants experiencing one or more episodes. Multiple factors contribute, including occlusion friction, and maceration. When the barrier has been compromised by these factors, chemical or biologic irritants in urine and feces continue to drive the dermatitis. Further irritants such as soaps, diaper wipes, and numerous topical products perpetuate the problem.

Treatment and Prognosis

Prevention of DD starts with maintenance of skin barrier function and prevention of irritation. The use of disposable diapers, particularly the superabsorbent variety, has been shown to be better at preventing DD than the use of cloth diapers (Lane et al., 1990). Avoiding prolonged skin contact with soiled diapers is an important aspect of basic infant care. Potential irritants or sensitizers should be identified and removed. Commercial diaper wipes are a common culprit. Barrier pastes such as zinc oxide ointment and zinc paste as well as petroleum jelly are bland skin protectants. Thick layers of these protective agents should be applied, and with diaper changes, only a partial layer should be removed to protect the underlying skin. Mineral oil provides a way to remove zinc preparations without rubbing the skin excessively. In the absence of *Candida* infection, a low-potency topical steroid can be helpful. If *Candida* infection is suspected, an anti-*Candida* agent can be added. Daily bathing is acceptable, but harsh soaps and scrubbing should be avoided. DD is self-limited and episodic.

Harlequin Color Change

Clinical Findings

Harlequin color change is a rare but benign event. It was first reported in 1952. It represents an abrupt deep vascular red color change to one side of the body, with contralateral blanching and sharp midline demarcation. It can involve the entire body or can be more localized to a single body area. It occurs most commonly in the newborn and is more often seen in premature infants.

Diagnosis

Diagnosis is by clinical appearance. Rapid resolution is the norm.

Etiology

Harlequin color change is thought to be due to the immaturity of the hypothalamic control center in newborns, which causes asymmetric control of the sympathetic peripheral vascular tone, resulting in skin color change with a sharp midline demarcation.



• **Fig. 106.6** Subcutaneous Fat Necrosis. Brawny, indurated, erythematous plaque on the back of an otherwise healthy infant with associated hypercalcemia.

Treatment and Prognosis

No treatment is required for this entity. It is benign and rapidly resolves on its own. Recognition and education are important as harlequin color change is a surprising and alarming occurrence for parents. Harlequin color change is unrelated to harlequin ichthyosis.

Subcutaneous Fat Necrosis

Clinical Findings

Subcutaneous fat necrosis is a relatively uncommon condition affecting full-term infants within the first several weeks after birth. It presents as single or multiple, poorly demarcated, tender nodules and plaques that are firm and have an underlying dusky reddish-purple hue (Fig. 106.6). The lesions are located in areas with fat pads, including the buttocks, thighs, arms, face, and shoulders. Calcification of the lesions can occur and can be associated with hypercalcemia.

Diagnosis

Skin biopsy will reveal subcutaneous granulomatous infiltration with multinucleated giant cells and damaged lipocytes containing characteristic needle-shaped clefts. Soft tissue necrosis and inflammation may cause local production of vitamin D, leading to hypercalcemia. Differential diagnosis includes sclerema neonatorum, which is a rare life-threatening panniculitis characterized by diffuse, progressive skin hardening.

Etiology

Subcutaneous fat necrosis is thought to be caused by some form of intrauterine or perinatal trauma. It has been associated with maternal cocaine use, hypothermia, meconium aspiration, and perinatal asphyxia (Rubin et al., 2015).

Treatment and Prognosis

This condition is often self-limited, but care must be taken to monitor calcium levels because of the risk of severe hypercalcemia

and to assess the newborn for evidence of renal injury because of nephrocalcinosis (Tuddenham et al., 2015). Appropriate immediate treatment should be instituted if there is evidence of either of these complications. Resolution of subcutaneous fat necrosis occurs over a period of weeks to months. Bisphosphonates have been suggested for use in subcutaneous fat necrosis, but appropriate controlled clinical trials are still lacking.

Cutis Marmorata

Clinical Findings

Cutis marmorata is a common finding, particularly in premature infants. It presents with a netlike violaceous reticular blanching pattern affecting the extremities more than the trunk. It is exaggerated with cooling and resolves with warming.

Diagnosis

Diagnosis is made by clinical observation. Findings should resolve with warming, and the condition itself should resolve by approximately 1 month of age. Persistent cutis marmorata past 1 month of age can be associated with genetic abnormalities such as Down syndrome, trisomy 18, and Cornelia de Lange syndrome. Cutis marmorata that persists after warming and is localized and asymmetric could represent cutis marmorata telangiectatica congenita, a distinctive vascular anomaly that can be associated with limb atrophy. Another diagnostic possibility is livedo reticularis, which presents with a clear vascular pattern that can be related to underlying vascular inflammation/vasculitis; however, this condition is quite rare in infants.

Etiology

Cutis marmorata occurs because of transient shifts in skin blood flow.

Treatment and Prognosis

Warming should resolve the cutaneous findings. If cutis marmorata is unresponsive to warming or persists past several months of age, further work-up for underlying causes may be required.

Dermal Melanocytosis

Clinical Findings

Dermal melanocytosis (DM), previously known as *Mongolian spots*, is an exceedingly common congenital cutaneous finding in children of African-American, Asian, and Hispanic descent. It presents as well-demarcated, deep blue to gray pigmented macules and patches that are most commonly seen on the buttocks and low back (Fig. 106.7). DM is usually an isolated finding and is not typically associated with tissue malformations or systemic disorders. There are very rare case reports of DM associated with inborn errors of metabolism (e.g., GM1 gangliosidosis) and other potential associations with a vascular nevus called *phakomatosis pigmentovascularis*, which is a form of twin spotting that occurs when two genetically distinct patches involve nearby or corresponding body areas (Franceschini and Dinulos, 2015).



• **Fig. 106.7** Dermal Melanocytosis. Gunmetal gray macules and patches on the back of a healthy infant consistent with dermal melanocytosis (aka *Mongolian spots*).

Diagnosis

Diagnosis is by clinical appearance. The differential diagnosis includes postinflammatory hyperpigmentation, nevus of Ito, vascular malformation, and child abuse. Bruising concerning for child abuse is suggested when the color of the lesion changes over time from blue green to brown and if the lesion is tender to palpation. Biopsy of DM is rarely performed but would show increased numbers of dermal melanocytes.

Etiology

DM is caused by an excessive number of dermal melanocytes. The bluish-gray coloration is due to the Tyndall effect (light reflection off the dermal-based melanocytes).

Treatment and Prognosis

No treatment is required for this benign and self-limited condition. DM tends to fade over time, with studies showing that 42% of cases disappear by 1 year of age (Gupta and Tappa, 2013). Features that portend persistence past 1 year of age are multiple dark and/or large patches and presence in locations other than the sacrum. Most of these lesions fade by several years of age. Laser treatment of the lesions is not recommended. Because of possible confusion with bruising as mentioned earlier, it may be prudent to document DM when it is first encountered.

Aplasia Cutis Congenita

Clinical Findings

Aplasia cutis congenita (ACC) is a rare congenital skin defect that is localized and is most often seen on the scalp as an isolated lesion (Tollefson, 2012). It presents with a localized loss of skin of variable

thickness and is rarely associated with underlying skeletal abnormalities. Findings can include an open ulceration or a healed atrophic scar. The clinical finding of a “hair collar sign” that presents as a ring of darker, longer hair surrounding the lesion represents an increased risk of an underlying neurodevelopmental abnormality.

Diagnosis

Diagnosis is clinical. Imaging of the central nervous system should be performed if a hair collar sign is present. Variants of ACC include focal dermal hypoplasia, or Goltz syndrome. Scalp ulcerations can be seen in infectious processes such as herpes simplex virus infection or can be due to perinatal trauma such as scalp electrode placement. Extensive ACC may be associated with limb hypoplasia (e.g., Adams–Oliver syndrome) or a variant of epidermolysis bullosa (e.g., congenital localized absence of skin, aka *Bart syndrome*).

Etiology

There are numerous theories regarding the cause of ACC. These include primarily intrauterine events such as intrauterine trauma, vascular abnormalities, intrauterine infection, and maternal medications (Browning, 2013). No firm cause has been elucidated to date.

Treatment and Prognosis

The lesions of ACC will heal without intervention but scarring will be present. Identification and management of the rare underlying neurodevelopmental abnormality are important aspects of care for these patients. In especially large lesions, surgical intervention may be required to close the defect. Scar revision can be performed later in life if desired.

Suggested Readings

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Complete references used in this text can be found online at www.expertconsult.com

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Cutaneous Congenital Defects

DEEPTI GUPTA AND ROBERT SIDBURY

KEY POINTS

- Infantile hemangiomas (IHs) are the most common soft tissue tumor of infancy, with a reported incidence of 4%–5% in mature newborns and up to 23% in extremely preterm infants.
- There is often parental concern regarding the risk of hemorrhage in IHs, but serious bleeding rarely occurs, and there is not a risk of coagulopathy or Kasabach–Merritt phenomenon.
- Propranolol is a nonselective beta blocker that was serendipitously discovered to effectively treat IHs.
- Port-wine stains can occur in isolation but also can be related to an underlying genetic disorder, of which *Sturge–Weber syndrome* is the most common. Laser therapy can yield remarkable improvement for many port-wine stains, minimizing the emotional pain that accompanies facial disfigurement.
- Localized areas of hypopigmentation on the skin of the newborn may be isolated phenomena or they may be markers of extracutaneous abnormalities.
- Congenital melanocytic nevi are hamartomas derived from neural crest cells that form in utero and are often classified by the largest diameter of their adult projected size. The risk of melanoma in small and intermediate-sized nevi is low, but for large and giant congenital melanocytic nevi, the risk of melanoma is higher, ranging from 4.5%–10%.

The spectrum of congenital cutaneous defects can be organized by the type of tissue or cell of origin or on the basis of the location within the skin. This chapter presents information on the most common and clinically significant congenital cutaneous defects.

Vascular Anomalies

There has been long-standing confusion regarding the nomenclature and pathogenesis surrounding cutaneous vascular anomalies, which has led to delays in diagnosis and improper treatment and management. Terms such as *cavernous hemangioma* were used to describe both vascular tumors and vascular malformations, and terms such as *hemangioma* have been used indiscriminately to encompass various vascular tumors of differing behavior, prognosis, morphology, and treatment. A classification system for vascular anomalies was originally proposed by Mulliken and Glowacki (1982) and was most recently updated in 2015 (Wassef et al., 2015) by the

International Society for the Study of Vascular Anomalies to allow more precise diagnosis, categorization, and management. Vascular anomalies can be largely divided into two groups—vascular tumors and vascular malformations, with a smaller third group of provisionally unclassified vascular anomalies (Table 107.1). Vascular tumors demonstrate cellular hyperplasia, while vascular malformations comprise a single malformed and dysplastic vessel or a combination of such vessels (Enjolras and Mulliken, 1997, and Finn et al., 1983). Vascular tumors can be further subdivided into benign, locally aggressive, and malignant tumors. Vascular malformations are subdivided into categories based on the predominant type of anomalous channel, arising either from a single abnormal channel type or a mixed malformation comprising a combination of capillary, venous, lymphatic, or arterial vessel involvement. Simple and combined vascular malformations can be further divided according to their flow characteristics. Vascular malformations can also be classified by their association with other clinical features or associated syndromes (Table 107.2). A complete list of the various vascular tumors and malformations is given in Table 107.1, with highlighting of the most common vascular anomalies described within the text.

Vascular Tumors

Infantile Hemangiomas

Infantile hemangiomas (IHs) are the most common soft tissue tumor of infancy. They have a reported incidence of 4%–5% (Haggstrom et al., 2007; Kilcline and Frieden, 2008; Munden et al., 2014) in mature newborns, and incidence rates of up to 23% have been reported in preterm infants weighing less than 1000 g (Goelz and Poets, 2015). Infantile hemangiomas have a female (2.3 to 2.9 times higher) and white predominance (Esterly, 1995). The most significant risk factor for the development of IHs is low birth weight, with the risk increasing by 40% for every 500-g decrease in birth weight (Drolet et al., 2008). Additional risk factors include prematurity, multiple gestation, preeclampsia, placental abnormalities, advanced maternal age, and in vitro fertilization (Kilcline and Frieden, 2008; Munden et al., 2014).

Clinical Features

IHs have a characteristic and unique growth pattern that is similar in both full-term and premature infants. At birth, IHs are either absent or barely evident. Within the first few weeks after birth, a precursor lesion is present, which can appear as either a pale area of vasoconstriction or a telangiectatic red macule or a “bruise-like”

TABLE 107.1 Classification of Vascular Anomalies

| Vascular Tumors | Vascular Malformations |
|---|--|
| Benign: <ul style="list-style-type: none">- Infantile hemangioma- Congenital hemangioma<ul style="list-style-type: none">- Rapidly involuting congenital hemangioma^a- Noninvoluting congenital hemangioma- Partially involuting congenital hemangioma- Tufted angioma (with or without Kasabach–Merritt phenomenon)^{a,b}- Pyogenic granuloma (aka <i>lobular capillary hemangioma</i>) Others: <ul style="list-style-type: none">- Glomeruloid hemangioma- Targetoid hemangioma | Simple/single vessel: Slow-flow malformations <ul style="list-style-type: none">- Capillary malformation- Venous malformation- Lymphatic malformation High-flow malformations <ul style="list-style-type: none">- Arteriovenous malformation- Arteriovenous fistula Primary lymphedema |
| Locally aggressive: <ul style="list-style-type: none">- Kaposiform hemangioendothelioma (with or without Kasabach–Merritt phenomenon)^{a,b}- Retiform hemangioendothelioma- Papillary intralymphatic angioendothelioma- Kaposi sarcoma | Mixed/combined malformation: <ul style="list-style-type: none">- Capillary malformation–venous malformation- Capillary malformation–lymphatic malformation- Capillary malformation–arteriovenous malformation- Lymphatic malformation–venous malformation- Capillary malformation–lymphatic malformation–venous malformation- Capillary malformation–lymphatic malformation–arteriovenous malformation- Capillary malformation–venous malformation–arteriovenous malformation- Capillary malformation–lymphatic malformation–venous malformation–arteriovenous malformation |
| Malignant vascular tumors: <ul style="list-style-type: none">- Angiosarcoma- Epithelioid hemangioendothelioma | |

^aMay be associated with thrombocytopenia or consumptive coagulopathy.
^bExpert consensus is that these lesions lie along a spectrum, rather than being distinct entities.
ISSVA Classification of Vascular Anomalies ©2014 International Society for the Study of Vascular Anomalies Available at "issva.org/classification" Accessed June 2016.



• **Fig. 107.1** A hemangioma precursor sign presenting as a pale vasoconstrictive halo surrounding two faint erythematous macules on the lumbosacral region of an infant.

area (Fig. 107.1). There is a short latency period of 1 to 3 weeks before initiation of a rapid proliferation phase in most hemangiomas. This rapid growth phase occurs within the first 3 months after birth, with most of the growth occurring between 5 and 8 weeks of age (Tollefson and Frieden, 2012). It is important to note that

up to 80% of IHs have completed their growth by 3 months of age. Their growth is also limited to increasing volume within a predefined area rather than exhibiting a true radial growth phase, which is seen more characteristically in other neoplasms (Chang et al., 2008). After a more rapid growth phase, IHs can continue to grow more slowly up until 9 to 12 months of age. There is then an observed phase of relative stabilization followed by spontaneous regression. Regression of a hemangioma occurs slowly over many years, with approximately 90% regression noted by 4 years of age (Bauland et al., 2011). Clinical signs of regression include dulling of the bright red color to a more purple color and a central gray-white discoloration that spreads centrifugally. If the white discoloration occurs in infants younger than 3 months, it can sometimes be a marker of impending ulceration. Regression does not indicate complete resolution and normalization of the underlying skin in all cases. There can be remaining telangiectasias, distortion of facial anatomy, and permanent textural changes characterized by residual fibrofatty tissue and skin laxity because of loss of elastic fibers causing fine wrinkling of tissue. Permanent scarring can also result from hemangiomas that were previously ulcerated.

While proliferation is often a key characteristic of IHs, it should not be considered an absolute defining feature. There is a unique subset of IHs known as *abortive hemangiomas* or *infantile hemangiomas with minimal or absent growth* in which the proliferation phase is absent (Suh and Frieden, 2010; Fig. 107.2). These hemangiomas present preferentially on the lower extremities and sometimes can be confused with capillary malformations (CMs). Although they lack the proliferation phase, they do spontaneously regress.

TABLE 107.2 Syndromes Associated With Vascular Malformations

| Vascular Malformation Type | Associated Syndrome | Clinical Features | Other Features |
|--|--|--|--|
| Capillary malformation/ port-wine stain | Sturge–Weber syndrome | <ul style="list-style-type: none"> - Facial port-wine stain (often involving forehead or V1 distribution) - Ipsilateral eye abnormalities (choroidal vascular anomalies, increased ocular pressure, buphthalmos, glaucoma) - Leptomeningeal and brain abnormalities | Mutations in <i>GNAQ</i> in syndromic and nonsyndromic port-wine stains identified |
| | Nova syndrome | <ul style="list-style-type: none"> - Capillary malformation of the glabella - Neurologic malformations (Dandy–Walker malformation, hydrocephalus, cerebellar agenesis, mega cisterna magna) | |
| | Phakomatosis pigmentovascularis | <p>Combination of cutaneous vascular and pigmentary abnormalities</p> <p>Classification into five subtypes:</p> <ol style="list-style-type: none"> 1. Capillary malformation, epidermal nevus 2. Capillary malformation, dermal melanosis, with or without nevus anemicus 3. Capillary malformation, nevus spilus, with or without nevus anemicus 4. Capillary malformation, dermal melanosis, nevus spilus, with or without nevus anemicus 5. Cutis marmorata telangiectasia congenita, dermal melanosis | Phakomatosis pigmentovascularis has been associated with other vascular anomaly syndromes, including Sturge–Weber syndrome and Klippel–Trenaunay syndrome |
| | Beckwith–Wiedemann syndrome | <ul style="list-style-type: none"> - Prominent nevus simplex/capillary malformation of the philtrum and glabella - Hemihypertrophy - Visceromegaly - Macroglossia - Omphalocele | Intelligence is often not impaired. Overgrowth syndrome, therefore increased risk of Wilms tumor—screening is recommended |
| | Macrocephaly–capillary malformation syndrome/ megalencephaly–capillary malformation– polymicrogyria syndrome | <ul style="list-style-type: none"> - Macrocephaly - Capillary malformation most prominent of the central face (philtrum and glabella) but can occur anywhere - Overgrowth of the body and brain (megalencephaly, hemihypertrophy) - Brain malformations (polymicrogyria, hydrocephalus) - Digital abnormalities (syndactyly, polydactyly) - Joint laxity | Overgrowth syndrome, therefore increased risk of Wilms tumor—screening is recommended. <i>AKT3</i> , <i>PIK3CA</i> , <i>PIK3R2</i> mutations detected. Developmental delay and seizures often occur. |
| Venous malformations | Blue rubber bleb nevus syndrome (Bean syndrome) | Multifocal venous malformations of the skin, mucosa, and gastrointestinal tract. Present as small black-blue papules and skin-colored nodules. Involvement of the palms and soles is common. Gastrointestinal bleeding is common. | Gastrointestinal bleeding is common and is a unique feature of Blue rubber bleb nevus syndrome. Bleeding can result in chronic anemia and can require transfusions |
| | Glomuvenous malformation syndrome | Small to large segmental venous malformations with cobblestoned appearance and bluish-purple color. Often painful to palpation | Autosomal dominant inheritance or sporadic, mutations in glomulin gene (<i>GLMN</i>) |
| | Familial venous malformation cutaneous and mucosal | Small venous malformations mainly involving skin and mucosa but can also involve gastrointestinal tract, brain, and skeletal muscle. Usually asymptomatic | Autosomal dominant inheritance. Often associated with <i>Tie2</i> mutation. |
| | Maffucci syndrome | <ul style="list-style-type: none"> - Venous malformation like skin nodules, rare presentation in infancy - Enchondromas, benign cartilage-forming tumors within the medullary cavity of the bone leading to bony distortion, fragility, and shortening of the affected limb. Hands and feet are involved 90% of the time. | Malignant transformation of the enchondromas can occur over time. Somatic mosaic mutations in <i>IDH1</i> and <i>IDH2</i> have been identified in cases |
| | Cutis marmorata telangiectatica congenita | <ul style="list-style-type: none"> - Capillary malformation, reticulated, can have focal areas of atrophy and ulceration (see Fig. 107.34) - Limb asymmetry of affected limb. | Other reported abnormalities: glaucoma and other ocular anomalies, cardiac defects, syndactyly, brain and spinal cord abnormalities |

Continued

**TABLE
107.2****Syndromes Associated With Vascular Malformations—cont'd**

| Vascular Malformation Type | Associated Syndrome | Clinical Features | Other Features |
|--|--|--|--|
| Lymphatic malformation | Gorham syndrome | <ul style="list-style-type: none"> - Multiple lymphatic malformations present in the bone, skin, and soft tissue - Progressive destruction of the bone associated with lymphatic malformation | |
| Arteriovenous malformation | Capillary malformation–arteriovenous malformation syndrome | <ul style="list-style-type: none"> - Multiple capillary malformations, congenital and acquired - Arteriovenous malformations of the central nervous system and soft tissues | Autosomal dominant Mutation in <i>RASA1</i> |
| | Cobb syndrome | <ul style="list-style-type: none"> - Spinal arteriovenous malformation - Vascular malformation of the skin (extremities or trunk) | |
| | Hereditary hemorrhagic telangiectasia | <ul style="list-style-type: none"> - Telangiectasias of the skin and mucosa - Epistaxis - Arteriovenous malformation of the lungs, brain, and liver | Autosomal dominant Infants with hereditary hemorrhagic telangiectasia can present with intracranial hemorrhage in the neonatal period |
| Vascular malformation and overgrowth syndromes | Beckwith–Wiedemann syndrome | <ul style="list-style-type: none"> - Prominent nevus simplex/capillary malformation of the philtrum and glabella - Hemihypertrophy - Visceromegaly - Macroglossia - Omphalocele | Intelligence is often not impaired. Overgrowth syndrome, therefore increased risk of Wilms tumor—screening is recommended |
| | Macrocephaly–capillary malformation syndrome/megalencephaly–capillary malformation–polymicrogyria syndrome | <ul style="list-style-type: none"> - Macrocephaly - Capillary malformation most prominent of the central face (philtrum and glabella) but can occur anywhere - Overgrowth of the body and brain (megalencephaly, hemihypertrophy) - Brain malformations (polymicrogyria, hydrocephalus) - Digital abnormalities (syndactyly, polydactyly) - Joint laxity | Overgrowth syndrome, therefore increased risk of Wilms tumor—screening is recommended. <i>AKT3</i> , <i>PIK3CA</i> , and <i>PIK3R2</i> mutations detected. Developmental delay and seizures often occur. |
| | Klippel–Trenaunay syndrome | <ul style="list-style-type: none"> - Combined slow-flow vascular malformation (capillary malformation, capillary malformation–venous malformation, capillary malformation–lymphatic malformation–venous malformation) in a characteristic geographic morphology - Venous varicosities - Overgrowth or undergrowth of the affected limb - Leg length discrepancy | At risk of coagulopathy, pulmonary embolism, contractures |
| | Parkes Weber syndrome | <ul style="list-style-type: none"> - Fast-flow vascular malformation (arteriovenous malformation) and arteriovenous shunts - Vascular stain - Overgrowth of the affected limb - Leg length discrepancy | Can occur as part of capillary malformation–arteriovenous malformation syndrome due to <i>RASA1</i> mutation, therefore family history should be obtained |
| | CLOVES syndrome | <ul style="list-style-type: none"> - Combined vascular malformation (capillary malformation, capillary malformation–lymphatic malformation–venous malformation, lymphatic malformation, arteriovenous malformation) - Epidermal nevus - Lipomas/truncal lipomatosis - Skeletal anomalies/scoliosis - Sandal gap deformity of toes | <i>PIK3CA</i> mutation. Risk of Wilms tumor |
| | Proteus syndrome | <ul style="list-style-type: none"> - Slow-flow vascular malformation (capillary malformation, capillary malformation–lymphatic malformation–venous malformation, lymphatic malformation) - Connective tissue nevi | Growth is progressive. |
| | <i>PTEN</i> hamartoma tumor syndrome (Cowden syndrome, Bannayan–Riley–Ruvalcaba syndrome) | <ul style="list-style-type: none"> - Vascular malformation (arteriovenous malformation, lymphatic malformation) - Epidermal nevus - Collagenomas - Lipomatosis | Segmental overgrowth. At risk of internal malignancies such as breast, thyroid, and endometrial cancers. Syndrome has characteristics of penile lentiginos, developmental delay, and macrocephaly |



• **Fig. 107.2** Infantile hemangioma with minimal or absent growth. A 4-month-old male with a reticulated vascular patch on the lower back.



• **Fig. 107.3** Infantile hemangioma, superficial type, located on the upper arm of an infant.



• **Fig. 107.4** Infantile hemangioma, deep type, located on the lower back of an infant.



• **Fig. 107.5** Infantile hemangioma, mixed type, with both superficial and deep components.

The clinical appearance of an infantile hemangioma is dictated by the depth of involvement, which can also lend itself to differences in the timing of growth. Superficial hemangiomas (Fig. 107.3) are located in the upper dermis and present as elevated bright-red, well-demarcated, papules or plaques, which are sometimes in lay language referred to as *strawberry hemangiomas*. In their early proliferation phase, small bright-red papules can be seen arising from fine telangiectatic vessels, which can be a distinguishing feature between IHs and CMs that can have a similar appearance in early infancy. Deep infantile hemangiomas (Fig. 107.4) are confined to the deep dermis and subcutis and present as bluish, dome-shaped tumors or nodules, with ill-defined borders. They can present in isolation or more commonly as a mixed infantile hemangioma (Fig. 107.5) with both superficial and deep components. The deep component of IHs often has a distinct growth pattern in which they tend to appear later, around 2 to 3 months of age, and grow for a longer period, sometimes even over years, as compared with their superficial counterparts (Brandling-Bennett et al., 2008; Chang et al., 2008). Hemangiomas are further characterized by their shape, pattern, and extent of involvement—whether focal or segmental. Focal hemangiomas seem to grow from a single point, whereas

segmental hemangiomas are thought to arise from an embryonic developmental unit or placode and comprise a pattern on the skin correlating with these developmental units. Segmental hemangiomas are at risk of associated anomalies and syndromes. Large facial or scalp hemangiomas should be evaluated for the possibility of PHACE (posterior fossa abnormalities, infantile hemangioma, arterial abnormalities, coarctation of the aorta, eye abnormalities) syndrome, which can also have associated midline defects such as supraumbilical raphe and sternal cleft abnormalities and therefore are sometimes referred to as PHACES. Perineal hemangiomas or segmental hemangiomas in the lumbosacral area should be evaluated for the possibility of underlying spinal and genitourinary abnormalities with possible LUMBAR (lower body hemangioma, urogenital abnormalities, ulceration, myelopathy, bony deformities, anorectal malformations, and renal abnormalities) syndrome, also known as SACRAL (spinal dysraphism, anogenital abnormalities, cutaneous, renal and urologic abnormalities, associated with angioma of the lumbosacral localization) or PELVIS (perineal hemangioma, external genitalia malformations, lipomyelomeningocele, vesicorenal abnormalities, imperforate anus, and skin tag) syndrome. Segmental hemangiomas, like deep IHs, can sometimes have a prolonged growth phase, which in rare cases may last for years.

There is often parental concern regarding the risk of hemorrhage in IHs, and parents should be reassured that serious bleeding rarely occurs. Coagulopathy and risk of Kasabach–Merritt phenomenon (KMP) was originally thought to be associated with large infantile hemangioma, but it has been shown that KMP does not occur with IHs and is seen only with other vascular tumors, such as tufted angiomas and kaposiform hemangioendotheliomas (Enjolras et al., 1997).

Pathogenesis

IHs form as a result of dysregulation of both vasculogenesis and angiogenesis. There are many proposed theories as to the trigger that initiates this dysregulation, without any one unifying cause, but it is likely that hypoxia plays an important role. There is strong evidence in support of IHs being derived from endothelial stem and progenitor cells (Khan et al., 2008). Isolation of CD133⁺ endothelial progenitor cells from infantile hemangioma tissue and injection of these progenitor cells into nude mice lead to formation of tumors with the unique immunohistochemical and growth characteristics of IHs (Khan et al., 2008). The endothelial cells that were isolated from IHs were also found to be clonal in nature, suggesting that these tumors were caused by a somatic mutation in one or more genes regulating endothelial cell proliferation (Boye et al., 2001). The endothelial cells in infantile hemangioma express a unique phenotype of cell surface markers with positive staining for glucose transporter 1 (GLUT1), merosin, and antigen Lewis Y, which is also expressed by placental endothelial cells, but are not present in any other tumor or vascular malformation (North et al., 2001). Histologically, IHs have increased cellularity and form small sinusoidal vascular channels. It has been suggested that IHs may result from ectopic placental tissue given the similarity in their endothelial cell profiles and the fact that the placenta and IHs share a similar life cycle (North et al., 2000). Further evidence suggestive of this link relates to the increased risk of development of IHs in low birth weight infants—particularly those whose mothers had preeclampsia or placenta previa, both of which are associated with placental hypoxia. Hypoxia likely plays a large role in the development of IHs and is thought to be an important triggering signal for their development (Drolet and Frieden, 2010; Léauté-Labrèze et al., 2017), with in utero hypoxia as a risk factor for localized hemangiomas and regional hypoxia from arterial abnormalities as a risk factor for segmental hemangiomas. It is likely that this hypoxic stress acts as a triggering signal and leads to overexpression of vascular endothelial growth factor via the hypoxia-inducible factor α pathway, which then leads to proliferation of CD133⁺ endothelial progenitor cells that are present in fetal tissue and causes them to differentiate into immature endothelial cells, along with pericytes, dendritic cells, and mesenchymal cells with adipogenic potential. The adipogenic potential may play a role during regression—when blood vessels are replaced with fibrofatty tissue. During the involution phase, endothelial cells are also noted to express caspases, which are known markers of apoptosis.

Diagnosis

In most cases a hemangioma can be diagnosed by its clinical appearance and characteristic pattern of evolution. A lesion that deviates from this typical picture presents a diagnostic dilemma. Doppler ultrasound examination can be easily performed and may be helpful in distinguishing between an infantile hemangioma and another low-flow malformation or nonvascular tumor. Other imaging modalities—magnetic resonance imaging (MRI) or angiography—may be indicated for large or obstructive lesions

(e.g., ocular, upper airway) to help define the extent of involvement or associated abnormalities (e.g., PHACE or LUMBAR syndrome) (Baker et al., 1993; Esterly, 1995). Skin biopsy is diagnostic for nonvascular tumors, which can mimic vascular birthmarks (e.g., pilomatricoma, juvenile xanthogranuloma [JXG], Langerhans cell histiocytosis, infantile myofibromatosis, rhabdomyosarcoma). IHs can also be differentiated from other vascular tumors by staining with GLUT1, an immunohistochemical marker that is highly selective and specific for IHs. GLUT1 is also expressed at the blood–brain barrier and in placental tissue but has not been found in any other vascular tumors, including congenital hemangiomas. Its discovery has helped in making the correct diagnosis of these vascular tumors, especially in cases with atypical presentations (North et al., 2000).

Complications

While the majority of IHs are uncomplicated and spontaneously regress without any need for intervention, in approximately 10%–15% of cases, complications can arise requiring treatment.

Local Complications

Ulceration. Ulceration is the most common complication observed in IHs and can be seen in up to 30% of cases (Maguiness and Frieden, 2012; Fig. 107.6). It can cause significant pain and discomfort and permanent scarring in the area of ulceration. Ulceration has been described to be more likely to occur during two points during the life cycle of the hemangioma: either just before the rapid proliferation phase, which can be the presenting sign of the hemangioma, or at the end of the growth phase (usually around 4 months of age). The exact mechanism causing ulceration is unknown but is thought to be related to tissue hypoxia, with the tumor outgrowing its blood supply (Chamlin et al., 2007). Ulceration is more commonly seen in large hemangiomas, those with segmental distribution, and those with mixed morphology with both superficial and deep components (Morelli et al., 1994; Mulliken et al., 1995; Chamlin et al., 2007). Areas of friction or those exposed to moisture for a long time, such as the lower lip, neck, intertriginous areas, and anogenital and diaper region, are at high risk of ulceration. Nearly one-third of all ulcerated hemangiomas are found in the diaper region (Kim et al., 2001). Various treatment modalities have been shown to be effective in treating ulcerated hemangiomas, but all ulcerated hemangiomas benefit from local wound care, and combination therapy is more effective than monotherapy (McCuaig et al., 2013). Treatment options



• Fig. 107.6 Painful ulcerated infantile hemangioma on the neck.

included topically administered brimonidine, 0.2% or timolol, 0.5%, topical antibiotics with mupirocin or metronidazole, pulsed dye laser treatment, and systemic therapy with propranolol. Topical analgesics can also be useful to minimize pain and discomfort (Cheng and Friedlender, 2016).

Disfigurement. In addition to the transient disfigurement, hemangiomas located on the central face, large hemangiomas, and those with a significant superficial component can predispose the affected child to permanent scarring. IHs involving the nasal tip are at risk of a bulbous nasal tip or a “Cyrano” deformity (Fig. 107.7). This is caused by splaying of the alar cartilage during the proliferative growth phase. Early intervention with initiation of propranolol therapy can preserve the contour of the nose (Perkins et al., 2014). If a persistent deformity develops, surgical debulking

may be needed. Similarly, IHs located on the lip are at risk of deformity and disruption of the natural contours of the lips in addition to being at high risk of ulceration and permanent scarring (Fig. 107.8; Yanes et al., 2016). Large hemangiomas of the central chest or hemangiomas involving the breast tissue in females can also be quite disfiguring, leading to permanent breast hypoplasia in some cases (Fig. 107.9; Theiler et al., 2016). Disfigurement alone, regardless of threat to function, is a reasonable indication for medical therapy in certain cases and should be considered. Regression of hemangiomas does not always ensure complete normalization of the underlying skin and therefore can lead to lifelong psychosocial and emotional sequelae experienced by both the family and the patient (Zweegers and Van der Vleuten, 2012).



• **Fig. 107.7** (A–B) Nasal tip infantile hemangioma with both superficial and deep components. Nasal tip hemangiomas are at high risk of causing disfigurement.



• **Fig. 107.8** Partial segmental infantile hemangioma of the lip and nose with ulceration of the lip.



• **Fig. 107.9** Infantile hemangioma of the breast involving the nipple and areola.

Functional Complications

Periocular Hemangiomas. Hemangiomas located on the lid or around the orbit are at risk of causing visual impairment and can lead to amblyopia in severe cases. Amblyopia can be caused by direct pressure on the globe, causing astigmatism or myopia, or because of the size of the hemangioma there can be visual axis obstruction or strabismus (Spence-Shishido et al., 2015). Larger and segmental hemangiomas in the periorbital area pose the greatest risk of ocular complications. Deep retrobulbar IHs may present with proptosis and can also cause strabismus and visual acuity changes. Deep and mixed infant hemangiomas can also cause tear duct obstruction and exposure keratopathy. Aggressive and early initiation of treatment along with evaluation by a pediatric ophthalmologist can help prevent some of these complications (Spiteri Cornish and Reddy, 2011; Xue and Hildebrand, 2013). Further imaging, such as MRI, may be needed to assess the extent of the hemangioma or the presence of a deeper component.

Auricular Hemangiomas. With infantile hemangiomas of the ear there is high risk of physical deformity of the ear, cartilage destruction, ulceration, potential infection when ulceration is present, and potential hearing alterations. Segmental hemangiomas in this region have a higher rate of complications, and with them there can also be a risk of sensorineural and conductive hearing loss.

Potential Life-Threatening Complications

Airway Hemangiomas. Airway obstruction by an infantile hemangioma is a life-threatening complication that requires immediate evaluation and treatment. Airway hemangiomas can occur with or without the presence of cutaneous IHs. The highest risk occurs with hemangiomas located in a “beard distribution” specifically involving the left or right preauricular areas, chin, lower lip, and anterior part of the neck (Orlow et al., 1997). Involvement with IHs at four or more of these sites is associated with a 63% risk of a symptomatic airway hemangioma. A greater number of lesions in the beard distribution leads to a higher risk of airway involvement. Segmental hemangiomas involving facial segment 3 have a 29% risk of airway involvement. Clinically, airway hemangiomas present most commonly between 6 and 12 weeks of age with biphasic stridor or a hoarse, croup-like cry. The subglottis is the most common site of involvement, but the oral cavity, oropharynx, hypopharynx, larynx, and upper trachea can also be involved. Referral to a pediatric otolaryngologist is important for evaluation of the airway, and systemic treatment should be started immediately (O et al., 2009). Treatment of airway hemangiomas may require a combination of multiple medical and surgical treatments depending on the extent of involvement, which may include propranolol, oral and intralesional corticosteroids, vincristine, interferon alpha, surgical excision, and laser therapy.

Hepatic Hemangiomas. The presence of an IH in the liver can lead to potential serious complications such as congestive heart failure and consumptive hypothyroidism. Individuals with five or more cutaneous IHs (Fig. 107.10) of any size and in any location should be screened for the possibility of a hepatic IH (Horii et al., 2011). This study showed that in patients with five or more cutaneous hemangiomas, 16% of them had evidence of a hepatic hemangioma versus none in the group with fewer than five cutaneous hemangiomas. Individuals with large or segmental IHs do not seem to be at greater risk of hepatic IHs (Boon et al., 1996a; Horii et al., 2010).

Parotid Hemangiomas. Parotid hemangiomas can be isolated to the parotid gland or can be present as part of a segmental IH in the maxillary distribution of facial segment 3. Parotid



• **Fig. 107.10** Diffuse hemangiomatosis presenting with multifocal infantile hemangiomas on the skin and in the liver.

hemangiomas have a unique pattern of growth as they can have a longer proliferative growth phase than typical IHs. This can lead to longer treatment courses (Brandling-Bennett et al., 2008). They have also been associated with functional complications relating to deformity of adjacent structures such as the ear and lip and conductive hearing loss caused by narrowing of the external auditory canal. Life-threatening complications can also arise, including an association with subglottic hemangiomas, and less commonly congestive heart failure and consumptive hypothyroidism (Greene, 2004; De Corti et al., 2015).

Treatment

Most IHs are uncomplicated and spontaneously regress, but there is a subset of approximately 10%–15% of IHs that result in complications requiring treatment (Mulliken et al., 1995; Hoeger et al., 2015). Treatment is indicated in cases complicated by disfigurement or risk of disfigurement, ulceration, or functional compromise. There are various treatment modalities, including topical or systemic medications, surgery, or laser therapy, that are chosen on the basis of various factors, including the stage of growth, location, potential complications, and associated conditions. The various treatment modalities are outlined in the following sections. In general, pharmacologic treatment is the first-line treatment, with surgical and laser therapies being considered as adjunctive or second-line treatment following medical interventions.

Active Nonintervention. For most lesions the initial treatment of choice is “active nonintervention.” This is reserved for hemangiomas that are not at risk of causing disfigurement, ulceration, or causing any functional impairment. These often tend to be small focal hemangiomas in nonfacial locations. Anticipatory guidance should be discussed with the family in regard to the natural course of IHs and the initial period of rapid growth in the first few months of life and then slow rate of involution over many years. Demonstration of before-and-after photographs of growing and involuted hemangiomas in other children can help demonstrate the natural course and diminish parental concern. There is also a common concern of risk of significant hemorrhage. This is rare in IHs. Minor episodes of bleeding can result from trauma and respond to short-term compression, like any superficial wound.

Although the large majority of IHs do not require treatment and spontaneously regress, the disfigurement associated with the residua of IHs should not be underestimated, and treatment should be considered on the basis of the location and size of the infantile

hemangioma. Up to 40% of hemangiomas leave permanent skin changes that can be disfiguring (Enjolras and Mulliken, 1993). Psychological and social problems may result from facial or other visible deformities. A small study found that patients with untreated involuted facial hemangiomas had higher levels of social anxiety and decreased social initiative as compared with children with treated facial IHs (Costa et al., 2016). Therefore early intervention should be considered for lesions with a higher potential for complications.

Topical Therapies. Topically administered timolol maleate is a nonselective β -adrenergic receptor blocker that was first reported to be used in IHs in 2010 and has been shown to be most effective for superficial and thin hemangiomas (Püttgen et al., 2016). It is approved for use in pediatric glaucoma, but its use topically on the surface of the IHs is off-label. The preferred formulation is timolol gel forming solution, 0.5%. In general, timolol is well tolerated, without significant systemic side effects. However, a recent prospective study showed systemic absorption with use of one drop twice a day—although the concentrations detected were below 0.2 ng/mL, which is below the level at which systemic effects may begin to be seen (Weibel et al., 2016). Caution should be used in preterm infants with postmenstrual age less than 44 weeks and low birth weight infants weighing less than 2500 g at the time of initiation of treatment as there have been a few case reports of symptomatic bradycardia in this group (Frommelt et al., 2016). However, in these reported cases, the doses of timolol being used exceeded 0.2 ng/mL, and the treatment areas had variable or increased absorption (e.g., thin-skinned areas such as eyelid, mucosal surfaces, and ulcerated sites). Absorption within mucosal sites and ulcerated hemangiomas is variable, and therefore caution should be used, but there have been reports of use in these areas without any adverse side effects (Cante et al., 2012).

Systemic Therapies

Propranolol. Propranolol is a nonselective β -adrenergic receptor blocker that in 2008 was discovered by chance to treat IHs (Léauté-Labrèze et al., 2015; Sans et al., 2009). It had been used at higher doses in children with cardiac disease for many decades previously. In 2014, Hemangeol (propranolol hydrochloride, Pierre Fabre, Parsippany, New Jersey, United States) was approved by the US Food and Drug Administration as the only approved systemic treatment for IHs, and propranolol become first-line systemic treatment for IHs. The dosages for IHs range from 1 to 4 mg/kg per day in divided doses (two or three times daily), with a 98% response rate at a mean dosage of 2.1 mg/kg per day (Marqueling et al., 2013). There have now been three randomized controlled trials (Hogeling et al., 2011; Léauté-Labrèze et al., 2013, 2015) examining the efficacy of propranolol for IHs, with the largest trial showing the highest efficacy at dosages of 3 mg/kg per day for a 6-month course (Léauté-Labrèze et al., 2015). Despite this, most practitioners use maintenance dosages of 2 mg/kg per day because dosing regimens of more than 2 mg/kg per day did not show a significant increase in effect but did show an increase in the rate of adverse events (Wedgeworth et al., 2016). The most common adverse side effects of the medication reported are gastrointestinal disturbance, sleep disturbance, and acrocyanosis. These side effects were overall mild and reversible. Serious adverse effects such as symptomatic hypotension, hypoglycemia, bradycardia, and bronchospasm occurred infrequently and in a randomized control trial were reported at a similar frequency in the placebo group (Léauté-Labrèze et al., 2015). Once the medication is discontinued, rebound growth can occur, but this was more likely if the hemangioma was treated for less than 9 months versus a course of 12 to 15 months (Shah et al., 2016).

Corticosteroids. Oral corticosteroids were the first-line treatment before the discovery of propranolol. Prednisone or prednisolone dosages of 2 to 5 mg/kg per day were used, with the most optimal effects reported at a dosage of around 3 mg/kg per day (Esterly, 1995; Mulliken et al., 1995). Within 1 to 2 weeks, 30% of hemangiomas would show a dramatic response, but 40% would respond equivocally. Many side effects were noted in these patients, most notably cushingoid appearance, gastroesophageal reflux, insomnia, irritability, transient growth retardation, hyperglycemia, and rarely adrenal insufficiency, hypertension, and osteoporosis. Studies comparing propranolol with oral corticosteroids showed that clinical response to propranolol was more rapid and effective, with need for fewer surgical interventions, and that overall propranolol was better tolerated (Bertrand et al., 2011). Corticosteroids as a monotherapy are no longer used for IHs but rather are now reserved for complex and refractory cases and can be used in conjunction with propranolol or other therapies (Ranchod, 2005).

Intralesional corticosteroid injections may be used in small, localized hemangiomas in cosmetically sensitive areas with high rates of morbidity such as the lip, nasal tip, and eyelid (Couto and Greene, 2014; Herlihy et al., 2016). This can be used as a therapy adjuvant to other topical or systemic treatments. Complications include cutaneous atrophy, skin necrosis, and, for intralesional periocular injections, ophthalmic artery occlusion and blindness. Thus periocular intralesional steroid injection should be performed only by experienced pediatric ophthalmologists.

Other Therapies. Historically, interferon alpha and vincristine were used with variable effect for treatment of IHs. These therapies have potential adverse effects and are now reserved only for refractory lesions (Barlow et al., 1998; Wörle et al., 1999; Perez Payarols et al., 1995). Orally administered sirolimus, a mammalian target of rapamycin (mTOR) inhibitor, has now been considered in a few such cases (Kaylani et al., 2013).

Surgical Therapies

Laser Therapy. Pulsed dye laser treatment has been used in various settings with IHs. There have been some controversial data regarding its use as a monotherapy for uncomplicated hemangiomas, and it seems to be more efficacious when used in conjunction with propranolol or timolol (Asilian et al., 2015). It has also been used to heal ulcerations (David et al., 2003) and to decrease residual erythema and telangiectasias in an involuting hemangioma. However, a randomized prospective controlled trial of 121 infants found that pulsed dye laser treatment in uncomplicated hemangiomas is no better than watchful waiting (Batta et al., 2002). Pulsed dye laser treatment cannot prevent the preprogrammed growth pattern of hemangiomas and has a limited role during the proliferative phase. One risk of laser use during the proliferative phase can be ulceration. In contrast, it can be useful during involution, if there is residual redness or telangiectasias, which may be resolved faster than with the natural course of involution, but has no effect on fibrofatty residuum.

Surgical Excision. Surgical excision can be done in cases of medically refractory hemangiomas that are symptomatic and proliferating, in an emergency situation where there is life-threatening functional compromise, or in situations with recurrent profuse bleeding. It may also be beneficial in situations where there is a large disfiguring pedunculated lesion that will leave behind significant fibrofatty residual or scar.

Wound Care. Ulceration is a therapeutic challenge, but all ulcers benefit from local wound care and potential occlusive dressings. The type of dressing chosen depends on the amount of exudate

**TABLE
107.3****Indications for Work-Up of Extracutaneous Anomalies Associated With Infantile Hemangiomas**

| Clinical Presentation of Hemangioma | Association | Evaluation |
|---|---|---|
| Large facial hemangioma/segmental facial hemangioma >5 cm | PHACE syndrome | MRI/MRA of the brain and neck, echocardiogram, ophthalmology evaluation |
| Multifocal hemangiomas ≥5 cm | Extracutaneous hemangiomas, especially hepatic hemangiomas | Abdominal ultrasound examination with Doppler imaging |
| Periocular hemangioma | Ocular complications | Ophthalmology evaluation |
| Parotid hemangioma | Consumptive hypothyroidism, congestive heart failure, airway hemangioma | |
| Lumbosacral hemangioma >2.5 cm | LUMBAR syndrome | MRI of lumbar spine and pelvis, neurosurgical evaluation |
| Large lower trunk or lower extremity hemangioma | PELVIS syndrome | MRI of lumbar spine and pelvis, neurosurgical evaluation, urologic evaluation |
| Beard distribution hemangiomas | Evaluation for respiratory distress | Otolaryngology evaluation and MRI of the neck |
| Breast hemangioma/large segmental chest hemangioma | Breast hypoplasia | Consider systemic treatment |
| Midline hemangiomas (with other cutaneous markers) | Spinal dysraphism | Spinal ultrasound examination at <3 months of age, MRI of the spine |
| Segmental proximal upper extremity hemangioma with extension onto the chest | PHACE syndrome or cardiac abnormality | Echocardiogram |

MRA, Magnetic resonance angiography; MRI, magnetic resonance imaging.

and on the location of ulceration. For sites that are difficult to dress, frequent and liberal application of petrolatum jelly is effective. Various dressing materials, including petrolatum-impregnated gauze and seaweed-derived alginate dressings, are often recommended. Off-label use of becaplermin gel, a recombinant human platelet-derived growth factor, has reportedly shown dramatic healing in a small case series. The product now carries a warning from the Food and Drug Administration, which must be taken into consideration before its use (Metz et al., 2004; Yan, 2008). Agents for pain control should be considered, including topical anesthetics (being mindful of the percentage of body surface area covered) and oral analgesics such as acetaminophen or ibuprofen. A high index of suspicion should be maintained for secondary infection, with appropriate use of topical or oral antibiotics as needed. If conservative therapy is unsuccessful, pulsed dye laser treatment may relieve pain and speed reepithelialization (Achauer and Vander Kam, 1991; Morelli et al., 1991, 1994). The ulcers will heal but will inevitably leave scars.

Work-Up and Associations. A complete list of associations and the work-up needed for specific hemangiomas on the basis of their size and location is given in Table 107.3. Here we will highlight a few other special circumstances.

Visceral Involvement/Hepatic Hemangiomas. Diffuse neonatal hemangiomatosis (see Fig. 107.10) manifests itself as widely scattered, small superficial hemangiomas. Infants with this pattern of cutaneous involvement may have lesions limited to the skin, known as *benign neonatal hemangiomatosis*. However, associated hemangiomatosis of the liver, gastrointestinal tract, lungs, and/or central nervous system (CNS) can be complicated by visceral hemorrhage, hepatomegaly, high-output cardiac failure, or unexplained anemia or thrombocytopenia, with a significant mortality rate (Byard et al.,

1991). Congestive heart failure can also occur with a large, isolated hepatic hemangioma (Mulliken et al., 1995).

Segmental Hemangiomas

PHACE Syndrome. PHACE syndrome is characterized by a large (>5 cm) segmental hemangioma of the face, scalp, or neck with associated anomalies and developmental defects (Frieden et al., 1996). The acronym PHACE is used to describe the constellation of anomalies related to this syndrome: posterior fossa malformations, hemangioma, arterial anomalies, cardiac anomalies/coarctation of the aorta, and eye abnormalities. Midline defects such as sternal cleft and supraumbilical raphe can also be seen, and therefore sometimes this disorder is referred to as *PHACES*. The diagnostic criteria for PHACE syndrome were formalized by a consensus panel, and clinical features were grouped into major criteria. Definitive diagnosis of PHACE syndrome requires a facial segmental IH or an IH larger than 5 cm on the face or scalp in addition to one major or two minor criteria (Metry et al., 2009). This was revised in 2016 to also include a segmental hemangioma of the neck, upper trunk, or trunk and proximal upper extremity plus two major criteria (Garzon et al., 2016). The exact incidence of PHACE syndrome is unknown, but it may be more common than Sturge-Weber syndrome (SWS) (Metry et al., 2006). There is a striking female predominance in PHACE syndrome, with a female-to-male ratio of 9:1. A prospective study for PHACE syndrome found that 31% infants with facial IHs with a surface area of 22 cm² or greater met the PHACE diagnostic criteria, which is based on expert consensus, and approximately 90% of affected infants had more than one extracutaneous finding (Haggstrom et al., 2010). Children with frontotemporal and frontonasal IHs (known as segments 1 and 4) have a higher correlation with structural cerebral and cerebrovascular anomalies. Those with

mandibular (segment 3) or beard distribution lesions are at higher risk of cardiac abnormalities and IHs in the airway (Waner et al., 2003). The maxillary face (segment 2) appears to be a lower-risk segment for association with PHACE syndrome. The most common and potentially devastating sequelae of PHACE syndrome are neurologic, including structural brain anomalies and abnormalities of cerebral vasculature. Progressive stenoses and occlusions of cerebral arteries can also be seen. Both moyamoya-like vasculopathy and arterial ischemic strokes have been reported (Drolet et al., 2006; Heyer et al., 2006). Other comorbidities have recently been reported in patients with PHACE syndrome, including headaches, endocrine abnormalities, hearing abnormalities, speech delay, dysphagia, and dental anomalies (Garzon et al., 2016).

Because of the associated anomalies, patients suspected of having PHACE syndrome require the following:

- Echocardiogram to assess the patient for coarctation of the aorta or other structural cardiac abnormalities with possible cardiac MRI/magnetic resonance angiography (MRA) if the echocardiogram is abnormal
- MRI with contrast medium and MRA of the head and neck evaluating the patient for cerebrovascular and structural anomalies
- Ophthalmology examination of the retina
- Complete physical examination with particular focus on ventral midline defects
- Additional studies as indicated on the basis of signs and symptoms

LUMBAR/SACRAL/PELVIS Syndrome. Similarly to PHACE syndrome, large hemangiomas of the lower body may be associated with underlying structural abnormalities. Sacral and lumbar hemangiomas may reveal spinal dysraphism, including tethered spinal cord, lipomyelomeningocele, imperforate anus, renal anomalies, or abnormal external genitalia (Tavafoghi et al., 1978; Albright et al., 1989; Goldberg et al., 1986; Laurent et al., 1998). Many acronyms have been coined, all describing a similar entity of a segmental hemangioma in the lumbosacral or perineal region with associated regional abnormalities. These include LUMBAR, SACRAL, and PELVIS (Girard et al., 2006; Stockman et al., 2007; Iacobas et al., 2010).

Exact diagnostic criteria have not yet been defined, but IHs in the lumbosacral or perineal area that are large, midline, segmental, or present with other cutaneous markers (i.e., lipoma, gluteal cleft deviation, skin tag, aplasia cutis) should be screened for underlying abnormalities. One prospective study noted that 35% of patients with lumbosacral hemangiomas larger than 2.5 cm had evidence of spinal dysraphism on MRI (Drolet et al., 2010). Segmental hemangiomas in the lumbosacral region typically present with minimal or absent growth and persist as a patch with coarse telangiectasias, which can lead to delays in diagnosis. These are also at high risk of ulceration.

Congenital Hemangiomas. Congenital hemangiomas are an uncommon and distinct type of vascular proliferation that are fully formed at birth. They do not undergo the characteristic proliferative growth pattern in postnatal life as seen with IHs, but rather their proliferative phase occurs in utero. They are also GLUT1 negative, unlike IHs. Congenital hemangiomas can be divided into three major subtypes of rapidly involuting congenital hemangiomas (RICHs), noninvoluting congenital hemangiomas (NICH), and partially involuting congenital hemangiomas (PICHs) on the basis of their clinical progression. Congenital hemangiomas are usually solitary in nature and are more common on the extremities and head and neck (Boull and Maguiness, 2016).

Rapidly Involuting Congenital Hemangiomas. RICHs can present clinically in three distinct ways: (1) a raised violaceous tumor with prominent peripheral vasculature, (2) a raised tumor with coarse overlying telangiectasias with a peripheral halo of vasoconstriction or pallor, or (3) a pink-purple tumor with deep infiltrative nodules. There can sometimes be overlying hypertrichosis (Boon et al., 1996b). Rapid involution often begins in the first few weeks of life and is completed by 14 months of age. After involution there may be some residual atrophy, telangiectasias, persistent and prominent vessels, or milia present. There is a rare subtype of RICH where complete involution occurs in utero called *RICH fetal involution type*. Complications of RICHs are that they can undergo ulceration and bleeding shortly after birth, which can be painful and leave a permanent scar in the area of ulceration. There have also been reports of transient coagulopathy with thrombocytopenia that can occur. The decrease in platelet count is brief and not progressive as seen in KMP. The presence of this phenomenon can lead to misdiagnosis of RICH with tumors associated with KMP, such as tufted angiomas and kaposiform hemangioendothelioma, and therefore biopsy may be required to confirm the diagnosis.

Noninvoluting Congenital Hemangiomas. NICHs (Fig. 107.11) present as vascular patches, plaques, or nodules with blue or pink-purple color with overlying coarse telangiectasias and peripheral rim of pallor and vasoconstriction (Lee et al., 2014). They persist over time and grow proportionally with the child, without spontaneous regression. Some can become more protuberant or develop an increase in draining veins over time. Symptoms may develop during pregnancy or puberty, most notably that of pain (Lee et al., 2014).

Partially Involuting Congenital Hemangiomas. There is a small subset of congenital hemangiomas that begin a phase of involution that lasts until 12 to 30 months of age, at which time the involution halts (Nasseri et al., 2014). The residual vascular lesion then persists lifelong morphologically, resembling a NICH. There have been no reported complications in patients with PICH. The existence of PICH suggests that all congenital hemangiomas may exist along a spectrum.

Kaposiform Hemangioendotheliomas and Tufted Angiomas. Kaposiform hemangioendotheliomas (KHEs) and tufted angiomas are rare vascular tumors with locally aggressive and benign growth potential, respectively. They are thought to exist along a spectrum and are associated with life-threatening KMP. KMP is a profound and life-threatening thrombocytopenia that results from intralésional platelet trapping with a consumptive coagulopathy evidenced by elevation of D-dimer levels, reduction in fibrinogen



• **Fig. 107.11** Noninvoluting congenital hemangioma presenting as an unchanged vascular plaque in a 1-year-old. There are coarse telangiectasias with a blue background and surrounding vasoconstrictive halo.

levels, and prolongation of prothrombin time and partial thromboplastin time. The severity of the coagulopathy is variable.

Tufted angiomas (Fig. 107.12) typically present in infancy or early childhood as a solitary dusky erythematous indurated vascular plaque with overlying hypertrichosis, hyperpigmentation, and/or telangiectasias (Herron et al., 2002; Osio et al., 2010). Tufted angiomas tend to not be as aggressive and do have as deep infiltration as their counterpart kaposiform hemangioma. Spontaneous regression has been reported in some congenital cases or cases with earlier onset (Browning et al., 2006).

KHE presents as a solitary ill-defined red-purple plaque during infancy or early childhood. It can extend to involve the viscera, chest wall, and retroperitoneum and may present without cutaneous findings. KHE is more commonly seen in neonates and infants as compared with older children and may be associated with an increased risk of KMP (Gruman et al., 2005).

Pyogenic Granulomas. Pyogenic granulomas, also known as *lobular capillary hemangiomas* because of their histologic appearance, are benign acquired vascular tumors that are commonly seen in infants and children (Patrice et al., 1991; Fig. 107.13). They have also been seen to develop within an existing port-wine stain. They present clinically as a rapidly growing solitary, red papule that can

sometimes be exophytic or pedunculated in nature. They often present with a crusted or eroded surface because of their friable nature. They do not spontaneously involute, and treatment is often pursued because of recurrent episodes of bleeding and their friability. Treatment options include simple curettage or shave excision with electrocautery of the base, which is most definitive and curative. Other treatment options include topical therapies, such as topically administered timolol therapy, a beta blocker, or imiquimod therapy, or pulsed dye laser treatment, which have variable success with prolonged treatment courses. Recurrence is possible.

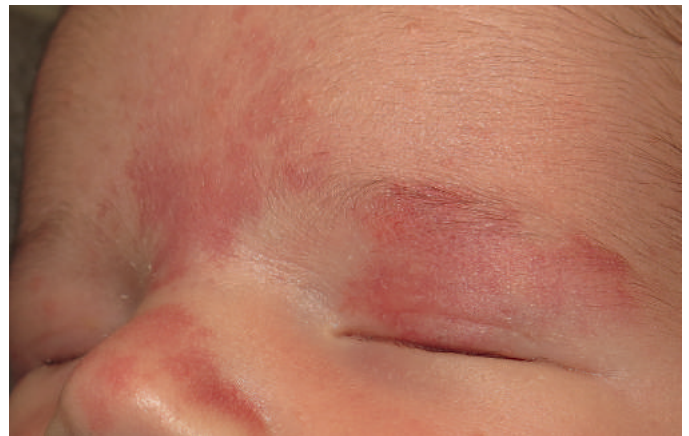
Vascular Malformations

Nevus Simplex

Nevus simplex, also known as a *salmon patch* or *fading capillary stain* and colloquially referred to as *angel's kiss* when it occurs on the forehead or eyelids and as *stork bite* when it occurs on the nape of the neck, is a very common capillary malformation that almost always spontaneously fades by 1 to 2 years of age (Fig. 107.14), although some malformations persist, most notably those at the nape of the neck (Fig. 107.15). It typically presents as a bilateral and symmetric faint pink patch with a feathery border and a



• **Fig. 107.12** Tufted angioma without Kasabach–Merritt phenomenon on the foot of a 4-month-old child. This was confirmed by biopsy.



• **Fig. 107.14** Nevus simplex on the glabella, nose, and eyelids of a newborn presenting as faint pink patches with feathery borders.



• **Fig. 107.13** Pyogenic granuloma on the cheek of a 8-month-old infant presenting as a bright-red glistening papule.



• **Fig. 107.15** Nevus simplex on the posterior occiput. Nevus simplex in this location can often persist.

midline predilection and most commonly occurs on the central forehead, glabella, upper eyelids, nose, upper lip, nape of the neck, posterior occiput, and the lower back. Nevus simplex located the midline in the lumbosacral area is usually a benign finding in isolation and not associated with underlying spinal dysraphism, but if there are any other cutaneous findings in this area (i.e., lipoma, hypertrichosis, faun tail, aplasia cutis) or abnormalities noted (i.e., gluteal cleft deformity), then further work-up is warranted. Prominent and persistent nevus simplex can be associated with underlying syndromes such as Beckwith–Wiedemann syndrome, macrocephaly–capillary malformation syndrome, and Nova syndrome (Burns et al., 1991; Table 107.2).

Capillary Malformation (Port-Wine Stain)

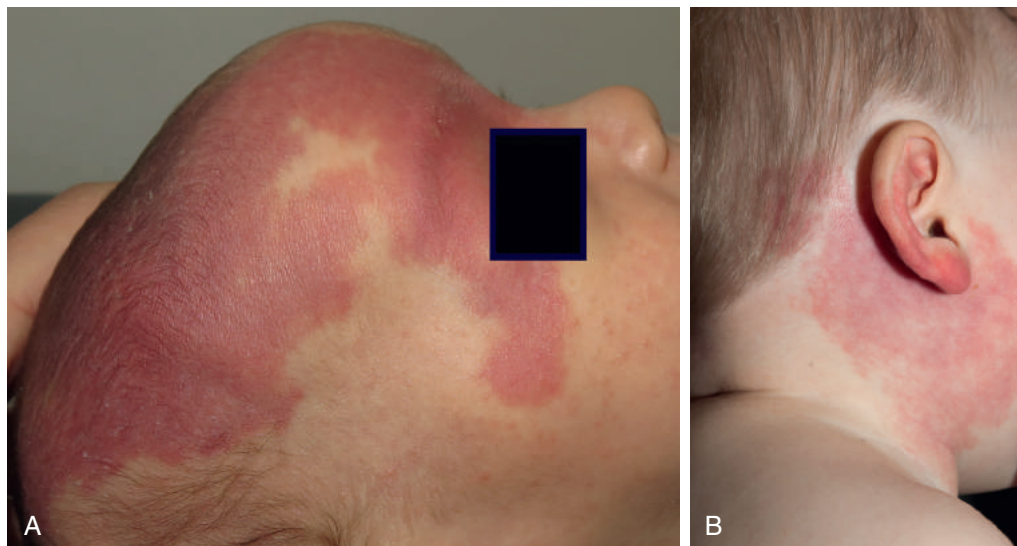
The terms *capillary malformation* and *port-wine stain* are used synonymously in the literature and refer to collections of malformed and ectatic dermal capillaries that persist over time (Fig. 107.16). They are present at birth and occur in 0.3% of neonates. They present as fairly well-demarcated red or pink patches that are often asymmetric, occur anywhere on the body, and may or may not be associated with localized overgrowth. They grow proportionally to the individual's somatic growth and over time can gradually darken from pink red to a darker red and deep purple. They also tend to thicken over time, developing a papulonodular surface change and can develop localized soft tissue or even bony hypertrophy. Nodular vascular growths called *pyogenic granulomas* (see earlier) can also develop within the existing port-wine stain and may require therapeutic intervention.

Port-wine stains can occur in isolation but also can be related to an underlying genetic disorder (Table 107.2). Of these syndromes, SWS is the most common. SWS is a sporadic neurocutaneous syndrome characterized by facial capillary malformation most commonly involving the upper face and forehead, leptomeningeal vascular malformation that can lead to seizures, hemiparesis, developmental delay, malformations of the choroid, and ophthalmologic abnormalities, most commonly glaucoma. SWS occurs in less than 30% of infants with facial port-wine stains. The risk is increased in infants with more extensive CMs of the face, notably those that have hemifacial or median forehead patterns of

involvement, previously thought to be located in the V1 trigeminal region. Patterns associated with SWS are now thought to correlate with patterns of mosaicism coined by Happle and Assim (2001) rather than trigeminal nerve distribution.

The degree of CNS involvement is variable in SWS, ranging from subclinical lesions to intractable seizures and intellectual impairment. Patients can still be at risk of glaucoma without SWS when the port-wine stain involves the forehead or upper or lower eyelid, V1 or V2 trigeminal pattern; therefore complete ophthalmologic examination is indicated for affected infants. Both SWS and isolated CMs have been found to be due to an activating somatic mosaic mutation in the *GNAQ* gene (Shirley et al., 2013). In SWS this mutation is also found in affected brain tissue, which is not seen in patients with isolated CMs. It is hypothesized that earlier timing of the mutation can lead to more extensive neurocutaneous involvement.

Treatment of an uncomplicated CM is aimed at minimizing disfigurement. With time these lesions can thicken and develop irregular surface changes, often with friable nodules. Most of the published data on laser treatment of port-wine stains in children come from studies using a pulsed dye laser (Kelly et al., 2002). Children require an average of 4 to 10 pulsed dye laser treatments for maximal lightening. The best results have been seen in children younger than 4 years, with studies recommending earlier onset of therapy to achieve the best results. Some studies describe use of laser treatment in patients as young as 4 weeks. In younger age groups, 20% can expect 95% clearing (Goldman et al., 1993). Pulsed dye laser therapy is less effective for facial port-wine stains that are close to the midline or those on the extremities (Garden and Bakus, 1993; Renfro and Geronemus, 1993). Laser therapy can yield remarkable improvement for many port-wine stains, minimizing the emotional pain that accompanies facial disfigurement. Unfortunately, none of the currently available lasers is capable of permanently erasing port-wine stains in most patients. There are also reports of darkening of port-wine stains many years after effective lightening (Huikeshoven et al., 2007). Laser treatment with the 1064-nm neodymium-doped yttrium aluminum garnet (Nd:YAG) laser and the 755-nm alexandrite laser has also been described to penetrate deeper vessels in more refractory port-wine



• **Fig. 107.16** (A) Port-wine stain of the periocular area, forehead, and scalp. (B) Port-wine stain involving the posterior auricular scalp, neck, cheek, and ear.



• **Fig. 107.17** A venous malformation on the sole of the foot.

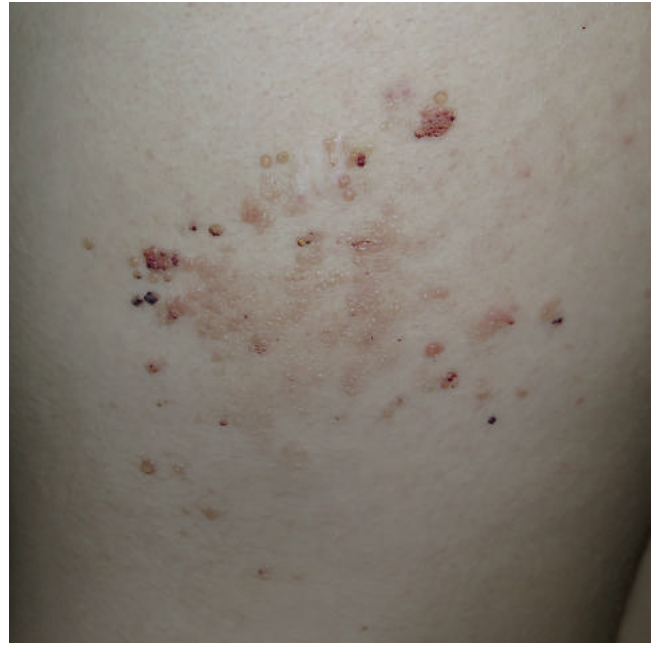
stains on the basis of the theory that the longer wavelength of the second laser may be able to better target deeper vessels (Jasim and Handley, 2007). Recently, the use of topically administered rapamycin (an mTOR inhibitor) in conjunction with pulsed dye laser treatment has been used for refractory port-wine stains, with the hypothesized mechanism being decreased angiogenesis after laser therapy (Marqués et al., 2015).

Venous Malformations

Venous malformations are slow-flow vascular malformations composed of ill-defined venous channels with abnormal vessel walls that lack smooth muscle cells. They are often evident at birth and present as soft, blue-purple tumors or plaques with tortuous vessels that increase in size with exertion or when in a dependent position (Fig. 107.17). Over time they may become symptomatic and larger with increased dilation of vessels. They may affect the skin, mucous membranes, subcutaneous tissue, muscles, and joints and can involve visceral organs. Most cases are sporadic, but familial cases have also been reported. Localized intravascular coagulation can occur within the venous malformation (VM) that leads to either formation of thrombi, which form into calcifications called *phleboliths*, which can be quite painful, or bleeding (Mazoyer et al., 2008). Glomuvenous malformations are a distinct subset of VMs that present more superficially as blue-purple cobblestoned plaques and nodules commonly located on the extremities. Glomus cells distinctly line the malformed veins. Familial cases caused by mutations of the glomulin gene (*GLMN*) are more common than sporadic cases.

Lymphatic Malformations

Lymphatic malformations (LMs) are slow-flow vascular malformations. They can be classified as microcystic when individual malformed channels are smaller than 1 cm and macrocystic (i.e., cystic hygroma) when individual channels are larger than 1 cm, combined, or rarely generalized. LMs are most often seen in the head and neck region and can cause life-threatening airway



• **Fig. 107.18** A superficial lymphatic malformation on the back presenting with characteristic hemorrhagic papules and vesicles.

compromise in neonates. They can occur as an isolated finding or can be seen in association with underlying syndromes (Table 107.2). Some isolated LMs and those related to overgrowth syndromes are caused by a mutation in the gene encoding phosphatidylinositol 4,5-bisphosphate 3-kinase, catalytic subunit alpha (*PIK3CA*) (Kirkorian et al., 2016). Therefore in some cases, rapamycin (the mTOR inhibitor also used for refractory port-wine stain) has been used as a medical therapeutic option. Other possible therapeutic interventions include surgical excision for localized lesions, sclerotherapy, and laser treatment with a pulsed dye laser and a long-pulse Nd:YAG laser for superficial lesions (Fig. 107.18).

Arteriovenous Malformations

Arteriovenous malformations (AVMs) are fast-flow vascular malformations that are characterized by direct shunting of blood between the arteries and veins with bypassing of the capillaries. AVMs may present as a capillary malformation on the skin with pulsatility and significant warmth. The formation of vascular blebs that can easily bleed may also be seen. They can present within the skin but also viscerally anywhere in the body. The head and neck region, including the brain, are common locations. AVMs can occur in isolation or in association with various genetic syndromes (Table 107.2). Recently, mutations in *RASAI*, associated with familial forms of AVMs, have been found in individuals with an AVM of an extremity (Parkes Weber syndrome) and in individuals with CM-AVM syndrome, where individuals present with multiple cutaneous CMs, many with a “thumb print”-like quality and potential AVMs in the brain and/or spine.

These lesions do not resolve spontaneously and are more likely to become larger and more symptomatic over time. Complications are related to the flow rate and extent of the lesion. Localized thrombosis and phlebitis occur in low-flow lesions; high-flow lesions can cause significant bleeding, destructive interosseous changes (Fig. 107.19), and high-output cardiac failure. Many centers now have collaborative multidisciplinary groups to help manage the

most complicated vascular malformations and vascular tumors, which typically require treatment by physicians in many specialties.

Lymphedema

Lymphedema is a term used to describe diffuse soft tissue swelling characterized by firm, pitting edema. Lymphedema can occur in the setting of anomalous lymphatic drainage. Congenital variants have been reported. Females are affected more frequently than males. The lower limbs are the most commonly affected sites, but other sites may also be involved, and rarely, chylothorax or ascites may be present. *Milroy disease* is an autosomal dominant condition that manifests itself with progressive lymphedema of the lower extremities. Lymphedema of the extremities also occurs in *Turner (XO) syndrome*. Disorders associated with lymphedema are listed in [Table 107.4](#).



• **Fig. 107.19** Arteriovenous malformation of the hand causing distortion and contracture of the hand.

Disorders of Pigmentation

Hypopigmented Lesions

Localized areas of hypopigmentation on the skin of the newborn may be isolated phenomena, or they may be markers of extracutaneous abnormalities. The degree of hypopigmentation and the distribution of the defect help distinguish among the different conditions. To properly evaluate a patient with hypopigmentation, a distinction must first be made between complete depigmentation and hypopigmentation. A depigmenting condition produces pure white lesions that are devoid of normal melanocytes. Even in fair-skinned infants the lesions can often be easily seen in ordinary daylight. This group of disorders includes tyrosinase-negative oculocutaneous albinism, piebaldism, and vitiligo, all of which are rarely seen in infancy. A hypopigmented lesion is often subtly lighter in color than the surrounding skin. Histologic examination reveals a normal number of melanocytes. In fair-skinned children these lesions may require Wood lamp illumination to become obvious. This group includes anomalies with a deficient amount of melanin or hemoglobin caused by vasoconstriction, causing pallor and decreased skin pigment. *Nevus anemicus* and *hemangioma precursors* are two examples of areas of pallor that result from diminished superficial blood flow. Here we will discuss disorders of cutaneous mosaicism presenting as patterned pigmentation on the skin.

Nevus Depigmentosus (Nevus Achromicus)

Nevus depigmentosus is an uncommon condition occurring in 0.4% of newborns ([Shih, 2007](#)). It presents as a well-demarcated hypopigmented patch with irregular borders that can involve a small isolated circular or rectangular area or a larger segmental region following the lines of Blaschko, which are embryonic lines of ectodermal cell migration ([Fig. 107.20](#)). The patches are not truly depigmented as the name suggests but actually have reduced melanin levels. *Nevus depigmentosus* is thought to be a form of cutaneous mosaicism and results in a postzygotic somatic mutation that leads to a population of cells with decreased melanogenesis potential. They usually present at birth or shortly thereafter and grow in proportion to the child's overall growth, thus maintaining its shape. They occur sporadically, with no familial pattern of inheritance. There have been case reports of seizures, cognitive delays, and ipsilateral extremity hypertrophy, but most affected individuals do not have any extracutaneous abnormalities ([Lernia, 1999](#)). Sometimes, lentigines can be seen within the hypopigmented area. These are thought to be areas within the nevus where there

TABLE 107.4

Hereditary Lymphedema Syndromes (Primary Lymphedema)

| Syndrome | Clinical Features | Genetics |
|---|---|---|
| Nonne–Milroy disease (primary congenital lymphedema) | Congenital lymphedema of the lower limbs. Associated with enlarged veins of the leg, hydrocele and recurrent cellulitis | Mutation in <i>VEGFR3</i> . Autosomal dominant |
| Meige disease/lymphedema–distichiasis (pubertal-onset lymphedema) | Most common type of lymphedema. Can be associated with distichiasis, yellow nails, ptosis, syndactyly, cleft palate, and cardiac septal defects | Mutation in <i>FOXC2</i> . Autosomal dominant |
| Hypotrichosis–lymphedema–telangiectasia | Lymphedema, sparse hair, and telangiectasias of the skin | Mutation in <i>SOX18</i> . Autosomal dominant |
| Hennekam syndrome (generalized lymphatic dysplasia) | Lymphedema and multiorgan involvement (intestinal lymphangiectasia, protein-losing enteropathy), developmental delay | Mutations in <i>CUBE1</i> (some cases). Autosomal recessive |



• **Fig. 107.20** Nevus depigmentosus presenting as a well-demarcated hypopigmented patch that tends to be circular or oval.



• **Fig. 107.21** Pigmentary mosaicism presenting as a segmental block-like hyperpigmented patch on the back of an infant.

is resolution of the mutation and a return of pigmentation (Bolognia et al., 1998).

Pigmentary Mosaicism

The term *pigmentary mosaicism* is representative of a group of heterogeneous disorders of hypopigmentation and/or hyperpigmentation that include hypomelanosis of Ito (incontinentia pigmenti achromians), linear and whorled nevoid hypermelanosis that present as unilateral or bilateral linear streaks and whorls of cutaneous pigment change oriented along the lines of Blaschko (Fig. 107.21). They can also present in a block-like or checkerboard pattern or a phylloid or leaf-like pattern. Affected individuals may have areas that are hyperpigmented or hypopigmented, or both. The pattern may be congenital or may become apparent within the first 2 years after birth.

This condition is a form of somatic mosaicism. Various extra-cutaneous abnormalities (CNS, ocular, cardiac, and musculoskeletal) (Alvarez et al., 1993; Devriendt et al., 1998; Dereser-Dennl et al., 2000; Tunca et al., 2000) have been reported in patients, but the vast majority of individuals have pigmentary changes confined only to the skin. CNS abnormalities, manifesting themselves as

• BOX 107.1 Syndromes Associated With Multiple Café au Lait Macules

Neurofibromatosis type 1 and type 2
Legions syndrome
Watson syndrome
McCune–Albright syndrome
Noonan syndrome
Noonan syndrome with multiple lentigines (LEOPARD syndrome)
Tuberous sclerosis
Bloom syndrome
Russell–Silver syndrome
Turner syndrome
Bannayan–Riley–Ruvalcaba syndrome
Ataxia–telangiectasia

seizures or developmental delay, most often present by the age of 2 years.

Ash Leaf Macules

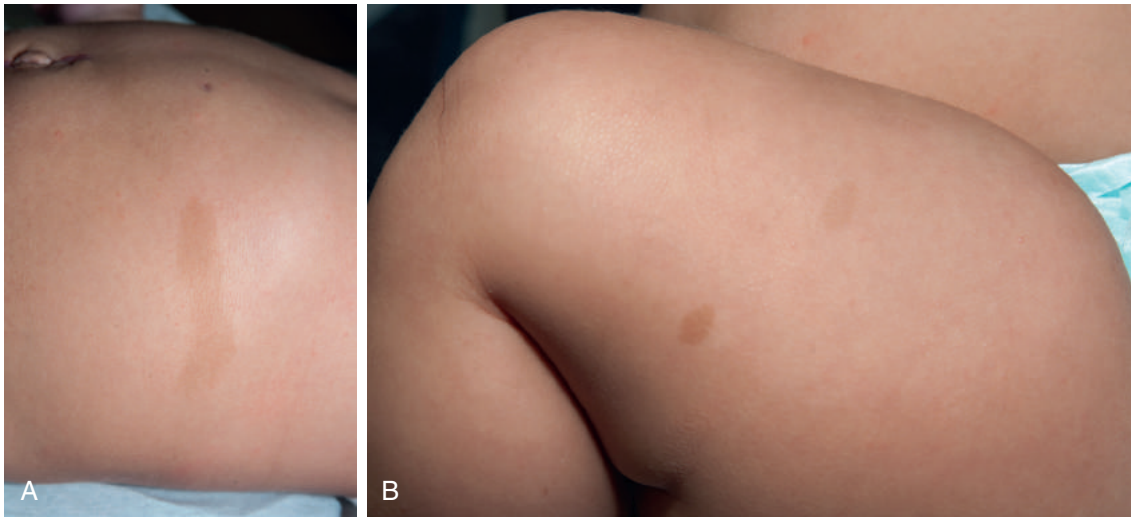
Ash leaf macules are small oval areas of hypopigmentation, named for their similarity in size and shape to a leaflet from a European mountain ash tree. They are one of the few congenital markers for infants with tuberous sclerosis. Tuberous sclerosis complex is a disorder of autosomal dominant inheritance with variable clinical manifestations characterized by the development of benign and malignant tumors in a variety of tissues, including the skin, CNS, and kidney (Gomez, 1991; Janninger and Schwart, 1993; Schwartz et al., 2007). This disorder is discussed in further detail in Chapter 104.

Hyperpigmented Lesions

Brown lesions usually reflect an increased number of melanocytic cells or an excess amount of melanin. Brown coloration can also be associated with a thickened epidermis. Most congenital brown lesions are isolated and benign, but it is important to recognize that some of them are syndrome associated and others may be potentially life threatening.

Café au Lait Macules

Café au lait macules (CALMs) are well-demarcated, oval or round, light brown macules or patches that differ in size, ranging from several millimeters to several centimeters in diameter (Fig. 107.22). They are commonly noted at birth or during infancy but may present in childhood as well. They cannot always be distinguished from melanocytic nevus on clinical grounds, but histologic examination is diagnostic, showing increased melanin levels within the basal keratinocytes, without melanocyte proliferation. CALMs differ within different ethnic groups, with a higher number and prevalence seen in darker-skinned individuals. The prevalence of CALMs is approximately 2.7% in the general population, 2% in white infants, and up to 12% in African-American infants. Large or segmental café au lait patches can be seen in McCune–Albright syndrome (Schwindinger et al., 1992; Shenker et al., 1993; Smith and Kirk, 2002), and multiple CALMs in the neonatal period may be an isolated finding but should alert the physician to the possibility of an associated syndrome, especially when six or more are present (Box 107.1; Fig. 107.23). Six or more café au lait lesions measuring 0.5cm or greater in diameter in prepubertal children and 1.5cm or greater in diameter in postpubertal children



• **Fig. 107.22** (A) Café au lait patch on the abdomen presenting as a tan brown well-demarcated patch. (B) Two café au lait spots presenting on the lateral thigh as well-demarcated tan brown macules.



• **Fig. 107.23** Multiple café au lait spots presenting on an infant with neurofibromatosis type 1. The presence of six or more café au lait spots larger than 0.5 cm in diameter on an infant and larger than 1.5 cm in adolescents is suggestive of the possibility of neurofibromatosis type 1. The presence of only café au lait spots does not meet the criteria for definitive diagnosis.

should alert the practitioner of the possibility of neurofibromatosis type 1 (NF1). Presence of café au lait lesions alone do not fulfill NF1 criteria (Ricardi et al.; Lazaro et al., 1994).

Lentigines

Lentigines are small tan-to-dark brown macules that most commonly appear sporadically in adulthood. They may be distinguished from other pigmented lesions by histologic examination that reveals elongated rete ridges, an increased number of singly dispersed

melanocytes along the basal layer, and increased melanization of the basal keratinocytes. Multiple or congenital lentigines are features of several syndromes.

Congenital Dermal Melanocytosis (Mongolian Spots)

More than 90% of African Americans, 81% of Asians, and 10% of whites (Pratt, 1953) are born with blue-gray macule of infancy, formerly known as *Mongolian spots*. These are brown, gray, or blue macules and patches, most commonly located in the lumbosacral area, but they can occur anywhere on the body. The macules may be single or multiple and range in size from a few millimeters to several centimeters in diameter. They often fade within the first few years after birth. Extensive lesions have been mistakenly attributed to abuse. Histologically, blue-gray macule of infancy is a collection of spindle-shaped melanocytes located deep in the dermis. Malignant change has never been reported.

Nevus of Ota/Ito

Nevus of Ota is a unilateral blue or gray discoloration involving the orbital and zygomatic areas, following the ophthalmic and zygomatic branches of the trigeminal nerve, including the sclera and fundus (Fig. 107.24). It is a sporadic condition, but it occurs with the highest frequency in Asians, affecting up to 1% of individuals in Japan (Kopf and Weidman, 1962), and has a female predominance. The discoloration is detected at birth in 60% of cases. Glaucoma is a frequent complication, occurring in 10% of individuals. A similar lesion, located in the deltotracheus area, is called *nevus of Ito* (Fig. 107.25). Spontaneous resolution does not occur, and over time these lesions can darken, increase in size, and potentially thicken. Individuals with periorbital involvement should undergo ophthalmologic examination. A rare association with malignant melanoma and uveal melanoma has been reported. Successful treatment has been achieved with Q-switched laser devices—ruby, alexandrite, and Nd:YAG.

Melanocytic Nevi

The category of nevocellular nevi includes congenital or acquired nevomelanocytic neoplasms. Nevomelanocytes are dendritic cells of neural crest origin. Nevocellular nevi have traditionally been categorized by the histologic position of the tumor nests within



• **Fig. 107.24** Nevus of Ota on the lateral right cheek of a girl presenting as a blue-gray patch of discoloration.



• **Fig. 107.25** Nevus of Ito on the left arm of a child presenting as a blue-gray patch.

the skin. *Junctional nevi* are the most superficial, located at the junction between the epidermis and dermis. These lesions appear clinically as macules. *Intradermal nevi* are located deep to the dermoepidermal junction and are usually papular. “*Blue*” *nevi* are a variant located in the deep dermis, made up of cells that have elongated, neural features. *Compound nevi* have both junctional and dermal nests of nevomelanocytes. Melanocytic nevi can be further subdivided by their time of onset: congenital, early onset (before the age of 2 years), and acquired (Williams, 1993; Kovalyshyn et al., 2009).



• **Fig. 107.26** (A) Congenital melanocytic nevus with hypertrichosis, small size. (B) Large congenital melanocytic nevus involving the neck and back with multiple smaller “satellite” nevi on the arms and lower back.

Congenital Melanocytic Nevi

Congenital melanocytic nevi (CMN) are hamartomas derived from neural crest cells that form in utero and are most often present at birth or within the first year (Fig. 107.26). They are classified by the largest diameter of their adult projected size. On the basis of size classification, they are categorized as small (<1.5 cm), intermediate sized (1.5 to 19.9 cm), large (20 to 40 cm), and giant (>40 cm). Delineations are based on adult data. Conversion factors have been developed to help predict ultimate size: for CMN on the head, multiply size by 1.7; CMN on the lower extremities, multiply size by 3.3; and for CMN on the trunk, upper extremities, and feet, multiply size by 2.8 (Marghoob et al., 1996). On the basis of these classifications the estimated incidence of small and intermediate-sized CMN is approximately 1%–6%, that of large CMN is approximately 1 in 20,000, and that of giant CMN is approximately 1 in 500,000 (Price, 2016). Melanoma in small and intermediate-sized nevi tends to be approximately 1% lifetime risk or less, nearing the risk within the general population, with

most cases occurring after puberty. In large and giant CMN, the risk of melanoma development ranges from 4.5%–10% (Kopf et al., 1979; Gari et al., 1988; Kufflik and Janniger, 1994; Ozturkcan et al., 1994; Williams and Pennella, 1994; Marghoob et al., 1996; Richardson et al., 2002), with 70% of melanomas diagnosed before the age of 10 years. Other malignancies have also been reported to arise from CMN. These include rhabdomyosarcoma, liposarcoma, malignant peripheral nerve sheath tumors, and other sarcomas. In addition to cutaneous malignancy, large and giant CMN have been associated with the development of neurocutaneous melanosis (NCM), which is characterized by CNS proliferation of melanocytes (Kadonaga and Frieden, 1991). Symptomatic NCM manifests itself with signs or symptoms of increased intracranial pressure and carries a very poor prognosis, with an increased incidence of CNS melanoma (Frieden et al., 1994; Sandsmark et al., 1994). NCM often becomes symptomatic within the first few years after birth. MRI can aid in the diagnosis (Barkovich et al., 1994). Although initial reports emphasized thickening of the leptomeninges, the most common MRI sign of NCM is the spin–lattice (T1) nuclear magnetic relaxation time in the parenchyma of the cerebellum or anterior temporal lobes (sometimes accompanied by the spin–spin [T2] relaxation time). Radiologic identification of malignant degeneration is difficult. Roughly half of asymptomatic infants and children with giant CMN have evidence of NCM on MRI (Frieden et al., 1994). The presence of asymptomatic NCM does not necessarily bode a poorer prognosis, and NCM can continue to be present and asymptomatic throughout life. The term *CMN syndrome* has now been used to broaden the scope of NCM to include more extracutaneous manifestations associated with CMN. Patients with NCM are at risk of developing CNS melanomas, but this risk is significantly higher in individuals with symptomatic NCM. The risk of developing NCM has been correlated with the number of accompanying CMN, historically termed *satellite lesions*, with patients who have more than 20 such lesions carrying a five times greater risk of NCM.

Conservative management of large congenital nevi by surveillance alone is complicated by the presence of features that may fit the screening ABCDE criteria (asymmetry, border irregularity, color variegate, diameter >6 mm, evolution) for a lesion that is suggestive of melanoma. Most of these nevi have an irregular surface appearance from birth and are variably thickened, hairy, verrucous, or nodular. Smaller, widely scattered satellite lesions are almost always present, but there have been no reports of melanoma in satellite lesions. Extracutaneous lesions have also been detected in several sites, including the meninges, lymph nodes, and placental villi (Hara, 1993). Often these nevi have atypical histologic features as well. For children who develop malignant melanoma within a giant nevus, the prognosis is very poor. However, many of the lesions that have an alarming appearance at birth do not exhibit malignant behavior or produce widespread metastases or cause death. In fact, congenital melanoma is very rare and is associated with congenital nevi in less than 50% of reported cases (Williams and Pennella, 1994).

The management of CMN remains controversial, with advocates for and against prophylactic excision. Routine excision is generally not recommended and may not eliminate the risk of melanoma completely given the presence of melanocytes deep with the fascia. Surgical removal is never an easy option. Multiple procedures are usually required, with the attendant high risks of significant morbidity, sometimes yielding results that are more disfiguring than the birthmark. Procedures performed in early childhood, such as tissue expansion (Vergnes et al., 1993) and partial-thickness resection (Sandsmark et al., 1993), may improve the aesthetic outcome but



• **Fig. 107.27** Nevus spilus presenting as a tan patch with multiple darker brown macules and papules within the patch.

have associated stigma and pain. The efficacy of such approaches in the prevention of malignancy has never been documented, and it is unlikely to eliminate the risk of malignancy completely.

Nevus Spilus

Nevus spilus (speckled lentiginous nevus) is a hyperpigmented lesion that consists of focal proliferation of melanocytes along the basal layer of the epidermis (dark spots) within a café au lait spot in the background (Fig. 107.27). Nevus spilus is considered a distinct subtype of a congenital melanocytic nevus, with the associated risk of melanoma being lower than with traditional CMN. There are no other associated abnormalities.

Congenital Tumors of Epithelial Origin

Epidermal Nevus

Epidermal nevus may manifest itself in the newborn period as a smooth hyperpigmented patch or rough, skin-colored plaque, most often on the trunk or extremities, frequently oriented along the lines of Blaschko (Fig. 107.28). With time, epidermal nevi may enlarge, usually within the first few years after birth, and most become verrucous and hypertrophic over time. Treatment is difficult because recurrence is common after destruction or excision.

Nevus Sebaceous

Nevus sebaceous presents as an alopecic tan, yellow, to salmon colored plaque appearing most often on the scalp or face (Fig. 107.29). It may be nodular at birth and again after puberty, flattening during childhood. A variety of neoplasms, both benign and malignant, including basal cell carcinoma, develop in up to 15% of patients with sebaceous nevi. Development of neoplasms rarely occurs before puberty.

Like other mosaic disorders, epidermal and sebaceous nevi are believed to be localized manifestations of somatic genetic mutations that would be lethal if fully expressed. A subset of patients with epidermal nevi are genetic mosaics for an autosomal dominant form of ichthyosis called *epidermolytic hyperkeratosis* (or *bullous ichthyosiform erythroderma*) (Paller et al., 1994). These individuals may be at risk of having offspring with total body involvement.

The striking appearance of epidermal nevi has inspired descriptive nomenclature. *Nevus verrucosus* is a solitary plaque. *Nevus unius lateris* is an extensive linear lesion that is unilateral, following the lines of Blaschko. Both keratinocytic and sebaceous components may occur in the same patient, the former more commonly involving the trunk and extremities and the latter more often involving the head and neck. The term *ichthyosis hystrix* refers to extensive, bilateral involvement with epidermal nevus.

Skin biopsy will rule out other conditions, distinguish between epidermal nevus and nevus sebaceous, and detect the diagnostic histologic features of epidermolytic hyperkeratosis. Small epidermal nevi do not require treatment. Nevus sebaceous carries a small risk of malignant degeneration. It may be excised at any time, depending

on the size of the lesion, the benefits of surgical intervention versus the risks of anesthesia, and the preference of the patient and family but rarely becomes symptomatic or much of an issue until after puberty. Recent studies suggest that the risk of basal cell carcinoma, the most common malignancy to arise in nevus sebaceous, is closer to 1%, much lower than was reported in the past (Cribier et al., 2000).

There is no optimal therapy for larger lesions or those that are disfiguring. Full-thickness excision, including the subcutaneous tissue, is recommended to decrease the risk of recurrence. Laser therapy has been performed with ablative lasers, and there are a few reports suggesting that the Q-switched Nd:YAG laser holds some promise as another therapeutic option. Topically applied keratolytic agents may be palliative. Genetic counseling about the risk for offspring of fully expressed disease should be considered for individuals with epidermal nevi that reveal the histologic features of epidermolytic hyperkeratosis.

Epidermal (Linear Sebaceous) Nevus Syndrome

In less than 10% of affected people, epidermal nevi and sebaceous nevi (especially those involving the head) are associated with a variety of extracutaneous abnormalities, mainly ocular (in 33% of cases), neurologic (in 50%), and skeletal (in 70%), a condition referred to as *epidermal nevus syndrome*. Bone abnormalities include vertebral anomalies, kyphoscoliosis, limb shortening, and hemi-hypertrophy. CNS disorders include seizures, mental retardation, and hemiparesis; ocular abnormalities include eyelid/conjunctival nevus, coloboma, corneal opacity, and nystagmus. Malignancies also occur in this syndrome with a greater than expected frequency, including Wilms tumor, nephroblastoma, gastrointestinal carcinomas, and rhabdomyosarcoma (Marghoob et al., 1993).

Congenital Tumors of Dermal and Subcutaneous Origin

Juvenile Xanthogranuloma

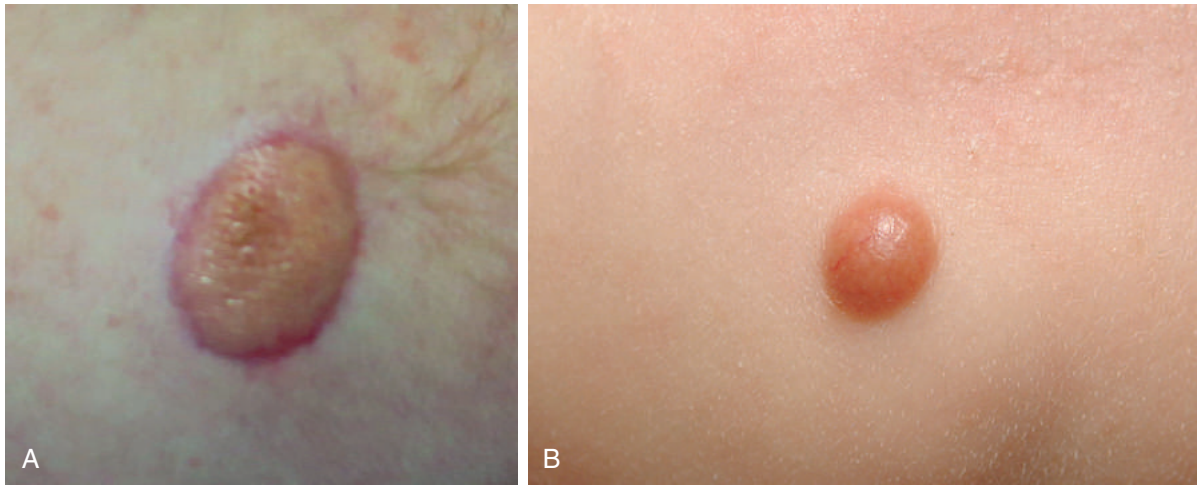
JXG is a benign, self-healing, non-Langerhans cell histiocytic tumor of infancy (Fig. 107.30). JXG is derived from dermal dendrocytes



• **Fig. 107.28** Epidermal nevus presenting as verrucous tan brown papules coalescing in a curvilinear plaque.



• **Fig. 107.29** (A) Nevus sebaceous in an adolescent male presenting as a thickened yellow-pink alopecic plaque with wart-like changes. (B) Nevus sebaceous presenting as an alopecic plaque with verrucous or wart-like changes.



• **Fig. 107.30** (A) Juvenile xanthogranuloma with characteristic tan yellow color and rim of erythema in an infant. (B) Juvenile xanthogranuloma presenting as a yellow-orange papule.

and is present in normolipemic individuals without abnormalities in their lipid metabolism (Newell et al., 1973; Gianotti and Caputo, 1985; Hernandez-Martin et al., 1997). JXG may be congenital in approximately 30% of cases, with 75% presenting within the first year of life (Nomland, 1959). Early on, cutaneous lesions present as erythematous papules with minimal yellow-orange color. As they mature they become more characteristically yellow in color and may develop overlying telangiectasias. They often present on the head, neck, and trunk and can be categorized by their size: “micronodular” (<10 mm) and “macronodular” (>10 mm) forms and rarely giant JXG, which can be up to 5 to 10 cm in size. They may present as a solitary lesion or as multiple lesions, with the solitary presentation being most common, occurring in up to 90% of patients. JXG may also be rarely localized to the eye or mucous membranes (De Raeve et al., 1994). Skin biopsy is usually diagnostic, revealing characteristic foamy histiocytes and Touton giant cells within the dermis.

The vast majority of infants with JXG are otherwise healthy. Giant JXG can have an alarming appearance and may be confused with other types of histiocytic tumors (Magana et al., 1994). JXGs have two clinically significant associations. The first is ocular JXG and its associated complications. Less than 0.5% of children with skin lesions have ocular involvement, but half of children with ocular JXG have cutaneous lesions (Chang et al., 1996). Ocular examination in individuals younger than 2 years with multiple JXGs is recommended. Ocular tumors may manifest themselves as unilateral glaucoma, uveitis, heterochromia iridis, or proptosis, and ocular JXG is the most common cause of hyphema in infancy (Zimmerman, 1965; Gaynes and Cohen, 1967). The iris is the most frequently affected ocular tissue (Hamdani et al., 2000).

The second significant association is related to reports of a rare triad of JXG, juvenile myelomonocytic leukemia (JMML), and neurofibromatosis type 1 (NF-1). The appearance of JXG usually precedes the diagnosis of leukemia or occurs concurrently at the time of diagnosis, and there are often multiple JXGs (Cooper et al., 1984; Sherer et al., 1993). Routine screening for JMML is not recommended in NF-1 patients with or without JXG, but evidence of hepatosplenomegaly, lymphadenopathy, or pallor should prompt appropriate work-up (Burgdorf and Zelger, 2004). Fewer than 20 patients with intracranial JXG without cutaneous manifestations have been described (Schultz et al., 1997; Bostrom et al., 2000).

Most cases of JXG are asymptomatic and self-limited, but ulceration and bleeding of cutaneous lesions can occur. Giant lesions have a similar prognosis (Magana et al., 1994). Ophthalmologic evaluation is indicated for children who present in the first 2 years after birth with multiple lesions. Parents should be provided with anticipatory guidance about the ocular complications (Chang et al., 1996). JXG typically involutes within 3 to 6 years (Hansen, 1992). Cutaneous lesions can leave behind residual anetoderma, mild atrophy, and/or hyperpigmentation. Recurrence has been documented after surgical excision, a therapy that is indicated only for lesions that are frequently traumatized, symptomatic, or are more disfiguring than the resultant scar would be.

Mastocytosis

Mastocytosis comprises a group of disorders characterized by increased numbers of tissue mast cells (Fig. 107.31). The skin is the most common site of involvement, but the lymphoreticular system, gastrointestinal tract, and bone marrow also may be affected. Symptoms result from the local or generalized effects of the release of histamine and other mast cell mediators. Pruritus, edema, blistering, and flushing are common. Abdominal pain, diarrhea, and vomiting are unusual. Hypotension is rare (Kettelhut and Metcalfe, 1991). If rubbed or traumatized, skin affected by mastocytosis will develop a diagnostic wheal (Darier sign). The site may blister or become hemorrhagic in a neonate.

Urticaria pigmentosa is the name given to the most common form of mastocytosis in infants, featuring multiple, small (1 to 3 cm in diameter) papules usually located on the trunk. The disease may be congenital but usually manifests itself within the first 6 months after birth (Kettelhut and Metcalfe, 1991). A single, localized lesion is known as a *solitary mastocytoma*. These tumors can range in size from approximately 2 to 6 cm. Diffuse cutaneous mastocytosis is an unusual condition that may manifest itself at birth with widespread blistering or diffuse thickening of the skin. Systemic mastocytosis is more commonly seen in adults and is defined by multifocal lesions in the bone marrow or other extracutaneous organs, together with signs of systemic disease. It is further subdivided into indolent systemic mastocytosis, systemic mastocytosis with an associated clonal hematologic non-mast cell lineage disease,



• **Fig. 107.31** Solitary mastocytoma presenting as a tan brown plaque with characteristic peau d'orange (orange peel-like) surface change.

aggressive systemic mastocytosis, and mast cell leukemia (Valent et al., 2001).

The diagnosis may be confirmed by a skin biopsy, which reveals mast cell hyperplasia within the dermis. Aminocaproate esterase is the most specific enzyme marker for identification of mast cells. Immunohistochemical stains for tryptase and c-Kit are also sensitive and specific markers for mast cells (Li, 2001). Mutations in *KIT*, the gene encoding the receptor for stem cell factor, may play a significant role in the biology of mast cell malignancies (Gupta et al., 2002). Plasma histamine levels are elevated in most children with mastocytosis, sometimes to remarkably high levels. Further work-up for evidence of systemic involvement should be limited to pediatric patients with extracutaneous signs and symptoms or those who require general anesthesia (Kettelhut and Metcalfe, 1991).

Caregivers should be educated to avoid exposing infants to factors that trigger mast cell degranulation, such as friction, pressure, temperature extremes, and substances that promote mast cell degranulation (aspirin, alcohol, narcotics, amphotericin B, or iodine-containing contrast media). If general anesthesia is required, perioperative administration of histamine receptor blockers is recommended (Lerno et al., 1990).

For patients with limited skin involvement, application of potent topical corticosteroids may hasten involution of lesions. Symptomatic patients may benefit from a classic histamine H₁ receptor blocker such as hydroxyzine or cyproheptadine. An H₂ blocker, such as ranitidine, or orally administered disodium cromoglycate may be added in the presence of gastrointestinal symptoms. Hypotension requires corticosteroids in addition to H₁ and H₂ antihistamines and intensive supportive care. Solitary mastocytomas usually involute by school age. Lesions of urticaria pigmentosa typically resolve by puberty.

Connective Tissue Nevus (Connective Tissue Hamartoma)

Connective tissue nevi are hamartomas comprising dermal collagen, elastic fibers, or a combination of the two. They are benign lesions that can occur in isolation or in relation to a genetic syndrome

such as tuberous sclerosis or Proteus syndrome (Smith and Kirk, 2002). They present as flesh-colored dermal plaques that can be subtle on examination to hypertrophic with a cobblestoned and cerebriform appearance. They are usually asymptomatic and do not often require any intervention. Diagnosis is often made clinically, but if the diagnosis is unclear, a skin biopsy can be performed.

Neurofibroma

Neurofibromas are benign tumors that are composed of neuromesenchymal tissue. They appear as brown or red-brown soft papules or papulonodules. They often present in young adulthood, but there is a variant of a plexiform neurofibroma that can present earlier in infancy and can resemble a congenital melanocytic nevus. Neurofibromas can occur as isolated tumors in an association with an underlying genetic disorder, such as NF-1. They can be surgically excised if they become symptomatic or are cosmetically disfiguring.

Developmental Anomalies of the Skin

Developmental anomalies are present at birth and represent a heterogeneous group of disorders that are caused by a disruption in the formation of vital structures within the skin. They may occur in isolation or they may be a marker of extracutaneous abnormalities and thus require further evaluation.

Midline Anomalies

Congenital midline defects are a distinct group of diagnostically and therapeutically challenging conditions. These anomalies can be markers for potential neural tube dysraphism occurring at the cranial or caudal midline. These markers include dimples, sinuses, skin tags, capillary malformation, hemangiomas, nodules, lipomas, dermoid cysts or sinus, and midline circumscribed or annular hypertrichosis that may represent a marker for an underlying CNS problem or an intracranial connection (Hayashi et al., 1984; Martinez-Lage et al., 1992). A midline mass in the nasal area may represent a dermoid cyst or sinus, encephalocele, or glioma (Paller et al., 1991). Midline scalp lesions include aplasia cutis congenita, dermoid cyst or sinus, encephalocele, meningocele, and heterotopic brain tissue. The “hair collar sign” may mark ectopic neural tissue and underlying CNS malformations (Commens et al., 1989; Drolet et al., 1995). Biopsy of a midline mass should not be performed unless an imaging study, computed tomography, or MRI has been performed to help clarify the nature of the lesion and any intracranial connections, although small intracranial connections may still be missed. If the possibility of an intracranial connection exists, referral to a neurosurgeon is indicated (Martinez-Lage et al., 1992).

Markers of occult spinal dysraphism exist in the lumbosacral region and include various cutaneous midline lesions such as lipomas, skin tags, pseudotails and tails, dimples and sinuses, hypertrichosis, aplasia cutis congenita, dermoid cyst or sinus, pigmentary changes, hemangiomas, CMs, and telangiectasias. The presence of two or more stigmata indicates a higher risk of the presence of occult spinal dysraphism. Box 107.2 stratifies findings on the basis of their risk for occult spinal dysraphism (Sewell et al., 2015). There are no evidence-based guidelines for imaging recommendations for spinal dysraphism. In general, in high-risk situations, MRI is the preferred imaging modality but is limited by its cost, availability, and need for sedation. In infants up to 6 months old, high-resolution ultrasonography may be performed before the ossification of the vertebral bodies, but it has decreased sensitivity

• BOX 107.2 Risk Stratification of Markers of Occult Spinal Dysraphism

High Risk

- Two or more cutaneous stigmata
- Lipoma
- Acrochordon, pseudotail, true tail
- Aplasia cutis and congenital scars
- Dermoid cyst or dermal sinus
- Infantile hemangioma ≥ 2.5 cm

Intermediate Risk

- Atypical dimple >5 mm in diameter, >2.5 cm from the anal verge
- Infantile hemangioma <2.5 cm
- Hypertrichosis (faun tail or silky down)

Low Risk

- Hyperpigmentation or hypopigmentation
- Melanocytic nevi
- Simple dimple ≤ 5 mm in diameter, ≤ 2.5 cm from the anal verge
- Teratomas
- Port-wine stain (capillary malformation) or telangiectasias

(Modified from Sewell MJ, Chiu YE, Drolet BA. Neural tube dysraphism: review of cutaneous markers and imaging. *Pediatr Dermatol*. 2015;32:161–170.)

when compared with MRI and has large variability in accuracy depending on the person performing the study. If the ultrasound results are abnormal or inconclusive, MRI should be performed. It is suggested that simple dimples (Gibson et al., 1995; Robinson et al., 2005) and isolated low-risk lesions do not require a screening ultrasound examination (Guggisberg et al., 2004). On the neck a midline pit or nodule may represent a congenital midline cervical cleft (Eastlack et al., 2000) or thyroglossal duct cyst or sinus. A bronchogenic cyst or sinus cyst presents as a nodule or pit at the suprasternal notch at birth and is due to remnant respiratory epithelium. Branchial cleft anomalies, cysts, or sinuses do not present at the midline and are located in the preauricular region and lateral neck. They are at risk of infection and therefore can be surgically removed.

Preauricular Pits and Sinuses

Preauricular pits are common congenital abnormalities that present as small depressions or dells, often 1 to 3 mm in size located adjacent to the external ear, usually at the anterior margin of the ascending limb of the helix, but have also been reported along the lateral surface of the crus of the helix and the superior posterior margin of the helix, tragus, or lobule. There may be a sinus tract connected to the pit in the skin, in which case fluid or pus may drain from the opening. Preauricular sinuses are usually asymptomatic and isolated but can be multiple and bilateral in 25%–50% cases. The bilateral sinuses tend to be inherited in an autosomal dominant pattern. There have been associations with a few genetic syndromes as well (Box 107.3).

Preauricular sinuses are rarely associated with deafness or renal problems. Renal ultrasound screening is recommended only if there are other worrisome features present, such as the presence of dimorphic features, a family history of deafness, a maternal history of gestation diabetes, and auricular and/or renal malformations (Wang et al., 2001). Hearing impairment occurs at a higher rate in individuals with accessory tragi, and screening is

• BOX 107.3 Preauricular Pits and Sinuses and Associated Genetic Syndromes

Branchiootorenal syndrome
Branchiootic syndrome
Branchiootoureteral syndrome
Branchiooculofacial syndrome
Branchiootocostal syndrome
Waardenburg syndrome
Goldenhar syndrome (oculoauriculovertebral syndrome)
Cat eye syndrome

• BOX 107.4 Accessory Tragi and Associated Genetic Syndromes

Goldenhar syndrome (oculoauriculovertebral syndrome)
Treacher Collins syndrome
Townes–Brocks syndrome
VACTERL syndrome
Wolf–Hirschhorn syndrome



• Fig. 107.32 Accessory tragus.

recommended if a newborn hearing screen was not performed (Roth et al., 2008). Symptomatic sinuses that drain fluid or become infected should be cultured and treated with appropriate antibiotics. If recurrent infection occurs, surgical excision by an experienced surgeon is recommended because the sinus can extend to the periosteum with the auditory canal (Scheinfeld et al., 2004).

Accessory Tragus

Accessory tragi, inaccurately referred to as *preauricular tags*, are relatively common congenital malformations of the external ear. They present as soft, flesh-colored pedunculated papules that can occur anywhere from the preauricular area to the angle of the mouth following the embryonic fusion line of the mandibular and maxillary branches of the first branchial arch (Fig. 107.32). They are present at birth and can be multiple and/or bilateral. In most

cases they occur as an isolated developmental defect, but association with other abnormalities of the first and second branchial arch or branchial arch syndromes can occur (Box 107.4; Bahrani and Khachemoune, 2014). Association with deafness and renal abnormalities is controversial. The renal ultrasound and hearing screening guidelines are the same as mentioned with preauricular pits and sinuses earlier. Accessory tragi can be limited to the dermis but can also contain cartilage or can be contiguous with the external ear canal. Therefore if surgical excision is performed, it should be done by an experienced surgeon. Suture ligation should not be done and can result in complications.

Congenital Cartilaginous Rests of the Neck (Cervical Tabs, Wattles)

Congenital cartilaginous rests of the neck, also known as *cervical tabs* or *wattles*, are present at birth and are formed from a remnant of the branchial arch or ectopic auricular tissue (Figs. 107.33–107.34).



• **Fig. 107.33** Congenital cartilaginous rests of the neck, aka *wattles*, presenting as a small flesh-colored papule on the anterior part of the neck.



• **Fig. 107.34** Cutis marmorata telangiectasia congenita.

They present as soft, flesh-colored papules or nodules that can occur anywhere on the neck but most commonly appear over the lower half of the sternocleidomastoid muscle. They may contain cartilage and can be multiple and bilateral. They do not have a deeper connection or sinus or tract associated with them and therefore do not require further imaging or treatment. Surgical excision could be considered, but largely for aesthetic purposes.

Supernumerary Digits (Rudimentary Polydactyly)

Supernumerary digits can range in their presentation from subtle small pedunculated papules to full-sized digits. They arise from the lateral surface of the normal digit and may occur on any digit, with the ulnar surface of the fifth finger being the most common site. They often contain cartilage, nerves, and nail. Therefore surgical excision with nerve dissection can be undertaken to remove these accessory digits. Ligation of the supernumerary digit without complete dissection of the nerve can result in a traumatic neuroma, skin necrosis, and infection (Frieden et al., 1995; Leber and Gosain, 2003).

Supernumerary Nipples (Polythelia, Accessory Nipples)

Supernumerary nipples can be found anywhere along the embryologic milk lines that course from the axilla to the inner thigh. They present as brown or reddish-brown pedunculated papules and are often bilateral. In the newborn period their clinical presentation is very subtle as a small light-brown macule. They may present with or without true extramammary tissue and mammary glands. They can be mistaken for melanocytic nevi, warts, neurofibromas, or acrochordons. Usually no further evaluation or treatment is necessary unless glandular tissue is present, in which case complete surgical excision is recommended because of enlargement at puberty causing pain and social stigma.

Median Raphe Cysts (Congenital Sinus and Cysts of Genitoperineal Raphe, Mucous Cysts of the Penile Skin, Parameatal Cysts)

Median raphe cysts present as small white to flesh-colored papules that can be found anywhere on the ventral penis, scrotum, and perineum in a midline position. They can be solitary or multiple. They are believed to result from congenital alterations in embryologic development of male genitalia and are due to incomplete closure of the urethral and/or genital folds or result from outgrowths of the embryologic epithelium after primary closure of the folds (Park et al., 2006). They are usually asymptomatic in most cases, and there have been reports of spontaneous regression over time. If they persist, become symptomatic, or infected, simple excision with primary closure can be undertaken (Krauel et al., 2008).

Suggested Readings

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Eye and Vision Disorders

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KEY POINTS

- “Early visual experience drives the architecture of the visual brain.”
- Screening eye examinations are important in all infants, regardless of gestational age. All neonates should have an examination of the red reflex before discharge from the newborn nursery.
- The absence of visual responsiveness by 2 months of age should prompt an urgent ophthalmologic evaluation.
- Most full-term infants establish normal ocular alignment within the first 2 months.
- Nystagmus can be a manifestation of a congenital motor entity, secondary to visual pathway defects or neurologic disease. Nystagmus caused by defective vision does not develop until approximately 3 months of age.
- Successful treatment of congenital cataracts is highly dependent on early diagnosis and prompt referral.
- The successful management of retinoblastoma depends on the ability to detect the disease while it is still intraocular; disease stage correlates with delay in diagnosis.
- Neonatologists have both a clinical and a medicolegal responsibility to arrange timely diagnostic retinopathy of prematurity examinations for preterm babies in the neonatal intensive care unit and to communicate to parents the vital importance of keeping outpatient ophthalmology appointments.

The fast pace of development of the visual system in the neonatal period makes the recognition of ocular abnormalities extremely important. As early as 4 to 6 months after birth, some visual functions are permanently set and if impaired cannot be fully restored to normalcy. For example, a visually significant congenital cataract must be surgically addressed before the third month of life to avoid potentially irreversible vision loss. This urgency makes the neonatologist an invaluable player in the recognition and management of neonatal eye diseases. Screening eye examinations are important in all infants, regardless of gestational age and whether they occur in the neonatal intensive care unit (NICU), the newborn nursery, or the primary care provider's office. Healthcare professionals caring for newborns need to be familiar with indications for referral to a pediatric ophthalmologist, and in premature infants the added risk of retinopathy of prematurity (ROP) mandates that neonatologists ensure timely

diagnostic examinations by an ophthalmologist with expertise in ROP.

General Examination Techniques

The Newborn Eye Examination:
Approach and Equipment

Effective physical examination of the newborn eye begins with familiarity with eye anatomy. Armed with a baseline understanding of “normal,” the pediatrician can identify abnormalities or asymmetries in the structure or function of the eyes by following a simple examination framework: *I-ARM* (inspection, acuity, red reflex, and motility) (Simon and Calhoun, 1998). Detailed information on each of these components follows a discussion of general considerations when one is examining the eyes of a young infant.

A thorough eye examination, although necessary, can be stressful and sometimes painful for a newborn or a young infant. ROP examinations in particular, which often necessitate the use of an eyelid speculum to retract the eyelids and scleral indentation to visualize the peripheral retina, have been associated with an increase in pain as assessed by validated pain scores (Premature Infant Pain Profile [PIPP] and CRIES, an acronym for the variables in the scale: Crying, Requires O2 for Sat >95, Increased vital signs, Expression, Sleepless) (Belda et al., 2004). The procedure has also been associated with other adverse effects, including episodes of desaturations, bradycardia, hypertension, and prolonged crying times (Rush et al., 2004). Swaddling, nesting, nonnutritive sucking, and oral administration of sucrose before, during, and after an examination can be very helpful in this regard (Gal et al., 2005; Grabska et al., 2005., Boyle et al., 2006), particularly for premature babies undergoing serial ROP examinations. The most distressing aspects of the eye examination are generally the bright light of the ophthalmoscope and the insertion of the speculum. The use of a topical anesthetic, such as proparacaine hydrochloride 0.5% or tetracaine 0.5% drops, reduces the discomfort but is not always sufficient and should be supplemented with other measures, such as sucrose, pacifiers, and nesting (Marsh et al., 2005; Dempsey et al., 2011). In addition, NICU and office staff should be aware that the repetitive use of topical anesthetics can result in corneal ulceration and melting, so bottles should be properly disposed of

and not confused with other medications, such as topical lubricants or antibiotics. Use of an indirect ophthalmoscope without a speculum produces significantly less pain response than that noted during examination with a speculum or with a contact fundus camera, such as the RetCam (Clarity Medical Systems, Pleasanton, California, United States) (Mehta et al., 2005). Nevertheless, a speculum is often necessary to adequately visualize ocular structures and should always be used if adequate visualization of the fundus is otherwise not possible. Supportive measures can also be used at the time of administration of dilating eye drops. Cohen et al. (2013) analyzed masked videotapes from before, during, and after eye drop administration and found that one-third of infants have a significant pain response to mydriatic eye drops. Finally, particular note should be made of the oculocardiac reflex, a dysrhythmia, typically bradycardia, resulting from direct manipulation of the eye during and immediately after the examination; monitoring by an assistant during and after the examination is therefore important. Nevertheless, in the experience of the authors, it is extremely rare for a properly supported infant to be unable to tolerate a quick fundus examination, the discomfort of any observers notwithstanding. Therefore we recommend that eye examinations not be postponed unilaterally by the neonatology team without their first discussing with the ophthalmologist the risk of ROP in each specific infant.

The infant is often examined best while lying supine. If he or she is in an incubator, the top must be raised to allow the examiner to examine the eyes closely and from different directions. A parent or a nurse may cradle the baby for examination as well. In slightly older infants, visual tracking and motility are best assessed with the infant in an upright position. It usually takes more than one set of hands to examine an infant's eyes. As discussed earlier, assistance with comforting the baby and monitoring vital signs is important. In addition, the head and body of the infant may need to be securely held to allow both a detailed and a safe anatomic examination. Such restraint should be applied carefully but firmly and is best left to the end of the examination, because once the child is upset, it will become difficult to assess certain parts of the ophthalmologic examination, such as visual function, motility, or intraocular pressure. Under some circumstances, it may become necessary for an ophthalmologist to perform an examination with the infant under sedation or general anesthesia.

Examination of the eye requires adequate light and magnification. Even a simple flashlight or penlight and a magnifying glass are helpful and worth using if specialized instrumentation is not available. The ideal light source provides a variable, very bright and focused beam. The portable slit lamp microscope combines such a light source in combination with excellent binocular magnification. The light beam width is controllable, allowing three-dimensional cross sections of the anterior segment structures of the eye, including the conjunctiva, cornea, anterior chamber, iris, and lens. Although the slit lamp is an extremely useful instrument, it is expensive, and practice is needed to develop facility. Again, any simple magnifier and bright light source will prove invaluable for an eye examination in the NICU, emergency department, or primary care provider's office. The direct ophthalmoscope, typically available in all three settings, can serve this purpose and is discussed in the next paragraph. An additional method of identifying disease of the ocular surface is application of fluorescein ophthalmic solution, which will stain areas of denuded corneal and conjunctival epithelium. Once the solution has been applied, a blue light source must be shone on the eye, and the examiner can identify corneal abrasions, which appear as patches of staining; corneal dryness, which appears as punctate staining; and multiple other conditions, such as dendritic herpetic

lesions. Most direct ophthalmoscopes have a blue light source, which may be used in conjunction with a magnifier. Alternatively, a Wood's lamp may be used.

The direct ophthalmoscope is an important instrument for examining a newborn's or an infant's eyes. It provides a light source to evaluate light perception and pupillary reactivity and to be used together with a magnifier for examination of the anterior structures of the eye. Red reflex testing with a direct ophthalmoscope (described in detail later) is a mandatory element of all newborn and well-baby physical examinations. Numerous vision-threatening and even life-threatening ocular diseases are primarily identified by the checking of red reflexes, and this technique is the principal method of detecting disease in the posterior segment of the eye. The direct ophthalmoscope may, of course, be used to directly visualize the optic nerve head and retina, but particular considerations and limitations must be kept in mind. Pupil size must be maximal; typically this means pharmacologic dilation (discussed later). If mydriatics are not used, dimming ambient light and, in older children, having the child fixate in the distance will maximize pupil dilation. The examiner must be very close to the infant (a few centimeters at most). Approaching from a slightly lateral angle and following the "arrows" of branching vessels back to the nerve head will help to identify the optic disc. Most important, the field of view or "spot size" of the direct ophthalmoscope is approximately the size of the optic nerve head, which represents but a tiny fraction of the ocular fundus. Therefore it is not possible to adequately evaluate the retina for ROP or other peripheral retinal disease with a direct ophthalmoscope; instead an indirect ophthalmoscope is needed. This instrument requires both greater skill and a handheld lens to use but provides binocular viewing with depth perception and a much wider field of view. Pharmacologically dilated funduscopic examination with an indirect ophthalmoscope is required for ROP and other retinal diagnostic examinations, such as for retinoblastoma or retinal hemorrhage (RH) in suspected abusive head trauma (AHT).

Pharmacologic dilation with mydriatic eye drops is commonly performed by consulting ophthalmologists, typically on all new patient evaluations and at all ROP examinations. Occasionally, a pediatrician may find it helpful to dilate the pupils to better visualize the red reflex or optic nerve head. Use of drops is better deferred if an ophthalmology consultation will be requested, because dilating the pupils may make it difficult or impossible to accurately assess the pupils, ocular alignment, intraocular pressure, or iris. Dilating eye drops include sympathomimetic drugs (e.g., phenylephrine) and anticholinergic drugs (e.g., tropicamide, cyclopentolate, atropine). Potential side effects of these drugs include elevated blood pressure, increased heart rate, cardiac arrhythmias, feeding intolerance, slowed gastric emptying, urticaria, contact dermatitis, and seizures (Isenberg et al., 1985; Bonthala et al., 2000; Chew et al., 2005). Bradycardic and apneic episodes following administration of these eye drops are pain response reactions and not a direct effect of the drugs. Adverse effects are potentially of greater concern in preterm infants, who are of lower weight and typically require multiple doses to achieve adequate dilation, as do many children with dark irides. Therefore it may be prudent to use reduced concentrations of mydriatics in premature infants, particularly cyclopentolate. In a randomized masked trial, Chew et al. (2005) concluded that cyclopentolate 0.2% with phenylephrine 2.5% is the mydriatic of choice in preterm infants with dark irides, because higher concentrations of cyclopentolate (0.5%, 1.0%) were more likely to result in increased mean blood pressure or feeding intolerance. In all children, systemic absorption of eye drops can be

minimized by compression of the lacrimal sac for 1 to 2 minutes after instillation. It is also recommended to wipe all the overflow drops from the skin as local vasoconstriction and pallor of the periorbital skin is frequently observed in premature neonates in whom the epidermal permeability barrier is still incompetent (Alpay et al., 2010). These authors typically use three drops of Cyclomydril (cyclopentolate hydrochloride 0.2% and phenylephrine hydrochloride 1%) or tropicamide 1% and phenylephrine 2.5% separated by 5 minutes in each eye, 30 to 60 minutes before the examination. Multiple doses are necessary because in premature and newborn babies, adequate pupil dilation is often difficult to obtain because of the immaturity of the dilator muscle of the pupil; the pupils of babies with dark irides, in particular, may be more difficult to dilate because of pigment binding of the mydriatic drugs, and higher concentration or more frequent administration may be required. We recommend avoiding the use of cyclopentolate 1% in children younger than 6 months.

Inspection (the I in I-ARM)

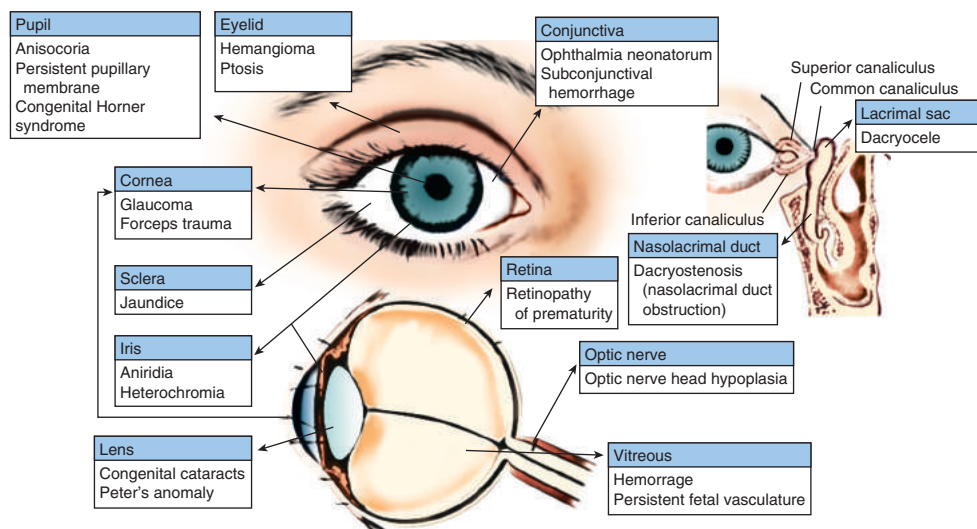
Inspection of anatomy is the primary method of detecting eye disease. The eyes should be closely examined with proper lighting and magnification (as described earlier), and the periocular and ocular structures should be approached in a systematic manner. One option is to begin with the external structures and work inward and posteriorly, looking at each eye carefully and comparing the two eyes with each other. As a guideline, any abnormality or asymmetry noted on examination should be referred to a pediatric ophthalmologist for further management. The urgency with which to seek consultation depends on the specific finding, and guidelines appear throughout this chapter. However, one should err on the side of urgency, because the neonatal period is a critical period in visual development. To adequately inspect the eyes, familiarity with normal anatomy is required. A general overview of these structures follows (Fig. 108.1).

The eyelids protect the eyes. The eyelids contain numerous glands, which produce tears to keep the ocular surface well lubricated. Blinking spreads the tears and actively pumps the tears into the lacrimal drainage system. An inability to adequately close the eyes presents a major problem and can result rapidly in surface drying, corneal epithelial breakdown, and vision- or eye-threatening

complications, such as ulceration, infection, or scarring. Use of an overhead heater should be avoided for infants who have poor eyelid closure (e.g., congenital eyelid abnormalities, neurologic problems), as the ocular surface can rapidly decompensate in such cases; alternative sources of maintaining body temperature should be used, and an ophthalmology consultation should be requested to manage the ocular surface. The nasolacrimal duct provides a means of egress for tears, which pass through the puncta, canaliculi, and lacrimal sac to the duct. The duct is blocked at birth in 5%–10% of newborns, resulting in epiphora and discharge in an otherwise white and quiet eye; 90% of such blockages clear by 1 year of age. Of note, congenital glaucoma can manifest itself with epiphora as well (see [Corneal Clouding](#)).

The conjunctiva is a translucent membrane that overlies the surface of the eye and the inside of the eyelids. The sclera is the white fibrous wall of the eye. It is relatively flexible at birth and gradually toughens over the first few years of life. The cornea is continuous with the sclera, which it meets at the limbus, and is a clear dome-shaped structure in the center of the globe (eye). The cornea provides two-thirds of the refractive power of the eye, is richly supplied with sensory nerves, and must be kept well lubricated to prevent surface breakdown. The cornea is a multilayered structure approximately 0.5 mm thick, kept clear by active pumping out of fluid by its endothelium. Any process that affects the clarity or integrity of the cornea can result in loss of vision.

The iris is a donut-shaped structure posterior to the cornea. The anterior chamber of the eye lies between the cornea and the iris and is best visualized with slit lamp examination (see the earlier discussion). The anterior chamber angle lies at the juncture of the cornea and iris peripherally and contains the aqueous fluid drainage structures of the eye; if these structures are occluded, a rapid rise in intraocular pressure and sight-threatening glaucoma can ensue. Such angle-closure glaucoma is uncommon in children. The iris contains muscle structures to constrict and dilate the pupil, which is a central aperture under involuntary control and changes size with variations in incident light, focusing effort (accommodation), emotions, and medications. The iris is an immature structure at birth. The color tends to be gray or blue and may become darker as the pigmented layer of the iris stroma becomes more fully developed, which typically occurs by about 6 months of age. *Heterochromia* refers to differences in the color of the iris between



• Fig. 108.1 Anatomic structures and common neonatal diseases by anatomic site.

or within eyes and can be seen in congenital Horner syndrome (with mild ptosis and a miotic pupil), as well as syndromic conditions such as Waardenburg syndrome or Hirschsprung disease. Behind the iris lies the crystalline lens, which provides one-third of the eye's refractive power. The lens width and therefore refractive power can be varied by contraction of the ciliary muscle of the eye to provide accommodation or "focusing-in" ability. An opacity in the lens is referred to as a cataract (see [Leukocoria and Abnormal Red Reflex](#)). The uvea is composed of the iris, the ciliary body, and the choroid. Inflammation of the uvea, uveitis, is diagnosable with a slit lamp and dilated fundus examination by directly visualizing white blood cells in the anterior chamber, as well as multiple other signs in the anterior and posterior segments of the eye. The ciliary body produces aqueous humor, which maintains eye pressure and nourishes the anterior segment structures. The choroid is a vascular structure that lies between the sclera and the retina; it supplies the retina and is the most densely packed blood supply in the body.

The retina is a multilayered, complex, highly metabolically active structure that lines the inside surface of the globe and contains photoreceptors that receive light and generate neuronal signals that are ultimately perceived as visual images. The macula is the central, posterior retina, between the superior and inferior temporal retinal vascular arcades, and the fovea is the very central retina containing the highest concentration of photoreceptors and producing central, high-resolution vision. Visual signals are transmitted through the optic nerve, whose cell bodies lie in the most anterior retina and which is composed of approximately 1 million individual nerve fibers. The proximal end of the optic nerve is visible as a normally golden disc approximately 15 degrees nasal and just superior to the fovea. The optic nerves lead to the optic chiasm and continuing visual and pupillary pathways in the brain. The anterior portion of the eye contains aqueous humor, and the posterior segment contains vitreous humor, which is well formed at birth but which undergoes gradual liquefaction (syneresis) with age. The vitreous is attached to the retina throughout the fundus (posterior inner surface of the eye), all the way to the retina's anterior extent or ora serrata, where the retina meets the ciliary body; however, it has particularly strong attachments at the ora serrata (vitreous base), at the optic nerve head, and along the retinal vessels in the most superficial layers of the retina.

Knowledge of normal eye structures and certain growth parameters in the newborn is important because a deviation from the averages can be associated with significant disease. For example, in congenital glaucoma, the corneal diameter is increased, and the axial length (sagittal length) of the eye is a parameter that is carefully followed by the ophthalmologist, with the aid of an ultrasound examination, to determine if the intraocular pressure is adequately controlled. At birth the eyeball is 70% of the adult size (average axial length in a newborn is 17 mm) and reaches 95% of the adult size by age 3 years. The corneal horizontal diameter is usually 9.5 mm at birth, which is 80% of the adult diameter. Corneal diameter can be assessed by simple bedside examination.

Acuity and Visual Development (the A in I-ARM)

The visual system is immature at birth. The fovea is not completely differentiated until 15 to 45 months ([Hendrickson and Yuodelis, 1984](#)), and myelination of the optic nerve is not completed until about 1 year of age ([Magoon and Robb, 1981](#)). The eye continues to develop synapses in the visual cortex during the first 10 years after birth, and although visual acuity reaches normal adult ranges by 2 years of age, this period continues to be important because

any abnormality can lead to amblyopia (see the definition later). Color vision improves greatly during the first 3 months after birth, and most normal 3-month-olds have at least some color vision; color visual processing mechanisms continue to mature throughout the first year of life ([Brown, 1990](#); [Yang et al., 2015](#)).

Because of the immaturity of the system at birth, qualitatively estimating the vision of a newborn is seldom attempted, and other clues to the status of visual function are more commonly used. However, clinical and laboratory techniques can be used to estimate vision in special situations. These include eliciting a nystagmus response with optokinetic targets (striped patterns), Teller cards for preferential forced looking (a test that depends on an infant's preference to look at patterns—grating of black and white stripes—rather than homogeneous fields), visual evoked potentials, and electroretinogram. Each of these techniques uses different stimuli and yields somewhat different results, but they all demonstrate a dramatic improvement in "acuity" during the first year of life. Most commonly, visual function in a newborn is assessed by detection of light aversion, which implies light perception. A bright light is shone into each eye or even through the thin eyelids to elicit closing or squeezing of the lids. Although visual fixation may be intermittently present soon after birth, it is not well developed until after the second month; in contrast, a blink response to light is already present at 26 weeks' postmenstrual age (PMA) ([Robinson and Fielder, 1990](#)), and head turning to a diffuse light starts around 32 weeks' PMA. A blink in response to an approaching object (visual threat response) does not develop until approximately 16 weeks' postnatal age. An infant should fix on and follow a face or object by 2 to 3 months of age. A clever technique that is based on the observation that neonates and infants will attend to the reflection of their own faces in a mirror has been described ([Bowman et al., 2010](#)). Measurement of the "mirror distance" is a simple and reliable technique to estimate visual acuity in infants that can be used as a screening test similar to the traditional "fix and follow" (see later) and is a useful additional tool for detecting impaired visual function at this early age ([Bowman et al., 2010](#)). Generally, the absence of visual responsiveness by a developmental or corrected age of 2 months should be taken seriously and prompt an urgent ophthalmologic evaluation.

Poor vision or blindness should be suspected in any infant with absent or poor pupillary responses, paradoxical pupillary response (initial brisk constriction of the pupil when the lights are turned off) ([Frank et al., 1988](#)), and nystagmus or roving eye movements, although these are not usually present until 2 to 3 months of age. Constant poking or rubbing the eyes can also be a sign of poor vision.

Causes of congenital blindness or poor vision include Leber congenital amaurosis (an early and severe form of retinitis pigmentosa), other retinal dystrophies (achromatopsia and congenital stationary night blindness), congenital cataract, glaucoma, aniridia, albinism, optic nerve abnormalities (hypoplasia and coloboma), chorioretinal colobomas, high refractive errors, and congenital infections. In most babies the cause of poor vision is obvious after complete ophthalmologic examination. Occasionally, further investigation is necessary and may include electrophysiologic testing and neuroimaging. Babies with cerebral (central or cortical) visual impairment (CVI) have normal eye examination findings, including normal pupillary responses and no nystagmus. CVI is used to describe children with visual impairment as a result of neurologic disease, which may be congenital or acquired. Perinatal causes include intrauterine infection, cerebral dysgenesis, asphyxia, hypoglycemia, intracranial hemorrhage, periventricular leukomalacia,

hydrocephalus, trauma, meningitis, and encephalitis. In developed countries, CVI is the single greatest cause of visual impairment in children, and most of these children have an associated neurologic deficit (usually epilepsy or cerebral palsy), which places a major burden on children's special services in these countries.

Amblyopia is defined as a reduction in best corrected vision that cannot be attributed directly to any structural abnormality of the eye or proximal visual pathway. Amblyopia is maldevelopment of the visual centers of the brain as a result of abnormal visual experience early in life. It includes three etiologic categories, which often overlap. Deprivational amblyopia results from obstruction at any point in the visual axis that causes the retina to perceive poor-quality, distorted, or no images. The causes of deprivational amblyopia include congenital or acquired cataract, corneal opacity, ptosis, and vitreous hemorrhage. Strabismic amblyopia results from a child's preferring one eye over the other when the visual axes are misaligned. Refractive amblyopia is a consequence of either a significant inequality of the refractive error in each eye or very high refractive errors in both eyes. Any of these forms of amblyopia can be encountered in the first few months of postnatal life. Because amblyopia is responsible for more cases of unilaterally reduced vision in childhood than all other causes combined, and because it is highly preventable with early detection and treatment, all newborns and infants suspected of having any of these conditions should be referred promptly to an ophthalmologist.

Pupillary Response and the Red Reflex (the *R* in *I-ARM*)

The onset of the pupillary light reflex (constriction in response to light) occurs around 30 to 34 weeks' gestation (Robinson and Fielder, 1990) and is not fully developed until the first month after birth. The pupils should be examined for size, shape, symmetry, reactivity to light, and afferent defects.

As the light passes through the pupils and is reflected through the normal clear media of the anterior and posterior segments of the eye, a characteristic red reflex is produced. The reflex is generated not from the retina, which is transparent, but from the choroidal pigmentation and vasculature. The red reflex test is vital for early detection of vision and potentially life-threatening conditions such as cataract, glaucoma, retinoblastoma, retinal abnormalities, systemic diseases with ocular manifestations, and high refractive errors. Red reflex assessment is an essential component of every neonatal and infant physical examination. The American Academy of Pediatrics currently recommends that all neonates have an examination of the red reflex before discharge from the neonatal nursery. In addition, the test should be performed during all subsequent routine health supervision visits (Donahue, 2016a; Donahue, 2016b).

The red reflex test should be performed in a darkened room, projecting the largest white-light circle light of the ophthalmoscope onto both eyes of the infant—first simultaneously, from approximately 18 inches away, while looking through the aperture of the ophthalmoscope. Once the reflexes have been assessed together to allow comparison, specific abnormalities can be more closely inspected by examination of each eye separately at a nearer distance. Since neonates and infants are frequently asleep and it is difficult to open their eyes and keep them open, the red reflex test may be challenging. A useful technique to encourage a neonate to spontaneously open the eyes is the use of a primitive reflex seen in neurologically healthy children up to 6 months old: the child is supported by a hand on the thorax at approximately a 45-degree angle from

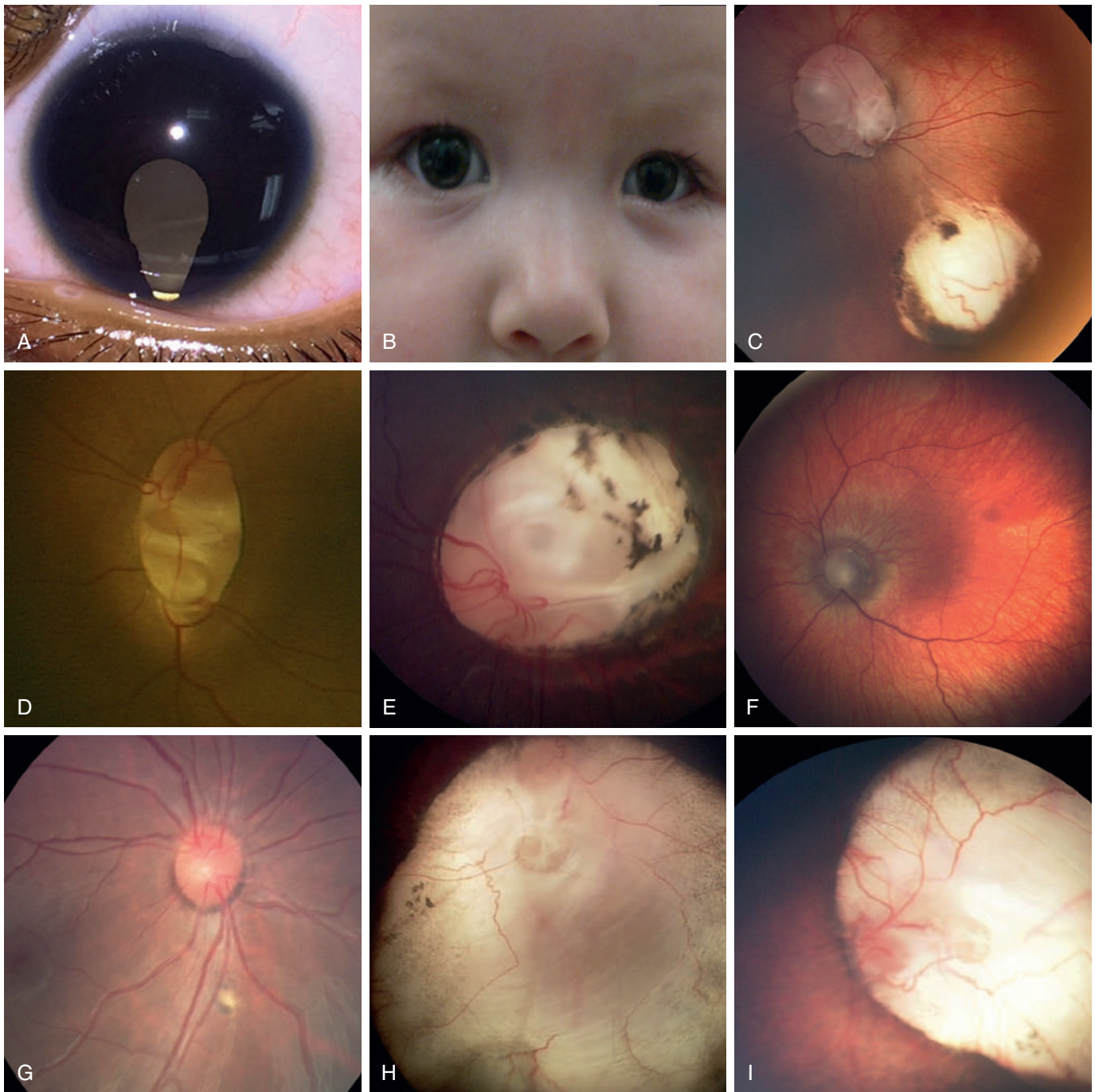
the horizontal with use of the other hand to jiggle the child's bottom (McLaughlin and Levin, 2006).

It is not necessary to pharmacologically dilate the pupils, although the reflexes are easier to assess with larger pupils; indeed, the most common cause of an absent reflex is small pupils. Darkening the room or changing the light beam of the ophthalmoscope will usually overcome this problem. Although traditionally the normal reflex has been described as "red," it can often be yellow, orange, or maroon. The symmetry of the color, clarity, and intensity of the red reflex between the eyes is usually more useful than the qualitative assessment of each red reflex independently, as there may be significant variation in normalcy. A markedly diminished or dim reflex, the presence of a white reflex (leukocoria), dark spots in the reflex, or an asymmetric reflex between the eyes is an indication for immediate referral to an ophthalmologist experienced in the examination of children (Donahue, 2016a). An exception to this rule is a transient opacity in the tear film from mucus or a foreign body that is mobile and completely disappears with blinking or manual opening and closing of the eyelids, after which the red reflex should appear normal. Unequal or high-refractive errors (need for glasses) and strabismus may also produce abnormal or asymmetric red reflexes. An additional observation is the position of the light reflex on the corneal surface. Asymmetric positioning of this reflex can indicate misalignment of the eyes (strabismus; see later).

In addition, the shape and regularity of each pupillary aperture should be assessed to look for colobomas (Fig. 108.2A) and other congenital abnormalities (see Common Diagnostic Problems). Asymmetry of pupil size (anisocoria) can be a sign of Horner syndrome, trauma, or a congenital third nerve palsy. The tunica vasculosa lentis is a plexus of vessels that crosses the pupil, visible in preterm babies up to 34 weeks' PMA. The extent of this anterior lens capsule vascularity can be used to estimate gestational age in babies between 27 and 34 weeks' gestation (Hittner et al., 1977; Krishnamohan et al., 1982; Zimmer et al., 1983). A failure of these vessels to regress can occasionally be seen as a persistent pupillary membrane, which appears as a regular arrangement of vessels looping into the pupillary axis in front of or behind the lens.

Motility and Alignment (the *M* in *I-ARM*)

Although the extraocular muscles are formed by 12 weeks' gestation and fetal eye movements can be detected as early as 16 weeks' gestation, the supranuclear eye movement system is not fully developed until after birth in full-term neonates. The eyes of a neonate commonly appear misaligned. It is not unusual to see the eyes shift from straight (orthotropia) to crossed inward (esotropia) to outward (exotropia). Transient deviations (neonatal ocular misalignments) occur very commonly in the first month of life in visually normal infants. At this age it is not possible to distinguish those infants who will progress to develop pathologic strabismus from those who will develop normal binocular vision (Horwood, 2003a). Exotropia is commonly observed in newborn nurseries and has been reported to occur in up to 33% of infants (Nixon et al., 1985); however, most exodeviations will usually resolve with development of the fixation reflex and are rarely observed beyond 6 months of age (Sondhi et al., 1988). In contrast, transient esodeviations in patients who do not go on to develop infantile esotropia do not usually persist beyond 10 weeks of age (Sondhi et al., 1988; Horwood, 2003b). Most full-term infants establish normal ocular alignment within the first 8 weeks of life. In some



• **Fig. 108.2** Wide spectrum in presentation of colobomas of the eye. (A) Iris coloboma, notice the difference in red reflex at the lens equator. (B) Microphthalmos with coloboma (the left eye is smaller because of a uveal coloboma). (C) Noncontiguous optic nerve and chorioretinal coloboma. (D–F) Optic nerve colobomas. (G) Small chorioretinal coloboma inferior to the optic disc. (H) Large chorioretinal coloboma involving most of the posterior pole. (I) Large chorioretinal coloboma involving the macula and optic disc.

babies the epicanthal folds of the eyelids hide the medial aspect of the sclera, creating the appearance of strabismus; however, in these cases the visual axes are not misaligned. This common condition is referred to as *pseudostrabismus* (Fig. 108.3B) and can be confirmed by the directing of a bright light to both eyes simultaneously and observing that the reflection of the light on the corneas appears symmetric between the eyes with respect to the center of

the pupil; the reflexes will appear asymmetric between the eyes if a true misalignment exists (see Fig. 108.3A).

Most cases of strabismus in infants are not paralytic in origin, but congenital third, fourth, and sixth nerve palsies can occur, as well as early acquired cranial neuropathies due to trauma, infection, and other central nervous system abnormalities. When there is doubt, any apparent misalignment after 3 to 4 months of age



• **Fig. 108.3** (A) Inward misalignment of the eyes in a premature child with infantile esotropia. (B) Pseudoesotropia or pseudostrabismus (a wide nasal bridge and prominent epicanthal folds give the appearance of esotropia; note the position of the corneal light reflex centered in the pupil of both eyes).

should be considered pathologic and referred for evaluation (Donahue, 2016a). This is especially important as, in some cases, strabismus can also be the first sign of serious ocular or systemic disorders. Premature and low birth weight infants are at increased risk of developing strabismus and other amblyogenic conditions throughout their childhood. Perinatal stroke is also associated with a high incidence of strabismus, early head turn, visual field cuts, and other vision abnormalities. Such high-risk infants as well as those with a strong family history of strabismus or amblyopia should be referred for evaluation.

It is recommended that all newborns have an ocular motility assessment (Donahue, 2016b). In addition, since vision in young nonverbal children is mostly assessed by evaluation of the child's ability to fix on and follow an object, this test provides information on the status of the visual and extraocular muscle systems. A standard assessment strategy is to determine whether each eye can independently fixate on the object, maintain fixation on it for a short period, and then follow it as it is moved in various directions. Neonates and young infants particularly fix and follow the human face or its likeness. This assessment should be performed binocularly and then monocularly in an awake and alert child. In neonates the following may be a jerky saccadic pursuit movement, which represents a series of hypometric saccades to localize the target. If following cannot be demonstrated, it should be verified that the motor system is intact. Range of motion and the ability to generate a saccade may be assessed by inducement of vestibular nystagmus by rotation of the child. If poor fixation and following are noted after 3 months of age, a significant ocular or neurologic abnormality is suspected and should be referred for evaluation (Donahue, 2016b).

Common Diagnostic Problems

Leukocoria and Abnormal Red Reflex

The term *leukocoria* means “white pupil.” It is often used more broadly to encompass a spectrum of opacities and abnormalities. On inspection, the pediatrician may grossly visualize a white lesion in the pupillary space or identify an abnormal red reflex. A white or abnormal reflex may also be identified in recreational photographs taken by family members (Cha et al., 1993; Russell, 2011; Abdolvahabi et al., 2013). The differential diagnosis for leukocoria includes vision- and life-threatening conditions, and leukocoria in an infant or older child requires urgent ophthalmologic evaluation. These conditions include cataract, retinoblastoma, chorioretinal or optic nerve head coloboma, retinal detachment, vitreous hemorrhage, ROP, persistent fetal vasculature, Coats disease, familial exudative vitreoretinopathy, toxocariasis, and uveitis. The distribution differs widely with the population studied. In one series, 60% of 71 children who presented to a tertiary referral center with leukocoria had cataract, 28% had retinoblastoma, and 12% had other retinal abnormalities (Haider et al., 2008).

Cataract

A cataract is any opacification of the normally clear crystalline lens of the eye. Although congenital cataract is much less common than age-related cataract, it is still responsible for approximately 10% of childhood blindness worldwide (Gilbert et al., 1993). Prevention of visual impairment caused by congenital cataract is an important component of the World Health Organization's international program for the elimination of avoidable blindness by 2020 (Thylefors, 1998). Congenital cataracts may be isolated, seen in association with another ocular developmental abnormality, or associated with systemic diseases. The incidence of congenital cataract is approximately 2 in 10,000 live births. Some subtypes of congenital cataracts are small and nonprogressive; dense central opacities larger than 3 mm are considered visually significant. Successful treatment of congenital cataracts may be extremely difficult, and intervention must occur very early in life; therefore early diagnosis is essential. Useful vision can be restored if the surgery is completed within the first 6 weeks after birth. Beyond this time, visual restoration becomes progressively more difficult because of irreversible deprivation amblyopia (Lundvall and Kugelberg, 2002; Birch et al., 2009). The appearance of nystagmus before surgery is an ominous sign of poor visual outcome and adds further urgency for surgical intervention (Lambert et al., 2006). Therefore it is essential that all newborns and young infants have screening eye examinations by a pediatrician, because visual prognosis is directly tied to timely ophthalmologic referral. Cataracts can develop or progress with time, so examination for an abnormal red reflex should be repeated at each well-child visit even if prior examination findings appeared normal.

After surgery, intensive visual rehabilitation strategies must be implemented. If the infant is left aphakic (without a lens), optical correction is achieved with special contact lenses or glasses. Adherence to use of contact lenses, glasses, and, in the case of monocular cataracts, aggressive amblyopia treatment by penalizing (patching) of the sound eye is critical and directly affects the child's ultimate visual outcome. Aphakic glaucoma is one of the most common sight-threatening complications of cataract surgery in infants. It can present soon after surgery or many years later, and it can be very difficult to treat, often requiring multiple surgical procedures.

The pathophysiology of aphakic glaucoma is not well understood, but important risk factors include early age at surgery and smaller corneal diameter (Swamy et al., 2007; Freedman et al., 2015).

Most patients with isolated nonsyndromic cataract have no identifiable cause. Although teratogenic agents (e.g., rubella) may account for a proportion of cases, such insults normally give rise to other systemic malformations in addition to cataract. Genetic mutation is likely to be the most common cause, particularly for bilateral cataract, but the proportion of cases with a genetic basis is still unclear (Reddy et al., 2004; Pichi et al., 2016). Genetic inheritance is most commonly autosomal dominant and rarely autosomal or X-linked recessive (Reddy et al., 2004; Hejtmančík and Smaoui, 2003). Multiple genes are involved in lens development, and numerous genetic loci have been identified (Hejtmančík and Smaoui, 2003; Reddy et al., 2004; Pichi et al., 2016). Marked variability can be present even within the same pedigree, and children with a family history of infantile or juvenile cataracts should be examined early by a pediatric ophthalmologist. The same is true of children with one of the numerous systemic conditions associated with cataracts: intrauterine infections (rubella, varicella); metabolic and endocrine disorders (galactosemia, neonatal hypoglycemia, diabetes mellitus, and hypoparathyroidism); fetal alcohol syndrome; chromosomal disorders (trisomy 21, Turner syndrome, trisomies 13 and 15); dermatologic diseases (congenital ichthyosis, ectodermal dysplasia); skeletal and connective tissue disorders (Smith–Lemli–Opitz, Marfan, Conradi, and Weill–Marchesani syndromes); renal disorders (Lowe, Alport, and Hallermann–Streiff–François syndromes); neurofibromatosis; and myotonic dystrophy. A selective diagnostic evaluation may be pursued in infants with cataract, particularly bilateral cataracts, and may include TORCH titers (including syphilis); urine tests for reducing substance (galactosemia); plasma urea, electrolyte, and urinary amino acid levels (Lowe syndrome); complete blood count and ferritin, blood glucose, calcium, and phosphate levels; quantitative amino acid levels and red blood cell enzyme levels (galactokinase, galactose 1-phosphate uridylyltransferase); genetic consultation; chromosome analysis and next-generation sequencing (focused on the 115 cataract-causing genes known to date); and ocular examination of parents and siblings. Infants with isolated unilateral cataracts often do not have a family history and rarely have associated systemic disorders (Reddy et al., 2004).

Retinoblastoma

Retinoblastoma is the most common ocular malignancy of childhood and accounts for 3% of all childhood cancers. The average age-adjusted incidence rate of retinoblastoma in the United States and Europe is 2 to 5 per million children or 1 in 14,000 to 18,000 live births (Parkin et al., 1988) but is higher in India and Africa (resulting in approximately 9000 new cases per year, of which fewer than 300 are in the United States). Seventy-five percent of patients have unilateral retinoblastoma, and 25% have bilateral retinoblastoma. The successful management of retinoblastoma depends on the ability to detect the disease while it is still intraocular; disease stage correlates with delay in diagnosis. Untreated retinoblastoma is almost uniformly fatal, and the long-term survival rate for disease diagnosed after it has spread outside the eye is less than 50%. In contrast, 5-year survival rates are greater than 90% when timely recognition and referral to centers specializing in retinoblastoma treatment occur (<http://www.1rbw.org>).

Two-thirds of patients receive a diagnosis before 2 years of age, and 95% receive a diagnosis before the age of 5 years (Parkin et al.,

1988). The earliest age at diagnosis reported is 21 weeks' gestation by prenatal ultrasound examination (Maat-Kievit et al., 1993). Abramson et al. (2003) reviewed 1831 consecutive cases of retinoblastoma. The most common presenting sign was leukocoria (54%), followed by strabismus (19%), poor vision (4%), family history with request for early examination (5%), and red eye (5%). The presenting sign was identified first by a family member or friend in 80% of cases, a pediatrician in 8% of cases, and an ophthalmologist in 10% of cases. Among patients presenting with leukocoria, the sign was first identified by a family member in 90% of cases. These findings stress the importance of routine red reflex testing for all children seen by pediatricians, beginning with newborns. Any child found to have leukocoria should be referred to an ophthalmologist for urgent evaluation.

The diagnosis of retinoblastoma can often be made by direct visualization. Imaging studies may provide useful information, including ocular ultrasonography to identify mass lesions and calcifications; magnetic resonance imaging (MRI) to image the globes and identify orbital and central nervous system involvement; and computed tomography (CT) scans to identify calcification, although MRI is now favored over CT for its ability to image soft tissues and to avoid ionizing radiation (Balmer et al., 2006). Treatment modalities include enucleation; systemic chemotherapy; intra-arterial chemotherapy, in which drug is delivered locally via catheterization of the ophthalmic artery; direct intravitreal chemotherapy injection; and focal destructive therapies such as retinal laser photocoagulation or cryotherapy. Radiation therapy, including brachytherapy, stereotactic conformal radiotherapy, and accelerated proton beam irradiation, is also used (Balmer et al., 2006; Shields et al., 2013), but external beam radiotherapy is now rarely indicated.

The retinoblastoma gene, *RBI*, located on chromosomal region 13q14, was the first tumor suppressor gene to be described. Five percent of patients with bilateral disease carry a large deletion involving the 13q14 locus. In those cases, retinoblastoma is part of a more complex syndrome characterized by facial dysmorphic features (thick anteverted earlobes, high and broad forehead, prominent philtrum, and short nose), skeletal abnormalities, mental retardation, and motor impairment. Children with germline mutations are at increased risk of developing nonocular tumors. Genetic counseling of affected parents is critical to estimate the risk of transmitting the disease to their children. Regardless of the clinical presentation, it is recommended that all patients undergo genetic testing.

Persistent Hyperplastic Primary Vitreous (Persistent Fetal Vasculature)

During embryogenesis and fetal development, the “primary vitreous” contains the hyaloid vasculature system, which fills the posterior segment of the eye and comes forward to surround the lens. This system normally disappears, and a spectrum of abnormalities can be seen when these structures fail to regress, ranging from persistent pupillary strands to a vascular stalk remnant to a retrolenticular membrane and retinal disorganization or detachment. Involved eyes are typically microphthalmic, and an abnormal red reflex or leukocoria may be identifiable. Depending on the extent, surgical intervention may help to avoid recurrent hemorrhage, glaucoma, and phthisis bulbi (atrophy and degeneration of a blind eye, which can become painful), and in some cases useful vision can be achieved (Dass and Trese, 1999). Persistent hyperplastic primary vitreous (PHPV) is the most common retinoblastoma-simulating lesion,

followed by Coats disease and presumed ocular toxocariasis (Shields et al., 1991).

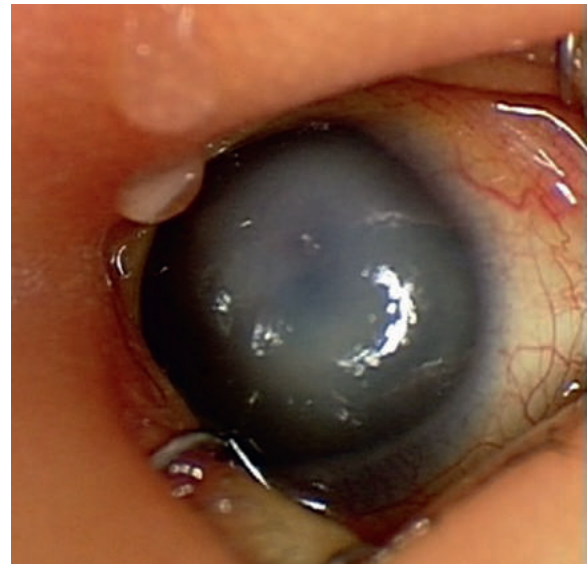
Coloboma

An ocular coloboma is a congenital anatomic defect or cleft that results from failure of the optic fissure to close during embryogenesis (Gregory-Evans et al., 2004). The result is essentially an area of missing tissue in the eye, most commonly in the inferonasal quadrant. Depending on the population studied, its incidence ranges from 0.5 to 7.5 per 10,000 births and accounts for 3%–11% of blind children worldwide (Gregory-Evans et al., 2004). Involved structures can include the iris, ciliary body, retina, choroid, and optic nerve (see Fig. 108.2). An iris coloboma appears as an irregular “keyhole,” or “cat’s-eye” pupil (see Fig. 108.2A). A chorioretinal or optic nerve head coloboma, depending on the size, will appear as an abnormal red reflex or leukocoria. The affected eye may be microphthalmic (see Fig. 108.2B). The visual prognosis depends on whether the central macula is involved, and children may have good central vision despite upper visual field defects if the macula is spared. Long term, there is a variable risk of complicating retinal detachment or choroidal neovascularization associated with retinal and optic nerve colobomas (Gregory-Evans et al., 2004). An ocular coloboma can be isolated or syndromic. There are numerous ocular abnormalities and systemic findings associated with coloboma, and more than 200 syndromes have been described. Examples include the CHARGE association, 22q11 deletion, and Treacher Collins, Walker–Warburg, and Aicardi syndromes. The systemic diagnostic testing in patients with apparently isolated bilateral or unilateral uveal coloboma should include a kidney ultrasound examination, audiometry, and spine radiographs. In addition, an echocardiogram and neuroimaging could be considered (Nakamura et al., 2011; Huynh et al., 2013).

Corneal Clouding

Slightly cloudy corneas can be seen in otherwise normal healthy newborns and normally clear within 1 to 2 days. Clouding is commonly seen in small for gestational age and premature infants, and it is proportional to the degree of prematurity. At 26 weeks’ gestation the degree of cloudiness is significant enough to prevent the evaluation of the iris details underneath, and along with an immature hyaloid vascular system, it prevents a detailed view of the posterior segment structures (retina and optic nerve). This is an important consideration when the ophthalmologist is asked to evaluate premature infants soon after birth for intraocular spread of systemic infections (endophthalmitis) or structural anomalies in suspected genetic diseases, etc. Slowly, as the child matures, the cornea establishes transparency and most of the time is sufficiently clear to allow retinal examinations for ROP by 30 to 32 weeks’ postconceptional age.

Persistent opacification of the cornea in a newborn may be the result of congenital glaucoma, corneal dystrophies, developmental abnormalities of the cornea and/or other anterior segment structures (Fig. 108.4), infection, iatrogenic trauma, or metabolic disorders. Close inspection with magnification will identify opacification in a focal, regional, or diffuse pattern, depending on the cause. The cornea is normally clear all the way to its border with the sclera (the limbus), with iris details easily visible underneath; these details will be obscured when the cornea is opacified. A large or central opacity will also result in an abnormal red reflex. When identified, congenital corneal opacification requires urgent ophthalmologic



• Fig. 108.4 Cloudy cornea secondary to anterior segment dysgenesis.

evaluation to rule out congenital glaucoma, infection—which could become eye or life threatening—or an opacification that is amenable to surgical correction during the early critical period in visual development.

Glaucoma is an optic neuropathy usually associated with raised intraocular pressure. In contrast with adult- or juvenile-onset glaucoma, which may be successfully managed medically, congenital glaucoma is a surgical disease that requires prompt intervention, frequently in the neonatal period. Vision loss from glaucoma is typically irreversible. Key signs to identify include corneal clouding (not always), corneal and eye enlargement (buphthalmos), tearing, blepharospasm (blinking), Haab striae (tears in the Descemet membrane, seen as lines in the red reflex), and photophobia. It is worth highlighting that tearing is a sign of glaucoma, not just of a blocked tear duct. Finally, congenital glaucoma has multiple systemic associations, such as Sturge–Weber syndrome, neurofibromatosis, Lowe syndrome, congenital rubella, and Rubenstein–Taybi disease.

Sclerocornea is characterized by cornea that is opacified and white like the sclera, with which it is developmentally continuous. Typically, these opaque areas are located at an indistinct corneoscleral limbus (border), can extend centrally, and contain superficial vascularization. It can occur in isolation or can be associated with cataract, microphthalmos, and/or infantile glaucoma (Nischal, 2007). In contrast to sclerocornea, *Peters anomaly* classically has a central corneal opacity with clear cornea peripherally, absence of posterior corneal stroma, and variable attachments between the posterior corneal surface and the iris and/or lens (Yang et al., 2009). The encompassing term *anterior segment dysgenesis* is sometimes preferred to describe many of these developmental anomalies of the cornea and anterior segment that result in congenital corneal opacities and congenital glaucoma as there is significant overlap and variability in their presentation (see Fig. 108.4).

Infectious keratitis can be herpetic with characteristic epithelial dendritic ulcers and corneal stromal inflammation. *Bacterial keratitis* is less common in countries that practice routine administration of conjunctivitis prophylaxis at birth. However, a bacterial corneal ulcer can begin as a corneal abrasion (see the later discussion) and then quickly enlarge, resulting in corneal thinning or perforation, endophthalmitis, and even bacterial sepsis. In the authors’

TABLE 108.1 Diagnostic Features and Management of Neonatal Conjunctivitis

| Etiologic Agent | Onset | Clinical Characteristics | Diagnosis | Treatment |
|---|-----------|---|--|---|
| Chemical | 24 h | Noninfectious Lid edema, watery discharge | History of exposure, self-limited in <48 h | None |
| Gonococcal (see Fig. 108.5) | 3–4 days | Bilateral, hyperacute purulent conjunctivitis, marked lid edema, copious discharge Can perforate cornea | Cell culture and Gram stain Gram-negative intracellular diplococci | Ceftriaxone 25–50 mg/kg daily intravenously Topical irrigation Topical antibiotics useful only if corneal ulcer present |
| <i>Chlamydia trachomatis</i> (most common) | 5–7 days | Mild mucopurulent nonfollicular conjunctivitis, lid edema, pseudomembrane formation Pneumonitis after 3–12 weeks | Cell culture, Giemsa stain, direct immunofluorescent assay, enzyme-linked immunoassay, PCR Basophilic intracytoplasmic inclusions in epithelial cells | Orally administered erythromycin, 12.5 mg/kg every 6 h for 2 weeks or azithromycin suspension, 20 mg/kg orally daily for 3 days |
| <i>Staphylococcus</i> , <i>Streptococcus</i> , and other bacteria | 5–14 days | Nosocomial, mucoid discharge, conjunctival hyperemia, and chemosis | Cell cultures, Gram stain | Broad-spectrum topical antibiotic (e.g., polymyxin B–trimethoprim, one drop every 4 h for 7 days) |
| Herpes simplex virus | 6–14 days | Unilateral or bilateral conjunctivitis (nonfollicular), serous discharge, associated lid vesicles, and, occasionally, corneal epithelial dendritic defects that stain with fluorescein with or without systemic involvement | Cell cultures, direct fluorescent antibody staining, enzyme immunoassay detection, PCR Multinucleated giant cells with intracytoplasmic inclusions | Acyclovir, 60 mg/kg per day in three divided doses for 2 weeks (3 weeks if CNS or disseminated disease) plus topical drops (1% trifluridine, 0.1% iododeoxyuridine, or 3% vidarabine) |

CNS, Central nervous system; PCR, polymerase chain reaction.

experience, this chain of events can also occur in reverse, with bacteremia seeding the eyes and ultimately manifesting itself as a keratitis. The findings include a white corneal stromal infiltrate with an overlying corneal epithelial defect that stains with fluorescein. Aggressive topical and sometimes systemic antibiotics are required, and any infant with a red eye and corneal opacity or abnormality should be referred for immediate ophthalmologic evaluation.

Iatrogenic trauma may result in corneal injury and may be the result of amniocentesis or forceps delivery. Injuries from the latter are characterized by linear breaks in the Descemet membrane, which are more likely to be vertical or diagonal than the horizontal breaks seen with glaucoma, and may be accompanied by other signs of trauma.

Corneal dystrophies, such as congenital hereditary endothelial dystrophy and posterior polymorphous dystrophy, may also result in cloudy corneas. The former always manifests itself at birth, whereas the latter may or may not be present at birth (Nischal, 2007). Metabolic disorders, such as some mucopolysaccharidoses, may cause progressive clouding but often do so later in life and rarely during the neonatal period.

Red Eye/Eye Discharge

The most common and important causes of a red eye in neonates include infectious conjunctivitis, subconjunctival hemorrhage, foreign bodies, and vascular malformations.

The incidence of neonatal conjunctivitis (ophthalmia neonatorum) has decreased dramatically since the introduction of prophylaxis in 1881. Despite this, ophthalmia neonatorum still blinds thousands of babies annually worldwide (Isenberg et al.,

1996). The etiologic cause of conjunctivitis in the newborn can be chemical, bacterial, or viral (Table 108.1). Although infections are usually transmitted to the infant by direct contact during passage through the birth canal, organisms can ascend to the uterus, so even infants born via cesarean delivery can be infected, particularly in the setting of prolonged rupture of membranes but even with intact membranes (Gallardo et al., 2005). Prophylactic agents include 1% silver nitrate, 0.5% erythromycin ointment, 1% tetracycline ointment, and 2.5% povidone–iodine (Isenberg et al., 1995, 1996). No prophylactic agent completely eliminates the risk of developing an infection, and a high index of suspicion should be maintained, in particular in those patients with risk factors (maternal infection, lack of prenatal care, or premature rupture of membranes). Gentamicin ointment should be avoided, as it is associated with periocular ulcerative dermatitis when used on the newborn eye (Binenbaum et al., 2010).

Despite common teaching, the timing of the onset of conjunctivitis is not a reliable diagnostic clue, because significant overlap exists among the different etiologic agents. For this reason, conjunctival cultures (in Thayer–Martin agar, blood agar, and chocolate agar) and conjunctival scraping for Gram and Giemsa staining are mandatory and should be performed without delay. It is not necessary to wait for an ophthalmology consultation to initiate laboratory investigation and treatment, because delays in treatment of gonococcal conjunctivitis can have devastating consequences. *Neisseria gonorrhoeae* can penetrate an intact corneal epithelium, rapidly leading to perforation of the globe within hours if treatment is not initiated; therefore all forms of conjunctivitis must be considered bacterial until proven otherwise. Appropriate treatment should be instituted once the results of cultures are known but should never be delayed (Fig. 108.5).



• **Fig. 108.5** A 6-day-old newborn with gonococcal conjunctivitis. Note the marked lid edema and copious purulent eye discharge.

The infant with mucopurulent discharge must be distinguished from the infant who exhibits only excessive tearing and a relatively white eye. The latter is most likely to have nasolacrimal duct obstruction, although the possibility of congenital glaucoma must always be ruled out. Congenital obstruction of the nasolacrimal duct is present in about 5% of infants (Paul and Shepherd, 1994) but usually resolves spontaneously by 12 months of age. Usually a thin mucosal membrane at the distal end of the nasolacrimal duct is the cause. Symptoms become manifest by 1 month of age in 80% of cases, with tearing or sticky mucoid discharge. As a consequence of chronic obstruction, secondary infection in the lacrimal sac may occur, a condition known as *dacryocystitis*. Pressure on the lacrimal sac causes a reflux of mucopurulent material from the punctum. This tends to be a low-grade chronic infection although occasionally it can progress to blepharoconjunctivitis and cellulitis. In contrast, acute dacryocystitis, more commonly seen with congenital dacryocystoceles, is a more severe infection that requires prompt intravenous antibiotics (see later). Simple nasolacrimal duct obstruction (NLDO) usually resolves spontaneously within the first year of life; only conservative management is indicated in most cases. This consists of digital massage downward from the lacrimal sac over the nasolacrimal duct on the side of the nose. The massage empties the sac, reducing the opportunity for bacterial growth. Topical broad-spectrum antibiotic drops or ointment, such as bacitracin zinc and polymyxin B sulfate ophthalmic ointment or drops, can be used if there is conjunctival injection and discharge. Referral to an ophthalmologist should be considered if the condition has not resolved toward the end of the first year, because probing and/or intubation of the lacrimal sac is likely necessary to relieve the obstruction (Katowitz and Welsh, 1987), although the optimal timing of surgery remains controversial. A more recent study reported that there was no age-related decline in the success rate of surgical treatment for congenital NLDO in children treated up to 36 months of age (Pediatric Eye Disease Investigator Group et al., 2008).

Preseptal and orbital cellulitis can also manifest with conjunctival injection, chemosis, and discharge. In addition, orbital cellulitis may cause altered ocular motility and pupillary reflexes and proptosis. Both conditions are typically unilateral and can rarely occur in the first month of life, sometimes secondary to acute dacryocystitis from an infected dacryocystocele (see *Ptosis and Other Eyelid and Lacrimal Abnormalities*).

Subconjunctival hemorrhage is seen as a bright red discoloration under the conjunctiva, obscuring the white scleral background, and is common in the perinatal period. In most cases it is caused by elevated venous pressure in the head and neck produced by compression during uterine contractions. Later in life, subconjunctival

hemorrhage may be a feature of child abuse, although it can also occur spontaneously. Subconjunctival hemorrhage in isolation is completely innocuous and usually resolves in 10 to 14 days.

Motility Abnormalities and Nystagmus

Various eye motility and alignment abnormalities can be present in the first month of life. The most common form of strabismus in early infancy is infantile or essential esotropia (see Fig. 108.3A). This form of convergent strabismus is rarely actually congenital, so this term has been abandoned. It is usually present by age 3 to 4 months and is characterized by a large-angle deviation. These children tend to cross-fixate (use the left eye to view the right visual field and vice versa), simulating an abduction deficit, because it is hard to get the child to follow an object to the ipsilateral field. This can easily be mistaken for bilateral sixth nerve palsy. However, sixth nerve palsies in the neonatal period are extremely rare, and an abduction movement can sometimes be elicited by the patching of one of the child's eyes to force the other eye to abduct in search of an object or by use of the doll's-eye (the eyes lag behind the turning of the head from side to side) or optokinetic nystagmus maneuvers. Infantile esotropia is a condition that is not likely to resolve spontaneously without surgical correction. Because of a high associated risk of amblyopia, prompt referral of these patients to a pediatric ophthalmologist within the first few months of life is appropriate. Even if the child's age or systemic condition is not appropriate for surgical correction, patching and other treatments should be initiated as early as possible. Ideally, surgical correction is accomplished early, by 6 to 12 months of age.

Congenital motor nystagmus (infantile nystagmus syndrome) is an involuntary, bilateral, conjugate oscillation of the eyes that develops within the first 6 months of life. Despite the name, the eye movement abnormalities are rarely noticed at birth. Disorders of the anterior visual pathways resulting in blindness or severe visual deprivation very early in life can also result in nystagmus (sensory deprivation nystagmus), which can be manifest as large-amplitude "wandering" eye movements or with a smaller-amplitude, faster movement that resembles the congenital motor form. Nystagmus caused by a visual deficit does not develop until about 3 months of age. Any form of bilateral (and sometimes unilateral) visual deprivation, including cataracts, corneal abnormalities, glaucoma, optic nerve problems, and chorioretinal colobomas, can manifest themselves with nystagmus, and a prompt ophthalmic evaluation is needed to rule out these conditions, which are treatable in some cases. Other conditions such as albinism, achromatopsia (congenital absence of the retinal cones), and aniridia can also result in nystagmus, which is often very similar to motor nystagmus. Since the ocular findings in these conditions are more subtle and easily missed, an electroretinogram is usually recommended before the diagnosis is confirmed. Because of the overlap with identifiable causes, motor nystagmus should always be considered a diagnosis of exclusion, as other ocular and neurologic disorders can manifest with the same clinical characteristics. Certain specific forms of nystagmus are commonly associated with neurologic dysfunction; these include upbeat nystagmus, see-saw nystagmus, and even monocular nystagmus. However, these forms have all been associated with sensory loss, and an ophthalmologic examination is necessary even when neuroimaging studies rule out intracranial disease.

Other transient disorders of the ocular motor system include flutter-like, high-frequency, small-amplitude movements that in newborns may be self-limiting and resolve spontaneously within the first few weeks of life. This saccadic oscillation should not be

confused with nystagmus. Some infants can also exhibit transient downward deviation of the eyes (Hoyt et al., 1980). This disorder can be distinguished from the more serious sun-setting sign, associated with hydrocephalus, by demonstration of intact upgaze movements using vestibular–ocular responses. Neuroimaging fails to demonstrate any underlying neurologic disorder, and the deviation tends to reduce gradually over the following months. A true upgaze palsy and convergent strabismus can be seen in premature infants who sustained intraventricular hemorrhage (Tamura and Hoyt, 1987). Paroxysmal tonic upgaze of childhood is a rare and distinctive syndrome characterized by episodes of sustained conjugate upward deviation of the eyes. Symptoms normally appear in babies younger than 1 year and are characterized by an upward stare with the eyes rolled back, while the chin is held low (possibly to compensate for the abnormal eye position). The horizontal eye movements are normal, as are the rest of the neurologic examination and imaging findings. The condition tends to improve with time (Ouvrier and Billson, 2005).

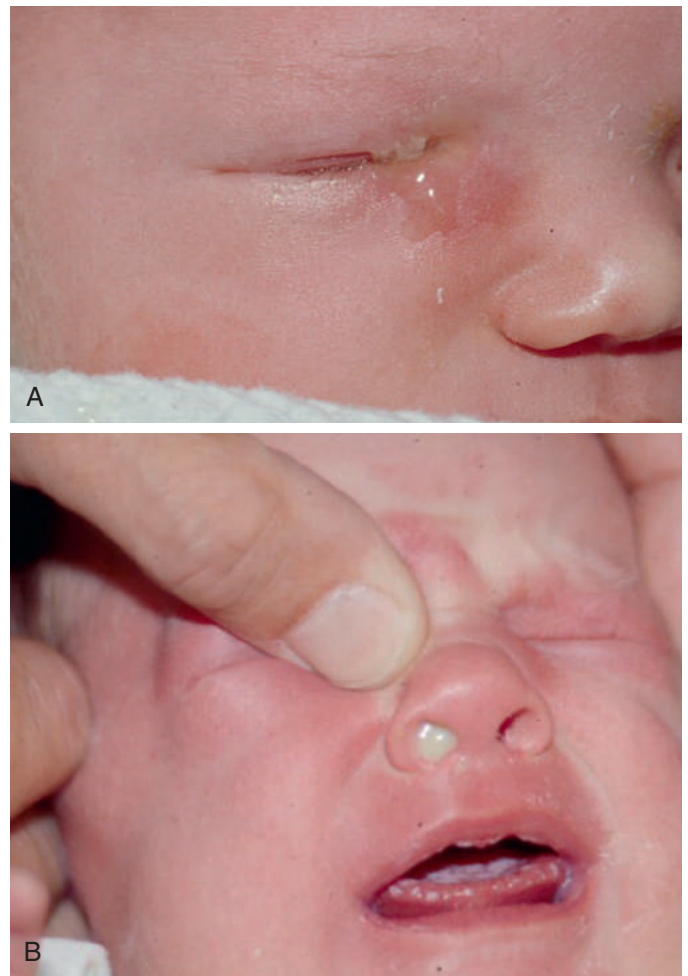
Ptosis and Other Eyelid and Lacrimal Abnormalities

External inspection of the eyes and eyelids should be performed in every newborn. The eyelids are fused usually until 25 weeks' gestation, but they may rarely remain fused until 30 weeks' gestation. Eyelid colobomas are rare congenital full-thickness defects of missing eyelid, usually involving the upper eyelid border. Eyelid colobomas may be associated with Goldenhar syndrome. The consequent lack of ocular surface coverage places the infant at high risk of corneal abrasion, ulcer, scarring, and vision loss. Such infants require urgent ophthalmology consultation and should not be placed under overhead heaters without adequate ocular protection.

Most cases of congenital ptosis are caused by an isolated developmental anomaly of the levator palpebrae muscle. Other causes include congenital third nerve palsy, Horner syndrome, blepharophimosis syndrome, and Marcus Gunn jaw-winking ptosis, which is a synkinesis between the levator palpebrae and the muscles of mastication. This type of ptosis is characterized by elevation of the lid associated with sucking or chewing.

Because form deprivation amblyopia can occur as a result of complete obstruction of the visual axis, this condition must be managed aggressively by an ophthalmologist familiar with the treatment of eyelid disorders and amblyopia. Even though this type of amblyopia is the most severe and concerning to the neonatologist, it is important to remember that even without complete obstruction of the pupil, amblyopia can occur secondary to the blur induced by astigmatism produced by the ptotic eyelid on the cornea. If vision is not threatened, surgery may be deferred until 4 or 5 years of age, although close follow-up to detect amblyopia is necessary, and many surgeons have argued the benefits of earlier surgery (Katowitz, 2002).

A number of eyelid tumors can be present at birth or shortly thereafter. Capillary hemangiomas are usually not present at birth and appear in the first couple of weeks of life. The lesions can have a superficial component, which is red and sometimes dimpled, resembling a strawberry, and a deeper component, which appears as a deep diffuse purplish mass. The hemangioma grows during the first 6 to 8 months of life and then stabilizes, regresses, and involutes over several years. These lesions are amblyogenic because they can cause mechanical ptosis, obstruct the visual axis, or cause significant astigmatism. Systemic nonselective beta-blockers (e.g., propranolol) have become first-line treatment, showing remarkable



• **Fig. 108.6** (A) Dacryocystocele in a newborn. (B) Manual decompression of mucopurulent material. (Photo courtesy of Robert Kersten, University California San Francisco Department of Ophthalmology, Ocular Plastics and Reconstructive Surgery San Francisco, California.)

effectiveness in causing regression of these tumors (Léauté-Labrèze et al., 2008, 2015). Topically administered timolol can be effective for superficial lesions as well (Chambers et al., 2012). Other treatment options include topical, intralesional, or systemic steroids; interferon alfa; vincristine; and surgical resection.

Capillary hemangiomas should be distinguished from other capillary vascular malformations (such as port wine stains) that are present at birth and do not exhibit regression. These are sharply circumscribed lesions that are usually unilateral. In these patients, Sturge–Weber syndrome should be suspected. When the skin lesion involves the eyelid, an ophthalmologic consultation should be requested to rule out glaucoma (occurring in about 50% of patients) and vascular abnormalities of the choroid.

A dacryocystocele is formed when a proximal and a distal obstruction coexist in the lacrimal sac and the lacrimal sac becomes distended. Clinically it is manifested as a bluish, nontender mass just inferior and nasal to the medial canthus (Fig. 108.6). The bulging of the mucosa at the lower end of the nasolacrimal duct into the nasal cavity can significantly compromise the airway and should be ruled out by inspection of the nasal passage (Paysse et al., 2000). A secondary infection of the distended sac, or dacryocystitis (Fig. 108.7), is not uncommon and can have potentially serious consequences in a neonate, including meningitis and



• **Fig. 108.7** (A) Bilateral dacryocystoceles. (B) Infected dacryocystocele with erythema in medial canthal area and a firm tender mass. (C, D) Dacryocystocele with progression to preseptal cellulitis. (E, F) Dacryocystitis with localized abscess before and after nasolacrimal duct probing with endonasal cyst marsupialization.

septicemia. Intravenous antibiotics should be administered followed by decompression of the sac after 24 to 48 hours if the dacryocystitis shows no abatement. A dacryocystocele should be distinguished from a frontal encephalocele and can occasionally be confused with a hemangioma or a dermoid cyst.

Ocular Trauma in the Neonatal Period

Newborns may experience injuries to the eye as a result of birth, more commonly difficult births, such as those involving prolonged delivery, cephalopelvic disproportion, vacuum extraction, or forceps use. Intrauterine injuries may result from amniocentesis, with discovery at birth. Some common injuries will heal without long-term visual or ocular sequelae (Holden et al., 1992). However, identification of birth-related eye trauma necessitates an ophthalmologic consultation to perform a complete dilated examination, identify all injuries, and assess the need for treatment.

Subconjunctival hemorrhage results from rupture of blood vessels on the surface of the eye. It is distinguished from conjunctival

injection due to an inflammatory or infectious cause by characteristically well-demarcated borders between spots of blood underneath the conjunctiva and completely white sclera directly adjacent to the blood. Subconjunctival hemorrhages will resolve spontaneously with time and do not require treatment. However large diffuse hemorrhages can be a sign of more significant trauma underneath the hemorrhage or within the eye, and a complete eye examination, including dilated fundusoscopic examination, may be indicated. Recurrent subconjunctival hemorrhage could be a sign of a coagulopathy or platelet abnormality, and an appropriate work-up should be pursued in such cases.

Hyphema is the presence of red blood cells in the anterior chamber of the eye (the space between the cornea and the iris), most commonly the result of trauma, including birth trauma. It is often associated with other ocular injuries. Clot or hemorrhage of differing magnitude may be seen on close inspection, ranging from a subtle crescent of blood at the corneoscleral limbus to an eye completely filled with blood, obscuring any view of the structures underneath. The red reflex is commonly abnormal. Hyphema is

a potentially vision-threatening condition and requires close management by an ophthalmologist. The immediate concern is obstruction of the aqueous fluid drainage angle structures, resulting in an acute rise in intraocular pressure (IOP) or acute glaucoma. High IOP is painful enough to cause vomiting in children and adults and may manifest as crying, irritability, grimacing, poor feeding, or emesis in an infant. If the IOP is high enough for any prolonged period, irreversible optic nerve damage can ensue, with permanent vision loss. Corneal blood staining may also occur, which takes many months to clear, and can result in amblyopia.

An open globe injury (ruptured globe) refers to a full-thickness break in the eyewall (e.g., sclera, cornea). These injuries can occur as a laceration or as a rupture, in which pressure is applied to the front of the eye, and the eye wall breaks open at its weakest points, such as the corneoscleral limbus or extraocular muscle insertion sites. Key signs include an obvious break with protrusion of intraocular contents (e.g., the iris), which typically appear brown; extensive subconjunctival hemorrhage; a flat anterior chamber (the iris and cornea have come together); hyphema; vitreous hemorrhage, which may be seen as a poor red reflex; and abnormal eye contour or intraocular foreign body on orbital imaging. An open globe is a surgical emergency.

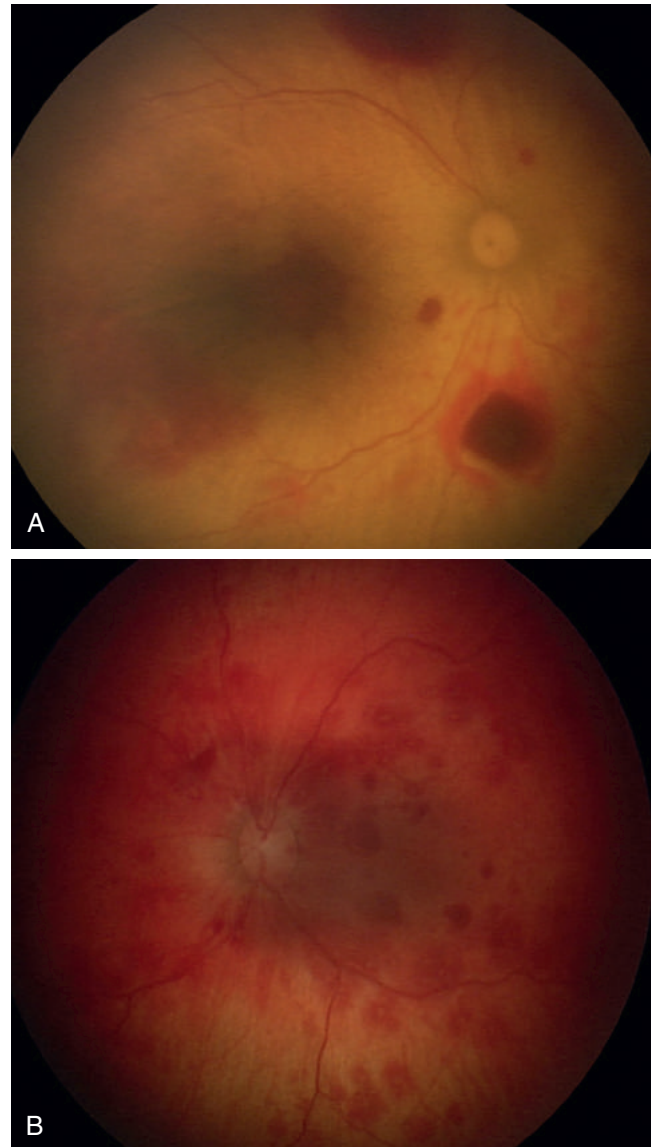
A *corneal abrasion* is an epithelial defect on the corneal surface. Staining with fluorescein will identify areas of missing epithelium, which appear green under a blue light source and magnification. Treatment includes topical antibiotic ointment and close follow-up to ensure complete healing and to monitor the patient for the development of bacterial keratitis (see the earlier discussion of corneal clouding). In a neonate a corneal abrasion must be taken very seriously, because complications can include the sequential development of keratitis, endophthalmitis, septicemia, and meningitis (O'Keefe et al., 2005).

Other injuries include RHs (see the later discussion); corneal trauma from forceps, resulting in tears of the Descemet membrane, visible as vertical or circumferential lines on red reflex examination, corneal thickening and opacification from edema, and corneal scarring; eyelid or adnexal injuries requiring surgical repair; choroidal rupture, which requires dilated fundus examination for diagnosis; and even traumatic optic neuropathy.

Retinal Hemorrhages and Abusive Head Injury

RH from the birth process is very common. The incidence is estimated to be between 10% and 40% of all newborns, depending on the examiner, time from birth, and specific population studied (Emerson et al., 2001; Hughes et al., 2006; Callaway et al., 2016). The rate in infants delivered with vacuum assistance is even higher, at 75%–78% (Emerson et al., 2001; Hughes et al., 2006). The association between vacuum-assisted delivery and RH supports a role for mechanical trauma to the retinal vessels (e.g., from direct compression of the globe); however, additional factors are likely involved, because head circumference and the duration of labor are not significant factors, and RH occurs after cesarean delivery, albeit at decreased frequency (7%) (Emerson et al., 2001; Callaway et al., 2016). RHs are usually identified by indirect ophthalmoscopy, although an abnormal red reflex may be noted in some severe cases.

The long-term visual consequences of birth-related hemorrhages remain unknown. Central or large hemorrhages can interfere with visual development in some cases (Fig. 108.8A), but the occurrence of significant visual deficits caused by RH is probably low. It is important to understand the characteristics that distinguish birth-related RH from those associated with AHT, as there may be



• **Fig. 108.8** Retinal hemorrhages in neonates. (A) Birth-related hemorrhages. Note the large hemorrhage covering the central macula in this neonate with thrombocytopenia. This patient developed severe myopia and amblyopia. (B) Diffuse intraretinal hemorrhages (dot blot, flame shape, and white centered) secondary to nonaccidental head trauma.

considerable overlap. The great majority of birth-related RHs are intraretinal; subretinal and preretinal hemorrhages are less frequent. Such hemorrhages include superficial flame and deeper blot hemorrhages, and larger hemorrhages may frequently contain white centers, which are a completely nonspecific finding (Emerson et al., 2001; Hughes et al., 2006; Callaway et al., 2016). Although commonly located in the posterior pole of the eye, they may also extend to the mid- and far-retinal peripheries and range in number from a few to too numerous to count (Emerson et al., 2001; Hughes et al., 2006). They may be bilateral, unilateral, or asymmetric.

The preceding characteristics are similar to those of the RH seen in AHT (see the later discussion), although the severity is usually greater in AHT. A key characteristic therefore is the time to resolution of birth-related RH. Most intraretinal hemorrhages clear within the first 2 or 3 weeks after birth, many within the first few days (Emerson et al., 2001; Hughes et al., 2006). In most

series, all intraretinal hemorrhages have cleared by age 4 weeks, whereas preretinal or subretinal hemorrhages may take 6 weeks or more to clear (Emerson et al., 2001). Therefore multiple intraretinal hemorrhages present past 1 month of age should be considered not related to birth. Isolated, resolving intraretinal hemorrhage or persistent preretinal or subretinal hemorrhage past 1 month of age may rarely be related to birth, but this pattern of RH would not at any age by itself be considered specific for any cause, including abuse.

Pediatric AHT is a leading cause of death in infancy. Previous names have included *shaken baby syndrome*, *inflicted or nonaccidental head trauma*, and *inflicted childhood neurotrauma*. AHT is characterized by intracranial hemorrhage with or without RHs and/or additional injuries, including bony fractures. Affected children are less than 3 years of age, with the great majority younger than 1 year (Duhaime et al., 1998; Binenbaum et al., 2009). The mechanism of trauma is believed to be repetitive acceleration–deceleration of an infant's head, with or without blunt impact. RHs are present in 50%–100% of victims of AHT (Duhaime et al., 1998; Binenbaum et al., 2009). The presence of any RH in an infant is highly associated with abuse, and increasing RH severity correlates with increasing likelihood of abuse (Binenbaum et al., 2009; Bhardwaj et al., 2010). In AHT, RH may range from none to a few intraretinal hemorrhages confined to the posterior pole to bilateral, “too numerous to count,” intraretinal, subretinal, and preretinal hemorrhages, extending to the far periphery or ora serrata (termination of the retina) (Levin, 2010; Binenbaum et al., 2014). The hemorrhages may be unilateral or markedly asymmetric. Macular retinal folds and hemorrhagic macular retinoschisis (splitting of the retinal layers) may be seen at the severe end of the spectrum. In comparison, RHs associated with accidental head trauma are typically few in number, intraretinal, and limited to the posterior pole (Bechtel et al., 2004; Binenbaum et al., 2009) (see Fig. 108.8). However, more severe RH and even retinal folds may be seen with unambiguous severe accidental trauma, such as fatal motor vehicle crashes (Kivlin et al., 2008).

In addition to accidental head trauma, the differential diagnosis of RH in infancy includes birth-related RH (see the earlier discussion), coagulopathy, septicemia, leukemia, anemia, and glutaric aciduria. These conditions may be recognized with use of various diagnostic tests and are often distinguishable from the patterns of RH seen in AHT. Studies have demonstrated that prolonged chest compression with cardiopulmonary resuscitation very rarely results in RH, and when present, these RHs are a few isolated posterior pole intraretinal hemorrhages. RHs associated with raised intracranial pressure are limited to small splinter hemorrhages on a swollen optic disc or superficial, flame-shaped, intraretinal hemorrhages adjacent to a swollen optic disc (Binenbaum et al., 2013). Vaccination injections, convulsions, and severe coughing do not cause RH (Binenbaum et al., 2015).

Children with AHT may present with a history of minor blunt head trauma, such as a short fall, or no trauma history at all and exhibit lethargy, seizures, increased or decreased tone, vomiting, poor feeding, breathing difficulties, or apnea (Duhaime et al., 1998). Head CT identifies subdural or subarachnoid hemorrhage, sometimes with a combination of chronic and acute features, but very rarely the findings may initially be normal; brain MRI provides a better look at the soft tissue and brain parenchyma to identify features such as hypoxic–ischemic injury; plain film radiographs may reveal a skull fracture; and a skeletal survey is a critical modality for identifying other bony injuries. Because the history provided is often vague or unreliable, a high index of suspicion and a low

threshold for obtaining diagnostic tests and specialist consultation must be maintained.

Ophthalmologic examination should occur within 48 hours, preferably within 24 hours, because RH can begin to resolve within days (Binenbaum et al., 2016). A dilated fundus examination with an indirect ophthalmoscope is required to adequately visualize the retina. Ophthalmologic consultation should not be delayed because of an inability to pharmacologically dilate the pupils because of neurologic pupil examination checks; the ophthalmologist can still attempt to view a portion of the retina and return for a dilated examination later. It is very important to document the type(s), number, location(s), and laterality of all RHs, by the use of explicit descriptive terms and thorough use of diagrams.

Fundus photographs, for example, those taken with a RetCam camera, should be obtained when possible. Such photographs provide important documentation and are often easily obtained at the time of sedation administered for MRI or other tests. However, the authors stress that the presence of RH is not descriptive enough, and the extent and detailed characteristics of the hemorrhages are vitally important. Cameras may identify and characterize posterior pole RH, but they do not always provide adequate visualization of the retinal periphery and are not a substitute for an indirect examination. RHs have been reported as a result of RetCam use in a premature infant undergoing ROP screening, but not in other circumstances (Adams et al., 2004). Notably, this 25-week-gestational-age infant was less than 34 weeks' PMA at the time of the examination and had immature retinal vasculature, without smooth muscle, elastin, or collagen layers and poor autoregulation, unlike the mature vasculature of a few-months-old infant evaluated for AHT (Adams et al., 2004). Further, other investigators have failed to identify any RHs with the routine use of the RetCam for ROP screening (Azad et al., 2004). Nevertheless, whenever possible an indirect ophthalmoscopic examination should be performed and documented before a contact fundus camera is used.

Visual impairment in children with AHT is thought to be due, most often, to cortical damage. However, persistent macular or vitreous hemorrhage, retinoschisis, and other scarring conditions may result in significant deprivation amblyopia, induced myopia and anisometropic amblyopia, or photoreceptor damage limiting visual function. The overall mortality rate in AHT has been reported to be between 13% and 36% (Barlow et al., 2005). Approximately two-thirds of the survivors have long-term neurologic deficits. In a prospective study of 25 children with a mean follow-up of 5 years, Barlow et al. (2005) found that 68% had neurologic and cognitive impairment, and half of these had severe disabilities and were totally dependent.

Retinopathy of Prematurity

ROP, a disease of the developing retinal vasculature, first became a significant cause of blindness in children in the 1950s with increased survival of premature infants as a result of improved neonatal care, in particular the use of supplemental oxygen in industrialized countries. With restriction of oxygen use in the mid-1950s, there was a reduction in the incidence of blindness from ROP, but this was associated with increased rates of death and cerebral palsy in premature babies. During the late 1960s, despite more accurate methods to monitor oxygen supplementation and improved management of perinatal complications, blindness from ROP began to reemerge because smaller and less mature babies were surviving. Surgical treatment of established disease

TABLE 108.2**International Classification of Retinopathy of Prematurity—Revisited (2005)**

| | | |
|---------------------------------------|---|---|
| Anterior–posterior location | | <p>Zone I: retinal area within a circle centered on the disc and with a radius of twice the estimated disc–foveal distance</p> <p>Zone II: retinal area extending from the edge of zone I to a circle with a radius from the disc to the nasal ora serrata</p> <p>Zone III: a crescent-shaped retinal area extending beyond zone II to the ora serrata</p> |
| Severity | Stage of ROP | <p>Stage 1: a thin, sharp line of demarcation between vascularized central retina and more peripheral avascular retina</p> <p>Stage 2: an intraretinal elevation (ridge) at the junction between vascularized and avascular retina</p> <p>Stage 3: a ridge with fibrovascular extension into the vitreous</p> <p>Stage 4: partial retinal detachment; stage 4A, does not involve the fovea; stage 4B: involves the fovea</p> <p>Stage 5: total retinal detachment</p> |
| Dilation | Aggressive posterior ROP | <p>Aggressive posterior ROP recognized by:</p> <ol style="list-style-type: none"> 1. Marked dilation and tortuosity of posterior pole vessels 2. Difficulty in documenting the stage of ROP at the junction between vascularized and avascular retina 3. Occurs in zone I or posterior zone II |
| Extent | | Number of clock hours of ROP along the circumference of the vascularized retina; this aspect is no longer used for treatment decisions |
| Posterior pole vascular abnormalities | <p>Plus disease</p> <p>Pre-plus disease</p> | <p>Presence of dilated and tortuous vessels of the posterior pole, in comparison with a standard photograph, present in two or more quadrants</p> <p>Abnormal vascular dilation and tortuosity that are insufficient for diagnosis of plus disease</p> |

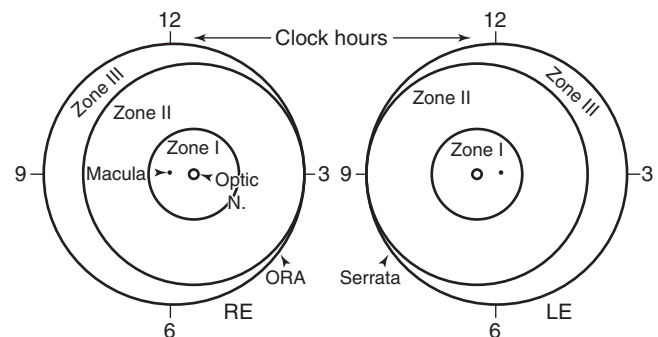
ROP, Retinopathy of prematurity.

and improved neonatal care are probably the major factors responsible for the reduction of the incidence of blinding ROP observed during the 1980s and 1990s in developed countries such as the United States (Gibson et al., 1989). However ROP-related blindness continues to be an important public health problem among middle-income countries, in which the capability to save small babies has developed, but oxygen use is often not controlled, and the resources to screen babies for and treat ROP are lacking (Gilbert et al., 2005).

Classification of Retinopathy of Prematurity

The International Classification of Retinopathy of Prematurity (ICROP) was published in 1984 (International Committee for the Classification of Retinopathy of Prematurity, 1984) and expanded in 1987 (International classification of ROPm, 1987), providing a standard nomenclature for the clinical findings and staging of ROP. As shown in Table 108.2, this classification takes into account four components of the ocular findings: anterior–posterior location of the retinopathy (zone), severity (stage), extent of the disease at the circumference of the vascularized retina (in clock hours), and the presence or absence of so-called plus disease. *Plus disease* is defined as engorged and tortuous vessels of the posterior pole and indicates a more serious form of ROP. The ROP status of an eye is determined by the highest stage and the lowest zone observed, along with the noting of the presence or absence of plus disease.

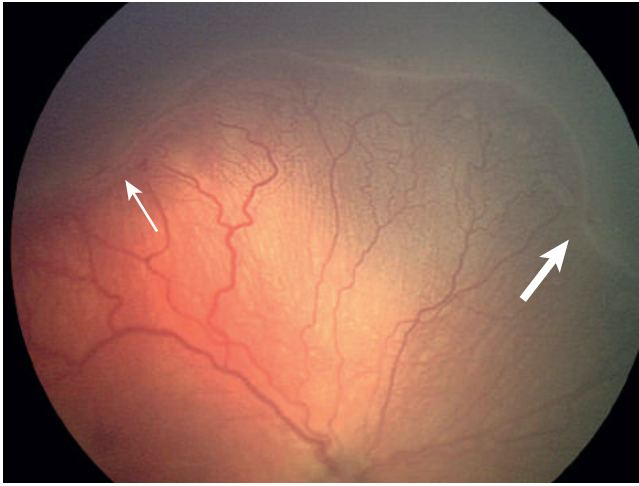
In ICROP the retinal surface is divided into three concentric “zones” centered on the optic nerve (Fig. 108.9). Most severe retinopathy occurs in zone I or posterior zone II, most retinopathy occurs in zone II, and ROP that occurs in zone III tends to be mild. The severity of retinopathy is designated on a scale of 1 to 5 by “staging” of the retinopathy at the junction between vascular and avascular retina (Fig. 108.10). The first two stages indicate mild and moderate disease; the extension of vessels into the vitreous indicates stage 3 ROP, a more serious disease. Stages 4 and 5



• **Fig. 108.9** Retina of the right eye (RE) and left eye (LE) showing zone borders and clock hours used to describe the location and extent of retinopathy of prematurity. N., Nerve. (Reproduced from *Arch Ophthalmol.* with permission.)

indicate the presence of retinal detachment. The term *plus disease*, a clinical diagnosis made by comparison with reference photographs used in clinical treatment trials in the United States (Good et al., 2005), indicates marked tortuosity and/or dilation of the peripapillary arterioles and venules in at least two quadrants. *Pre-plus disease* designates the presence of vessels that are not normal appearing but are not sufficiently abnormal to be designated as plus disease. Other signs that frequently accompany serious ROP include vitreous haze, iris vascular engorgement, and pupillary rigidity, but these signs are not required to make the diagnosis of plus disease.

In 2005 aggressive posterior ROP was added to ICROP, defining the observation of a particularly aggressive form of ROP observed with increasing frequency in the smallest premature babies and that may be more difficult to recognize, because the peripheral retinopathy is not as remarkable even though the posterior pole vascular abnormalities of dilation and tortuosity are quite marked



• **Fig. 108.10** Fundus photograph showing the ridge between vascularized and avascular retina characteristic of stage 2 retinopathy of prematurity (large arrow). Stage 3 retinopathy of prematurity is present in the left-hand portion of the photograph (small arrow).

(International Committee for the Classification of Retinopathy of Prematurity, 2005).

Prevalence and Incidence of Retinopathy of Prematurity

Large natural history studies have shown that, in most cases, ROP begins at 31 to 33 weeks' PMA (Fielder et al., 1992; Good et al., 2005) with progression during the next 2 to 5 weeks. Spontaneous regression occurs in eyes with stage 1 and stage 2 ROP and early stage 3 ROP (Palmer et al., 1991; Repka et al., 2000). Blindness or severe visual impairment commonly results from progression of the retinopathy to retinal detachment or severe distortion of the posterior retina.

ROP occurs in most babies with birthweights less than 1500 g (very low birth weight [VLBW]), with an even greater proportion of babies developing ROP in the less than 1000 g birthweight category (extremely low birth weight, ELBW) and in the less than 750 g birthweight category (Fielder et al., 1992; Good et al., 2005). As neonatal services continue to improve, a greater proportion of VLBW and ELBW babies survive, with a resultant increase in the population of babies at risk (Gilbert et al., 2005).

Pathogenesis of Retinopathy of Prematurity

ROP develops as a result of poor retinal vascularization, leading to retinal hypoxia and pathologic neovascularization. With premature birth, relative hyperoxia, exacerbated by exogenous oxygen supplementation, damages existing retinal blood vessels and suppresses retinal secretion of vascular endothelial growth factor (VEGF), a hypoxia-induced vasoactive molecule responsible for normal and pathologic blood vessel development and growth in the body (Pierce et al., 1996; Hellstrom et al., 2001; Smith et al., 2003, 2008). Premature birth also results in a loss of maternal insulin-like growth factor (IGF)-1, a somatic growth factor secreted by the liver but derived primarily from maternal sources until the third trimester (Hellström et al., 2003). As retinal development progresses, relative hypoxia ensues, but VEGF-induced vessel growth is suppressed by low serum levels of IGF-1 (Hellstrom et al., 2001). Finally, as the infant's innate production of IGF-1 rises after 30 to 33 weeks' PMA, heightened local concentrations of VEGF are activated, causing pathologic neovascularization.

Prediction of Retinopathy of Prematurity

Following from the pathologic sequence described previously, important risk factors for ROP include the degree of prematurity (early gestational age at birth and low birth weight); hyperoxia early in the postnatal course, especially from excessive oxygen supplementation; and low postnatal serum IGF-1 level in the postnatal period up to 33 weeks' PMA (Perez-Munuzuri et al., 2010). Slow postnatal weight gain is a reliable surrogate measure for serum IGF-1 levels (Hellstrom et al., 2001; Hellström et al., 2009, 2010). Numerous other factors have been associated with ROP, such as sepsis, acidosis, nutritional deficiencies, and necrotizing enterocolitis; however, many of these factors may influence serum IGF-1 levels in a common mechanistic pathway (Löfqvist et al., 2006).

Current screening guidelines focus solely on gestational age at birth and birthweight as predictors of ROP. Because slow postnatal weight gain is highly correlated with IGF-1 levels and has been shown to be a reliable predictor of subsequent ROP development (Löfqvist et al., 2006, 2009a; Wu et al., 2010; Binenbaum et al., 2011), multiple postnatal weight gain predictive models have been developed in an effort to improve the efficiency of ROP screening in highly developed NICU systems. These models include WINROP (Weight, Insulin-like growth factor-I, Neonatal, ROP) (Wu et al., 2012; Lundgren et al., 2013), ROPScore (Eckert et al., 2012), the Premature Infants in Need of Transfusion ROP model (Binenbaum et al., 2011), the Children's Hospital of Philadelphia ROP model (Binenbaum et al., 2012), and the Colorado ROP model (Cao et al., 2016). All of these models have exhibited very high sensitivity for predicting ROP, while reducing the number of infants who would have required examinations when applied in retrospective studies. Soon, slow postnatal weight gain could be incorporated into current screening guidelines if the ongoing larger validation studies provide precise estimates of sensitivity for predicting severe ROP (Binenbaum et al., 2013).

Prevention of Retinopathy of Prematurity

Medical treatments designed to prevent ROP hold promise to markedly decrease development of serious ROP. Interventions such as steroids given to expectant mothers immediately before preterm birth and surfactant administered to the neonate shortly after birth decrease the incidence of respiratory distress syndrome, which would be expected to decrease the incidence of serious ROP. However, these medical advances have also increased the survival rate of VLBW babies (Soll, 2000). Currently there are no interventions that have been proven to prevent the development of severe ROP in VLBW infants. Strict regulation of the use of supplemental oxygen in the first few weeks of life has been recommended as a strategy to reduce the incidence of severe ROP; however, this can result in increased morbidity and mortality. In an effort to determine the optimal oxygen saturation target for premature infants, several randomized clinical controlled trials have been conducted recently: the Surfactant, Positive Pressure, and Oxygenation Randomized Trial (SUPPORT) in the United States, the Canadian Oxygen Trial (COT), and the Benefits of Oxygen Saturation Targeting II (BOOST II) trial conducted in the United Kingdom, Australia, and New Zealand. In a metaanalysis of these three trials, the risk of developing severe ROP was not significantly different between the restricted oxygen group (SpO₂ targets of 85%–89%) and the liberal oxygen group (SpO₂ targets of 91%–95%) (risk ratio [RR] 0.72, 95% confidence interval [CI] 0.50–1.04); however, sensitivity analysis using a fixed-effect model (where BOOST II had a larger weight assigned) moved the estimated effect in favor of restricting

oxygen use (RR 0.75, 95% CI 0.63–0.88). Higher frequencies of necrotizing enterocolitis and death before hospital discharge were, however, found in the restricted oxygen group. The study authors concluded that the level of certainty for the ROP outcome is low, and there is still significant uncertainty about the optimal target range for SpO₂ (Manja et al., 2015).

Vitamin E supplementation, dietary supplementation with omega-3 polyunsaturated fatty acids, systemic and topical beta-blockers and intravenously administered IGF-1 and IGF-binding protein are currently under investigation (Raju et al., 1997; Löfqvist et al., 2009b; Bührer, 2015).

Detection of Serious Disease

Because the benefit of treatment of serious ROP has been shown in randomized clinical trials (*Cryotherapy for Retinopathy of Prematurity Cooperative Group*, 1990, 1991, 2001; *Early Treatment for Retinopathy of Prematurity Cooperative*, 2003), it is essential to identify the at-risk baby so that timely examinations can be performed to prevent blindness or at least decrease its likelihood. In the United States the recommended guidelines for detection of serious ROP indicate that diagnostic examinations should be performed on infants with birthweights less than or equal to 1500 g or of 30 weeks' gestation, along with babies in the 1501–2000-g birthweight group thought to be at high risk by the neonatologist (Reynolds et al., 2002; Fierson et al., 2013). In Latin American countries and in urban centers of newly industrializing countries in Asia and eastern Europe, the same screening criteria do not apply, because evidence suggests that larger, older babies are also at risk in these settings, and national or regional guidelines need to be developed (Azad and Chandra, 2003; Trinavarat et al., 2004; Fortes Filho et al., 2007; Vedantham, 2007; Varughese et al., 2008).

The first examination should generally occur at 31 weeks' PMA or chronologic age of 4 weeks, whichever occurs later (Reynolds et al., 2002; American Academy of Pediatrics Section on Ophthalmology et al., 2013). Examinations usually continue on an every-other-week basis unless ROP develops, at which time examination frequency may be increased depending on the severity of the disease. In general, examinations continue until ROP is observed to regress, ROP progresses to treatment severity, or vessels are observed on at least two occasions to have progressed into zone III in the absence of ROP. Coordination of this schedule among neonatal care providers, ophthalmologists, nursing staff, and parents is essential. If outpatient appointments are not kept or proper information is not conveyed when the baby is transferred to another facility, potentially treatable disease may be missed, with disastrous consequences (Mills, 2009).

Telemedicine and Retinopathy of Prematurity

Although direct examination by an ophthalmologist experienced in examining babies at risk of ROP continues to be the optimal standard of care, retinal digital imaging has emerged as a promising modality for ROP screening. The increased workload because of the increase in survival of premature infants worldwide (Gilbert et al., 2005) coupled with the reduced availability of ophthalmologists with sufficient ROP experience has required the development of alternative screening approaches. The published sensitivity of digital fundus imaging as a screening technique for ROP ranges from 57% to 100% (with wide 95% CIs) when compared with the reference standard (indirect ophthalmoscopy). In 2015 the American Academy of Pediatrics and the American Academy of Ophthalmology published a review of the current status of telemedicine in ROP (Fierson et al., 2015) and concluded that retinal

digital imaging is a reasonable alternative to but not a substitute for direct examination by an on-site ophthalmologist. However, more studies are needed to determine the overall validity of the approach.

Notwithstanding the lack of consensus, ROP telemedicine is becoming increasingly available in the United States and other countries. The Stanford University Network for Diagnosis of Retinopathy of Prematurity (SUNDROP) has reported its experience evaluating infants meeting ROP screening criteria with images taken in the NICU by trained nurses and remotely evaluated by an ophthalmologist. In this program all infants undergo one direct examination after discharge. In a recent report of 608 infants examined during a 6-year period, sensitivity was reported as 100%, with specificity and a negative predictive value of 99.8% and 100%, respectively (Wang et al. 2015). Comparable results were reported by Weaver and Murdock (2012) on infants imaged in rural Montana where there was no ophthalmologist within 200 miles of the hospital. As in the SUNDROP program, all infants who underwent telemedicine evaluations also underwent an outpatient diagnostic examination within 2 weeks of discharge.

Recently, the results of the National Eye Institute of the NIH-sponsored Telemedicine Approaches to Evaluating Acute-Phase ROP (e-ROP) study were released. This is the largest study to date, with more than 1200 infants with birthweights less than 1251 g enrolled (Quinn et al., 2014). In e-ROP, nonphysicians obtained a series of wide-field images from each eye using the RetCam Shuttle (Clarity Medical System, Pleasanton, California, USA). Image sets were then uploaded to a central server for grading by nonphysician readers who had undergone extensive training (Daniel et al., 2015). The sensitivity of detecting ROP that warranted referral on image grading was 90% (95% CI 85%–94%), the specificity was 87% (95% CI 84%–90%), and the negative predictive value was 97.3% in this study. The reported adverse event rate (apnea, bradycardia, emesis, and reintubation shortly after e-ROP procedures) was noted in 4.9% of infants and 0.8% of study procedures. Adverse events were more likely to occur when the infant was receiving mechanical ventilation, regardless of whether the procedure was an eye examination or retinal imaging.

Other reports have focused on the logistics of the implementation of ROP telemedicine in various settings throughout the world. The Karnataka Internet Assisted Diagnosis of Retinopathy of Prematurity model provides ROP screening in more than 80 units in the Karnataka region of India, using technicians who obtain and grade the images at the bedside and report the need for an evaluation to an ophthalmologist, who can then give treatment if needed (Vinekar et al., 2014). This model has demonstrated that ROP screening services can be provided in regions of the world with limited access, but it is still highly dependent on the availability of ophthalmologists to provide treatment and high levels of training and technical expertise. Gilbert et al. (2016) have stressed the need to shift the emphasis on examination to remote imaging in low- and middle-income regions of the world where ROP remains a leading cause of childhood blindness yet the expertise, availability and physical resources for adequate ROP screening are quite limited. In these countries this approach has the potential to expand services dramatically as increasingly small birthweight and low gestational age infants survive worldwide. In more developed healthcare systems the potential role of telemedicine as a stand-alone screening tool is currently undergoing intensive scrutiny, highlighting a number of complex issues for implementation (limited number of available imaging systems, high cost of equipment; personnel training

requirements, quality control issues, coordination of services, accountability, etc.) that should be resolved before a successful and safe implementation of such a screening modality.

Treatment of Retinopathy of Prematurity

Laser ablation of the peripheral avascular retina can prevent progression to blinding disease in patients with severe ROP and is currently the standard of care for treatment. The evidence of treatment effectiveness and the indications for treatment (severity level) have evolved through the last 30 years as multiple multicenter clinical trials have been conducted to evaluate different treatment modalities and preventative strategies. Landmark studies include the Cryotherapy for Retinopathy of Prematurity (CRYO-ROP) study, the Early Treatment for Retinopathy of Prematurity (ET-ROP) study, the Supplemental Therapeutic Oxygen for Prethreshold Retinopathy of Prematurity (STOP-ROP) study, the Effects of Light Reduction on Retinopathy of Prematurity study, and the Bevacizumab Eliminates the Angiogenic Threat for Retinopathy of Prematurity (BEAT-ROP) study.

Current treatment guidelines are based on the findings of the ET-ROP study, so treatment intervention is recommended on the development of “type 1” prethreshold ROP, defined as *any stage with plus disease in zone I, stage 3 without plus disease in zone I, or stage 2 or 3 with plus disease in zone II*. It is important to highlight that the number of clock hours of disease previously used in the CRYO-ROP definition of “threshold” is not considered anymore, and treatment should not be delayed until “threshold” ROP is reached.

Today, in most centers, laser photocoagulation is preferred because of its advantages over cryotherapy, including less discomfort intraoperatively and postoperatively, less pigmentation resulting from the therapy, and direct visualization of the area during treatment. For advanced disease (i.e., retinal detachment, stages 4 and 5), studies have shown mixed results, but treatment is in general much less successful (Jabbour et al., 1987; Hirose et al., 1993; Trese, 1994; Andrews et al., 1999). For eyes with total detachment, good functional outcome is not likely to be achieved (Quinn et al., 1991; Hirose et al., 1993; Quinn et al., 1996). Laser treatment concerns include the risk of intubation and sedation frequently required for the procedure, the prevalence of high myopia and peripheral visual field reduction, and poor visual acuity outcomes in patients with posterior or aggressive ROP.

In the past decade there has been increased interest in the use of anti-VEGF drugs for the treatment of ROP. The effectiveness of these drugs, reported in multiple observational studies, was confirmed in a multicenter randomized controlled trial (Mintz-Hittner et al., 2011), where intraocular bevacizumab injection was shown to result in a lower retreatment rate at 54 weeks’ PMA than laser photocoagulation in babies with zone I and posterior zone II type 1 ROP (6% vs 42% in the zone I subgroup). As a result, intravitreal injections of bevacizumab and other anti-VEGF agents (e.g., ranibizumab, aflibercept) are being considered more and more often as a first-line treatment alternative, in particular for eyes with zone I ROP because laser photocoagulation is relatively ineffective in decreasing retinal detachment in those eyes. At the 6-year outcome of the ET-ROP study, more than 30% of zone I type 1 eyes had visual acuity of 20/200 or less, and more than 20% had macular folds or retinal detachments. The BEAT-ROP study reported that at 2.5 years children treated with bevacizumab have a significantly lower incidence of high myopia than those treated with laser ablation. Very high myopia occurred in 3.8% of zone I eyes and 1.7% of zone II eyes that received bevacizumab

compared with 36.4% of zone I eyes and 51.4% of zone II eyes that received laser treatment ($P < .001$) (Geloneck et al., 2014). However, visual function outcomes have not been reported yet, and long-term systemic effects have not been adequately studied. The concerns over the ocular and systemic antiangiogenic consequences of these drugs are based on the fact that VEGF is a critical factor in neurodevelopment as well as vasculogenesis in other developing organ systems, including the lungs and kidneys. It has been shown that systemic absorption of the medication after intraocular injection is significant and leads to a reduction in systemic VEGF levels, lasting at least 60 days after injection.

The BEAT-ROP (bevacizumab) trial was too small to assess safety and effects on future development of brain and other tissues. In addition, subsequent reports described late and sometimes severe recurrences with the use of bevacizumab monotherapy that might not have been captured in the original trial. Lepore et al. (2014) reported vascular and macular abnormalities detected on fluorescein angiogram 9 months after ROP treatment with bevacizumab. More recently, reports from Canada and Taiwan described increased risk of motor impairment in ROP patients treated with bevacizumab (Lien et al., 2016; Morin et al., 2016; Quinn and Darlow, 2016). In the Canadian study, the authors attempted to control for many possible factors that could affect neurocognitive development, such as SNAP II scores, bronchopulmonary dysplasia, sepsis, intraventricular hemorrhage, gestational age, and maternal education, and found that at age 18 months, bevacizumab-treated infants were two to three times more likely to display unfavorable developmental outcomes compared with those that underwent laser ablation. However, these studies are still limited by their observational retrospective nature, which may not adequately control for other unknown possible confounders. It is acknowledged that ROP in itself is an independent predictor of adverse long-term neurodevelopment, and in many instances the indication for anti-VEGF treatment over laser ablation is confounded by the severity of disease or the underlying systemic condition of the infant (confounding by indication). Nevertheless, this early evidence increases the concern regarding the long-term safety of anti-VEGF treatment and calls for more research in this area. When one is considering all the treatment options, careful consideration and a thorough discussion with the parents and neonatology team are recommended. None of the anti-VEGF drugs have been approved by the US Food and Drug Administration for the treatment of ROP, and therefore detailed consent for their off-label use must be obtained. Premature infants treated with anti-VEGF injections must be followed up very closely for extended periods, at least until complete retinal vascularization is observed.

Common Ophthalmic Manifestations of Systemic Diseases

Tables 108.3–108.5.

Role of the Neonatal Healthcare Provider

Neonatal and primary pediatric healthcare providers clearly play a central role in the ophthalmologic care of the newborn and young infant. The first few weeks after birth constitute a critical period of visual development in the brain, and the opportunity and responsibility to screen the newborn for ocular disease rests in the hands of those providers, whether neonatologist, pediatrician, family physician, or nurse practitioner. With use of the I-ARM

**TABLE
108.3****Ophthalmic Manifestations of Systemic Diseases With Neonatal Findings**

| Etiologic Disorder | Inheritance | Ophthalmic Manifestations |
|---|---|--|
| Aicardi syndrome | X-linked dominant disorder characterized by triad of callosal agenesis, infantile spasms, and chorioretinal lacunae OMIM 304050 | Bilateral or unilateral, multiple, chorioretinal lacunae (most constant feature), optic disk/retinal/iris colobomas, optic nerve head hypoplasia, microphthalmos, retinal detachment (Aicardi, 2005) |
| Alagille syndrome (1 and 2) | Autosomal dominant disorder characterized by neonatal jaundice secondary to hepatic cholestasis OMIM 118450 and 610205 | Posterior embryotoxon, optic disc drusen, and retinal pigmentary changes sometimes in associations with rod–cone dystrophy |
| Albinism | Heterogenous disorder of melanin metabolism associated with abnormal development of the retina and visual pathways Ocular forms: type 1 (X-linked) Oculocutaneous forms: types 1–4, Hermansky–Pudlak and Chediak–Higashi syndromes (autosomal recessive, autosomal dominant) More than 13 genes involved | Reduced vision, delayed visual maturation, nystagmus, strabismus, iris transillumination, fundus hypopigmentation, foveal hypoplasia, misrouting of optic nerve fibers, anomalous optic chiasm |
| CHARGE syndrome (coloboma, heart defect, choanal atresia, retarded growth, genital hypoplasia, and ear anomalies) | Nonrandom cluster of congenital abnormalities Mutation of <i>CHD7</i> on 8q12.1 (Vissers et al., 2004 ; Jongmans et al., 2006) OMIM 214800 | Unilateral or bilateral uveal colobomas (retinal more frequent than iris), microphthalmos, Bell palsy |
| 22q11.2 deletion syndrome | Contiguous gene deletion syndrome (encompasses DiGeorge syndrome, velocardiofacial syndrome, conotruncal anomaly–face syndromes) Autosomal dominant | Posterior embryotoxon, tortuous retinal vessels, eyelid hooding, strabismus, ptosis, sclerocornea (Forbes et al., 2007 ; Binenbaum et al., 2008) |
| Down syndrome | Trisomy 21 OMIM 19605 | NLDO, refractive errors, nystagmus, strabismus, retinal abnormalities, keratoconus, cataracts (Creavin and Brown, 2009) |
| De Morsier syndrome (septo-optic dysplasia) | Heterogeneous disorder defined by the combination of optic nerve hypoplasia, pituitary dysfunction, and midline abnormalities of the brain (absence of the corpus callosum and septum pellucidum) OMIM 182230 | Optic nerve hypoplasia (unilateral or bilateral), strabismus, nystagmus, optic chiasm hypoplasia. Severe cases may have microphthalmos or anophthalmos |
| Galactosemia (classic) | Metabolic disorder caused by mutation in the galactose 1-phosphate uridylyltransferase gene OMIM 230400 | Congenital cataracts (“oil-droplet cataracts”) |
| Goldenhar syndrome (oculoauriculovertebral dysplasia, hemifacial microsomia) | Craniofacial birth defect involving first and second branchial arch derivatives OMIM 164210 | Epibulbar dermoid (unilateral or bilateral), coloboma of the upper lid, ptosis, strabismus, microphthalmos, nasolacrimal duct obstructions |
| Homocystinuria | Autosomal recessive metabolic disorder caused by cystathionine β -synthetase deficiency OMIM 236200 | Progressive ectopia lentis (lens subluxation), pupil block glaucoma, progressive myopia, optic atrophy, retinal detachment (Harrison et al., 1998) |
| Kabuki syndrome | Congenital mental retardation syndrome OMIM 147920 | Long palpebral fissures with eversion of the lateral third of the lower eyelids, ptosis, strabismus |
| Möbius sequence | Sporadic disorder characterized by congenital facial weakness and abduction deficits OMIM 157900 | Abnormal tearing, esotropia with limited abduction (unilateral or bilateral CN VI palsy), horizontal gaze palsy, other CN palsies (III, IV, V), ptosis |
| Myasthenic syndromes (congenital and neonatal myasthenia gravis) | Neuromuscular disorders. The congenital form is a nonautoimmune autosomal dominant disorder. The neonatal form is secondary to maternal antibodies | Bilateral or unilateral ptosis, any pattern of strabismus, limited ocular motility |
| Incontinentia pigmenti (Bloch–Sulzberger syndrome) | X-linked dominant disorder with characteristic skin lesions OMIM 308300 | Strabismus, nystagmus, cataracts, optic atrophy, corneal abnormalities, retinovascular abnormalities (may resemble ROP), retinal detachment |

Continued

**TABLE
108.3****Ophthalmic Manifestations of Systemic Diseases With Neonatal Findings—cont'd**

| Etiologic Disorder | Inheritance | Ophthalmic Manifestations |
|---|--|--|
| PHACES syndrome (<i>posterior fossa</i> brain malformations, <i>hemangiomas</i> of the face, <i>arterial cerebrovascular anomalies</i> , <i>cardiovascular anomalies</i> , <i>eye anomalies</i> , <i>sternal defects</i>) | Heterogeneous associations that occur in patients with large segmental cervicofacial hemangiomas OMIM 606519 | Microphthalmos, Horner syndrome, retinal vascular abnormalities, optic nerve atrophy, iris hypoplasia, cataracts, sclerocornea, lens coloboma, strabismus, choroidal hemangiomas, congenital third nerve palsy, morning glory deformity, and glaucoma (Coats et al., 1999; Hartemink et al., 2009) |
| Stickler syndrome (hereditary arthro-ophthalmopathy) (1 and 2) | Autosomal dominant disorder of collagen synthesis OMIM 108300 and 604841 | Congenital myopia, vitreous abnormalities, cataracts, retinal detachment |
| Trisomy 13 | Chromosomal abnormality most consistently associated with severe ocular defects | Anophthalmos, cyclopia, microphthalmos, uveal colobomas, cataracts, corneal opacities, retinal dysplasia, intraocular cartilage |
| Trisomy 18 | Chromosomal abnormality with frequent ocular defects | Microphthalmos, short palpebral fissures, ptosis, hypertelorism, iris coloboma, corneal opacities, cataracts |
| Tuberous sclerosis | Autosomal dominant multisystem disease characterized by hamartomatous growths in multiple organs OMIM 191100 and 605284 | Retinal hamartomas, vitreous hemorrhage, chorioretinal punched-out lesions, papilledema, optic nerve atrophy, strabismus (Rowley et al., 2001) |

CN, Cranial nerve; NLDO, nasolacrimal duct obstruction; OMIM, Online Mendelian Inheritance in Man; ROP, retinopathy of prematurity.

**TABLE
108.4****Eye Manifestations of Intrauterine and Perinatal Infections**

| Infection | Ophthalmic Findings | Diagnosis |
|--|--|--|
| Toxoplasmosis (<i>Toxoplasma gondii</i>) | Retinitis, vitritis, choroiditis and anterior uveitis; flat atrophic retinal scars | Present in 75% of newborns with toxoplasmosis, 10% have only ocular findings |
| Syphilis (<i>Treponema pallidum</i>) | Chorioretinitis (salt-and-pepper appearance or pseudoretinitis pigmentosa), anterior uveitis, glaucoma, interstitial keratitis | Clinical ELISA Positive IgM assay result (maternal IgM does not cross the placenta) |
| Rubella | Nuclear cataracts, glaucoma, uveitis, microphthalmos, retinopathy with salt-and-pepper appearance, pseudoretinitis pigmentosa | Bilateral interstitial keratitis is the classic ophthalmic finding; it occurs in 10% of patients but manifests itself later in childhood or adulthood. |
| Cytomegalovirus infection | Retinochoroiditis, optic nerve atrophy, microphthalmos, cataracts, uveitis, strabismus (Coats et al., 2000) | 50% of children with rubella have ocular findings, bilateral 70% Severe postoperative inflammation after cataract surgery |
| Herpesvirus infection (HSV 1 and 2, EBV) | Vesicular skin lesions, keratoconjunctivitis, retinochoroiditis, cataracts | Most common intrauterine viral infection in the United States Only 10% of infants show clinical features of CMV infection, with ophthalmologic abnormalities in less than 30% of these. |

CMV, Cytomegalovirus; EBV, Epstein-Barr virus; ELISA, enzyme-linked immunosorbent assay; FTA-ABS, fluorescent treponemal antibody absorption; HSV, herpes simplex virus; IgM, immunoglobulin M; PCR, polymerase chain reaction; VDRL, Venereal Disease Research Laboratory.

TABLE 108.5 Eye Findings After In Utero Exposure to Teratogens

| Teratogen | Ophthalmic Manifestations |
|--|---|
| Alcohol (fetal alcohol syndrome) | Short palpebral fissures, microphthalmos, epicanthal folds, optic nerve head hypoplasia, tortuosity of retinal vasculature, strabismus, cataracts (Strömland and Hellström, 1996) |
| Cocaine | Optic nerve abnormalities, delayed visual maturation, ROP-like fundus abnormalities (Teske and Trese, 1987), prolonged eyelid edema (Good et al., 1992) |
| Anticonvulsants (fetal hydantoin syndrome) | Ptosis, trichomegaly, hypertelorism, strabismus, retinal coloboma, microphthalmos, optic nerve head hypoplasia (Hoyt and Billson, 1978; Hampton and Krepostman, 1981) |
| Methadone | Nystagmus, delayed visual development, abnormal VEPs at birth (McGlone et al., 2013, 2014) |
| Warfarin and coumadin | Optic atrophy, cataracts, anterior segment dysgenesis, microphthalmos (Hall et al., 1980) |
| Thalidomide | Strabismus, Duane syndrome, ptosis, paradoxical gustulacrimal tearing, Möbius sequence (Miller et al., 2009) |
| Misoprostol | Möbius sequence (Miller et al., 2009) |

ROP, Retinopathy of prematurity; VEPs, visual evoked potentials.

framework as a guide, referral to a pediatric ophthalmologist should be made for any abnormality or asymmetry in an atomic structure or visual function. Some conditions require a particularly urgent referral, such as an abnormal red reflex, cloudy cornea, and ocular infection or trauma. Identification of a sight-threatening cataract or life-threatening retinoblastoma is most commonly made by an astute pediatric clinician in the NICU, nursery, or primary care office who carefully evaluates the red reflex in both eyes. In the case of neonatal conjunctivitis, diagnostic cultures and treatment should be undertaken without delay, but ophthalmology consultation is still necessary to rule out corneal or intraocular involvement. Premature infants at risk of ROP are a particularly vulnerable population. It is the neonatologist's clinical and medicolegal responsibility to arrange timely diagnostic ROP examinations in the NICU and to communicate to parents the absolute necessity of keeping ophthalmologist outpatient appointments after the infant has been discharged from the hospital. Such appointments should be scheduled before discharge. A delay in care by even 1 week could result in a disastrous visual outcome.

The neonatal healthcare and primary care pediatric teams should provide ongoing eye-related education and support. Reducing the risk of pediatric AHT is one important topic to be discussed with all parents and caregivers. Viewing of educational videos on shaken baby syndrome is mandatory in an increasing number of municipalities, and reductions in AHT incidence have been demonstrated. Pediatricians should discuss crying and parental stress at office visits during the first few months of life; the Period of PURPLE Crying educational materials (<https://www.dontshake.org/purple-crying>) are very useful in this regard. Pediatricians can also provide support for the families of children with visual impairment, ensuring early, anticipatory referral to state commissions for the blind and

early intervention services, and ongoing encouragement for parents to maximally utilize such resources when they are available.

Rapid visual development occurs in the months following the neonatal period. Primary care eye examinations and vision assessments continue to be vital for the detection of conditions that may result in visual impairment or blindness, lead to problems with school or social performance, signal the presence of a serious systemic disease, or threaten the child's life. Children should have an eye examination at each well-child visit. Children at high risk of eye disease should be referred for examination by a pediatric ophthalmologist, including children with a history of prematurity or metabolic or genetic diseases; significant developmental delay or neurologic problems; systemic diseases associated with eye abnormalities; a family history of retinoblastoma, childhood cataracts or glaucoma, inherited retinal disorders, or blindness in childhood; and those whose parents needed glasses at a very young age.

Suggested Readings

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Ear and Hearing Disorders

BETTY VOHR

KEY POINTS

- Hearing loss is one of the most common congenital abnormalities, occurring in approximately 1.5–2 per 1000 newborns.
- Hearing loss may be present at birth or have delayed onset.
- A comprehensive workup is indicated to identify if the etiology is related to genetic or nongenetic causes and whether the hearing loss is progressive or associated with other abnormalities.
- Early diagnosis and intervention within the first 6 months of life provide the best opportunity for normal language development.

Normal Hearing

To understand the spectrum of permanent and transient hearing disorders that can be identified, it is necessary to understand the normal hearing pathway, which consists of transmission of sound energy from the environment via the outer ear through the middle ear, inner ear, auditory nerve, brainstem, and, finally, the temporal lobe cortex, where the sound's electrical energy is interpreted as language (Table 109.1). Dysgenesis, disruption, or injury at any point in the pathway may result in a hearing loss that can have its onset in fetal life or during childhood. The auditory pathway develops embryologically very early in gestation. The inner ear develops first, at about 22 days, and is derived from the otic placodes on either side of the developing head. The middle ear and ossicles derive from the first and third pharyngeal pouches and begin to develop at about day 33. The outer ear develops from the dorsal portion of the first pharyngeal cleft. As a result, congenital hearing loss is often seen in conjunction with structural abnormalities of the head, eyes, ears, nose, and throat. In fact, the odds of having a hearing loss is significantly increased if a preauricular skin tag or pit are present (Roth et al., 2008). Atresia or microtia of the pinna are also strongly associated with a permanent hearing loss. Table 109.2 shows the area of pathology associated with the types of hearing loss. A standard medical assessment for every newborn/child should include both a family history of hearing loss and a comprehensive physical examination for minor or major congenital abnormalities and syndromes associated with hearing loss (Wang et al., 2001). The primary provider should be familiar with risk factors associated with hearing loss, particularly a family history of permanent hearing loss. Approximately 50% of children with permanent hearing loss, however, will not have associated stigmata. Every physical examination in childhood should include an evaluation for the presence of transient middle ear fluid. Once

a child is diagnosed with a permanent hearing loss by an audiologist, additional consultation with ophthalmology, otolaryngology, genetics, and developmental behavioral specialists and a parent support group are recommended to ensure that both the hearing loss and associated diagnoses and challenges are addressed (American Academy of Pediatrics, 2007).

Permanent Hearing Loss—The Challenge

Hearing loss is one of the most common congenital abnormalities, occurring in approximately 1.5–2 per 1000 newborns. Subgroups of infants with risk factors for hearing loss, especially those requiring neonatal intensive care unit (NICU) care, are at increased risk of hearing loss (Vohr et al., 2000; Gaffney et al., 2010). When undetected, permanent hearing loss in infants is associated with significant delays in language development, literacy, and academic success (Yoshinaga-Itano et al., 2001; Moeller et al., 2007; Harrington et al., 2010). Before newborn screening, children in the United States in the 1980s who were deaf or hard of hearing achieved reading comprehension skills at the third grade level at the time of graduation from high school (Schildroth and Karchmer, 1986). A method of early identification trialed in the 1980s was the use of a high-risk register. Physicians would be informed by the birthing hospital of infants who were considered high risk for hearing loss. However, approximately 50% of infants with a permanent hearing loss identified in the newborn period do not have a known risk factor for hearing loss. Newborns were not routinely screened with technology for hearing loss at the time, because there was no effective physiologic method available. This changed after a demonstration project (White et al., 1993) in Rhode Island, funded by the Department of Education and Health and Human Services in 1990–1991, which demonstrated the feasibility of a newborn hearing screening with a new objective physiologic technique, called otoacoustic emissions (OAE). This was followed in 1993 by a National Institutes of Health consensus conference recommending universal newborn hearing screening (NIH Consensus Statement, 1993). Data from the Centers for Disease Control (CDC) indicate that in 2013 more than 97% of newborns were screened in the United States. http://www.cdc.gov/ncbddd/hearingloss/2013-data/2013_ehdi_hsf_summary_e.pdf.

Hearing Disorders

A hearing loss is secondary to interference in the transmission of sound from the outer ear (pinna and ear canal) to the middle ear

TABLE 109.1 Anatomy of the Normal Hearing Pathway

| Structure | Components | Function |
|---|---|--|
| Outer ear | Pinna/auricle, ear canal and the outer layer of the tympanic membrane | Sound waves travel through the air and are conducted through the ear canal to the tympanic membrane where vibrations enter the middle ear. |
| Middle ear | Three ossicles (malleus, incus, and stapes) | Vibrations enter the middle ear and are amplified and transmitted via the ossicles to the fluid within the cochlea (inner ear). Acoustic energy in air is converted to compression waves in fluid in the cochlea. |
| Inner ear | Cochlea Vestibular system | The cochlea converts sound pressure patterns into electrochemical impulses that are passed on to the auditory nerve. A part of the cochlea, the organ of Corti, consists of sensory epithelium and hair cells, which transform fluid waves to nerve signals that regulate balance. |
| Eighth cranial nerve Vestibulocochlear nerve contains both the auditory nerve and the vestibular nerve | Auditory nerve is also called the acoustic nerve or cochlear nerve | The auditory nerve carries auditory sensory information from the cochlea to the brainstem. The vestibular nerve transmits sensory information. |
| Brainstem | Superior olive, to inferior colliculus, to thalamus | Continued transmission of electrical energy on journey to temporal lobes |
| Temporal lobes | Superior temporal gyrus and transverse temporal gyrus (Heschl gyrus) | Sounds are processed and interpreted as language. |

TABLE 109.2 Types of Hearing Loss

| Type | Characteristics |
|--|--|
| Sensorineural | Pathology involving the eighth nerve, outer hair cells, and inner hair cells of the cochlea that impairs neuroconduction of sound energy to the brain stem |
| Permanent conductive | An anatomic obstruction of the outer (atresia) or middle ear (fusion of ossicles) that blocks transmission of sound |
| Neural or auditory neuropathy or auditory dyssynchrony | Pathology of the myelinated fibers of the eighth cranial nerve or the inner hair cells that impairs neuroconduction of sound energy to the brainstem. The function of the outer hair cells remains intact. |
| Transient conductive | Debris in the ear canal or fluid in the middle ear that blocks the passage of sound waves to the inner ear |
| Mixed hearing loss | A combination of sensorineural or neural hearing loss with transient or permanent conductive hearing loss |

(tympanic membrane, ossicles, middle ear space, and eustachian tube opening), the inner ear (cochlea and vestibular system), the central auditory pathway (nerve pathways that transmit to the brainstem and cortex), and the auditory cortex (temporal lobes) where sound information is processed. There are anatomic or neural problems that can develop at any point in this pathway that may interfere with the transmission of sound energy and result in a change in the hearing threshold. There are three types of permanent

hearing loss that may be present in the neonate: sensorineural hearing loss, auditory neuropathy (neural hearing loss), and permanent conductive hearing loss, in addition to transient conductive hearing loss (middle ear fluid or debris in the ear canal). Some infants may have a mixed hearing loss that is a combination of two or more types of hearing loss. Types of permanent and transient hearing loss are shown in [Table 109.2](#).

Degrees of hearing loss are determined using clinical audiograms, which evaluate the softest hearing level (HL) thresholds, in decibels (dB). Thresholds between -10 and $+20$ dB HL are considered to be in the normal range, while thresholds above 20 dB are considered diagnostic for mild (20–34 dB), moderate (35–49 dB), moderately severe (50–64 dB), severe (65–79 dB), and profound (>80 dB) hearing loss. Ear and frequency-specific behavioral response hearing testing using a visual reinforcement audiometry (VRA) protocol (conditioned response) can be accomplished beginning at approximately 5 to 6 months of age if this is consistent with the infant's developmental status. Behavioral testing using conditioned play audiometry can be used in children with developmental ages of 3 to 5 years. The official definition of deafness from the Individuals with Disabilities Education Act is “a hearing impairment that is so severe that the child is impaired in processing linguistic information through hearing, with or without amplification.” *Hard of hearing* refers to a hearing loss where there may be enough residual hearing that an auditory device provides adequate assistance to process speech.

Methods for Newborn Hearing Screening

The current methods readily available for newborn screening do not differentiate permanent hearing loss from transient conductive hearing loss. Since the primary objective of newborn hearing screening is to identify permanent hearing loss, this limitation results in an ongoing challenge of false-positive newborn screens and, at times, a delay in the diagnosis of a permanent hearing loss.

**TABLE
109.3****Comparison of Otoacoustic Emissions and Automated Auditory Brainstem Response Screens**

| Characteristics | PHYSIOLOGIC SCREENING METHODS | |
|---|---|--|
| | Otoacoustic Emissions | Automated Auditory Brainstem Response |
| Measurement | Physiologic response of outer hair cells of cochlea | Response in cochlear and auditory neural function to brainstem |
| Detects sensorineural hearing loss | Yes | Yes |
| Detects auditory neuropathy | No | Yes |
| Detects transient and permanent conductive hearing loss | Yes | Yes |
| OAE threshold for fail | 30–35 dB HL | |
| AABR threshold for fail | | 40–45 dB HL |
| Recommended for NICU screens | No | Yes |
| Recommended for well baby screens | Yes | Yes |

AABR, Automated auditory brainstem response; OAE, otoacoustic emissions; NICU, neonatal intensive care unit.

There are currently two objective, noninvasive physiologic measures that are available and reliable for screening newborns and young infants: OAE, either transient or distortion product, and automated auditory brainstem response (AABR) (Cone-Wesson et al., 2000; Gravel et al., 2000; Norton et al., 2000a, 2000b; Johnson et al., 2005).

There are important differences between the two measures as shown in Table 109.3. OAE measures a physiologic response from the cochlear outer hair cells, while AABR measurements reflect both cochlear status, as well as auditory neural function beyond the cochlea to the brainstem. Thus the AABR response reflects activity from a greater portion of the auditory pathway than does the OAE and will detect auditory neuropathy, whereas OAEs do not. Therefore AABR is recommended for screening in the NICU because of the higher incidence of auditory neuropathy among NICU infants (American Academy of Pediatrics, 2007; Maris et al., 2011). Both methods will screen positive for sensorineural hearing loss and permanent conductive hearing loss. Permanent conductive hearing loss is caused by abnormalities of the ossicular chain or Eustachian tube anatomy. Relative to screen fail rates, AABR is more likely to miss borderline or mild hearing loss because of the higher threshold for a pass result (van Zyl et al., 2009).

In addition, both methods screen false positive for permanent hearing loss in the presence of transient retained fluid in the ear canal or middle ear fluid with transient conductive hearing loss (Levit et al., 2015). Uncertainty also may arise with mixed hearing loss, which is a combination of sensorineural or neural hearing loss in conjunction with transient conductive hearing loss. Therefore although the methods continue to improve, limitations remain, supporting the need for ongoing surveillance by the primary care provider. Hearing loss missed by both methods may include mild hearing loss, hearing loss in an isolated frequency range, progressive hearing loss, and late-onset hearing loss (Johnson et al., 2005; Young et al., 2011). A recent study (Levit et al., 2015) of a large longitudinal cohort in the United Kingdom of over 17,000 neonates born from 2013 to 2014 reported that 24% of infants who did not pass a transient evoked OAE screen but passed an AABR screen were subsequently diagnosed with a hearing loss greater

than 45 dB HL using diagnostic ABR. Although current screening methods have limitations, tremendous headway has been made in lowering the age of diagnosis, and newborn hearing screening is now recommended for all infants before discharge from the birthing hospital. In an effort to facilitate diagnosis of the highest risk infants in a timely fashion, a current recommendation is for NICU infants who do not pass their hearing screen to have a diagnostic ABR performed before discharge. This approach facilitates the current Joint Committee on Infant Hearing (JCIH) early hearing detection and intervention (EHDI) recommendation to screen by 1 month, diagnose by 3 months, and provide intervention services by 6 months of age (American Academy of Pediatrics, 2007).

Risk Factors for Permanent Hearing Loss in Infants and Children (Table 109.4)

Although approximately 50% of infants with an identified hearing loss have a risk factor for hearing loss, 50% do not. Therefore ongoing surveillance and rescreening of all infants/toddlers are recommended, with added diligence to those who pass the screen but have a risk factor. Most birthing hospitals currently report risk factors to the primary care provider. However, it may be incomplete since family history is often reported after discharge, and diagnosis of a congenital syndrome may occur postdischarge. Therefore a complete physical and comprehensive review of history of hearing loss with the family after discharge is recommended for all infants.

Although all infants require ongoing surveillance of auditory and speech language skills, infants who pass the newborn screen but have a risk factor will benefit from enhanced surveillance and follow-up with an audiologist experienced in assessing children by 7–12 months of age. This approach is especially important since it is well documented that the rate of identified childhood deafness and hearing loss increases from approximately 1.2 per 1000 in newborns to 3 per 1000 in early school-age children. In a recent report the prevalence of children confirmed as deaf or hard of hearing by school age was 3.65 per 1000 (Prieve et al., 2015).

TABLE 109.4 Risk Factors for Permanent Hearing Loss

| | |
|--|---|
| Family factors | 1. Caregiver concern regarding hearing, speech, language, or developmental delay (Roizen, 1999) 2. Family history of permanent childhood hearing loss (Morton, 1991; Cone-Wesson et al., 2000; Carey and Palumbos, 2016) |
| Neonatal | 3. Neonatal intensive care of more than 5 days or any of the following regardless of length of stay including ECMO (Fligor et al., 2005) 4. In utero infections, such as CMV, herpes, rubella, syphilis, and toxoplasmosis (Vohr et al., 2000; Boppana et al., 2005; Yamamoto et al., 2011) |
| Stigmata, syndromes, and neurodegenerative disorders | 5. Craniofacial anomalies, including those that involve the pinna, ear canal, ear tags, ear pits, and temporal bone (Wiley et al., 2011) 6. Physical findings, such as white forelock, that are associated with a syndrome known to include a sensorineural hearing loss or permanent conductive hearing loss (Nance, 2003; Wiley et al., 2011; Alford et al., 2014) 7. Syndromes associated with hearing loss or progressive or late-onset hearing loss, such as neurofibromatosis, osteopetrosis, and Usher syndrome (Huang et al., 2012); other frequently identified syndromes including Waardenburg, Alport, Pendred, and Jervell and Lange-Nielson (Nance, 2003; Huang et al., 2012; Carey and Palumbos, 2016) 8. Neurodegenerative disorders, such as Hunter syndrome, or sensory motor neuropathies, such as Friedreich ataxia and Charcot-Marie-Tooth syndrome (Wiley et al., 2011) |
| Neonatal or postneonatal | 9. Culture-positive postnatal infections associated with bacterial and viral (especially herpesviruses and varicella) meningitis (Roizen, 2003; Hille et al., 2007) |
| Postneonatal | 10. Head trauma, especially basal skull/temporal bone fracture that requires hospitalization (Bergemalm, 2003; DeMarcantonio and Choo, 2015) 11. Chemotherapy (Gruss et al., 2012) |

CMV, Cytomegalovirus; ECMO, extracorporeal membrane oxygenation.

Family Factors

Risk Factor 1

Parent or caregiver concern about child hearing, speech, language, or developmental delay has consistently been shown to be a reliable predictor of hearing loss (Roizen, 1999).

Risk Factor 2

A history of family members who are deaf or hard of hearing with onset in childhood (Morton, 1991; Cone-Wesson et al., 2000; Alford et al., 2014; Carey and Palumbos, 2016) has high predictive value and is associated with known genetic causes including connexin 26 mutations. It has been shown that young parents may not be informed of a family history until after their infant is diagnosed with a hearing loss. Monitoring is based on etiology, with a diagnostic evaluation recommended by no later than 7–9 months of age (Nance and Kearsey, 2004; Morton and Nance, 2006; Bush et al., 2014).

Neonatal

Risk Factor 3

Includes infants who require care in the NICU or special care nursery for more than 5 days, which is used as an indicator of illness severity (American Academy of Pediatrics, 2007). This broad category encompasses most other medical risk factors including prematurity, perinatal asphyxia, and a requirement for extracorporeal membrane oxygenation. All infants with hyperbilirubinemia requiring an exchange transfusion, regardless of length of stay, are included in this risk category (Shapiro, 2003; Shapiro, 2005). Use of aminoglycosides for less than 5 days is not a risk factor.

Risk Factor 4

Includes in utero infections. Follow-up of herpes, rubella, syphilis, and toxoplasmosis is recommended by 7–9 months of age.

Cytomegalovirus (CMV) infection is a leading cause of congenital infection occurring in 0.2%–2% of live births worldwide and is a leading cause of nongenetic unilateral or bilateral early, progressive, and delayed-onset sensorineural hearing loss (Yamamoto et al., 2011; Cannon et al., 2014; Ross et al., 2014). The recommendation for CMV infection is longitudinal follow-up and assessment of language and hearing skills by the primary care provider and audiologist.

Stigmata, Syndromes, and Neurodegenerative Disorders

Risk Factors 5 and 6

Craniofacial anomalies, including those that involve the pinna, ear canal, ear tags, ear pits, and temporal bone anomalies (Bergemalm, 2003; Wiley et al., 2011), and physical findings such as white forelock that are associated with a syndrome known to include a sensorineural hearing loss or permanent conductive hearing loss, have a significant amount of overlap, and infants with these findings are at increased risk of neonatal and later onset hearing loss.

Risk Factors 7 and 8

Syndromes associated with hearing loss or progressive or late-onset hearing loss, such as neurofibromatosis, osteopetrosis, and Usher syndrome (Roizen, 2003), and other frequently identified syndromes include Waardenburg, Alport, Pendred, and Jervell and Lange-Nielson. There are more than 400 syndromes and genetic disorders associated with hearing loss (Morton, 1991; Morton and Nance, 2006; Schrijver and Gardner, 2006; Siemerling et al., 2006). Neurodegenerative disorders, such as Hunter syndrome, and sensory-motor neuropathies, such as Friedreich ataxia and Charcot-Marie-Tooth syndrome, are strongly associated with permanent hearing loss (Roizen, 2003). Consultation with a

geneticist is indicated for all infants diagnosed with congenital hearing loss for whom there is not a clear etiology.

Neonatal or Postneonatal

Risk Factors 9, 10, and 11

May occur in the neonatal period or postdischarge.

Risk Factor 9

Culture-positive bacterial and viral (especially herpesviruses and varicella) postnatal infections including meningitis may result in rapid-onset hearing loss (Stevens et al., 2003; Westerberg et al., 2008; Bassler et al., 2009; Cohen et al., 2014). Widespread administration of conjugate vaccines has resulted in decreased rates of *Haemophilus influenzae* type B infection, pneumonia, measles, mumps, rubella, and childhood meningitis; however, encephalitis viral infections (especially herpesviruses) and varicella remain serious risk factors (Cohen et al., 2014). Children with cochlear implants remain at increased risk of postnatal infection (Biernath et al., 2006).

Risk Factors 10 and 11

Head trauma, especially basal skull/temporal bone fractures that require hospitalization, and particularly injury to the mastoid, is associated with trauma-associated hearing loss (Bergemalm, 2003). Hearing loss among pediatric cancer patients as a consequence of treatment with chemotherapy and radiation is common, and these children should be closely monitored for hearing loss (Gruss et al., 2012).

After Newborn Hearing Screening: Audiology Diagnostic Protocols

In 1999 the American Academy of Pediatrics endorsed EHDI, defined as screening by 1 month, diagnosis by 3 months, and appropriate intervention services by 6 months of age. The recommended audiology diagnostic assessment for infants who do not pass the newborn screen includes the following battery of tests (American Academy of Pediatrics, 2007):

1. Click-evoked ABR testing using both condensation and rarefaction single-polarity stimulus, if there are risk indicators for neural hearing loss (auditory neuropathy).
2. OAEs, either distortion product or transient evoked.
3. Tympanometry using a 1000-Hz probe tone.
4. VRA, not until 7–9 months of age.
5. Clinician observation of the infant's auditory behavior may be used as a cross check for the electrophysiologic measures. Behavioral observation alone is not adequate for determining whether hearing loss is present and is not adequate for the fitting of amplification devices.

Identification of an audiologist with skills in pediatric diagnostic assessment can be obtained from the EHDI–pediatric audiology links to services (PALS) website: <http://www.ehdipals.org/>. The report from the audiologist will include the type of hearing loss, whether the loss is unilateral or bilateral, and the degree of hearing loss.

Medical Workup for Hearing Loss

A visit should be scheduled with the family as soon as a diagnosis of hearing loss is made by the audiologist, to discuss the audiologist's report, provide information on community supports and intervention resources, and provide support for the family during a period

of stress. During the visit with the family, the physician reviews the pregnancy, neonatal, and family history for hearing loss and reexamines the child for evidence of any craniofacial abnormalities or a syndrome associated with hearing loss.

The evaluation of all children with a diagnosis of hearing loss or suspected of hearing loss should include a complete physical with thorough examination of the head, eyes, ears, nose, and throat and an evaluation of middle ear status. It is not uncommon for newborns and NICU graduates to have middle ear effusion or retained amniotic fluid, which can cause transient conductive loss and a false-positive screen. Persistence of middle ear fluid in the infant should be monitored and managed in the medical home. In persistent cases of middle ear fluid, myringotomy placement will be necessary to complete a valid diagnosis to rule out a concurrent permanent hearing loss. There is evidence that persistent middle ear fluid identified in the NICU is a risk factor for chronic otitis media with effusion in the first year (Doyle et al., 2004). Knowledge and review of the JCIH (American Academy of Pediatrics, 2007) risk factors of both syndromic and nonsyndromic hearing loss and associated physical abnormalities is essential. Once the diagnosis of a permanent hearing loss is established, it is necessary to discuss the benefits of early intervention (EI) services and amplification. The primary care physician therefore needs to be aware of all community resources and support the family choice of EI program and mode of communication.

Multidisciplinary Team

Consultations are recommended and facilitated with otolaryngology and genetics specialists. Every infant with confirmed hearing loss should be evaluated by an otolaryngologist with knowledge of pediatric hearing loss. The otolaryngologist conducts a comprehensive assessment to determine the etiology of hearing loss and provides recommendations and information to the family, audiologist, and primary care provider on candidacy for amplification, assistive devices, and surgical intervention, including reconstruction, bone-anchored hearing aids, and cochlear implantation.

The otolaryngologist will order required radiographic imaging. Temporal bone imaging is indicated as part of the work-up to aid in identifying the etiology, any malformations that may need surgical intervention, and appropriateness of amplification or cochlear implantation. High-resolution computed tomography is appropriate for assessing the osseous structures of the external auditory canal and middle ear, and magnetic resonance imaging (MRI) is optimal for evaluating soft tissue and cranial nerves.

Because of the prevalence of hereditary hearing loss, all family members of children with confirmed hearing loss should be offered a genetics evaluation and counseling. This evaluation can provide families with information on etiology, prognosis, associated disorders, and recurrence in offspring. The geneticist will review the family history for specific genetic disorders or syndromes and complete genetic testing for syndromes or gene mutations for nonsyndromic hearing loss such as *GJB2* (Connexin 26) (Erbe et al., 2004; Santos et al., 2005; Siemerling et al., 2006; Alford et al., 2014).

Because 30%–40% of children with confirmed hearing loss have developmental comorbidities, developmental milestones should be closely monitored and referrals initiated to developmental specialists for suspected disabilities as needed. It is also recommended that each child with a permanent hearing loss have at least one examination by an ophthalmologist experienced in evaluating infants because of the association of hearing loss with

TABLE 109.5 Characteristics of Modes of Communication

| Mode | Mechanism | Language Goal |
|-------------------------------|--|---|
| Auditory oral communication | The use of residual hearing and amplification with visual support (speech reading) | Spoken |
| Auditory verbal communication | Based on listening skills alone | Spoken |
| Cued speech | Uses visual communication and combines listening with eight hand shapes in four placements near the face | Spoken |
| Total communication | Combines all means of communication and encourages simultaneous use of speech and sign | Spoken and sign |
| American Sign Language | Visual and manual | ASL, English learned as a second language |

ASL, American Sign Language.

vision impairments and the importance of vision for children with hearing loss.

Communication Options

EI providers, including teachers of the deaf, the speech–language pathologist, and audiologist, will provide information to the family on communication options consistent with the child’s diagnosis. The family needs to decide on the communication mode that will work optimally for the child and family. There are five options described in [Table 109.5](#): auditory oral communication (speech reading), auditory verbal communication, cued speech, total communication, and American Sign Language (ASL). Parents may initiate with total communication and change mode subsequently as the child progresses depending on the child’s progress with communication. For example, the family of a child with profound hearing loss may initially choose total communication but, after a cochlear implant at 12 months of age, may use predominantly auditory verbal or auditory oral communication.

Amplification

Hearing Aids

Infants as young as 1 month of age can be fitted with hearing aids. Hearing aids are compact and worn either in-the-ear or behind-the-ear. Components are the microphone that picks up sounds and the amplifier. The audiologist uses computer programming to adjust the sound for an individual child’s needs. If the child has different degrees of hearing loss at different frequencies, the

audiologist adjusts the gain (loudness). The young growing infant needs to be seen by the audiologist approximately every 6 weeks to replace outgrown molds.

Frequency-Modulated Systems

Frequency-modulated (FM) systems were developed to enhance hearing in noisy environments. An FM system consists of a small radio transmitter attached to a microphone and a small radio receiver. A parent, EI provider, or teacher wears the FM transmitter and microphone while the infant/child wears the FM receiver. The FM transmitter sends a low-power radio signal to the FM receiver, which needs to be within 50 feet of the transmitter. The FM receiver receives the signal from the microphone and sends it to a personal hearing aid or cochlear implant. Listening to the FM signal is similar to listening to speech only inches away. Systems can be used in the home, while shopping, or at school.

Cochlear Implants

Children 12 months and older who are deaf or severely hard of hearing are approved by the US Food and Drug Administration to receive cochlear implants. As of December 2012, roughly 58,000 devices had been implanted in adults and 38,000 in children in the United States (<https://www.nidcd.nih.gov/health/cochlear-implants>). It is currently recommended that the child be adequately fitted with hearing aids for 3–6 months before determining implant candidacy. A lack of benefit in the development of auditory skills with amplification using hearing aids needs to be demonstrated to determine eligibility for an implant. There are increasing reports ([Philips et al., 2009](#); [Meister et al., 2015](#)) demonstrating the beneficial effects on speech and language for infants with bilateral profound hearing loss who are implanted before 12 months of age.

Because of an increased risk for bacterial meningitis, it is recommended that physicians monitor all patients with cochlear implants (particularly children whose implants have a positioner) for middle ear and other infections ([Reefhuis et al., 2003](#); [Wilson-Clark et al., 2006](#); [Rubin and Papsin, 2010](#)). *Streptococcus pneumoniae* is the most common pathogen causing meningitis in cochlear implant recipients ([Bluestone, 2003](#); [Centers for Disease Control and Prevention, 2003](#); [Biernath et al., 2006](#); [Glikman et al., 2009](#)). All children with cochlear implants should be vaccinated according to the American Academy of Pediatrics high-risk schedule (<https://www.aap.org/en-us/Documents/immunizationschedule2017.pdf>).

Comprehensive Early Intervention

All infants diagnosed with a permanent hearing loss should be referred and enrolled in EI by 6 months of age to minimize the deleterious effects of communication deprivation ([Moeller, 2000](#); [Yoshinaga-Itano, 2003a, 2003b](#); [Vohr et al., 2011](#)). The JCIH recommends that states should have a single point of entry into intervention specific for children with hearing loss, to ensure that, regardless of geographic location, all families who have infants or children with hearing loss receive information about a full range of options regarding amplification and technology, communication and intervention, and accessing appropriate counseling services ([American Academy of Pediatrics, 2007](#); [Yoshinaga-Itano, 2014](#)).

The JCIH published 12 best practice guidelines for EI programs ([Yoshinaga-Itano, 2014](#)). These include the following:

- The provision of timely referral to EI services with providers who have knowledge and skills in early childhood deafness and hearing loss.
- Infusion within the system of partnerships with parents as well as professionals who are (deaf/hard of hearing).
- Longitudinal developmental assessments for monitoring the child's development.
- Data management systems that include developmental outcomes.
- A process to monitor the fidelity of the intervention.
- Appropriate services for children with additional disabilities, those from non-English speaking families, and those from special populations, including unilateral hearing loss and auditory neuropathy/dyssynchrony.

As the number of bilingual children with hearing loss continues to increase, the question has arisen regarding the optimal way for EI providers to teach speech to children with hearing loss who are bilingual. A number of investigators have reported that children with hearing loss supported in both their home language and the majority culture language compared with children with hearing loss exposed to majority language alone acquired English and the primary home language at similar rates (Waltzman et al., 2003; Guiberson, 2014). Two studies (Bunta and Douglas, 2013; Bunta et al., 2016) investigated the effects of supporting both the home language (Spanish) and the language of the majority culture (English) on language outcomes in bilingual children with hearing loss who used cochlear implants and hearing aids compared with bilingual peers who received English-only support. Bilingual children who received dual-language support outperformed peers who received English-only support as measured by the Total Language and Expressive Communication language age scores of the Preschool Language Scales 4. Because of the high incidence of hearing loss

among Hispanics in the United States, it is likely there will be an increased need for provision of dual-language EI services (Mehra et al., 2009).

In addition, a total communication approach including ASL and speech is recommended at the initiation of EI services to ensure access to language. An expert opinion summary proposes consideration of the use of both sign and spoken language for all young children who are deaf to ensure access to language and linguistic competence. Napoli et al. (2015) reviewed findings demonstrating that sign language development is associated with both written and spoken language development (Knors and Marschark, 2012; Rinaldi et al., 2014).

Neonatal Intensive Care Unit Infants and Hearing Loss

The majority of infants cared for in an NICU have a risk factor for hearing loss. The relationship between risk factors and hearing loss in several studies of NICU infants is reviewed, and summaries are shown in Table 109.6. The study by Robertson et al. (2009) included a cohort of 1279 infants weighing 1250 grams and below or at 28 weeks' gestation screened with AABR followed by audiology diagnostic assessment and followed to 5 years of age. The rate of permanent hearing loss was 3.1%, rate of severe to profound was 1.9%, 28% had progressive hearing loss, and 73% had other disabilities. Risk factors included prolonged oxygen use, gastrointestinal surgery, patent ductus arteriosus ligation, and low socioeconomic status (SES).

Schmidt et al. (2003) published two studies examining the effects of the major neonatal morbidities of bronchopulmonary dysplasia (BPD), major brain injury, and severe retinopathy of

TABLE 109.6 Risk Factors and Rates of Hearing Loss Among Infants Cared for in a Neonatal Intensive Care Unit

| Author | Dates of Birth | Sample | Screen | Age of Diagnostic Follow-Up | Risk Factors for HL | Rates of Hearing Loss | |
|---------------------------|----------------|-----------------------|--------|-----------------------------|--|-----------------------|-------|
| Robertson et al., 2009 | 1974–2003 | ≤28 weeks and ≤1250 g | AABR | 3 years | BPD; gastrointestinal surgery; patent ductus ligation; low SES | All degrees HL | 3.1% |
| | | | | | | Severe to profound | 1.9% |
| Schmidt et al., 2003 | 1996–1998 | 500–1250 grams | AABR | 18 months | 3 major morbidities: BPD, brain injury, and severe ROP | BPD | 3.5% |
| | | | | | | Brain injury | 2.9% |
| | | | | | | Severe ROP | 3.8% |
| Schmidt et al., 2015 | 1996–1998 | 500–1250 grams | AABR | 5 years | # of morbidities: BPD, brain injury, and severe ROP | No risk factor | 1.3% |
| | | | | | | 1 risk factor | 3.6% |
| | | | | | | 2 risk factors | 5.4% |
| | | | | | | 3 risk factors | 25.0% |
| Van Dommelen et al., 2015 | 1998–2012 | <32 weeks | AABR | 2–4 months | Lower weeks of gestational age | 24.0–24.9 | 7.5% |
| | | | | | | 25.0–25.9 | 5.2% |
| | | | | | | 26.0–26.9 | 4.6% |
| | | | | | | 27.0–27.9 | 2.8% |
| | | | | | | 28.0–28.9 | 2.0% |
| | | | | | | 29.0–29.9 | 1.6% |
| | | | | | | 30.0–30.9 | 2.0% |
| | | | | | | 31.0–31.9 | 1.2% |
| Morris et al., 2008 | 2002–2005 | ≤1000 grams | ABR | 18–22 months | Hyperbilirubinemia with aggressive and conservative photo therapy (PT) | Aggressive PT | 1.0% |
| | | | | | | Conservative PT | 3.0% |

AABR, Automated auditory brainstem response; BPD, bronchopulmonary dysplasia; HL, hearing loss; ROP, retinopathy of prematurity; SES, socioeconomic status.

prematurity (ROP) on rates of hearing loss at 18 months in very low-birth-weight infants weighing less than 1250 grams. Rates of hearing loss compared with not having the risk factor were 3.5% versus 1.4% for BPD, 2.9% versus 2.2% for brain injury, and 3.8% versus 2.2% for severe ROP. In the second study children were followed to 5 years of age (Schmidt et al., 2015). Overall, 2% of the 1514 infants weighing between 500 and 1250 grams were described as deaf. Rates of deafness were related to the risk factors of BPD, serious brain injury, and severe ROP. In this report the investigators identified a relationship of rate of hearing loss with an increasing number of risk factors (no risk factors: 1.3%, one: 3.6%, two: 5.4%, and three: 25%).

Van Dommelen et al. (2015) reported on the relationship between gestational age and hearing loss in a large cohort of 18,564 preterm infants. The prevalence of hearing loss consistently increased with decreasing week of gestation from 1.25% at 31 weeks to 7.55% at 24 weeks. The study of Morris et al. (2008) examined the effects of two protocols to provide phototherapy to very preterm infants with hyperbilirubinemia. Compared with the conservative-phototherapy group, the aggressive-phototherapy group had significant reductions in the rate of severe hearing loss, from 3% to 1%. The conclusion was that the benefit to decreased impairment may be offset by the increased mortality in the smallest infants. All of the studies indicate that there are increased rates of hearing loss among NICU infants who are the most preterm and have the highest rates of neonatal morbidities.

The Brain, Language Outcomes, and Access to Language

The most important predictor of improved language outcomes is early age of identification. Age of identification reported in the 1980s and 1990s ranged from 19 months to 31–35 months (Coplan, 1987; Mace et al., 1991). Since 1997 the age of identification in some reports has dropped to 2–4 months (Vohr et al., 2002; Johnson et al., 2005). The National CDC EHDI data for 2013 indicated that 69% of newborns who did not pass the newborn screen and who were diagnosed with a permanent hearing loss were diagnosed by 3 months of age. This rate may in part be due to incomplete reporting but indicates how some parts of the country continue to struggle in achieving the EHDI 1–3–6 goal. (<https://www.cdc.gov/ncbddd/hearingloss/data.html>). Enrollment in EI by 6 months remains a critical EHDI goal as earlier age is consistently associated with improved outcomes for children who are deaf or hard of hearing (DHH).

Yoshinaga-Itano et al. (2001, 2010) have shown that early quality intervention services in the first year of life that provide access to language (either speech or visual [ASL]) improve the language and developmental outcomes for children who are deaf or hard of hearing. The longitudinal study of Moeller (2000) showed in adjusted analyses that enrollment in EI services and greater family involvement predicted higher vocabulary scores on the Peabody Picture Vocabulary test at 5 years of age among DHH children in Nebraska. The most recent CDC data for 2014 indicate that of those diagnosed with permanent hearing loss, 88% were referred to EI, 65% were receiving EI and of those 68% were referred by 6 months of age (<https://www.cdc.gov/ncbddd/hearingloss/data.html>).

The first 3 years of life are particularly critical for access to language since this period is a time of both rapid language and brain growth. In addition, it is not in the ears but the brain where language is processed and understood. During the first 3 years of

life, the brain grows and matures in complexity and connectivity at an incredible rate that is in part mediated by the infant's exposure to language. Sharma et al. (2002, 2005) have shown that earlier implantation of a cochlear implant is associated with more optimal development of the auditory pathway to the auditory cortex (temporal lobes). Children who are deaf and born to deaf parents have natural early exposure to visual language (ASL). However, more than 90% of children born DHH are born to hearing parents and do not have immediate access to either visual language (ASL) or auditory language (speech). Therefore early access to language and EI that meets the communication needs of the family is the key. The studies of Hart and Risley (1995) have shown that the amount and quality of language that all children are exposed to in the home in the first 3 years of life vary significantly, and the greater the input of child-directed quality language, the more optimal the child's language and cognitive outcomes.

Further evidence of the importance of language access comes from the studies of the neuronal maturation of the central auditory pathway (Sharma et al., 2002, 2005). In the absence of typical auditory stimulation, the auditory pathway and cortex remain maximally sensitive and plastic for development for about 3.5 years in response to placement of a cochlear implant. From 3.5 to 7 years response to a cochlear implant drops to 7%–30% and after 7 years to less than 5%. These data identify that the window of opportunity and plasticity for development of the auditory pathway and speech for a child with a severe to profound hearing loss is the first 3.5 years of life. In addition, MRI studies suggest that cross-modal reorganization of the auditory cortex occurs in DHH children without auditory stimulation, in response to visual and somatosensory stimulation. It is suggested that this modification in brain development may facilitate enhanced processing of skills in these domains for DHH (Sharma et al., 2005). Evidence of very early impact has been shown in infants who are DHH exposed to visual language, who develop sign babbling at the rate at which hearing infants develop verbal babbling (Petitto et al., 2004).

A study by Tomblin et al. (2015) on the usage characteristics of amplification identified that a combination of factors, including earlier age of fitting, higher daily duration of amplification, and greater audibility of the device, were all significantly associated with higher early language skills in a cohort of children with mild to severe hearing loss. Another important finding, however, was that children fitted with hearing aids after 18 months of age were able to improve their language abilities as a function of increased duration of hearing aid use. This finding again suggests that brain plasticity and the language learning system remain open to experience provided by increased access to linguistic input during the preschool years. Data from Tomblin et al. (2015) also indicate that the development of language for children with a more severe hearing loss is more challenging than in children with a mild loss. Within a cohort of children divided into mild (25–45 dB HL), moderate (45–60 dB HL), or moderate to severe (>60 dB HL) hearing loss, compared with age- and SES-matched controls, children with moderate to severe hearing loss consistently had the lowest language scores at 2–5 years of age. For all of the HL groups, a greater degree of hearing loss was associated with lower language abilities.

Additional factors shown to contribute to language success in children include enhanced maternal communicative intent (Vohr et al., 2010), higher SES, higher parent education level (Hart and Risley, 1995), less parental stress (Quittner et al., 2010), a more optimal home-language environment (Vohr et al., 2014), and access to deaf role models (Yoshinaga-Itano, 2014).

In summary, EHDI systems of care continue to improve access to timely screening, diagnosis, and EI services to ensure successful outcomes. It is expected that ongoing surveillance and facilitation of individualized coordinated care with the use of the electronic medical record, multidisciplinary partnering and implementation of both local and telemedicine services (Dharmar et al., 2015), and coordination with state EHDI programs will continue to contribute to improved outcomes.

Suggested Readings

- Alford RL, Arnos KS, Fox M, et al. American College of Medical Genetics and Genomics guideline for the clinical evaluation and etiologic diagnosis of hearing loss. *Genet Med*. 2014;16:347-355.
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